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DIETARY ACCUMULATION OF C_{12}- AND C_{16}-CHLORINATED ALKANES BY JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Abstract—Dietary exposures using juvenile rainbow trout (*Oncorhynchus mykiss*) were conducted with four ^{14}C-polychlorinated alkanes ($C_{12}H_{20}Cl_6$ [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]) in order to measure bioaccumulation parameters, metabolism, and tissue distributions. These chlorinated alkanes are found in industrial chlorinated paraffin (CP) products, although their method of synthesis is different than that of CPs. Trout were exposed for 40 d to nominal concentrations of 20 and 200 ng/g of each chlorinated alkane, as well as to 2,000 ng/g for $C_{16}H_{21}Cl_{13}$, followed by an elimination period of up to 173 d. Whole-body half-lives in the rainbow trout ranged from 37 ± 2 d for $C_{16}H_{31}Cl_3$ to 87 ± 11 d for $C_{12}H_{16}Cl_{10}$, and assimilation efficiencies of $C_{16}H_{31}Cl_3$ (33 to 35%) and $C_{12}H_{16}Cl_{10}$ (34 to 38%) were highest among the four alkanes. Biomagnification factors ranged from 0.44 for $C_{16}H_{21}Cl_{13}$ to 2.15 for $C_{12}H_{16}Cl_{10}$. Accumulation of $C_{16}H_{21}Cl_{13}$ (molecular weight = 674) may be sterically hindered due to its large molecular size. Lower chlorinated alkanes, e.g., $C_{16}H_{31}Cl_3$, had shorter half-lives than highly chlorinated alkanes, probably due to increased metabolism. High-performance liquid chromatography ^{14}C analysis of fish tissue extracts revealed that the chlorinated alkane mixtures were selectively biotransformed with certain unknown components persisting in tissues. Lower chlorinated alkanes had greater proportions of polar ^{14}C, which implies greater metabolism of these compounds. Highly chlorinated, short-carbon-chain ($C_{10–13}$) alkanes and lower chlorinated, medium-carbon-chain ($C_{14–18}$) alkanes appear to have the greatest potential for biomagnification among CP components. No reduced growth rates or hepatic monooxygenase enzyme induction were seen in any of the chlorinated alkane exposures when compared with controls.

Keywords—Chlorinated paraffins Polychlorinated alkanes Rainbow trout Bioaccumulation Biotransformation

INTRODUCTION

Chlorinated paraffins (CPs) are a class of polychlorinated alkanes that are used as plasticizers, flame retardants, high-pressure lubricants, and in a number of other industrial applications [1,2]. They vary in both carbon chain length (10 to 30 carbons) and chlorine content (35 to 69% chlorine by weight) and consist of thousands of possible structural isomers. Despite relatively large global production of CPs (300 kt/a) [3], there is relatively little information on their physical–chemical properties, bioaccumulation potential, aquatic toxicity, or environmental fate. A recent study in Sweden found CPs to be the most prevalent organochlorine in three terrestrial samples, two marine fish samples, and one freshwater fish sample [4].

Bioaccumulation data are needed for a complete ecological risk assessment [5]; however, this type of data for CPs is quite limited. Bengtsson and Ofstad [6] exposed bleaks (*Alburnus alburnus*) to three formulations of CPs differing in carbon chain length and degree of chlorination and found that they had different uptake and elimination rates. Furthermore, Bengtsson et al. [7] noted that CPs of short carbon chain length and low chlorination had the highest uptake rate in fish. High-molecular-weight (MW) CPs (MW >600) have been found to have low, or nonexistent, accumulation in fish [7–9]. Although these studies provide some broad information on CP accumulation in aquatic food webs, all of these experiments used industrial CP products and did not provide data on specific chlorinated alkanes of known carbon chain length or Cl content.

Chlorinated alkanes have low water solubilities [10] and high octanol/water partition coefficients (K_{ow}) [11] that vary with carbon chain length and chlorine content. Chemicals with physical–chemical properties similar to those of chlorinated alkanes, such as polychlorinated biphenyls (PCBs) [12], have been found to bioaccumulate [13] and biomagnify [14,15] in aquatic food chains. The main objective of this study was to determine bioaccumulation parameters (depuration rate, half-life, biomagnification factor [BMF], and assimilation efficiency) of four ^{14}C-polychlorinated alkanes in juvenile rainbow trout (*Onchorhynchus mykiss*) through dietary exposure. The four chlorinated alkanes used include two C_{12} compounds ($C_{12}H_{20}Cl_6$ [56% Cl by weight] and $C_{12}H_{16}Cl_{10}$ [69% Cl]) and two C_{16} compounds ($C_{16}H_{31}Cl_3$ [35% Cl] and $C_{16}H_{21}Cl_{13}$ [69% Cl]) found in commercial CP products. We hypothesize that, like PCBs [16] and chlorinated dioxins and furans (PCDD/Fs) [17], certain chlorinated alkanes may be more persistent as a result of the number of chlorines and the length of the carbon chain. These results provide the first bioaccumulation parameters for chlorinated alkanes of known carbon chain length and percentage Cl.

A second objective of this work was to evaluate the toxicity of chlorinated alkanes by monitoring growth rates and general health of the rainbow trout and to measure CYP1A1 mixed-function oxygenase (MFO) enzyme activity by measuring ethoxyresorufin-*O*-deethylase (EROD) activity in liver, since elevated EROD levels have been reported in female flounder (*Platichthys flesus*) exposed to high dietary concentrations of an industrial CP product [18].

MATERIALS AND METHODS

Chemicals and food preparation

All ^{14}C-polychlorinated alkanes were synthesized and purified using techniques outlined previously [19]. The [1-^{14}C] dode-

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Table 1. Growth parameters (mean ± 1 standard error) of juvenile rainbow trout exposed to four ^{14}C-chlorinated alkane compounds (significant differences [*t*-test, $p < 0.05$] in body and liver growth rates for all treatments are indicated by capital letters)

Chemical	Concn. in food (ng/g)	Uptake period (d)	Depuration period (d)	Growth rates[a] Body (10^{-3}/d)	Growth rates[a] Liver (10^{-3}/d)	% Lipid[b]	LSI[c] (%)	% Mortality
Control 1	–	40	173	10.4 ± 1.1 (0.74)^{D}	8.6 ± 1.4 (0.56)^{E}	6.1 ± 0.4	1.47 ± 0.35	0
Control 2	–	40	80	10.4 ± 2.5 (0.39)^{BD}	7.9 ± 2.9 (0.22)^{BE}	6.4 ± 0.3	1.37 ± 0.21	15.8
$C_{12}H_{20}Cl_6$	26	40	80	21.5 ± 3.1 (0.65)^{A}	16.5 ± 2.8 (0.57)^{A}	5.7 ± 0.2	1.16 ± 0.07	18.4
	242	40	120	14.0 ± 1.9 (0.63)^{BD}	11.8 ± 1.7 (0.61)^{CE}	6.2 ± 0.3	1.23 ± 0.07	0
$C_{12}H_{16}Cl_{10}$	21	40	120	16.2 ± 1.8 (0.71)^{AB}	12.8 ± 1.6 (0.68)^{ABC}	5.9 ± 0.3	1.29 ± 0.03	0
	222	40	120	15.3 ± 1.6 (0.76)^{ABC}	12.6 ± 1.3 (0.75)^{ABC}	5.7 ± 0.2	1.10 ± 0.07	0
$C_{16}H_{31}Cl_3$	29	40	120	15.8 ± 1.9 (0.72)^{ABC}	12.6 ± 1.4 (0.72)^{ABCD}	5.6 ± 0.3	1.23 ± 0.18	7.9
	296	40	120	10.1 ± 2.6 (0.59)^{D}	8.3 ± 1.6 (0.49)^{E}	5.7 ± 0.3	1.12 ± 0.08	5.3
$C_{16}H_{21}Cl_{13}$	21	40	173	11.6 ± 1.2 (0.72)^{D}	8.7 ± 1.3 (0.59)^{E}	5.9 ± 0.4	1.27 ± 0.15	0
	198	40	173	11.4 ± 1.1 (0.77)^{D}	9.2 ± 1.8 (0.46)^{E}	5.5 ± 0.3	1.20 ± 0.10	0
	2,000	40	173	11.6 ± 1.2 (0.74)^{DC}	9.9 ± 1.2 (0.70)^{DE}	6.9 ± 0.8	1.46 ± 0.10	0

[a] The growth rates (± 1 standard error) were calculated using the equation ln weight = $a + b$ time (d), where b is the growth rate (coefficient of determination for the model is shown in parentheses).
[b] The percent lipid is an average (± 1 standard error) of all fish in a treatment from day 5 until the end of the experiment.
[c] Liver somatic index (LSI) (± 1 standard error) calculated at day 40 of the uptake phase.

canes contained 55.9 and 68.5% chlorine (mean of 5.9 and 9.8 chlorine atoms/molecule, respectively). The [1-^{14}C] hexadecane had 34.1% chlorine (3.3 chlorine atoms/molecule), and the [U-^{14}C] hexadecane had 69% chlorine (13.4 chlorine atoms/molecule). For simplicity, the number of chlorine atoms in each compound has been rounded to the nearest integer.

Food was spiked by suspending a known quantity of each chlorinated alkane standard in 150 ml of hexane and 100 g of commercial fish food (Martin's Feed Mills Ltd., Elmira, ON, Canada) and slowly evaporating to dryness. Food was air-dried for 24 h and stored at 10°C. The fish food consisted of 41% protein, 14% lipid, and 3% fiber. Concentrations in the food were determined by the same analytical techniques used to determine levels in the rainbow trout tissue (see below) and are found in Table 1. Control food was treated in an identical manner but without the addition of a chlorinated alkane compound.

Experiment

Juvenile rainbow trout (*Oncorhynchus mykiss*) (initial weights 2 to 7 g) were exposed to the spiked food for 40 d, followed by 160 ($C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$, and $C_{16}H_{31}Cl_3$) to 173 d ($C_{16}H_{21}Cl_{13}$) of depuration. The daily rate of feeding was equal to 1.5% of the mean weight of the rainbow trout, corrected after each sampling period. Fifty fish were used in control tank 1 and the three $C_{16}H_{21}Cl_{13}$ treatments, and 36 fish were used in the remaining treatments. Three fish were sampled from each treatment for ^{14}C determination on days 5, 10, 20, 30, and 40 of the uptake period and on days 5, 10, 20, 40, 80, and 160 (or 173 for $C_{16}H_{21}Cl_{13}$ treatments) of the depuration period. Six additional fish in the $C_{16}H_{21}Cl_{13}$ high-concentration treatment were exposed to the chlorinated-alkane-spiked food for 80 d to follow the uptake for an extended period. Sampled fish were separated into liver, gastrointesinal (GI) tract (includes stomach, pyloric caeca, spleen, intestines, and adipose fat associated with these organs as well as gut contents), and carcass (whole fish minus liver and GI tract). Each tissue (including the carcass) was weighed and analyzed separately for ^{14}C radioactivity. At day 40 of uptake, three additional fish were sacrificed for EROD measurements from each of the high-concentration treatments for each chlorinated alkane and from the two control treatments. Liver samples were weighed (0.05 to 0.3 g) and homogenized in 0.5 to 2.0 ml *N*-2-hydroxyethylpiperazine-*N*′-2-ethane-sulfonic acid (HEPES)–KCl (0.02 M HEPES 0.15 M KCl, pH 7.5), depending on sample size, and homogenates were centrifuged for 20 min (15,600 *g*). All preparation steps were done in a cold room (2°C). The supernatants were frozen at −80°C until analyzed.

^{14}C Analysis

Fish samples were frozen, freeze-dried, and weighed prior to extraction. To extract ^{14}C, samples were homogenized in toluene, centrifuged, and the supernatant was then used to determine ^{14}C by adding a fraction of the toluene to fluor (Atomlight, Dupont Chemical Co., Boston, MA, USA). Counting was done on a Beckman LS 7500 liquid scintillation counter (LSC) (Beckman Instruments Inc., Irvine, CA, USA). ^{14}C counts were corrected for quench using a quench curve prepared from ^{14}C-toluene (Dupont Chemical Co.) and were automatically corrected for background by the LSC. Lipids were determined gravimetrically using 1 ml of the supernatant.

Toluene extracts of selected samples (day 40 of uptake and day 20 of depuration) were analyzed by reverse-phase high-performance liquid chromatography (HPLC) to assess the composition of the ^{14}C counts in the standard and fish extract. The day 40 uptake samples were chosen because they were expected to have the highest concentrations. The day 20 depuration samples were chosen because the concentrations of metabolites were expected to be higher than at later depuration dates.

Lipids were first removed from the samples using gel permeation chromatography (GPC) followed by elution through a Florisil column. The GPC columns (inner diameter, 29.5 mm; length, 400 mm, reservoir, 500 ml) were packed with 60 g (dry weight) of 200- to 400-mesh Bio-Beads® S-X3 beads (Bio-Rad Laboratories, Hercules, CA, USA) which had been soaked in dichloromethane (DCM):hexane (1:1) overnight. The column was eluted with 300 ml of DCM:hexane; the first 125 ml contained lipids and were discarded. The remaining eluate, containing the chlorinated alkanes, was evaporated to 1 ml for Florisil cleanup. After adding the GPC eluate to the Florisil column (8 g of 1.2% deactivated Florisil), the chlorinated alkanes were recovered by successive elution with 42 ml of hexane, 38 ml of 85% hexane:15% DCM, and 52 ml of 50% hexane:

50% DCM. The Florisil elutions were combined and then evaporated to near dryness under a gentle N_2 stream and made up in either methanol (C_{12}-chlorinated alkanes) or acetonitrile (C_{16}-chlorinated alkanes) for HPLC analysis. Due to the more hydrophobic nature of the C_{16}-chlorinated alkanes, a less polar solvent, acetonitrile, was substituted for methanol to ensure that the C_{16}-chlorinated alkanes completely dissolved. Samples were injected on a Varian 5000 liquid chromatograph (Varian Canada Inc., Mississuaga, ON, Canada) equipped with a Prep Nova pak HR C-18 column (Waters Division of Millipore®, Milford, MA, USA), an autosampler, and an automated fraction collector. The mobile phase used for the C_{12}-chlorinated alkane samples consisted of 90% methanol and 10% water; 3-min fractions were collected over a 60-min period. For the C_{16}-chlorinated alkane samples, a mobile phase of 90% acetontrile and 10% water was used, and 4-min fractions over 80 min were collected. Fractions were counted using LSC.

The remaining toluene was decanted from the tissue, and the tissue was washed and decanted twice with toluene and allowed to dry. A subsample of the air-dried, toluene-washed tissue was oxidized on a Packard Model 306 Oxidizer (Packard Instruments Co., Downers Grove, IL, USA) for determination of nontoluene-extractable ^{14}C.

Mixed-function oxidase assays

Analysis of liver samples for MFO enzyme activity was carried out with postmitochondrial supernatants as described previously [20]. The small size of the livers precluded preparation of microsomal fractions. The EROD activity was measured using the method of Pohl and Fouts [21] with several modifications [20]. The reaction was started by the addition of 10 μl of ethoxyresorufin in dimethylsulfoxide (0.04 mg/ml). The samples were incubated for precisely 2 min in a water bath at 25°C, and then the reaction was stopped by addition of 2.5 ml of methanol. The samples were centrifuged at 24,000 *g* to pellet the precipitated protein, and the amount of resorufin in the supernatant was determined spectrofluorometrically using an excitation wavelength of 530 nm and an emission wavelength of 585 nm. Protein was determined using the Lowry method as modified by Markwell et al. [22].

Data analysis

Growth rates were determined by fitting all fish and liver weight data to an exponential model (ln fish weight = $a + b$ time (d), where a is a constant and b is the growth rate) [20]. Chlorinated alkane concentrations were corrected for growth dilution and lipid-normalized for all bioaccumulation parameters. Assimilation efficiencies (α) were calculated by fitting the concentration data to the integrated form of the kinetic rate equation for constant dietary exposure [23] using iterative nonlinear regression:

$$C_{fish} = (\alpha F C_{food}/k_d) \times [1 - \exp(-k_d t)]$$

where F is the feeding rate (lipid corrected), C_{fish} is the concentration in the fish (lipid basis and growth corrected), C_{food} is the concentration in the food (on a lipid basis), and t is the time of uptake (d). Feeding rate (F) is assumed to be 1.5% of the body weight of the fish, corrected for the lipid percentage of the fish and the food (14%, determined in the same manner as the lipid percentage in the fish). Depuration rates (k_d) were calculated by fitting the depuration phase data to a first-order decay curve (ln concentration = $a + b$ time (d), where a is a constant and b is the depuration rate). Equilibrium BMF was predicted from the equation $BMF = \alpha \cdot F/k_d$.

Differences between growth rate constants among treatments and 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 elimination rate and growth rate constants at the $p < 0.05$ level of significance.

RESULTS

Effects

The growth rates of both $C_{12}H_{16}Cl_{10}$ treatments, the low-concentration $C_{12}H_{20}Cl_6$ treatment, and the low-concentration $C_{16}H_{31}Cl_3$ treatment were found to be significantly higher than those of the first control group, the high-concentration $C_{16}H_{31}Cl_3$ treatment, and the low- and medium-concentration C_{16} treatments (t test, $p < 0.05$) (Table 1). Based on the growth rates, it is unlikely that the chlorinated alkanes had any negative effect on the growth of juvenile rainbow trout. The same pattern holds for the liver growth rates, with the controls having the slowest growth rates (Table 1). A fin rot disease spread through the control 2, the low-concentration $C_{12}H_{20}Cl_6$ and both $C_{16}H_{31}Cl_3$ treatment tanks, causing a number of mortalities. However, none of the infected fish were used for data analysis, and since there is no pattern to the mortalities with respect to treatment and the affected tanks were side by side, it is unlikely that the disease was a result of the chlorinated alkane exposures. Liver somatic index (LSI = liver weight/whole fish weight × 100) and lipid percentages did not vary between treatments (Table 1), although the percentage lipid increased throughout the experiment.

The EROD levels in chlorinated-alkane-exposed rainbow trout were not higher than those in nonexposed rainbow trout on the last collection day (day 40) of uptake, corresponding to wet weight liver concentrations (ng/g) on day 40 of 16.2 ± 1.0 (mean ± 1 SE) for $C_{12}H_{20}Cl_6$, 23.4 ± 0.3 for $C_{12}H_{16}Cl_{10}$, 27.9 ± 3.1 for $C_{16}H_{31}Cl_3$, and 75.6 ± 5.0 for $C_{16}H_{21}Cl_{13}$.

Bioaccumulation parameters

Accumulation of all four chlorinated alkanes from food by juvenile rainbow trout was observed by day 5 of the uptake phase (Fig. 1). None of the four compounds reached steady state after 40 d of exposure (Fig. 1), and the $C_{16}H_{21}Cl_{13}$ did not reach steady state after 80 d of exposure (data not shown). The depuration rate in rainbow trout exposed to $C_{16}H_{31}Cl_3$ was significantly faster than the depuration rates of rainbow trout exposed to $C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$, and $C_{16}H_{21}Cl_{13}$ (Table 2). With the exception of the $C_{16}H_{31}Cl_3$, depuration rates for each chlorinated alkane did not differ significantly between concentrations ($p < 0.05$) (Table 2), although no comparison was made between the two $C_{12}H_{21}Cl_6$ treatments because data were not available for exactly the same time period of depuration for the lower-concentration exposure (26.2 ng/g). Trends of depuration rates in whole fish were consistent with depuration rates determined using concentration data only from carcass tissue. Whole-body half-lives varied from 37 ± 2 d in the high-concentration $C_{16}H_{31}Cl_3$ treatment to 87 ± 11 d in the low-concentration $C_{12}H_{21}Cl_{10}$ treatment (Table 2).

Assimilation efficiencies based on whole-body concentrations ranged from 9.4 ± 1.1% in the medium-concentration $C_{16}H_{21}Cl_{13}$ treatment to 37.6 ± 1.1% in the low-concentration $C_{12}H_{16}Cl_{10}$ treatment (Table 2). Whole-body BMFs varied from 0.44 in the medium-concentration $C_{16}H_{21}Cl_{13}$ treatment to 2.15 in the low-concentration $C_{12}H_{21}Cl_{10}$ treatment. Assimilation efficiency and

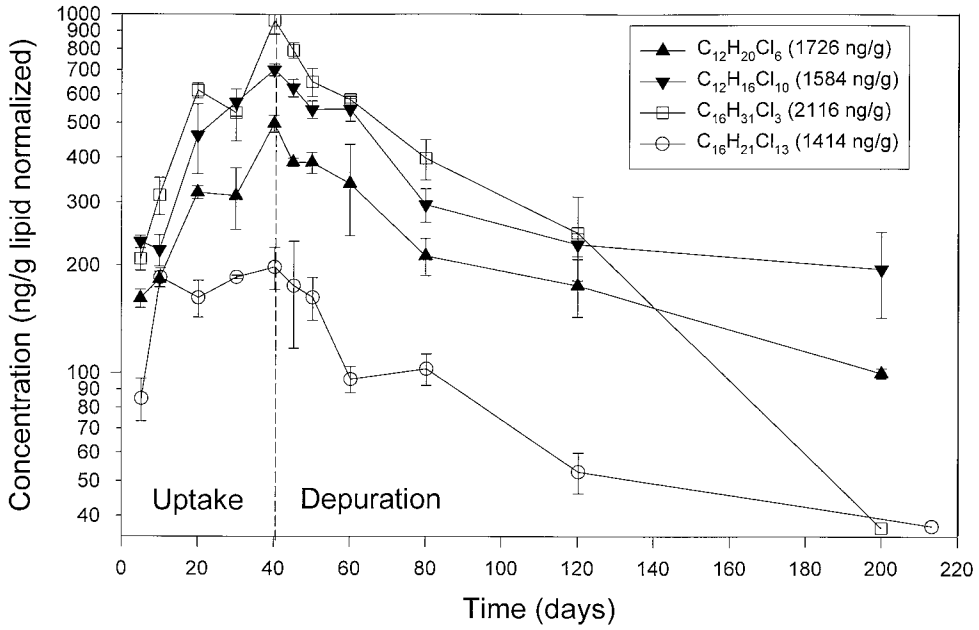

Fig. 1. Accumulation and depuration of four ^{14}C-polychlorinated alkane compounds through dietary exposure to juvenile rainbow trout. Each point is the mean ± 1 standard error of three fish. Concentrations are for whole fish, corrected for growth dilution and lipid content. Exposure concentrations (lipid corrected) are provided in the legend.

BMF values calculated with whole-body and carcass-tissue-only concentrations were similar and followed the same trends for all four chlorinated alkanes.

Tissue distribution and metabolic transformation

The carcass contained the greatest percentage of ^{14}C (including extractable and nonextractable) throughout the experiment for all four chlorinated alkanes, ranging from 50% to greater than 70% (Table 3). The relative proportion of ^{14}C increased in the carcass over time, mainly due to increasing amounts of nonextractable ^{14}C. There was a slight drop in relative amounts of extractable ^{14}C in the GI tract from the beginning until the end of the uptake phase, which was probably due to the greater proportion of ^{14}C in the GI tract resulting from undigested spiked food in the gut. Nonextractable ^{14}C decreased in the liver and GI tract over time, which could be explained by the high turnover of the liver and GI tract lining. Relative amounts in the liver were low because the liver accounted for only about 1.5%

Table 2. Bioaccumulation parameters of four ^{14}C-chlorinated alkanes from dietary exposures using juvenile rainbow trout data for whole-body concentrations (significant differences ($p < 0.05$) between whole-body depuration rates calculated with 160 d of depuration data are indicated with capital letters)

Chemical	Concn. in food (ng/g wet wt)	Length of depuration (d)	Depuration rate constant[a] (10^{-2}/d)	$t_{1/2}$[b] (d)	BMF[c]	Assimilation efficiency[d] (%)
$C_{12}H_{20}Cl_6$	26.2[e]	80	1.8 ± 0.2 (0.83)	39 ± 4	0.60	25.3 ± 2.5
	242	160	0.9 ± 0.1 (0.75)[C]	77 ± 9	0.93	20.7 ± 2.0
$C_{12}H_{16}Cl_{10}$	20.5	160	0.8 ± 0.1 (0.68)[C]	87 ± 11	2.15	37.6 ± 1.1
	222	160	0.9 ± 0.1 (0.75)[C]	77 ± 9	1.76	34.1 ± 1.3
$C_{16}H_{31}Cl_3$	28.9	160	1.4 ± 0.2 (0.73)[B]	50 ± 7	1.07	33.1 ± 2.0
	296	160	1.9 ± 0.1 (0.93)[A]	37 ± 2	0.90	35.1 ± 2.0
$C_{16}H_{21}Cl_{13}$	20.8[f]	80	1.2 ± 0.2 (0.62)	58 ± 10	0.72	30.0 ± 7.2
	198	173	1.1 ± 0.2 (0.69)[C]	63 ± 11	0.44	9.4 ± 1.1
	2,003	173	0.9 ± 0.1 (0.68)[C]	77 ± 9	0.50	11.7 ± 1.3

[a] Depuration rate constants (k_d) (±1 standard error) were calculated using the model ln concentration (lipid weight basis) = $a + b$ (time) for the elimination of toluene-extractable radioactivity for 160 d of depuration (coefficient of determination for the model is shown in parentheses).

[b] Half-life (± 1 standard error) is calculated from the equation $t_{1/2} = 0.693/k_d$.

[c] Biomagnification factor (BMF) is calculated from the equation BMF = $\alpha F/k_d$, where F is the feeding rate on a lipid basis.

[d] The assimilation efficiency (α) (± 1 standard error) is determined by fitting the data to the integrated form of the kinetic rate equation for constant dietary exposure using iterative nonlinear regression: $C_{fish} = (\alpha F C_{food}/k_d)\cdot[1 - \exp(-k_d t)]$, where C_{fish} is the concentration in the fish (lipid basis and growth corrected), C_{food} is the concentration in the food (on a lipid basis), and t is the time of uptake (d).

[e] Due to mortalities, no fish from this treatment were available for day 160 analysis.

[f] Depuration data of the low-concentration $C_{16}H_{21}Cl_{13}$ treatment did not significantly fit a linear relationship when day 160 was included; therefore, depuration rate was calculated with only 80 d of depuration data.

Table 3. Extractable (Ext) and nonextractable (Nonext) radioactivity as a percentage of the total fish radioactivity in the liver, gastrointestinal (GI) tract, carcass, and total fish of juvenile rainbow trout exposed to four ^{14}C-polychlorinated alkane compounds (U refers to the uptake phase and D the depuration phase)

		$C_{12}H_{20}Cl_6$		$C_{12}H_{16}Cl_{10}$		$C_{16}H_{31}Cl_3$		$C_{16}H_{21}Cl_{13}$	
Tissue	Day	Ext	Nonext	Ext	Nonext	Ext	Nonext	Ext	Nonext
Liver	10 U	1.2	1.5	1.9	1.4	1.7	1.7	16.0[a]	3.1[a]
	20 U	1.1	1.2	1.4	1.1	0.8	1.0	6.6[b]	2.6[b]
	40 U	0.6	2.2	0.7	1.1	0.6	0.8	5.1	28.7
	10 D	0.2	0.8	0.3	0.9	0.5	0.8	4.3	3.9
	40 D	0.3	0.6	0.1	0.7	0.2	0.3	1.4	1.4
	80 D	0.1	0.5	0.2	0.4	0.2	0.2	1.0	1.2
GI tract	10 U	31.0	19.9	29.7	15.3	26.8	14.9	26.2[a]	16.3[a]
	20 U	32.0	18.6	24.8	11.9	22.0	9.8	30.5[b]	15.0[b]
	40 U	26.4	14.1	26.1	6.4	21.4	10.2	15.8	12.2
	10 D	22.1	5.1	26.6	2.1	14.2	5.0	22.7	4.8
	40 D	26.3	3.2	24.2	3.8	16.9	1.8	24.7	2.8
	80 D	30.7	5.6	25.6	2.0	22.5	26.6	21.6	2.5
Carcass	10 U	32.0	14.3	41.8	9.9	36.1	18.8	27.1[a]	11.3[a]
	20 U	31.9	15.3	51.0	9.7	46.0	20.4	35.7[b]	9.6[b]
	40 U	37.0	19.6	51.5	14.3	44.2	22.8	25.6	12.6
	10 D	41.3	30.5	49.5	20.5	46.8	22.2	53.9	10.4
	40 D	45.8	23.8	48.0	22.9	49.7	31.1	50.9	18.8
	80 D	37.1	26.0	47.4	24.4	30.2	20.3	45.3	28.4
Total	10 U	64.2	33.7	73.4	26.6	64.6	35.4	69.3[a]	30.7[a]
	20 U	65.1	34.9	77.2	22.8	68.8	31.2	72.8[b]	27.2[b]
	40 U	64.0	36.0	78.3	21.7	66.2	33.8	46.5	53.5
	10 D	63.6	36.4	76.4	23.6	61.5	38.5	80.9	19.1
	40 D	72.4	27.6	72.3	27.7	66.8	33.2	77.0	23.0
	80 D	67.9	32.1	73.2	26.8	52.9	47.1	67.9	32.1

[a] Day 10 $C_{16}H_{21}Cl_{13}$ liver samples were lost; percentages represent day 5 liver samples.
[b] Day 20 $C_{16}H_{21}Cl_{13}$ liver samples were lost; percentages represent day 30 liver samples.

of the total fish weight. Both extractable and nonextractable ^{14}C decreased in the liver throughout the experiment, providing evidence that metabolic transformation of chlorinated alkanes may occur in the liver.

High-peformance liquid chromatography (HPLC) chromatograms of the toluene extracts differed from the analytical standards on day 40 of uptake for all four chlorinated alkanes (Fig. 2). This difference is most pronounced in the $C_{12}H_{20}Cl_6$ treatment, where a number of larger peaks in the fish extracts are minor in the analytical standard. After 20 d of depuration (no exposure to treated food), all four chlorinated alkanes showed chromatographic profiles markedly different from the analytical standards (Fig. 2). Toluene nonextractable residue is assumed to represent chlorinated alkanes which have been metabolically transformed and have become more polar and therefore unextractable with toluene. The more highly chlorinated C_{12}- and C_{16}-alkanes had a greater proportion of toluene-extractable ^{14}C, or parent compound (Table 3), implying less metabolic transformation of these compounds.

DISCUSSION

Chlorinated alkanes with 12 and 16 carbons and 35 to 69% Cl content (by weight) are accumulated through dietary exposure by juvenile rainbow trout despite relatively large molecular weight. As other authors have observed with CPs, accumulation of chlorinated alkanes is dependent on the carbon chain length [6], number of chlorines [3], and molecular size of the molecule [9]. The assimilation efficiency of the C_{12}-chlorinated alkanes in this experiment increased from a mean of 23 to 36% (whole fish) with the addition of four chlorine atoms, corresponding to an increase in K_{ow} (log K_{ow} of $C_{12}H_{20}Cl_6$ and $C_{12}H_{16}Cl_{10}$ is approx. 5.2 and 6.8, respectively; G.R.B. Webster, personal communication). However, the assimilation efficiency of the C_{16}-chlorinated alkanes decreased from a mean 34 to 11% when the number of chlorine atoms increased from three to 13, despite an increase in K_{ow} (log K_{ow} of $C_{16}H_{31}Cl_3$ and $C_{16}H_{21}Cl_{13}$ is approx. 6.9 and 7.4, respectively; G.R.B Webster, personal communication). The $C_{16}H_{21}Cl_{13}$ was found to have the highest K_{ow} of the four chlorinated alkanes but the lowest accumulation and assimilation efficiency. The reduced uptake of the $C_{16}H_{21}Cl_{13}$ may be due to its large size (MW = 674) [24–26]. Low uptake of long-chain CPs has also been observed by Zitko [9], who found that two industrial CP products with high molecular weights (MW = 579 to 922) showed little bioaccumulation in juvenile Atlantic salmon.

The assimilation efficiencies calculated for these chlorinated alkanes are higher than assimilation efficiencies reported for commercial CP products using bleaks [6]. An industrial CP mixture consisting of C_{10-12} CPs with 71% Cl had a ''mean effectiveness in uptake'' from food of only 6%, which was half the assimilation found for a less chlorinated (49% Cl) C_{10-12} CP commercial product [6]. We found that an increase in chlorination of C_{12}-chlorinated alkanes resulted in an increase in assimilation efficiency. The assimilation efficiency of the chlorinated alkanes used in this experiment are comparable to those of tetra- and pentachlorodibenzofurans under similar experimental conditions using juvenile rainbow trout [20,27] and to those of hexa- and octachlorbiphenyls in guppies (*Poecilia reticulata*) [28].

Half-lives of the chlorinated alkanes in this experiment are different than half-lives reported for other CPs [6,29]. Madeley and Maddock [29] reported a half-life range from 16.5 d in

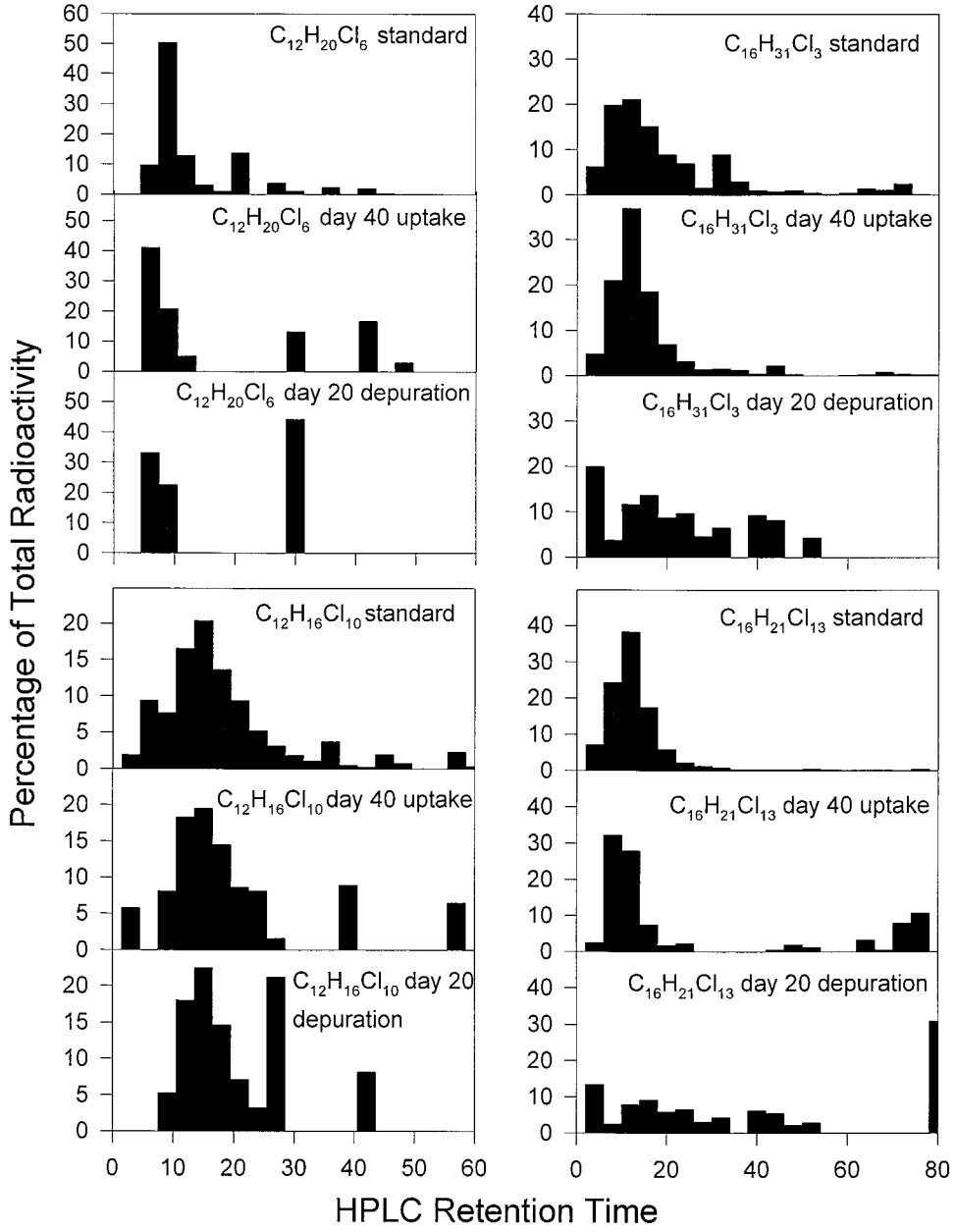

Fig. 2. High-performance liquid chromatography chromatograms of the ^{14}C-polychlorinated alkane standards and fish carcass toluene extracts from day 40 of uptake and day 20 of depuration. Each bar in the C_{12}-chlorinated alkane and C_{16}-chlorinated alkane chromatograms represent the radioactivity in a 3- or 4-min fraction, respectively, as a percentage of the total radioactivity.

dorsal muscle to 23.9 d in the viscera of rainbow trout exposed to waterborne short-chain (C_{10-12}) 58% CP, about half the value we calculated for the $C_{12}H_{20}Cl_6$. Bengtsson et al. [6] reported very rapid elimination ($t_{1/2}$ <7 d) of a 49% Cl short-chain (C_{10-13}) CP mixture. However, Bengtsson et al. [6] also reported that there was no elimination of a short-chain (C_{10-13}) CP with 71% Cl after a 316-d elimination period, much longer than the 77- to 87-d half-life calculated for the $C_{12}H_{16}Cl_{10}$ used in this experiment. It should be noted that the concentrations of CPs reported by Bengtsson et al. [6] were determined by measuring chlorine levels in the fish and not CPs. It may well be that the CPs in Bengtsson et al.'s [6] work were metabolically transformed but the Cl remained in the fish.

The $C_{12}H_{16}Cl_{10}$ would be expected to biomagnifiy in aquatic food webs based on its equilibrium BMFs of 1.76 and 2.15. A BMF value greater than 1 implies increasing concentrations along aquatic food webs, or biomagnification [15,27]. The low-concentration $C_{16}H_{31}Cl_3$ treatment was also found to have a BMF above 1 (1.07); however, the high-concentration treatment had a BMF of only 0.90. The addition of one or two chlorines to $C_{16}H_{31}Cl_3$ could reduce the depuration rate, by decreasing metabolism, and increase the assimilation efficiency, due to a higher K_{ow}, sufficiently to result in a BMF greater than 1. The $C_{16}H_{21}Cl_{13}$ and the $C_{12}H_{20}Cl_6$ would not biomagnify in aquatic food webs based on their predicted equilibrium BMFs of 0.44 to 0.50 and 0.60 to 0.93, respectively.

From the results of these experiments, it appears that the relationship of bioaccumulation and carbon chain length and chlorine content of chlorinated alkanes is complex. Although metabolism of short-chain CPs (C_{10-13}) with low chlorination may reduce accumulation, lower-chlorinated medium- (C_{14-18}) and long- (C_{19-30}) chain CPs may be less susceptible to metab-

olism because of the carbon chain length [30]. High chlorination of short-chain chlorinated alkanes results in sufficient accumulation for biomagnification but makes medium- and long-chain, highly chlorinated alkanes too large to diffuse through biological membranes without hindrance. The results suggest that, despite having some characteristics of bioaccumulative chemicals (i.e., high K_{ow}, low biotransformation rate), highly chlorinated (>60%), medium- (C_{14-18}) and long- (C_{19-38}) carbon-chain alkanes are not likely to biomagnify in aquatic food webs. A further confounding factor, to which we could not address with these ^{14}C-labeled chlorinated alkanes, is the position of the chlorines on the carbon chain. The HPLC chromatogram of the fish extracts showed that certain chlorinated alkanes within the standards were accumulating to a greater extent than others. Chlorine positioning on the alkane chain could explain the persistence of some alkanes, and low persistence of other chlorinated alkanes, with the same molecular formula. This differential bioaccumulation, which is observed in invertebrates, fish, and mammals with other chlorinated compounds such as chlordane [31], toxaphene [32], and PCBs [33], needs further study.

These experiments provide the first dietary bioaccumulation parameters for chlorinated alkanes with a single carbon chain length and known amount of chlorine, although the positions and exact number of chlorine atoms are not known. From Bergman et al. [19] and our own HPLC work, it appears that the number of chlorine atoms per compound in each standard is close to the integer value assigned in this report, although due to nonselective synthesis procedures for these ^{14}C compounds, these standards are likely composed of numerous compounds and positional isomers. Confounding this problem is the identical electron capture negative ion mass spectra of CP congeners containing similar carbon and chlorine atoms (G.T. Tomy, personal communication), making it difficult to identify separate chlorinated alkanes based on chlorine substitution patterns.

Evidence suggests that CPs are oxidized in fish [30,34], with short-carbon-chain (C_{10-13}) CPs being more susceptible to metabolism than medium- (C_{14-18}) and long-chain (C_{19-30}) CPs [30]. Åhlman et al. [35] have found sulphur-containing metabolites of CPs in rats, implying that CPs may covalently bind to biological macromolecules. Lower Cl substitution resulted in greater relative amounts of nontoluene-extractable ^{14}C for the C_{12} and C_{16} chlorinated alkanes. Because the ^{14}C was unextractable from the tissue with toluene, it is considered to represent a more polar compound than the standard (such as a hydroxlated or carboxylic acid substituted product) or has been incorporated with biological macromolecules.

The $C_{12}H_{20}Cl_6$ showed greater change from the analytical standard than the more highly chlorinated $C_{12}H_{16}Cl_{10}$, but this relationship is not as evident for the C_{16}-chlorinated alkanes. Madeley and Birtley [30] reported that short-chain ($<C_{13}$) CPs with low chlorination (<60%) are the most readily oxidized in microorganisms. However, it is obvious from the HPLC-^{14}C chromatogram of day 20 depuration that all the chlorinated alkanes used in this experiment are susceptible to metabolism. Therefore, the rate of metabolism of chlorinated alkanes is dependent on both chlorine substitution and carbon chain length but may also be dependent on chlorine position.

No toxic effects were observed in any of the treatments. Past toxicity studies on CPs have found that large doses are required for toxic effects in fish [29,36]. No EROD induction was observed for any of the treatments on day 40 of uptake, when burdens in the fish were at their highest. This is not surprising because chlorinated alkanes do not have the same planar ringed structure normally found in EROD-inducing organochlorines such as PCDD/Fs and coplanar PCBs [16,37]. However, in the only other work involving EROD induction and CPs, female flounder exposed to extremely high doses of an industrial CP mixture were found to have elevated EROD levels [18]. The EROD induction found in the flounder may be a result of impurities, such as chlorinated aromatics, which have been found within industrial CP mixtures [38].

In summary, the bioaccumulation parameters of chlorinated alkanes reported here differ from previous results on bioaccumulation of CPs in fish. These chlorinated alkanes are found in commercial CP products, although they are synthesized in a different manner, and this represents the first work that has used chlorinated alkanes of known carbon chain length and chlorine content for dietary bioaccumulation studies. The elimination rates, half-lives, and assimilation efficiencies of the chlorinated alkanes are similar to those of PCDD/Fs and PCBs. Reduced accumulation of lower chlorinated C_{12}-alkane could be attributed to metabolism, while uptake of highly chlorinated C_{16}-alkane may have been hindered because of its large molecular size. The $C_{12}H_{16}Cl_{10}$ would likely biomagnify in aquatic food chains based on a BMF of greater than 1 (1.76 and 2.15). Highly chlorinated short-chain (C_{10-13}) CPs and lower chlorinated medium-chain (C_{14-18}) CPs have the greatest potential for bioaccumulation by aquatic organisms.

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