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Accumulation and Depuration of Polychlorinated Biphenyls from Field-Collected Sediment in Three Freshwater Organisms

Jordana L. Van Geest,[†] Donald Mackay,[‡] David G. Poirier,[§] Paul K. Sibley,[†] and Keith R. Solomon[†,*]

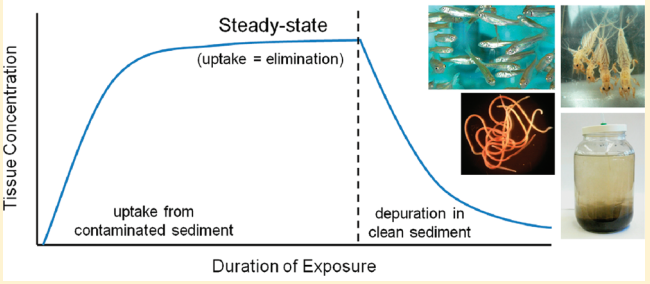
[†]School of Environmental Sciences, University of Guelph, Guelph, Ontario, Canada N1G 2W1

[‡]Environmental and Resource Studies, Trent University, Peterborough, Ontario, Canada K9J 7B8

[§]Laboratory Services Branch, Ontario Ministry of the Environment, 125 Resources Road, Etobicoke, Ontario, Canada M9P 3 V6

Supporting Information

ABSTRACT: As laboratory-based bioaccumulation methods are standardized and expanded to include other test species, kinetic studies assessing the major classes of contaminants with these species are needed to adequately select the standard duration for bioaccumulation tests. In the present study we measured the uptake (28-d exposure) of polychlorinated biphenyls (PCBs; total and selected congeners) from field-contaminated sediment in the oligochaete *Lumbriculus variegatus*, mayfly nymph *Hexagenia* spp., and fathead minnow *Pimephales promelas*. Depuration (25 d) of PCBs was measured in organisms that had been transferred to clean sediment after the 28-d exposure. Uptake and elimination of PCBs was rapid in *L. variegatus* and *Hexagenia* spp. Tissue residues reached steady-state concentrations within 28 d; elimination rates and biota-sediment accumulation factors (BSAFs) of the PCB congeners were not correlated with K_{OW}. Uptake and elimination rates of PCBs were slower in *P. promelas*, and it is not clear whether steady-state was reached in fish tissues. Elimination rates of the PCB congeners significantly decreased with increasing K_{OW} in fish. The appropriateness of a 28-d exposure for measuring steady-state concentrations in tissue of the invertebrates was confirmed, but further study is required for fish.



INTRODUCTION

The exposure duration in bioaccumulation tests with contaminated sediment is a critical aspect of experimental design, as estimates of steady-state concentrations in tissue are necessary to evaluate risks to wildlife and human health.[1] The time to achieve steady-state varies with compound, sediment characteristics, and the metabolic capacity of organisms. Steady-state can be assessed through time-series sampling and has been defined operationally as no significant change in tissue concentration for three successive sampling intervals.[2] Kennedy et al.[3] commented on the inconsistency of this approach due to the influence of selection of sampling time points, statistical methods, and analytical detection limits. Twenty-eight days has been recommended as a standard exposure period for sediment tests with benthic invertebrates as this typically results in tissue residues within 80% of steady-state concentrations.[2,4] This duration was also recommended in water-only bioconcentration tests with fish and mollusks.[5] However, uncertainty remains concerning how representative a 28-d exposure is of steady-state conditions, particularly for higher K_{OW} compounds and in organisms like fish, not in direct contact with sediment.[3,4]

A kinetic approach can be used to determine uptake and elimination rates and predict steady-state concentrations in tissue using a compartment-based model. Studies have estimated these kinetic parameters for different compounds in benthic invertebrates by fitting uptake data to an appropriate model.[6−9] The elimination rate, considered the key factor in determining the time to steady-state for a compound in an organism, is best determined from depuration studies where tissue residues are directly measured in previously exposed organisms following their transfer to a clean system.[10] Other studies have measured both uptake and depuration in invertebrates for polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, hexachlorobiphenyl, and dichlorodiphenyltrichloroethane (DDT, plus metabolites).[11−17] These studies were limited to a few species and compounds, primarily investigating the oligochaete *Lumbriculus variegatus* using spiked sediments. As laboratory-based bioaccumulation methods are standardized and expanded to include other test species, kinetic uptake and depuration studies assessing the major classes of contaminants with these species are needed to adequately select the standard duration for bioaccumulation tests, which additionally provides data to improve predictive bioaccumulation models.

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The Ontario Ministry of the Environment (OMOE) recently completed development and standardization of a 28-d laboratory protocol for measuring bioaccumulation of sediment-based contaminants in freshwater organisms. This included a critical review of bioaccumulation methods in the literature[18] and testing of data gaps regarding exposure techniques (*e.g.,* loading density[19]). This method considered procedures used in the American Society for Testing and Material[2] (ASTM) and U.S. Environmental Protection Agency[20] (U.S. EPA) 28-d bioaccumulation test with *L. variegatus* and those of Bedard et al.[21] The new OMOE protocol offers the use of two invertebrate species, the oligochaete worm *L. variegatus* and mayfly nymph *Hexagenia* spp. and one fish species, the fathead minnow *Pimephales promelas*. Validation of the method involved testing a variety of field-contaminated sediments to compare bioaccumulation potential between the three species exposed to different contaminants (*e.g.,* PCBs, PAHs, dioxins and furans, organochlorine pesticides, and selected metals)[22] and comparison of laboratory bioaccumulation data with available field data.[23] Although the literature provides considerable information on kinetic uptake and elimination of certain hydrophobic chemicals from sediment in *L. variegatus*,[16,17,24,25] there is far less for *Hexagenia* spp. and almost none for *P. promelas* or other fish species. Despite the extensive use of *L. variegatus* in bioaccumulation tests, there exists little kinetic data for polychlorinated biphenyls (PCBs), which are common contaminants in sediments. To address these data gaps and build upon the previous work with the three species, the new OMOE methods (modified as necessary) were used to measure the kinetic uptake and depuration of PCBs from additional field-contaminated sediments in the three species. Our primary objective was to assess the appropriateness of 28-d as a standard test duration, since data were particularly lacking for *Hexagenia* spp. and *P. promelas*. Also, examination of the kinetics of individual PCB congeners provides information on the relationship between bioaccumulation and properties such as hydrophobicity.

METHODS

Bioaccumulation and Depuration Tests. Oligochaetes and fish were raised at the OMOE laboratory from in-house cultures following standard operating procedures.[20,21,26] Mayfly eggs were obtained in the field from emerged adults (by J. Ciborowski, University of Windsor), stored, hatched, and reared in the OMOE laboratory.[21,27] All water used in culturing and testing was dechlorinated city of Toronto tap water.

Sediments were collected from locations in southwestern Ontario. Control sediments were from relatively uncontaminated areas, and PCB-contaminated sediments were from two industrialized areas where historical dumping of PCB-containing materials occurred. Sediment from Site 1 was passed through a 2-mm mesh sieve to remove large gravel and other debris but prevent the loss of pore water and fine particulates. Other samples did not require sieving based on physical characteristics, in accordance with existing guidelines.[28,29] Prior to use, all control and test sediments were homogenized and subsampled for full physicochemical characterization (Table S1). All samples were stored at ~4 °C, in the dark, prior to use.

All sediments were screened for acute toxicity using the three species in small-scale, 4-day tests prior to use in bioaccumulation tests.[20] Sediment from Site 1 was not acutely toxic, but mortality of *Hexagenia* spp. was observed in the bioaccumulation test and uptake and elimination of PCBs could not be fully characterized. Kinetic information for *Hexagenia* spp. was obtained by testing with sediment from Site 2. Only *Hexagenia* spp. tests were conducted with this sediment due to its limited volume. Sediments from Sites 1 and 2 were stored for 2 weeks and 4 months, respectively, prior to bioaccumulation tests.

One day prior to test initiation, 24 (21 for *Hexagenia*) 4-L glass jars were filled with sediment to provide a 27:1 ratio of total organic carbon (TOC) to organism dry weight (dw). This loading density was selected for the OMOE protocol based on previous research indicating no impact on organism survival, growth, and bioaccumulation compared to the 50:1 ratio used in the ASTM and U.S. EPA bioaccumulation methods with *L. variegatus*.[19] Sediment and water were added to test containers in a ratio of 1:4 (v/v).[21] At initiation of the bioaccumulation test approximately 5 g total wet weight (ww) of organisms was randomly added to each test container. Approximately 1.3-times the required amount of worm tissue was added to account for excess weight from water in the sample of nonblotted worms.[20] The average wet weight of mayfly nymphs was between 20 and 30 mg and for fish was between 250 and 500 mg. A (5 g) sample of organisms for each species was collected for pre-exposure analysis. Organisms were exposed to sediment under static conditions with aeration of overlying water.[21,28,29] Tests were conducted at 23 ± 2 °C, under a 16 h light, 8 h dark photoperiod of 500−1000 lx.[28,29] No food was provided to worms or mayflies, to avoid preferential ingestion of food instead of sediment, limiting the uptake of contaminants.[2] Since fish do not actively ingest sediment, they were fed a maintenance diet of ground Nutrafin fish food flakes at a rate of 1% wet body weight/d.[21] The food contained negligible amounts of PCBs, and the small rations were consumed immediately by the fish, thus supplemental feeding was unlikely to influence exposure and bioaccumulation.

On days 4, 7, 14, 25, and 28 (4, 12, 25, 28 for *Hexagenia*) of the uptake phase, organisms were recovered from three test containers for analysis of tissue residues (~250 μm and 1 mm mesh sieve used for worms[2,20] and nymphs, respectively). After 28 d of exposure, organisms were recovered from the remaining test containers and transferred into containers with control sediment for the depuration phase of the study. Controls were relatively uncontaminated, field-collected, sediments suitable for culturing/testing with these species. Organisms were recovered from three test containers after 4, 14, and 25 d of depuration (= d-32, -42, and -53 of the study). Organisms were recovered from one control replicate on d-7 (-12 for *Hexagenia*), -28, and -53, to assess the integrity of the test system and the general health of organisms. At each sampling interval, the recovered organisms from each replicate were held in 1.5 L of fresh water for 24 h to allow them to purge sediment from their guts. All other conditions were the same as in the test, but no food was provided to the fish. To reduce stress associated with continual swimming, mayflies were provided a mesh substrate and overhead lights were turned off. Organisms were rinsed, euthanized in CO_2-charged water, weighed, and frozen. Survival was recorded for nymphs and fish. Survival and growth for *L. variegatus* could not be reasonably determined due to the large number of worms, so total biomass (ww) was measured for this species. All pre-exposure, control, and test organisms were analyzed for PCBs and lipid content. Water quality (pH, conductivity, temperature, dissolved oxygen, and ammonia) was measured at the beginning of the tests and at each sampling interval using ion-specific meters, following OMOE standard operating procedures.

Physicochemical Analyses. All physicochemical analyses of sediment and tissue samples were conducted by the OMOE Laboratory Services Branch, following their standard operating procedures (described below).

Total organic carbon was determined by subtracting the total inorganic carbon from total carbon. Total carbon was measured by combustion using a LECO C-632 Carbon Determinator.[30] Inorganic carbon was determined by measurement of CO_2 produced by the reaction of carbonate with 2 N perchloric acid, using a coulometer.[31] Particle size composition in sediment was measured with a Coulter LS230 Particle Size Analyzer[32] expressed as percent sand, silt, and clay (Table S1).

For measurement of congener-specific PCBs, sediment samples were air-dried, extracted twice with acetone and 25% (v/v) dichloromethane in hexane using an ultrasonic bath, followed by the use of a vortex shaker. Thawed tissue samples were acid-digested using concentrated hydrochloric acid and then extracted using 25% (v/v) dichloromethane in hexane. Lipid content was determined gravimetrically using an aliquot of the final extract from each tissue sample. An aliquot of each extract (sediment or tissue) was cleaned up using preconditioned Florisil prior to analysis by a gas chromatograph equipped with electron capture detectors (detection limits: 0.6−11 ng/g dw sediment and 0.4−22 ng/g ww tissue; 74 to 98% recovery on matrix spikes).[33,34]

Data and Statistical Analyses. Total PCBs are the sum of total PCB congeners measured in the method, with congener values below reporting limits treated as zero. PCB concentrations in all pre-exposure and control organisms were at or below trace levels. All concentrations in tissue were normalized to lipid content. As growth of organisms did not vary considerably across sampling intervals (growth/sampling interval mean ± standard deviation: *L. variegatus* −32 ± 8%, *Hexagenia* spp. 31 ± 13%, and *P. promelas* 9 ± 10%), corrections for growth dilution were not necessary.

Steady-state for total PCBs was assessed by comparing tissue residues across sampling intervals of the uptake phase using an analysis of variance (ANOVA, $\alpha = 0.05$) and using a kinetic approach by calculating uptake and elimination rate constants. Apparent steady-state was defined as 95% of the equilibrium tissue residues,[2] and the theoretical time to reach steady-state (t_{95} in d) was calculated as $t_{95} = -\ln(1-0.95)/k_2$. Elimination rate constants (k_2) were calculated by fitting data from the depuration phase to a first-order decay curve (ln concentration = $a - k_2 \cdot t$, where a is a constant). Uptake rate constants (k_1) were derived for total PCBs by fitting all of the data from the uptake phase to a first-order bioaccumulation model using nonlinear regression

$$C_{org} = k_1/k_2 \cdot C_{sed} \cdot (1 - e^{-k2t})$$

where C_{org} = concentration in tissue (ng/g lipid), C_{sed} is the concentration in the sediment (ng/g organic carbon - OC), and t is time in days. Although expressing kinetic parameters on the basis of total PCBs violates the assumption of first-order kinetics of the model, this provides an initial, generalized assessment of uptake and elimination of contaminants. For the bioaccumulation model to be valid, it was assumed that the concentration in the sediment remained constant (verified by sampling on d-28). Data from the uptake phase were transformed (square root for invertebrates, cube root for fish) to meet the assumptions of normality and constant variance in the nonlinear regression. Biota-sediment accumulation factors were calculated using kinetic rate constants ($BSAF_{kin} = k_1/k_2$; where k_1 was calculated) or concentrations in organisms measured on d-28 of the exposure ($BSAF_{d\text{-}28} = C_{org}$ [lipid-normalized ww]$/C_{sed}$ [OC-normalized dw]).

To narrow the scope of which PCB congeners to examine, those congeners below the method detection limit in both sediment and tissue and with less than 1% contribution to total PCBs were first excluded (Figure S1; congener profiles). Congeners were selected that were common to both sediments tested (with some exceptions) with sufficient representation of the different congener groups (Table S3; selected congeners). Rate constants for elimination, half-lives, time to steady-state, and measured BSAFs were calculated for 13 congeners in *L. variegatus* and *P. promelas* and nine congeners in *Hexagenia* spp. The relationship between the logarithms of the elimination rate constant (k_2) and the octanol−water partition coefficient (K_{OW})[35] for the selected PCB congeners was assessed through linear regression. Tests for normality (Kolmogorov−Smirnov) and equal variance (Leven median) were incorporated in all analyses for total PCBs and selected congeners, with $\alpha = 0.05$ (SigmaStat v. 3.5). Errors reported for rate constants are standard errors.

RESULTS

Survival, Growth, and Lipid. Total biomass of worms decreased in the control and test sediments by 14 to 46% and has been observed to be a variable end point in this species.[14,17,22,36] Mean survival of *Hexagenia* spp. was ≥82% through 28 d of the test and in the control treatment on d-53. Survival of nymphs from the test treatments during the depuration phase was lower (77−79%). Mean survival of *P. promelas* was ≥97% through 32 d of the test and was more variable but remained ≥80% on d-42 and d-52. Growth of nymphs and fish was minimal and average wet weights of recovered organisms were within the range measured on d-0 (Table S2). Lipid content of worms decreased slightly from d-25 to d-32 but remained consistent (0.6−0.8%) during the remainder of the depuration phase. The lipid content of mayflies decreased between d-14 and d-32, and in fish lipid decreased between d-7 and d-25, but remained consistent thereafter (*Hexagenia* spp. 0.3−0.5%, *P. promelas* 3.1−3.4%; Table S2).

Total PCBs. PCBs in sediment from Site 1 were rapidly accumulated by *L. variegatus*, and apparent steady-state was reached in approximately 9 d (Figure 1). Tissue residues measured on the first sampling day (d-4) were 66 and 73% of the measured (d-28) and estimated (modeled) steady-state concentrations, respectively. Tissue residues were not significantly different between the five sampling intervals of the uptake phase. Depuration of PCBs was also rapid once worms were transferred to control sediment (Figure 1). The elimination rate was calculated with data from d-28 to d-42 only, with $k_2 = 0.3266 \pm 0.0333$ d^{-1} ($R^2 = 0.932$). This k_2 resulted in a half-life of 2.1 d, which corresponds with the observation that approximately 90% of the body burden was depurated within the first 4 d. The uptake rate was calculated to be $k_1 = 0.460 \pm 0.0108$ g OC g lipid^{-1} d^{-1} ($R^2 = 0.946$). The BSAF measured on d-28 ($BSAF_{d\text{-}28} = 2.1$) was 1.5-times higher than the resulting $BSAF_{kin}$ (= 1.4).

Bioaccumulation of PCBs in *Hexagenia* spp. was very rapid within the first 4 d of exposure to sediment from Site 2. Tissue residues reached a plateau between d-4 and d-25 (no significant difference across sampling intervals) but increased by a factor of 2 from d-25 to d-28 (Figure 1). This increase was only significant with data normalized to lipid. Time to steady-state was

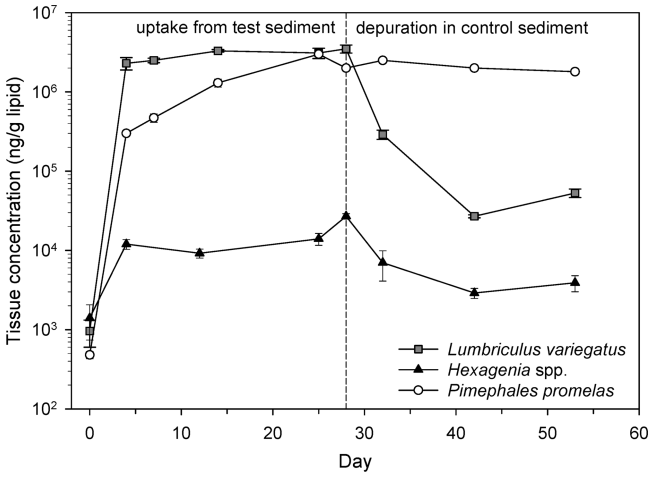

Figure 1. Accumulation and depuration of total PCBs in *Lumbriculus variegatus* and *Pimephales promelas* exposed to sediment from Site 1 and in *Hexagenia* spp. exposed to sediment from Site 2. Data are mean ($n = 3$) and standard errors.

approximately 8 d. Nymphs rapidly depurated PCBs once transferred to control sediment (Figure 1). When data from d-28 to d-42 were used to calculate k_2 the resulting half-life was 4.8 d. However, 74% of the body burden was lost in the first 4 d. When data from only d-28 and d-32 were used, $k_2 = 0.3699 \pm 0.0970$ d^{-1} ($R^2 = 0.784$) and the corresponding half-life was 1.9 d. The resulting uptake rate was $k_1 = 0.549 \pm 0.0397$ g OC g lipid^{-1} d^{-1} ($R^2 = 0.532$). The $BSAF_{d\text{-}28}$ (= 3.6) was 2.4-times higher than the resulting $BSAF_{kin}$ (= 1.5).

Bioaccumulation of PCBs in *P. promelas* was not as rapid as in the invertebrates, and concentrations in tissue increased significantly up to d-25 (Figure 1). Tissue residues measured on d-28 were significantly lower (30%) than on d-25, and the reason for this is not clear. Depuration of total PCBs from *P. promelas* was minimal with almost no change in tissue residues during the depuration phase (Figure 1). Tissue residues on d-32 were slightly higher than those measured on d-28. Data from only d-32 to d-53 were used to calculate $k_2 = 0.0143 \pm 0.0036$ d^{-1} ($R^2 = 0.697$), resulting in a half-life of 48 d. The uptake rate k_1 was calculated as 0.0567 ± 0.0038 g OC g lipid^{-1} d^{-1} ($R^2 = 0.646$). The resulting $BSAF_{kin}$ (= 4.0) was 2.2- and 3.4-times higher than the d-25 (= 1.8) and d-28 (= 1.2) BSAFs, respectively. This suggests that steady-state concentrations in tissue may not have been reached during the uptake phase. The time to steady-state was estimated to be 209 d.

PCB Congeners. The kinetics of PCBs in the three test species were further investigated by examining a number of different congeners. In each species, the shape of the uptake curve was similar between the selected congeners and that for total PCBs (Figure 2 for *L. variegatus*; Figures S2 and S3 for other species). The exception was PCB33 in *P. promelas*, for which tissue residues reached a maximum concentration on d-14 and declined thereafter. In contrast, depuration curves varied across the PCB congeners and did not always follow the pattern of total PCBs. Elimination rates (k_2) were calculated using data from the sampling intervals that best represented the ln-linear phase of depuration (Table S3). Half-lives ($t_{1/2}$) and time to apparent steady-state (t_{95}) were determined based on k_2. Elimination rates in *L. variegatus* ranged from 0.246 to 0.607 d^{-1} for most congeners. Half-lives were typically 1.1 to 2.8 d and time to

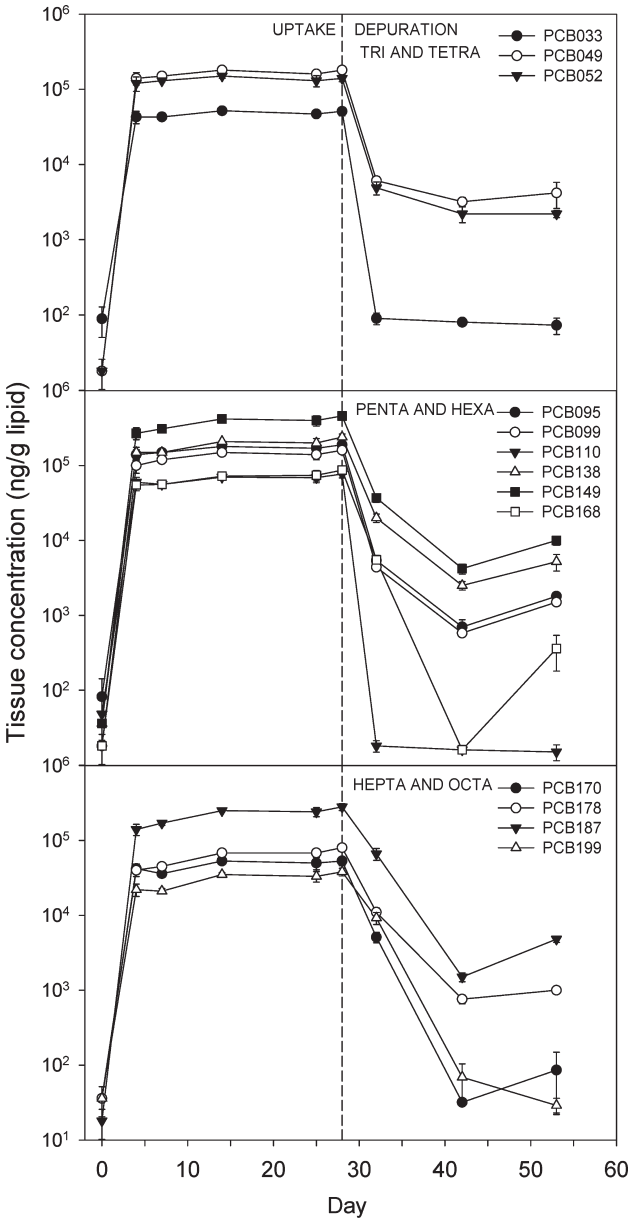

Figure 2. Accumulation and depuration of 13 PCB congeners in *Lumbriculus variegatus* exposed to sediment from Site 1. Data are mean ($n = 3$) and standard errors. See Figures S2 and S3 for other species.

steady-state was 5 to 12 d. The exceptions were PCB33 and PCB110 with elimination rates more than 2.5-times greater than for other congeners, resulting in time to steady-state of ≤2 d. Elimination rates in *Hexagenia* spp. ranged from 0.136 to 0.301 d^{-1} and were approximately 25 to 90% lower than those for *L. variegatus*. Half-lives were between 2.3 and 5.1 d. The time for individual congeners to reach steady-state ranged from 10 to 22 d; longer than the 8 d determined for total PCBs. In *P. promelas*, depuration of the selected congeners was minimal, similar to the observations with total PCBs. PCB33 was the exception and was almost completely depurated by d-53. Elimination rates in the fish were, on average, ≥ 90% lower than in the invertebrates and typically ranged from 0.0091 to 0.0372 d^{-1}. For many of the higher chlorinated congeners in the fish, regression analyses for elimination data were not significant. With the exception of

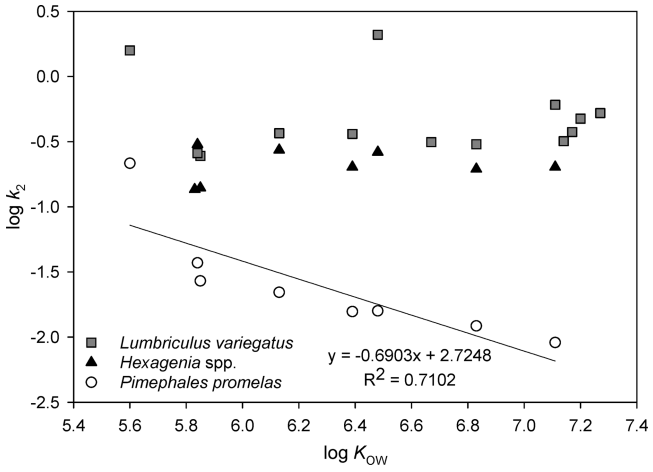

Figure 3. Relationship between elimination rate constants (log k_2) in three test species versus the octanol−water partition coefficient (log K_{OW}) for the selected PCBs congeners. Regression between log k_2 and log K_{OW} is significant for *Pimephales promelas* (p = 0.0086).

PCB33, half-lives in *P. promelas* were anywhere from 19 to 76 d and time to steady-state from 81 d to >320 d. A significant correlation between log k_2 and log K_{OW} was observed for *P. promelas* and was described by the relationship log k_2 = 2.2748 − 0.6903 · log K_{OW} (p = 0.0086, R^2 = 0.7102; Figure 3). There was no relationship between k_2 and K_{OW} for the invertebrates in the present study.

DISCUSSION

Rates of uptake and elimination and the time required to achieve steady-state of total PCBs were similar for *L. variegatus* and *Hexagenia* spp. In contrast, elimination rates for individual congeners were lower in *Hexagenia* spp. than *L. variegatus*. These relative differences for total PCBs and the selected congeners may be influenced by the relative contribution of different congeners to the measure of total PCBs. In *Hexagenia* spp., the low R^2 (= 0.532) of k_1 for total PCBs was likely due to the shape of the uptake curve in which tissue residues were slightly lower (by ~20%) on d-12 than d-4. In both invertebrates, the $BSAF_{d\text{-}28}$ for total PCBs was higher than the $BSAF_{kin}$, suggesting that k_1 may have been underestimated since the linear uptake phase was not captured in the sampling intervals used. Additionally, k_1 may also have been underestimated as the result of the model not considering the rapidly desorbing fraction of a compound, which was observed by Sun and Gosh[37] to influence bioaccumulation of PCBs in *L. variegatus*. Regardless of this, there is clear evidence that these organisms reached steady-state concentrations during the 28-d exposure, even for PCB congeners with a high K_{OW}.

Rates of uptake were an order of magnitude lower in *P. promelas* than in the invertebrates, as can be expected due to differences in their routes of exposure. The two invertebrates actively burrow into sediment and mainly accumulate (and possibly depurate) organic contaminants from ingestion of sediment.[12,38−40] Therefore, they have a higher exposure to PCBs in sediment and faster uptake than fish. Fish live in the water column but interact with the sediment surface through swimming and feeding behavior. They may accumulate contaminants dissolved in the water (via gills and skin) or through ingestion of suspended sediment and food, which are more indirect and lower sources of exposure. Although uptake was slower in *P. promelas*, by d-25 and d-28 tissue residues of total PCBs and most congeners reached concentrations of a similar magnitude to those in *L. variegatus*. This similarity in d-28 tissue residues was observed in an earlier test with a different sediment sample from this site.[22] This indicates that a thermodynamic equilibrium may have been achieved between the lipid of both species and the organic carbon of the sediment. This could also reflect a pseudosteady-state in the fish with desorption kinetics controlling uptake as the fish behavior causes (re)suspension of sediment particles. Less time may be required to achieve steady-state than that predicted by the t_{95} values estimated from the kinetics of depuration. There may be uncertainty associated with the k_2 values determined for both total PCBs and congeners in *P. promelas*, since the depuration phase was not of sufficient duration to observe a significant decline in tissue residues (other than for PCB33). The legitimacy of the uptake curve in depicting steady-state, when not corroborated by the depuration kinetics, remains questionable.

Elimination rates were also an order of magnitude or more lower in *P. promelas* than in the invertebrates. Loonen et al.[41] suggested that the low lipid content and relatively large surface area to volume ratio of *L. variegatus*, in addition to the intensive blood circulation through the skin, enhanced the exchange processes between the body and environment compared to that in fish, leading to large differences in uptake and elimination rates between these organisms. Enhanced depuration in clean sediment versus water-only has been observed in a number of invertebrates.[12,13,42,43] This is thought to occur from a change in fugacity as the organic compound partitions from the organism onto the uncontaminated sediment as it passes through the gut, although the exact mechanism is unknown.[42]

The bioavailability of a compound is related to its hydrophobicity (K_{OW}), and bioaccumulation (i.e., uptake rates, BSAFs) typically decreases with increasing K_{OW} because of greater partitioning to the organic carbon in sediment. Elimination rates may also be related to K_{OW}; however, in the present study, K_{OW} had little, if any, effect on elimination rates or BSAFs in both invertebrates. Fisk et al.[44] similarly observed that the chlorine content (and resulting K_{OW}) of polychlorinated alkanes had little effect on k_2 in *L. variegatus* exposed via spiked sediment. This was unexpected as k_2 had been observed to decrease with increasing K_{OW} in other aquatic invertebrates.[38,42] These other studies had observed this relationship across 4 to 6 different organic compounds in water-only exposures with the amphipod *Diporeia* spp. (depurated in sediment) and the midge larvae *Chironomus riparius* (depurated in water or sediment). Schuler et al.[15] observed that elimination rates of hexachlorobiphenyl (PCB153) were similar in the amphipod *Hyalella azteca* and the midge *Chironomus tentans* but more rapid than in *L. variegatus*. In contrast, they observed that uptake rates of the compound were significantly greater in *L. variegatus* than in the other two species. This demonstrates that differential accumulation and elimination can occur between benthic species, which could be attributed to different feeding rates, assimilation efficiencies, and contribution of different routes of exposure (e.g., pore water versus sediment). Landrum and Poore[11] projected that the fraction of the body burden (of benzo(a)pyrene) obtained from sediment would be ≥0.9 in *Hexagenia* spp., 0.34 to 0.67 in oligochaetes, and 0.39 in *Diporeia* spp., suggesting that the role of sediment as a source depends on the characteristics of the organism and the sediment. These differences may explain why the relationship between k_2 and

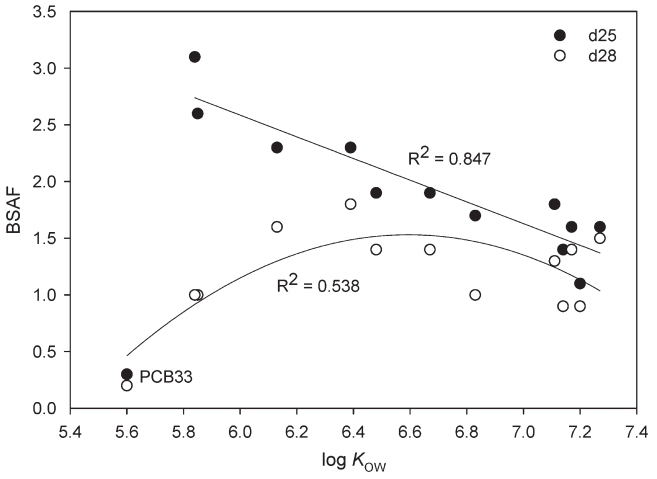

Figure 4. Biota-sediment accumulation factors (BSAF; normalized to lipid and organic carbon) measured in *Pimephales promelas* for the selected PCB congeners. Relationships are significant between BSAF and log K_{OW} (d-25, excluding PCB33 $p < 0.0001$; d-28, including PCB33 $p = 0.021$).

K_{OW} observed by others was not observed with the invertebrates in the present study.

In *P. promelas*, elimination rates of the PCB congeners selected in the present study were inversely proportional to the K_{OW}. It has been suggested that depuration rates decrease with increasing K_{OW} if passive diffusion is the dominant mechanism.[17] Thermodynamics may be driving elimination of these compounds from *P. promelas* but not for *L. variegatus* and *Hexagenia* spp. The elimination rate of PCB33 from the fish was much higher than for other congeners, and this congener was effectively eliminated from the fish by the end of the study. It is also possible that this congener was metabolized by the fish. As a result, the $BSAF_{d\text{-}28}$ for PCB33 was very low. Predictive modeling of PCB bioaccumulation by Gobas and Arnot[45] showed disparities regarding PCB33, in that it was measured in tissue samples from shiner surfperch, white croaker, and cormorants but was not detected in mussels, oysters, jacksmelt, or harbor seals. Similar to the present study, the measured BSAF for PCB33 in white croaker was observed to be lower than for other congeners. For the other congeners, tissue concentrations and BSAFs in *P. promelas* were higher on d-25 than on d-28. If PCB33 is excluded, the $BSAF_{d\text{-}25}$ for the selected congeners decreased significantly with increasing K_{OW} ($p < 0.0001$, $R^2 = 0.847$; Figure 4). The same trend was not observed for $BSAF_{d\text{-}28}$. There was a significant, although weaker, relationship between $BSAF_{d\text{-}28}$ and K_{OW} only when PCB33 was included ($p = 0.021$, $R^2 = 0.538$), and it exhibited a parabolic shape (Figure 4). This latter relationship corresponds with the notion that compounds with low or very high K_{OW} will have lower BSAFs than those with a midrange K_{OW}, due to the combination of different desorption rates, assimilation efficiencies, and elimination rates. Neither of these trends has been observed for d-28 BSAFs in bioaccumulation tests with other PCB-contaminated sediments that we have conducted with *P. promelas* or the two invertebrates.

The rapid uptake and elimination of PCBs in *L. variegatus* in the present study has been similarly observed in other studies. Sun et al.[46] measured depuration in *L. variegatus* after exposure to PCB-contaminated sediment and determined elimination rates for 30 PCB congeners (tri- to hexa-PCBs). Only PCB49 and 99 were common to both studies. Elimination rates were 0.575 and 0.460 d^{-1}, respectively, for these congeners, which was 2.3- and 1.2-times higher than values in the present study. The following range of k_2 values were reported for congeners in the various homologue groups: tri- 0.689−0.844 d^{-1}, tetra- 0.384−0.638 d^{-1}, penta- 0.430−0.546 d^{-1}, and hexa- 0.358−0.397 d^{-1}. Compared to the present study, these values were within a factor of 1.4 to 2.6 (higher and lower) for the respective homologues, with the exception that k_2 for PCB110 (hexa-) was 4.3-times higher in the present study. Sun et al. noted that elimination rates decreased by a factor of 2 with an increase in chlorination from tri- to hexa-PCBs; they did not, however, further investigate the relationship between k_2 and K_{OW}. In a depuration study using spiked sediment, Schuler et al.[15] calculated a k_2 value of 0.168 d^{-1} for PCB153 in *L. variegatus*. This was 1.8- to 3.6-times lower than the values determined for hexa-PCBs in the present study. Kukkonen et al.[9] calculated elimination rates for two PCB congeners in *L. variegatus* exposed to seven spiked sediments, based on the fit of uptake data to the first-order bioaccumulation model. The elimination rates ranged from 1.032 to 2.016 d^{-1} for PCB77 (tetra-) and 0.199 to 0.778 d^{-1} for PCB153. These values were 4- to 8-times higher than those for tetra-PCBs and encompass the range for hexa-PCBs determined in the present study. *Lumbriculus variegatus,* in particular, has been extensively used in bioaccumulation tests, including kinetic studies, with both field-contaminated and spiked sediments; however, it is apparent that even estimates of PCB congener-specific parameters vary between sediment and studies.

There have been much fewer studies examining bioaccumulation in *Hexagenia* spp. in general and the kinetics of uptake and elimination in particular. Landrum and Poore[11] measured the kinetic uptake and depuration of benzo(a)pyrene, phenanthrene, and PCB153 in *Hexagenia* spp. nymphs. Elimination rates for PCB153 (at 20 °C) ranged from 0.168 to 0.408 d^{-1}, which encompasses the values for PCB138 and 168 determined for *Hexagenia* spp. in the present study. Drouillard et al.[47] measured the kinetics of uptake of a variety of organic compounds from field-contaminated sediment in nymphs but did not investigate depuration of these compounds. They too observed that BSAFs (as well as k_1) were relatively independent of K_{OW} (for compounds with log $K_{OW} > 5.8$).

Even fewer laboratory studies have measured the bioaccumulation of contaminants from sediment into fish. Apart from a study by Schuytema et al.[48] that examined the kinetic uptake and elimination of hexachlorobenzene in *P. promelas* from water-only and sediment-water tests and one by Schrap and Opperhuizen[49] that examined the kinetic uptake of five chlorobenzenes in guppies from a sediment suspension, no other studies were found in the literature that have investigated the kinetics of the bioaccumulation of persistent organic pollutants in fish exposed to sediment.

The present study is the first to describe both the kinetic uptake and elimination of PCBs (total and congeners) from field-contaminated sediment in any of the three test organisms. Uptake of PCBs was rapid in both *L. variegatus* and *Hexagenia* spp., and tissue residues reached steady-state concentrations within 28 d. This further supports the selection of 28 d as a standard duration for bioaccumulation tests, which had been sufficiently demonstrated for *L. variegatus* elsewhere in the literature,[14,16,41] but not for *Hexagenia* spp. Uptake of PCBs was much slower in *P. promelas*, likely due to the more indirect routes through which they are exposed to contaminants from the

sediment. There remains uncertainty as to the time required to achieve steady-state concentrations in fish and the appropriateness of a 28-d test. Additional bioaccumulation tests with sampling before and after 28 d, assessing the operational definition of steady-state (i.e., no difference between three successive sampling intervals), should provide the information needed to evaluate the appropriateness of this test duration.

In the present study, elimination rates and BSAFs of the selected PCB congeners were not correlated with K_{OW} for either invertebrate species. This suggests that bioaccumulation in these organisms may be influenced by active uptake and elimination (via sediment ingestion) rather than solely thermodynamic processes and the hydrophobicity of these congeners. In contrast, elimination rates significantly decreased with increasing K_{OW} in *P. promelas*, suggesting that elimination may be passive and driven by thermodynamic partitioning due to the hydrophobicity of the compound. An inverse linear relationship was observed between d-25 BSAFs and K_{OW}, while the relationship for d-28 BSAFs exhibited a parabolic shape. The reasons for these differences are unknown, and these trends have not been observed in our previous work with any of these species.

■ ASSOCIATED CONTENT

Supporting Information. Table S1: physicochemical characteristics of test sediments. Table S2: additional end points from bioaccumulation tests. Table S3: kinetic parameters and BSAFs. Figure S1: profiles of PCB congeners. Figures S2 and S3: uptake and depuration curves of selected congeners for *Hexagenia* spp. and *P. promelas*, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
*Phone: (519) 824-4120 x58792. Fax: (519) 837-0442. E-mail: ksolomon@uoguelph.ca.

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