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ACCUMULATION AND DEPURATION OF SEDIMENT-SORBED C_{12}- AND C_{16}-POLYCHLORINATED ALKANES BY OLIGOCHAETES (*LUMBRICULUS VARIEGATUS*)

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Abstract—Oligochaetes (*Lumbriculus variegatus*) were exposed to sediment spiked with four ^{14}C-polychlorinated alkanes (PCAs) ($C_{12}H_{20}Cl_6$ [56% Cl by weight], $C_{12}H_{16}Cl_{10}$ [69% Cl], $C_{16}H_{31}Cl_3$ [35% Cl], and $C_{16}H_{21}Cl_{13}$ [69% Cl]) to measure bioaccumulation parameters and biotransformation. Chlorinated paraffins are industrial products that consist of thousands of different PCAs. Chlorinated paraffins are hydrophobic (log octanol–water partition coefficients [K_{ow}s] > 5.0) and are reported to have relatively high concentrations in sediment compared with other persistent organochlorines; however, no data exist on their bioavailability from sediment. The PCAs $C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$, and $C_{16}H_{31}Cl_3$ were readily available to sediment-ingesting oligochaetes, whereas $C_{16}H_{21}Cl_{13}$ had lower bioavailability. Uptake rates of the C_{12}-PCAs were greater than the C_{16}-PCAs, but half-lives ($t_{1/2}$s) were greater for the C_{16}-PCAs ($t_{1/2}$ = 30–33 d) than for the C_{12}-PCAs ($t_{1/2}$ = 12–14 d). Biota–sediment accumulation factors were >1 for $C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$, and $C_{16}H_{31}Cl_3$, but <1 for $C_{16}H_{21}Cl_{13}$. Comparison of toluene-extractable and -nonextractable ^{14}C suggest that PCAs were biotransformed in aerobic sediments and by oligochaetes, and that the susceptibility to degradation in sediments decreases with increasing chlorine content. The relative abundance of individual PCAs may differ between sediment and benthic invertebrates because of differences in the bioaccumulation and degradation of PCAs of varying carbon chain length and chlorine content.

Keywords—Polychlorinated alkanes Sediment bioavailability Biota–sediment accumulation factors Oligochaetes Biodegradation

INTRODUCTION

Chlorinated paraffins (CPs) are a class of polychlorinated *n*-alkanes (PCAs) that are used as plasticizers, flame retardants, high-pressure lubricants, and for a number of other industrial applications [1,2]. Chlorinated paraffins have carbon chain lengths between 10 and 30, with chlorine content varying from 35 to 70% by weight. Each commercial CP product has thousands of different compounds and isomers [3]. The global production of CPs has been estimated at over 300 kilotonnes/year, with a majority having medium carbon chain lengths ($C_{14–18}$) [2].

Chlorinated paraffins are very hydrophobic (log octanol–water partition coefficient [K_{ow}] > 5.0) [4], and are bioaccumulated from water and food by fish in laboratory experiments [5–9]. Although the amount of data is limited, CPs have been quantified in environmental samples [3,10–12]. Fisk et al. [12] found that concentrations of short-chain CPs (60–70% Cl) were similar to those of polychlorinated biphenyls (PCBs) in sediments and zebra mussels collected from the Detroit River and the western basin of Lake Erie. Jansson et al. [10] reported that CPs ($C_{10–13}$; 60% Cl) were the predominant organochlorines in a representative set of terrestrial and aquatic organisms from Sweden.

In aquatic systems, compounds of low aqueous solubility partition to a large extent onto suspended and bottom sediment. Once bound to sediment, compounds may not be available for direct uptake by fish and other nonfiltering aquatic invertebrates. Sediment-bound compounds can reenter the food chain through benthic organisms via interstitial water or by consumption of sediments. Therefore, the bioavailability of these sediment-sorbed chemicals controls their fate, and exposure to organisms, in aquatic ecosystems. For example, concentrations of organochlorines in Lake Ontario sculpin are thought to be derived from sediment via the benthic food chain [13].

Despite their low water solubility, high bioaccumulation potential, and relatively high environmental sediment concentrations, no data exist on the bioavailability of sediment-sorbed CPs. To address this knowledge gap, we exposed oligochaetes (*Lumbriculus variegatus*) to sediment spiked with four ^{14}C-chlorinated alkanes to determine uptake and depuration rates, and biota–sediment bioaccumulation factors (BSAFs). As well, K_{ow}s of these PCAs were determined experimentally using reverse-phase, high-pressure liquid chromatography (RP-HPLC) to clarify the results of the bioavailability experiments.

MATERIALS AND METHODS

Chemicals, sediment, and sediment spiking

The four ^{14}C-PCAs consist of two C_{12}- and two C_{16}-alkanes that were synthesized in a different manner from commercial CPs [14]. However, gas chromatography–mass spectrometry (GC-MS) analysis shows these ^{14}C-products contain a similar range of components to those in commercial products (G.T. Tomy, personal communication). The [1-^{14}C]dodecanes con-

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Table 1. Bioaccumulation parameters of four sediment-sorbed ^{14}C-polychlorinated alkanes for the oligochaete *Lumbriculus variegatus* (significant differences [$p < 0.05$] between depuration rates are indicated with capital letters)

Chemical	Concn.[a] (ng/g)	Log K_{ow}[b]	OC[c] (%)	Lipids[d] (%)	Uptake rate[e] ($\times 10^{-2}$ g/g/d)	Depuration rate[f] ($\times 10^{-2}$/d)	Half-life[g] (d)	Kinetic[h] BSAF
$C_{12}H_{20}Cl_6$	26.5	6.2	2.3	3.7 ± 0.6	22 ± 2.1 (0.77)	5.0 ± 0.7 (0.75)A	14 ± 2.0	4.4
	106		3.8	2.9 ± 0.3	51 ± 11 (0.38)	5.6 ± 0.9 (0.71)A	12 ± 1.9	9.1
$C_{12}H_{16}Cl_{10}$	124	6.6	3.6	3.6 ± 0.8	9.0 ± 0.8 (0.77)	4.8 ± 1.1 (0.50)A	14 ± 3.2	1.9
	442		3.1	3.4 ± 0.8	11 ± 1.2 (0.69)	5.8 ± 0.8 (0.75)A	12 ± 1.7	1.9
$C_{16}H_{31}Cl_3$	47.1	7.2	1.4	2.5 ± 0.3	9.3 ± 1.0 (0.68)	2.1 ± 0.7 (0.32)BC	33 ± 11	4.4
	135[i]		1.3	2.3 ± 0.2	7.6 ± 0.7 (0.71)	0.73 ± 0.5 (0.10)B	95 ± 65	10
$C_{16}H_{21}Cl_{13}$	264	7.4	1.5	2.0 ± 0.2	1.3 ± 0.1 (0.84)	2.3 ± 0.6 (0.51)C	30 ± 7.8	0.6

[a] Sediment concentrations are dry weight.
[b] Octanol–water partition coefficient (K_{ow}) values for $C_{12}H_{20}Cl_6$ and $C_{16}H_{31}Cl_3$ are a mean of two and four values determined by high-performance liquid chromatography, respectively.
[c] Organic carbon contents are for dry weight of sediment.
[d] Lipids percentage determined as the mean of all samples.
[e] Uptake rate constants (k_1) (±1 standard error) (coefficient of determination is shown in parentheses) were determined by fitting the data to the integrated form of the kinetic rate equation for constant uptake from sediment using iterative nonlinear regression: $C_{olgio} = [C_{sed}k_1/k_2)(1 - \exp(-k_2t)]$, where C_{olgio} is the lipid- and growth-corrected concentration in the oligochaetes, C_{sed} is the organic carbon-normalized concentration in the sediment corrected for the loss of toluene-extractable ^{14}C, k_2 is the depuration rate, and t is the time in days.
[f] Depuration rate constants (k_2) (±1 standard error) were calculated using the model ln concentration = $a + b$ (time) where b is the depuration rate (coefficient of determination for the model is shown in parentheses). Depuration rates for $C_{16}H_{21}Cl_{13}$ have been calculated from data for day 2 to day 56 only.
[g] Half-life (±1 standard error) is calculated from the equation $t_{½} = 0.693/k_2$.
[h] The kinetic biota–sediment accumulation factors (BSAFs) were determined from the equation BSAF = k_1/k_2.
[i] The depuration data for this treatment did not have a significant linear relationship with time.

tained 55.9 and 68.5% chlorine (mean of 5.9 and 9.8 chlorine atoms per molecule, respectively) (specific activities 16 mCi/mmol and 23 mCi/mmol, respectively). The [1-^{14}C]hexadecane had 34.1% chlorine (3.3 chlorine atoms per molecule) (specific activity 22.7 mCi/mmol), and the [U-^{14}C]hexadecane had 69% chlorine (13.4 chlorines per molecule) (specific activity 25 mCi/mmol). For simplicity, the number of chlorine atoms in each compound has been rounded to the nearest integer.

Sediment was collected with an Ekman dredge from Lake 468, an oligotrophic, uncontaminated lake at the Experimental Lakes Area (ELA), Ontario, Canada, in October 1995. Sediment was stored at 4°C under 1 cm of lake water until the experiment. Prior to spiking, the wet sediment was sieved with a 600-μm mesh sieve to remove large benthic invertebrates and debris (wood, stones, and other items).

For spiking, the sediment was added to a 6-L flask that contained ~5 L of distilled water, and the sediment–water slurry was mixed with a Teflon stir bar and mixer. ^{14}C-dichlorinated alkanes were added to the slurry in ~100 μl of acetone, and the sediment was mixed for 24 h. After mixing, the sediment was allowed to settle and the water was decanted leaving 1 cm of overlying water. The sediments were allowed to stand for 18 d prior to the beginning of the experiment.

Experiment

Oligochaetes (*L. variegatus*) were exposed to two concentrations (Table 1) of each ^{14}C-PCA in separate experiments (only one concentration was established for the $C_{16}H_{21}Cl_{13}$). One control experiment was performed with untreated sediment.

For each treatment 36 60-ml glass jars were filled with sufficient sediment to provide a 100:1 organic carbon to lipid ratio for 15 oligochaetes (~100 mg). Each jar received 15 oligochaetes and all the jars from a treatment were carefully placed in a 10-L aquarium that had flow-through ultraviolet (UV) and carbon dechlorinated Winnipeg (Manitoba, Canada) tap water at 11.6 ± 0.1°C (mean ± 1 SE). On days 1, 3, 7, 10, and 14 of uptake, three jars from each treatment were randomly selected and sieved to collect the oligochaetes. After 14 d of exposure, all jars were sieved and oligochaetes were placed into identical jars with untreated sediment to follow depuration. Three jars from each of the C_{16}-PCA exposures were maintained for 7 additional days (21 d in total) to attempt to achieve steady state. On days 1, 3, 7, 10, 14, 28, and 42 of depuration, oligochaetes were collected from three jars for each treatment. No food was added to any of the treatments. Oligochaetes were placed in water to rinse off sediment, and were then blotted dry, weighed, frozen, and freeze-dried.

Analysis of ^{14}C in oligochaetes

Freeze-dried oligochaetes were homogenized in toluene, centrifuged (10,000 rpm for 10 min), and a fraction of the supernatant was added to scintillation cocktail (Atomlight, Dupont, Boston, MA, USA) and counted on a Beckman LS 7500 liquid scintillation counter (LSC) (Beckman Instruments, Fullerton, CA, USA) to determine ^{14}C. The ^{14}C counts were corrected for quenching using a quench curve prepared from ^{14}C-toluene (Dupont), and were automatically corrected for background by the LSC. Lipids were determined gravimetrically using 25% of the supernatant.

The remaining toluene supernatant from the high-concentration PCA exposures was decanted, and washed and decanted with a second milliliter of toluene. The remaining tissue was oven dried at 80°C for 24 h and combusted on a Packard Model D306 Oxidizer (Packard Instruments, Downers Grove, IL, USA) for determination of nontoluene-extractable ^{14}C.

Analysis of ^{14}C in sediment and interstitial water

Sediment samples were collected on days 0 and 14 of the uptake phase for analysis of ^{14}C. Sediment was frozen and freeze-dried, and ^{14}C was determined by three methods. The toluene sonication method involved measuring a known quan-

tity of freeze-dried sediment (~0.5 g) into a test tube with 3 ml of toluene and sonicating for 30 min. After allowing the sediment and toluene to stand for 24 h, 1 ml of toluene was counted by LSC. The second method used Soxhlet extraction of freeze-dried sediment (~1.0 g) with 250 ml of dichloromethane (DCM) for 16 h. The 250 ml of DCM was rotoevaporated and transferred to approximately 1 ml of toluene and counted on the LSC. The third method involved direct combustion of a sample of unextracted freeze-dried sediment (~0.2 g) on the oxidizer. Recovery of PCAs, after spiking freeze-dried sediment ($n = 3$) and using the toluene sonication method, ranged from 82 to 97%. The organic carbon content of the sediments from each of the treatments was determined by high-temperature combustion with detection of CO_2 by thermal conductance, corrected for $CaCO_3$ [15]. The sediment size fractions of the sediment used for the C_{12}- and C_{16}-PCA exposures were determined by the second reading hydrometer method [16].

A portion of the sediment was centrifuged at 10,000 rpm for 30 min to collect interstitial water for determination of PCA concentrations. To obtain PCA concentrations in interstitial water, 1 ml of the interstitial water was counted. A second milliliter of interstitial water was eluted through a C_{18} Sep-Pak (Waters Division of Millipore, Milford, MA, USA) to determine freely dissolved and dissolved organic carbon-bound concentrations of the PCAs [17]. The Sep-Pak was then eluted with 15 ml of hexane to give the free dissolved concentrations of the PCA.

High-performance liquid chromatography analysis of sediment and oligochaete extracts

Toluene extracts from oligochaetes and sediments collected on day 14 of the uptake phase were evaporated to near dryness under a gentle N_2 stream and made up in acetone (~100 μl) for HPLC analysis. Samples were injected on a Varian 5000 liquid chromatograph (Varian Canada, Mississuaga, ON, Canada) equipped with a Prep Nova Pak HR C-18 column (Waters Division of Millipore), a Marathon autosampler (Varian Canada), and a Foxy 200 automated fraction collector (Canberra Packard Canada, Mississauga, ON, Canada). The mobile phase consisted of 85% acetontrile and 15% water, 4-min fractions were collected over a 60-min period. Fractions were counted using LSC.

Determinations of K_{ow}s and organic carbon–water partition coefficients

The K_{ow}s of the four PCAs were determined experimentally using RP-HPLC. Methods have been described in detail previously [18]. The HPLC was carried out with a Waters model 6000A pump, a U6-K injector, a model 490 multiwavelength UV detector, and a Whatman Partisil 10 μm ODS-3 C_{18} column (Waters Division of Millipore), using a methanol–water mixture for the mobile phase. An LKB 2111 Multirac Fraction collector was used to collect radiolabeled fractions of the PCAs.

A series of hydrophobic compounds (DDT, dibenzo-*p*-dioxin, 1-chlorodibenzo-*p*-dioxin [CDD], 2-CDD, 2,7-dichlorodibenzo-*p*-dioxin, 1,2,4-trichlorodibenzo-*p*-dioxin, octachlorodibenzo-*p*-dioxin, and polychlorinated biphenyl congeners 1, 2, 18, 29, 101, 130, 153, 185, and 194) with published K_{ow} values were used to construct a standard curve between log K_{ow} and the log of the HPLC retention time (R_t) and PCA log K_{ow} values were estimated by interpolation from the standard curve.

Organic carbon–water partition coefficients (K_{oc}s) were estimated from the equation

$$K_{oc} = K_p / f_{oc}, \quad (1)$$

where $K_p = C_{sed}$ (ng/g, dry weight)/$C_{\text{interstitial water}}$ (ng/ml), C_{sed} is the concentration in the sediment, $C_{\text{interstitial water}}$ is the concentration in interstitial water, and f_{oc} is the fraction of organic carbon in the sediment.

Data analysis

Growth rates were determined by fitting oligochaete weight data (total wet weight of oligochaetes in one jar) to an exponential model (ln oligochaete weight = $a + b\cdot$time [d], where a is a constant and b is the growth rate). All concentrations determined for oligochaetes were lipid normalized and corrected for growth dilution for bioaccumulation parameter calculations. Only toluene-extractable concentration data were used for bioaccumulation parameter calculation. Depuration rates (k_2) were determined by fitting the depuration phase data to a first-order decay curve (ln concentration = $a + b\cdot$time [d], where a is a constant and b is the depuration rate). Uptake rates (k_1), which represent uptake from interstitial water and through ingestion of sediment, were calculated by fitting the uptake phase concentrations to the integrated form of a first-order uptake model [19] for constant exposure to sediment-sorbed contaminant using nonlinear regression

$$C_{oligo} = (C_{sed}\cdot k_1/k_2) \times [1 - \exp(-k_2\cdot t)] \quad (2)$$

where C_{oligo} is the concentration in the oligochaetes (lipid corrected), C_{sed} is the concentration in the sediment (dry weight, organic carbon corrected, and corrected for loss of toluene-extractable ^{14}C assuming a first-order degradation rate [see Discussion]), and t is the time (d). Biota–sediment accumulation factors were calculated using kinetic rate constants ($BSAF_{kin} = k_1/k_2$), or concentrations when the oligochaetes had achieved a steady state ($BSAF_{ss} = C_{oligo}$ [lipid normalized, wet weight]/C_{sed} [organic carbon normalized, dry weight]).

Differences between depuration rates among treatments were examined by testing the homogeneity of slopes in an analysis of covariance. The Student's *t*-test was used to compare pairs of depuration rate constants at the $p < 0.05$ level of significance.

RESULTS

Organic carbon content of sediments

Although the sediment used for all exposures came from a single source, differences in organic carbon content were found between C_{12}- and C_{16}-PCA exposures (Table 1). All spiking procedures and times were kept consistent between treatments; however, the C_{12}-PCA experiments were carried out prior to the C_{16}-PCA experiments. This procedure resulted in a greater percentage of sand in the C_{16}-PCA experiment (76% sand, 21% silt, 3% clay) than in the C_{12}-PCA experiments (40% sand, 58% silt, 2% clay), and probably explains the lower organic carbon levels in the C_{16}-PCA experiments.

Oligochaete recovery, growth, and lipids

Recovery of oligochaetes ranged from 97 to 120% for all the treatments (mean of all sampling dates). Recoveries greater than 100% suggest that the oligochaetes reproduced. Growth rates ranged from 0.006 ± 0.003 to 0.014 ± 0.007/d (mean

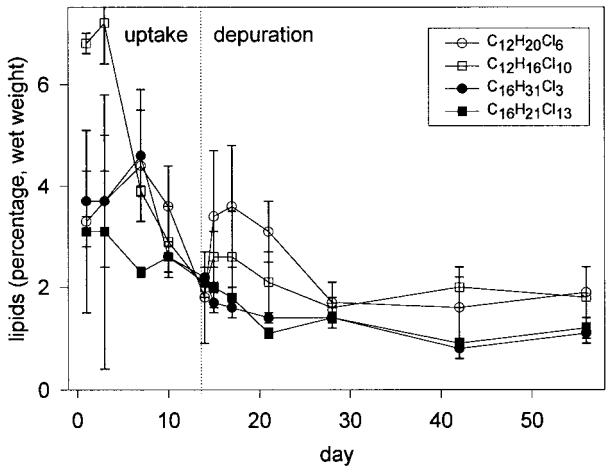

Fig. 1. Lipid percentages (wet weight) of oligochaetes exposed to four ^{14}C-polychlorinated alkanes. Each point is the mean lipid percentage ± 1 standard error of three samples of oligochaetes (~15 individuals). Data from the high concentration exposures were used.

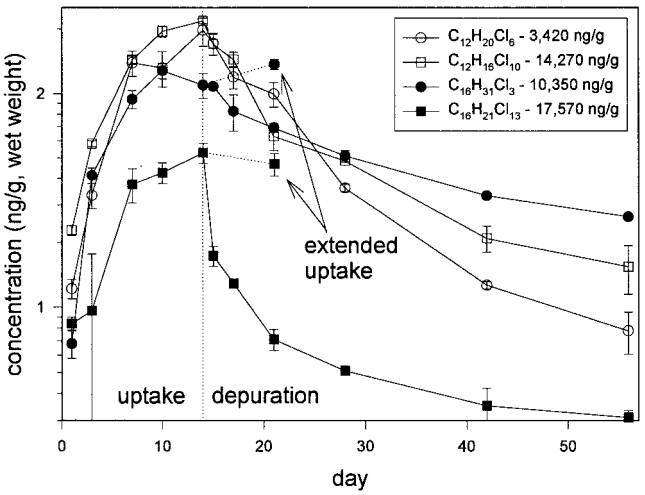

Fig 2. Accumulation and depuration of four sediment-sorbed ^{14}C-polychlorinated alkanes from the highest exposure treatments. Concentrations in the legend represent the sediment concentrations (organic carbon corrected). Each point is the mean concentration (wet weight) ± 1 standard error of three oligochaetes.

± 1 SE), but r^2 values were low (0.03–0.43) and no significant growth occurred in some treatments. Lipid content of the oligochaetes declined throughout the experiments but increased slightly upon transfer to new sediments at the beginning of the depuration phase (Fig. 1). Lipid percentages were greater in the oligochaetes exposed to C_{12}-PCAs than in those exposed to the C_{16}-PCAs (Table 1).

Accumulation of ^{14}C-PCA

All PCAs were detectable in oligochaetes after 1 d of exposure to spiked sediment (Fig. 1). The uptake rate of $C_{12}H_{20}Cl_6$ was the highest among the PCAs, whereas that of $C_{16}H_{21}Cl_{13}$ was the lowest (Table 1). Concentrations of $C_{16}H_{21}Cl_{13}$ in the oligochaetes decreased rapidly during the first day of depuration. This has been observed with other very hydrophobic compounds in similar sediment bioavailability tests [20], and is likely due to high concentrations of the $C_{16}H_{21}Cl_{13}$ bound to sediment in the gastrointestinal tracts of the oligochaetes that are not absorbed and are excreted during the depuration phase. Depuration rates for $C_{16}H_{21}Cl_{13}$ have been calculated with data from day 2 to 56 only. Depuration data for the $C_{16}H_{31}Cl_3$ high exposure did not have a significant linear relationship with time, and parameters for this treatment should be viewed with caution. With the exception of the $C_{16}H_{31}Cl_3$ high exposure and the $C_{16}H_{21}Cl_{13}$ exposure, no differences in depuration rates were observed for PCAs of the same carbon chain length; however, the C_{12}-PCAs were found to have significantly greater depuration rates than the C_{16}-PCAs (Table 1). Accordingly, half-lives ($t_{1/2}$s) of the C_{12}-PCAs were about one half of those of the C_{16}-PCAs ($t_{1/2}$ 12–14 and 30–95 d, respectively).

The C_{12}-PCAs did not achieve equilibrium between oligochaete and sediment after 14 d of exposure (Fig. 1), and therefore no $BSAF_{ss}$ could be calculated. The $BSAF_{kin}$ for $C_{12}H_{20}Cl_6$ ranged from 4.4 to 12, and for $C_{12}H_{16}Cl_{10}$ was 1.9 (Table 1). After 21 d of exposure the C_{16}-PCAs appeared to have achieved steady state between sediment and oligochaete (Fig. 2). However, the $BSAF_{ss}$s for these compounds were less than one half of the $BSAF_{kin}$ (Table 1). The compound $C_{16}H_{31}Cl_3$ had BSAFs of between 0.7 (steady state) and 4.4 to 10 (kinetic). The compound $C_{16}H_{21}Cl_{13}$ had BSAF values much less than one (0.2–0.6, steady state and kinetic BSAF, respectively).

High-performance liquid chromatography of oligochaete and sediment extracts

The HPLC chromatograms of toluene extracts of sediment and oligochaetes on day 14 of the uptake phase showed similar patterns of ^{14}C-PCA in oligochaete extracts and the PCA standards (Fig. 3). The chromatograms of the extracts from oligochaetes exposed to $C_{16}H_{21}Cl_{13}$ varied between the sediment and standard. However, concentrations of ^{14}C in these oligochaetes were below detection limits of ^{14}C in most HPLC fractions. These results suggest that toluene extracted the parent PCA compounds but little of the transformed compounds (see Discussion).

Values of K_{ow}s and K_{oc}s of PCAs

The standard regression curve relating RP-HPLC-adjusted R_t with published K_{ow} values was

$$\log K_{ow} = 3.13 \cdot \log R_t + 2.80 \quad (3)$$

The equation and fit of the line ($r^2 = 0.96$) are comparable to previous K_{ow} determinations by HPLC [18]. The PCA standards have broad HPLC peaks because they are synthesized in a manner that does not produce a single compound but rather a series of compounds with similar chlorine content and numerous positional isomers. Four peaks were resolved for $C_{16}H_{31}Cl_3$ resulting in a number of K_{ow} estimates (Table 2). A majority of the radioactivity for this PCA was found in the second peak, which corresponds to a mean K_{ow} value of 7.2 (Table 2). Log K_{ow}s of the PCAs determined by this equation ranged from 5.0 to 8.2, increasing with greater carbon chain length and chlorine content. The K_{ow}s of the PCAs determined using equations developed by Sijm and Sinnige [4] are in general agreement with the HPLC-derived K_{ow}s of the C_{16}-PCAs, but are higher than the C_{12}-PCA HPLC-derived K_{ow}s (Table 2).

Log K_{oc}s of PCAs ranged from 4.1 to 5.2, and as with K_{ow}, increased with greater carbon chain length and chlorine content (Table 3). Drouillard [21], using gas sparging methods, re-

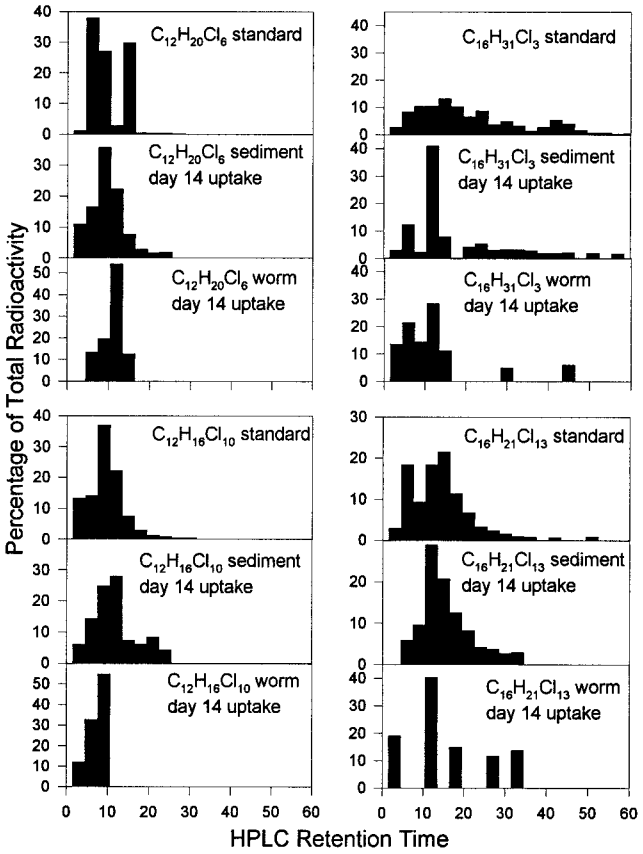

Fig 3. High-performance liquid chromatography chromatograms of the ^{14}C-polychlorinated alkane standards and sediment and oligochaete (worm) toluene extracts from day 14 of the uptake phase. Each bar represents the radioactivity in a 4-min fraction as a percentage of the total radioactivity.

ported log K_{oc} values of 4.6 to 6.0 for the same ^{14}C-$C_{12}H_{20}Cl_6$ used in this experiment. This suggests that our methods may have underestimated the true K_{oc} value of these compounds or that measured K_{oc} values are lower at higher sediment to water ratios. No other data have been published on K_{oc}s of PCAs.

Extractable and nonextractable ^{14}C

Concentrations of PCAs in sediment determined by toluene sonication and Soxhlet extraction with DCM showed good agreement for all treatments (Table 4). However, concentrations of chlorinated alkanes determined by oxidation were 1.6 to 4.4 times higher than those determined by sonication or Soxhlet extraction (Table 4). For the lower chlorinated alkanes, $C_{12}H_{20}Cl_6$ and $C_{16}H_{31}Cl_3$, the extractable ^{14}C accounted for only 23 to 25 and 32 to 40% of the total ^{14}C, respectively. For the higher chlorinated alkanes, $C_{12}H_{16}Cl_{10}$ and $C_{16}H_{21}Cl_{13}$, the extractable ^{14}C accounted for 63 to 64 and 58% of the total ^{14}C, respectively. Extractable to nonextractable ratios in sediment were similar to those observed in the oligochaetes during the uptake phase (Fig. 4).

DISCUSSION

Accumulation of C_{12}-PCAs (56 and 69% Cl, by weight) and C_{16}-PCAs (35 and 69% Cl) from sediments by oligochaetes was strongly influenced by carbon length, chlorine content, and K_{ow} (or K_{oc}). Therefore, relative abundance of individual components of CP mixtures in benthic invertebrates would be expected to be different than those observed in sediment or commercial products. Oliver [22] and Wood et al. [23] reported changes in relative abundance of PCBs congeners and polycyclic aromatic hydrocarbons between sediment and benthic invertebrates due to differences in bioavailability and biotransformation rates of individual compounds.

Table 2. Log octanol–water partition coefficient (K_{ow}) values for the ^{14}C-polychlorinated alkanes (PCAs) determined experimentally by reverse-phase high-performance liquid chromatography (HPLC) and using the equation of Sijm and Sinnige [4]. The PCAs produced broad HPLC peaks and estimates of K_{ow}. Mean values represent the mean retention time of the peak. Multiple estimates for $C_{16}H_{31}Cl_3$ represent resolved peaks

PCA	HPLC log K_{ow} peak range	HPLC log K_{ow} mean[a]	Estimated log K_{ow}[b]
$C_{12}H_{20}Cl_6$	5.0–7.1	6.2	6.8
$C_{12}H_{16}Cl_{10}$	5.0–7.4	6.6	7.3
$C_{16}H_{31}Cl_3$	4.7–6.6 6.6–7.8 7.8–8.0 8.0–8.3	5.9 (21) 7.2 (54) 7.9 (8) 8.2 (17)	6.9
$C_{16}H_{21}Cl_{13}$	6.9–7.8	7.36	7.5

[a] Values in parentheses represent the percentage of total radioactivity in each peak.

[b] Estimated log K_{ow} determined using the equation $\log K_{ow} = -0.386 + 0.600N_{tot} - 0.0113N_{tot}^2$, where N_{tot} is the total number of carbon and chlorine atoms [4].

Lipid content of the oligochaetes varied between treatments and decreased in all treatments over the course of the experiments. Therefore, all data were lipid-corrected prior to parameter calculations. Unfortunately this introduced error to all the parameters, in particular the depuration rates. The r^2 values for PCA depuration rates using data that were not lipid-corrected were >0.76 (data not shown), and were higher than the r^2 values generated with lipid-corrected data (Table 1). Although lipid-correction had a relatively small impact (generally <10%) on the parameters (small increases in k_1 and decreases in k_2), it did alter the relative magnitude of the uptake rates. This suggests that proper evaluation of these data required lipid-correction. The method used for lipid determination (solvent extraction and gravimetric determination) was not as accurate as methods to determine PCA concentrations because of the small mass and low lipid content of the oligochaetes.

The $t_{1/2}$s of C_{16}-PCAs were significantly greater than those of the C_{12}-PCAs; however, chlorine content did not have a significant effect on the half-life of PCAs (analysis of covariance, $p < 0.05$). Half-lives of tri- and tetrachlorobiphenyls in wild-collected oligochaetes (*Tubifex* sp. and *Limnodrilus hoffmeisteri*) ($t_{1/2}$s 26–43 d) were similar to the C_{16}-PCA $t_{1/2}$s but were greater than those for C_{12}-PCAs [24]. West et al. [25] reported that the $t_{1/2}$ of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in oligochaetes (*L. variegatus*) ranged from 13 to 21 d, after exposing the oligochaetes via spiked food. These results are greater than the $t_{1/2}$s of the C_{12}-PCAs but less than those of the C_{16}-PCAs. Loonen et al. [20] reported TCDD and octachlorodibenzo-*p*-dioxin $t_{1/2}$s in *L. variegatus* of 4 and 13 d, respectively, after exposure to spiked sediments. These results suggest that the $t_{1/2}$s of C_{12}- and C_{16}-PCAs in oligochaetes are similar to those of chlorinated aromatic contaminants of comparable K_{ow}. Therefore, benthic invertebrates could provide a pathway for transfer of PCAs, or CPs, from sediment into aquatic food chains.

Half-lives of the C_{16}-PCAs might have been larger if the

Table 3. Sediment (ng/g dry sediment) and interstitial water (ng/ml) concentrations (mean ± 1 SE), sediment sorption partition coefficients ($K_p = C_{sediment,\ dry\ wt.}/C_{water}$), and organic carbon–water partition coefficients ($K_{oc} = K_p/f_{oc}$; f_{oc} is the fraction organic carbon in sediment) of ^{14}C-chlorinated alkanes on day 14 of the uptake phase

Compound	Sediment (ng/g, dry wt.)	Total interstitial water[a] (ng/ml)	DOC and POC[a] (ng/ml)	Freely dissolved[a] (ng/ml)	K_p	Log K_{oc}
$C_{12}H_{20}Cl_6$	107 ± 1.8	3.0 ± 0.1	1.5 ± 0.1	0.2 ± 0.03	535	4.1
$C_{12}H_{16}Cl_{10}$	442 ± 19	1.1 ± 0.03	0.3 ± 0.1	0.3 ± 0.002	1,473	4.7
$C_{16}H_{31}Cl_3$	135 ± 4.6	2.4 ± 0.1	1.3 ± 0.1	0.1 ± 0.03	1,350	5.0
$C_{16}H_{21}Cl_{13}$	263 ± 5.1	0.8 ± 0.1	0.3 ± 0.04	0.1 ± 0.02	2,630	5.2

[a] A portion of the sediment was centrifuged at 10,000 rpm for 30 min to collect interstitial water for determination of polychlorinated alkane (PCA) concentrations. To obtain total interstitial water PCA concentrations, 1 ml of the interstitial water was counted. A second milliliter of interstitial water was eluted through a C_{18} Sep-Pak to determine freely dissolved and dissolved organic carbon concentrations of the chlorinated alkanes [15]. The Sep-Pak was then eluted with 15 ml of hexane to give the free dissolved concentrations of the PCAs. DOC = dissolved organic carbon; POC = particulate organic carbon.

lipid content of these oligochaetes had been the same as in the C_{12}-PCA exposures. Greater lipid percentage may result in lower depuration rates of hydrophobic compounds in invertebrates [26,27]. However, greater organic carbon content of sediments is also associated with slower elimination rates of hydrophobic compounds in benthic invertebrates over short periods (50 h) [28].

The fact that chlorine content had little effect on the $t_{1/2}$ of PCAs was unexpected based on the higher K_{ow} and lower metabolism of the higher chlorinated alkanes (see below). Half-lives have been observed to increase with increasing K_{ow} in aquatic invertebrates [26,28]. Fisk et al. [9], using the same ^{14}C-PCAs, observed longer $t_{1/2}$s for the more highly chlorinated C_{12}- and C_{16}-PCAs in rainbow trout.

The PCA uptake rates decreased with increasing K_{ow} or K_{oc}. Compounds of high K_{ow} or K_{oc} have been reported to have lower interstitial water concentrations and slower rates of desorption from sediment to interstitial water [29]. Assimilation of compounds from ingested sediment should also decrease with increasing K_{ow} or K_{oc}. Variations in PCA uptake rates may also be due to differences in the types of sediment particles that the PCAs were sorbed to [30]. It should be noted that the greater organic carbon content in the sediment, and lipid percentage in the oligochaetes, of the C_{12} exposures may have enhanced the C_{12}-PCA uptake rates [27].

Uptake of the PCAs from the sediment will occur through accumulation from interstitial water and through gut absorption from ingested sediments. However, bioconcentration factors (BCFs) of PCAs (BCFs = 7.4–7.9), determined from lipid-corrected concentrations in oligochaetes divided by freely dissolved interstitial water concentrations, were greater than their K_{ow} values for all PCAs but $C_{16}H_{21}Cl_{13}$ (BCF = 1), suggesting that additional accumulation occurred through ingestion of sediments. Ingestion of sediments has been identified as an important route of accumulation of sediment-associated hydrophobic contaminants [31,32]. Bioconcentrations factors decreased with increasing K_{ow}, which may be due to lower concentrations of the more hydrophobic PCAs in interstitial water and reduced assimilation in the gut.

Table 4. Sediment concentrations (mean ± 1 SE, $n = 3$) of ^{14}C-polychlorinated alkanes determined by toluene sonication, Soxhlet extraction, and oxidation. Sediment was collected on day 14 of the uptake phase

Compound	Toluene extractable concn. (ng/g, dry wt.)	Soxhlet extractable concn. (ng/g, dry wt.)	Oxidizer concn. (ng/g, dry wt.)	% Toluene extractable/ oxidizer concn.
$C_{12}H_{20}Cl_6$	36 ± 3.3	27 ± 1.8	159 ± 3.1	23
	113 ± 4.4	107 ± 1.9	424 ± 2.8	27
$C_{12}H_{16}Cl_{10}$	112 ± 3.5	124 ± 4.1	175 ± 2.1	64
	372 ± 9.1	442 ± 19	590 ± 4.8	63
$C_{16}H_{31}Cl_3$	51 ± 2.1	47 ± 3.9	128 ± 7.6	40
	128 ± 7.6	135 ± 4.6	399 ± 40	32
$C_{16}H_{21}Cl_{13}$	283 ± 6.7	263 ± 5.1	485 ± 69	58

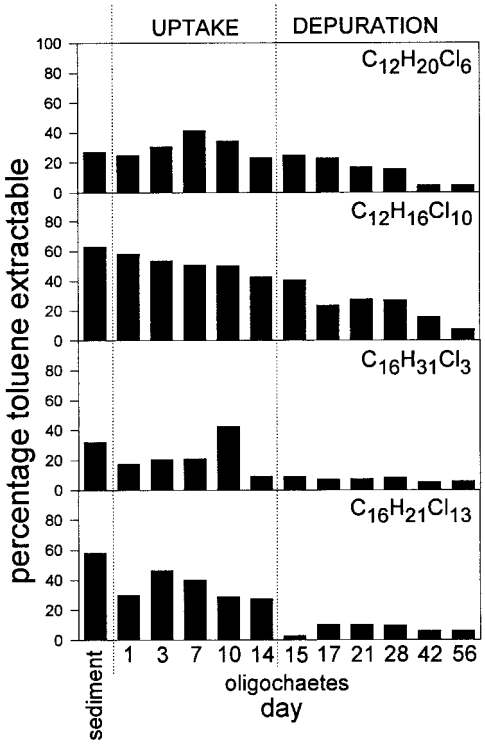

Fig 4. Percentage of toluene-extractable ^{14}C in ^{14}C-polychlorinated alkane-spiked sediment (day 14 uptake) and oligochaetes exposed to the sediment for 14 d followed by 56 d exposed to unspiked sediment.

The $BSAF_{kin}$s of $C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$, and $C_{16}H_{31}Cl_3$ were >1, suggesting that the concentrations of PCAs with low and medium chlorination (35–60% Cl) and short ($C_{10–13}$) and medium carbon chains ($C_{14–17}$) would magnify between sediments and sediment-ingesting invertebrates. The compound $C_{16}H_{21}Cl_{13}$ had a $BSAF_{kin} < 1$ suggesting that highly chlorinated medium-carbon chain PCAs, when associated with sediment, have lower bioavailability to benthic invertebrates than short-chain and medium-chlorinated medium-carbon chain PCAs. Based on these results, BSAFs of PCAs decrease with increasing K_{ow}. This is consistent with the results of Oliver [24], who in similar experiments using oligochaetes (*Tubifex* sp. and *L. hoffmeisteri*) and organochlorine compounds, observed decreasing BSAFs above a log K_{ow} of 6. It should be noted that BSAFs may not be well described by simple relationships with K_{ow} [29].

An unexpected result of this work were the relative amounts of toluene-extractable and -nonextractable ^{14}C in oligochaetes and sediments (Fig. 4). The ^{14}C-PCAs were efficiently recovered from spiked oligochaetes and sediment. Therefore, it is likely that ^{14}C that is not extracted by toluene, but is measured by complete combustion, is more polar, or more tightly bound, than the starting material. The nonextractable ^{14}C in the oligochaetes might be explained by biotransformation. However, amounts of nonextractable ^{14}C in sediment were also high and very similar to the ratios observed in the oligochaetes during the uptake phase. Fisk et al. [9] found a much smaller percentage of nonextractable ^{14}C in rainbow trout exposed to the same ^{14}C-PCAs. Therefore, it is more likely that PCAs are being transformed in the sediment prior to being accumulated by the oligochaetes. Alternatively, the oligochaetes are transforming greater amounts than fish, which seems unlikely. However, the proportion of toluene-extractable ^{14}C also decreased throughout the depuration phase for all the PCAs when oligochaetes were in PCA-free sediment, indicating that oligochaetes do metabolize PCAs. Further studies on the degradation and metabolism of CPs in sediments, invertebrates, and fish are needed to fully evaluate the fate of CPs in sediment.

Limited information is available on the degradation of PCAs in sediment. The susceptibility of PCAs to microbial degradation has been found to decrease with greater chlorine content and carbon chain length [33,34]. Madeley and Birtley [33] reported that microorganisms previously acclimated to specific CPs showed a greater ability to degrade the compounds. Based on the ratios of extractable to nonextractable ^{14}C in this work, lower chlorinated alkanes are more susceptible to degradation in the sediment. However, data are insufficient to make any conclusions about differences in susceptibility to microbial degradation due to carbon chain length or about the type of degradation products produced.

Estimates of PCA $t_{1/2}$s in sediment were made by assuming that the percentage of toluene-extractable ^{14}C on the first and 14th day of the exposure, 18 and 32 d after spiking, respectively, represented the amount of remaining parent compound. Assuming that the rate of degradation was a first-order process, the sediment $t_{1/2}$ of $C_{12}H_{20}Cl_6$ is 13 ± 3.6 d, of $C_{12}H_{16}Cl_{10}$ is 30 ± 2.6 d, of $C_{16}H_{31}Cl_3$ is 12 ± 0.9 d, and of $C_{16}H_{21}Cl_{13}$ is 58 ± 58 d. The r^2 value for the degradation rates were greater than 0.93 for $C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$, and $C_{16}H_{31}Cl_3$, but only 0.49 for $C_{16}H_{21}Cl_{13}$.

CONCLUSIONS

This work represents the first data on the bioavailability of sediment-associated PCAs. Bioaccumulation of sediment-associated PCAs by oligochaetes varies with carbon chain length, chlorine content, and K_{ow}. Short-chain ($C_{10–13}$) and medium-chlorinated (~60% Cl) medium-chain ($C_{14–18}$) CPs have BSAFs > 1, and are readily bioavailable when associated with sediments. Higher chlorinated (~70% Cl) medium-chain CPs have lower bioavailability when associated with sediment and a BSAF < 1. The PCAs were persistent in oligochaetes; $t_{1/2}$s of the C_{12}-PCAs ranged from 11 to 13 d and of the C_{16}-PCAs from 24 to 43 d and are similar to those of other persistent organochlorines. Sediment ingestion is an important exposure route for sediment-sorbed PCAs. The PCAs appear to be readily degraded in aerobic sediments. Estimated $t_{1/2}$s of the PCAs in aerobic sediment ranged from 10 to 27 d, with higher chlorinated PCAs having longer $t_{1/2}$s.

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