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Dietary accumulation and depuration of individual C_{10}-, C_{11}- and C_{14}-polychlorinated alkanes by juvenile rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Dietary exposures using juvenile rainbow trout (*Oncorhynchus mykiss*) were conducted with 19 polychlorinated alkanes (PCAs) with varying carbon chain length (C_{10}, C_{11} and C_{14}) and chlorine content (4–8 Cl atoms) to determine bioaccumulation parameters. Although these PCAs have the same carbon chain lengths and chlorine content as some chlorinated paraffin (CP) products, all are 1,2-Cl substituted and would not likely be prevalent in commercial CP mixtures. All of the PCAs were rapidly accumulated from the food and had high assimilation efficiencies. Half-lives of PCAs ranged from 7 to 53 d, but in general were much lower than expected for compounds of log K_{ow} of 6 or greater. Half-lives were positively correlated with K_{ow}, carbon chain length and chlorine content. All of the C_{14}-PCAs, and a number of the higher chlorinated C_{10}- and C_{11}-PCAs, had biomagnification factors (BMF) >1, implying a potential to biomagnify in aquatic food chains. BMFs increased with increasing K_{ow} and decreasing carbon chain length. Based on these results and previous work, highly chlorinated short-carbon-chain (C_{10-13}) PCAs and lower and medium chlorinated (40–60% Cl) medium-carbon-chain PCAs (C_{14-18}) have the greatest potential for biomagnification among PCAs or CPs. Cl position was also found to influence bioaccumulation parameters. Shorter-carbon-chain and lower chlorinated PCAs appear to be more susceptible to biotransformation by rainbow trout, compared with persistent organochlorines, such as PCBs, studied under identical conditions.

Keywords: Polychlorinated alkanes; Chlorinated paraffins; Dietary accumulation; Half-life; Biomagnification; Rainbow trout

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1. Introduction

Polychlorinated *n*-alkanes (PCAs) (C_{10-30}, 35–70% Cl by weight), also known as chlorinated paraffins (CPs), are used as high pressure lubricants, flame retardants, and plasticizers, and for a number of other industrial applications (Campbell and McConnell, 1980; Canadian Environmental Protection Act, 1993). Annual global production of CPs is approximately 300 kilotonnes, with a majority having medium-carbon-chain (C_{14-18}) length. Short-carbon-chain CPs (C_{10-13}) have been placed on the Priority Substance List under Canada's Environmental Protection Act and on the Environmental Protection Agency Toxic Release Inventory in the USA.

CPs have low water solubility and are accumulated by fish from water and food in laboratory experiments (Bengtsson et al., 1979; Fisk et al., 1996a). The toxicity of CPs to aquatic life is considered low. However, short- and medium-carbon-chain CP products have a potential to act as tumor promotors in mammals (Kato and Kenne, 1996). Data on environmental levels of CPs is scarce, but CPs have been measured at relatively high concentrations in biota from Sweden (Jansson et al., 1992), sewer films from Germany (Rieger and Ballschmiter, 1995), biota, sediment and water from Lakes Erie and Winnipeg, Canada (Fisk et al., 1996b; Tomy et al., 1997), and marine biota and human milk from the Canadian arctic (Stern et al., 1997).

Commercial CP products are complex mixtures which consist of thousands of congeners. For example, there are 4200 congeners theoretically possible (not including enantiomers) in one commercial CP product (C_{10-13}, 60% Cl). Unfortunately, standards for individual polychlorinated alkanes (PCAs) are not available and PCAs are not resolved by high resolution, capillary gas chromatography (GC) columns (Tomy et al., 1997). Therefore, to examine the dietary accumulation of individual CP compounds by juvenile rainbow trout, it was necessary to synthesize PCAs by chlorination of alkenes. In this study four chlorinated products were used (C_{11}-, C_{14}- and two C_{10}-PCAs) which contained PCAs with known Cl content and positioning, as well as PCAs with known Cl content but unknown positioning. These latter products were formed by free radical substitution of H by Cl. The objective of this work was to develop relationships between bioaccumulation parameters and carbon chain lengths, chlorine content and K_{ow} for PCAs with a range of carbon chain lengths and chlorine content.

2. Methods and materials

2.1. Chemicals

PCAs were synthesized by chlorination (Cl_2 gas) of four individual alkenes: 1,9-decadiene; 1,5,9-decatriene; 1,10-undecadiene; and 1,13-tetradecadiene (Fig. 1) (Tomy et al., 1997). The dominant product of each synthesis was an alkane derived by Cl addition to the double bonds (Fig. 1). As well, a number of additional PCAs were produced which had the same chlorine substitution as the dominant product but with additional Cl atoms due to free radical substitution (Figs. 1 and 2). PCAs with the same molecular formula but different Cl substitution patterns have been differentiated with a letter at the end of the chemical formula.

2.2. Experimental protocol

Two treatments, each containing two of the synthesized PCA standards, were established. Products of the 1,9-decadiene and 1,13-tetradecadiene chlorination were combined and will be referred to as standard DT (Fig. 2). The combined products of the 1,5,9-decatriene and the 1,10-undecadiene chlorination will be referred to as standard DU. All PCAs in DU and DT standards that were resolved by GC methods were quantified. Food was spiked by suspending a known quantity of each of the standards (DT and DU) separately in 150 ml of hexane and ~100 g of commercial fish food (41% protein, 14% lipid and 3% fiber) and slowly evaporating to dryness using a roto-evaporator under vacuum. Concentrations in the food were determined by the same analytical techniques used to determine levels in the

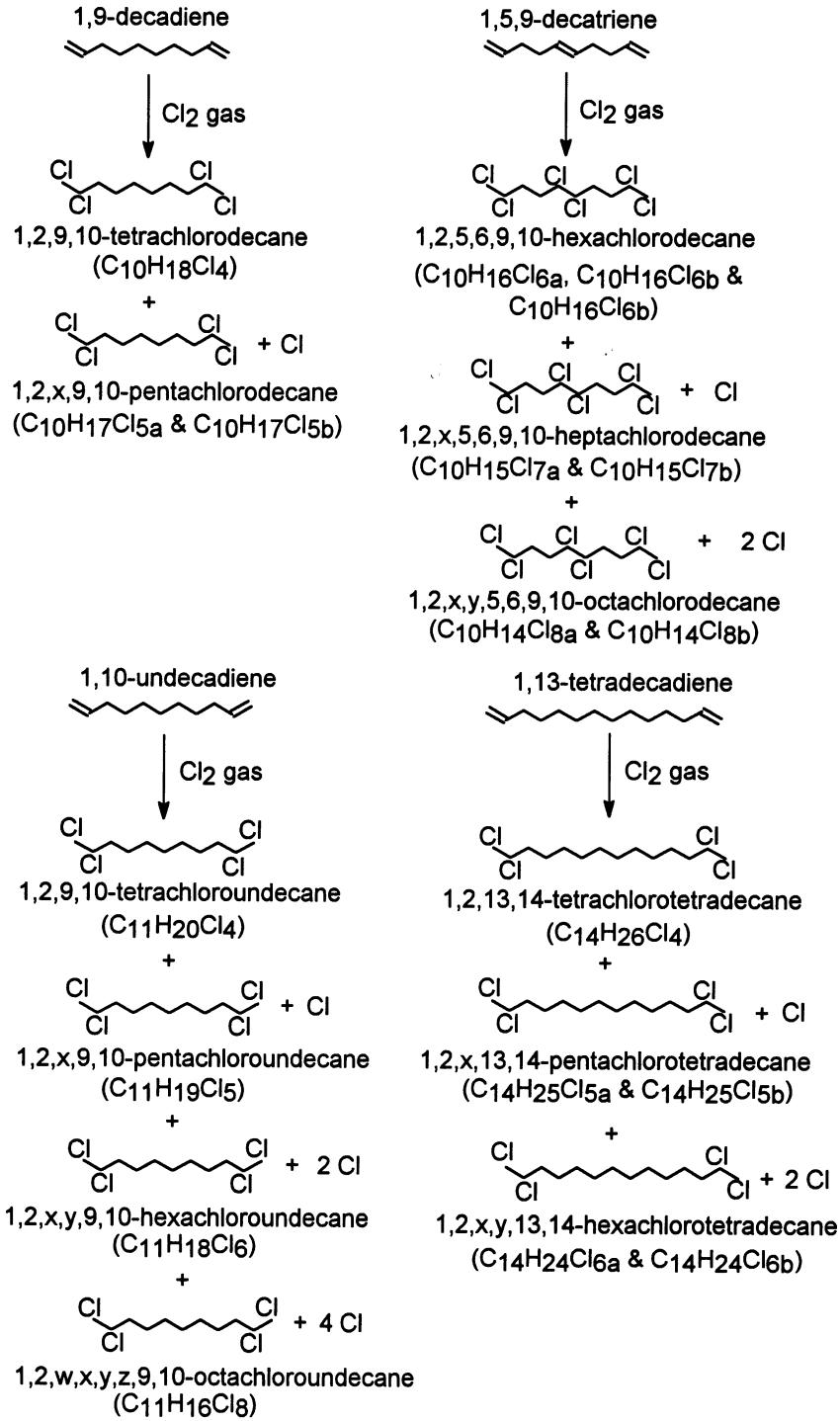

Fig. 1. Polychlorinated alkanes (PCAs), and their starting material, used for dietary accumulation experiments with juvenile rainbow trout. A letter in the chemical name represents a Cl atom whose position on the alkane is unknown. PCAs with the same molecular formula but different arrangements of Cl atoms are differentiated with a letter.

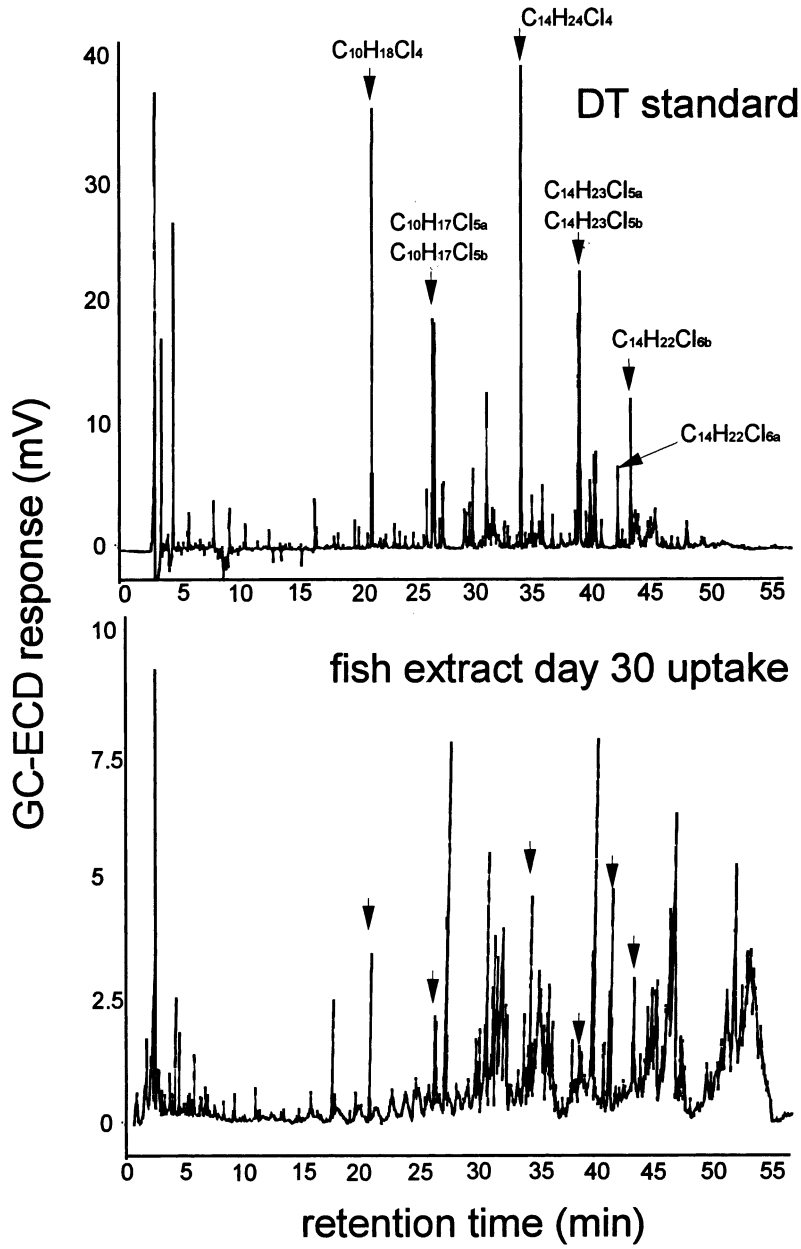

Fig. 2. GC-ECD chromatograms of the DT standard and fish extract after 30 days exposure to the DT standard in food. Chlorination of the alkene compounds produced PCAs with the same molecular formula but different Cl substitution patterns. These PCAs have been identified with a letter at the end of the chemical formula.

rainbow trout tissue (Table 1). Control food was treated in an identical manner, but without the addition of a chlorinated alkane compound.

Juvenile rainbow trout (*Oncorhynchus mykiss*) (initial weights 2–7 g) were exposed to the spiked food for 40 days followed by 80 days of depuration. Three fish were sampled from each treatment on days 5, 10, 20, 30 and 40 of the uptake period,

Table 1
Bioaccumulation parameters of chlorinated alkanes from dietary exposures using juvenile rainbow trout

Compound[a]	Log K_{ow}[b]	Concentration in food[c] ($ng \cdot g^{-1}$)	Depuration length[d] (days)	Depuration rate[e] (10^{-2})	Half-life[f] (days)	Assimilation efficiency[g] (%)	BMF^{h}_{CALC}	BMF^{i}_{EQUIL}	BMF^{j}_{SS}
$C_{10}H_{18}Cl_4$	5.8	412	40	8.3 ± 1.5 (0.73)	8.3 ± 1.5	23 ± 4.0 (0.73)	0.26	0.46	0.18
$C_{10}H_{17}Cl_{5a}$	6.1	251	40	8.9 ± 1.5 (0.74)	7.8 ± 1.3	13 ± 2.6 (0.66)	0.14	0.43	0.10
$C_{10}H_{17}Cl_{5b}$	6.1	737	40	9.7 ± 1.6 (0.73)	7.1 ± 1.2	76 ± 10 (0.82)	0.73	0.40	0.48
$C_{10}H_{16}Cl_{6a}$	6.3	1754	80	6.8 ± 0.6 (0.91)	10 ± 0.9	130 ± 35 (0.54)	1.5	0.57	1.4
$C_{10}H_{16}Cl_{6b}$	6.3	542	80	6.9 ± 0.6 (0.92)	10 ± 0.9	63 ± 19 (0.49)	0.71	0.56	0.64
$C_{10}H_{16}Cl_{6c}$	6.3	526	80	3.4 ± 0.9 (0.49)	20 ± 5.4	46 ± 12 (0.55)	1.1	1.1	0.66
$C_{10}H_{15}Cl_{7a}$	6.5	106	80	4.7 ± 0.5 (0.86)	15 ± 1.6	100 ± 29 (0.51)	1.6	0.82	1.5
$C_{10}H_{15}Cl_{7b}$	6.5	91	80	8.1 ± 0.5 (0.95)	8.5 ± 0.5	107 ± 30 (0.52)	1.0	0.48	1.1
$C_{10}H_{14}Cl_{8a}$	6.8	183	80	2.3 ± 0.4 (0.66)	30 ± 5.2	41 ± 12 (0.48)	1.4	1.7	1.1
$C_{10}H_{14}Cl_{8b}$	6.8	132	80	5.0 ± 0.4 (0.91)	14 ± 1.1	105 ± 45 (0.33)	1.6	0.77	0.67
$C_{11}H_{20}Cl_4$	6.1	590	80	6.4 ± 0.6 (0.87)	11 ± 1.0	54 ± 16 (0.50)	0.65	0.60	0.60
$C_{11}H_{19}Cl_5$	6.3	154	80	7.7 ± 1.2 (0.78)	9.0 ± 1.4	39 ± 11.2 (0.53)	0.39	0.50	0.64
$C_{11}H_{18}Cl_6$	6.5	592	80	4.1 ± 0.6 (0.75)	17 ± 2.5	29 ± 9.6 (0.43)	0.54	0.94	0.40
$C_{11}H_{16}Cl_8$	6.9	108	80	1.9 ± 0.5 (0.61)	37 ± 9.6	41 ± 12 (0.53)	1.7	2.0	1.0
$C_{14}H_{26}Cl_4$	6.8	92	80	1.8 ± 0.3 (0.74)	39 ± 6.4	33 ± 2.4 (0.94)	1.7	2.1	0.82
$C_{14}H_{25}Cl_{5a}$	6.9	66	80	1.3 ± 0.3 (0.58)	53 ± 12.3	51 ± 3.9 (0.94)	3.6	3.0	1.4
$C_{14}H_{25}Cl_{5b}$	6.9	54	80	1.5 ± 0.3 (0.69)	46 ± 9.2	46 ± 3.5 (0.93)	2.9	2.6	1.2
$C_{14}H_{24}Cl_{6a}$	7.1	63	80	2.4 ± 0.6 (0.55)	29 ± 7.2	130 ± 12 (0.92)	5.0	1.9	2.8
$C_{14}H_{24}Cl_{6b}$	7.1	40	80	1.6 ± 0.3 (0.69)	43 ± 8.1	27 ± 2.4 (0.91)	1.6	2.9	0.74

Parameters were calculated from carcass concentrations (three trout/sampling day), and were corrected for growth dilution and lipid normalized.

[a] Chlorination of the alkene compounds produced PCAs with the same molecular formula but different Cl substitution patterns. Therefore, these PCAs have been identified with a letter at the end of the chemical formula.

[b] Log K_{ow} values determined from the equation: $\log K_{ow} = -0.386 + 0.6 * N_{tot} - 0.0113 * N_{tot}^2$, where N_{tot} is the total number of carbon and chlorine atoms (Sijm and Sinnige, 1995).

[c] Concentration in food is given as wet weight.

[d] Only the first 40 days of data were used to calculate the depuration rate of $C_{10}H_{18}Cl_4$, $C_{10}H_{17}Cl_{5a}$, and $C_{10}H_{17}Cl_{5b}$, because these PCAs were not detectable on day 80.

[e] Depuration rate constants (k_d) (± 1 standard error) were calculated using the model ln concentration (lipid wt basis) $= a + b$ (time) for the elimination of toluene-extractable radioactivity for 120 days of depuration (coefficient of determination for the model is shown in parentheses).

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

[g] 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 non-linear regression: $C_{fish} = (\alpha F C_{food}/k_d) * [1 - \exp(-k_d t)]$ where F is the feeding rate on a lipid basis, 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 (days). r^2 are provided in the brackets.

[h] Biomagnification factor (BMF_{CALC}) is calculated from the equation $BMF = \alpha F/k_d$.

[i] BMF_{EQUIL} calculated assuming α is 0.5.

[j] $BMF_{SS} = C_{fish}$ (lipid corrected, not growth corrected)/C_{food} (lipid corrected) on day 40 of uptake. C_{14}-PCAs did not achieve steady state.

and days 5, 10, 20, 40 and 80 of the depuration period. Sampled fish were separated into liver, 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), and all tissue was weighed and frozen until analyzed.

2.3. Extraction and analysis

Extraction of PCAs was identical to the methods used by Fisk et al. (1998). In brief, carcass samples were freeze dried and homogenized in toluene. The extracts were exchanged into hexane, and a portion was used to determine lipids gravimetrically. Lipids were removed from the sample by gel permeation chromatography. The lipid-free eluate, containing the PCAs and other organochlorines, was evaporated to 1 ml and applied to a Florisil column (8 g, 1.2% deactivated). PCAs were eluted from the Florisil column by successive elution using 38 ml of hexane (F1), 42 ml dichloromethane (DCM):hexane (15:85) (F2), and 52 ml of DCM:hexane (1:1) (F3). F1 contained polychlorinated biphenyls and a number of other potentially interfering organochlorine compounds, but no PCAs, and was discarded. F2 and F3 contained the PCAs and were combined, evaporated, transferred to 2,2,4-trimethyl pentane and evaporated to approximately 100 ml for GC analysis.

Individual compounds in the DU and DT standards were quantified by comparing their electron ionization (EI)-mass spectrum (MS) response to the EI-MS responses of a series of $C_{10}H_{20}Cl_2$ standards of known concentration. These DU and DT standards were then used as external standards for quantification of samples by GC with an electron capture detector (ECD). Samples were analyzed on a Varian 3600-GC equipped with a 60-m × 0.25-mm DB-5 column and an 63Ni-ECD. The carrier gas was H_2. Responses of individual PCA compounds were monitored by analyzing DU and DT standards after every four samples. The limits of detection were approximately 1 $ng \cdot g^{-1}$.

2.4. Data analysis

Depuration rates (k_d) were determined by fitting the data to a first order decay curve (ln conc $= a + b$ time (d), were a is a constant and b is the k_d). Half-life (days) is $= \ln 2/k_d$. Assimilation efficiency (α) was determined by fitting the concentration data to the integrated form of the kinetic rate equation for constant dietary exposure using iterative non-linear regression (Bruggeman et al., 1981):

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

where F is the feeding rate (lipid corrected), C_{fish} is the concentration in the fish (lipid corrected), C_{food} is the concentration in the food (lipid corrected) and t is the time (d). Equilibrium biomagnification factors (BMFs) (BMF_{EQUIL}) were predicted from the equation $BMF = \alpha \cdot F/k_d$. Calculated BMFs (BMF_{CALC}) were determined with the same equation used for BMF_{EQUIL}, but assumed an α of 0.5. Steady state BMFs (BMF_{SS}) were determined from the equation $BMF_{SS} = C_{fish}/C_{food}$ (lipid corrected) using data from day 40 of the uptake phase.

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

3. Results and discussion

The compounds used in these dietary accumulation experiments are the first synthesized C_{10-14} PCAs with known carbon chain length, Cl content and Cl position. Commercial CP products are PCAs. However, the PCAs used in these experiments are not likely to be found at high proportions in commercial CP mixtures because they contain Cl on terminal and adjacent carbons, which is chemically and energetically unfavorable under free radical reaction conditions used in the production of commercial CP products. Unfortunately, PCA standards with known chlorine content and chlorine position that do not have

Table 2
Growth parameters of juvenile rainbow trout exposed to mixtures of polychlorinated alkanes

Treatment	Growth rate[a] (10−3/d)	% Lipid[b]	LSI[c] day 40	LSI[c] day 120	% Mortality
Control	16.7 ± 1.8 (0.76)	2.6 ± 0.1	1.6 ± 0.3	1.0 ± 0.1	0
DU	16.4 ± 1.9 (0.73)	3.0 ± 0.2	1.6 ± 0.8	0.9 ± 0.1	0
DT	15.4 ± 1.1 (0.87)	2.7 ± 0.1	1.3 ± 0.1	1.0 ± 0.0	0

Significant differences in growth rates are indicated by capital letters (ANCOVA, $P<0.05$).
[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. Control does not include day 80 or 120 fish.
[c] Liver somatic index (LSI) (1 ± standard error).

terminal chlorine substitution are currently not available. Nevertheless, these congeners share many physical-chemical properties and structural features of CP components (Drouillard, 1996).

Exposure to the PCAs did not appear to influence the health of the rainbow trout. Growth rates were not significantly different between PCA exposed and control populations (Table 2). Lipid percentages and liver somatic indices were also similar between treatments (Table 2). No deaths occurred in any of the treatments.

All of the PCAs were detected in the trout after 5 days of exposure (Figs. 3 and 4). No PCAs were detected in the control fish on any collection day. With the exception of the C_{14}-PCAs, most compounds achieved steady state between food and fish within 30 or 40 d (Figs. 3 and 4). The longer $t_{1/2}$'s of the C_{14}-PCA may explain why these compounds did not achieve steady state (Bruggeman et al., 1981).

Differences in bioaccumulation parameters between PCAs with the same molecular formula but different Cl positioning were observed for some PCAs (e.g., $C_{10}H_{15}Cl_{7a}$ and $C_{10}H_{15}Cl_{7b}$) but not others (e.g., $C_{10}H_{17}Cl_{5a}$ and $C_{10}H_{17}Cl_{5b}$) (Table 1). Positioning of most Cl on these alkene-derived PCAs was quite similar because they were synthesized from diene or triene starting material (Fig. 1). The positions of the free radical substituted Cl are unknown, so that we were unable to reach definitive conclusions on the importance of Cl position. Nevertheless, the results suggest that Cl position can, in some cases, have a significant influence on the bioaccumulation of PCAs.

Half-lives of PCA ranged from 7 d for $C_{10}H_{17}Cl_5$ to 53 d for $C_{14}H_{25}Cl_5$, but in general were much lower than expected for compounds of log K_{ow} of 6 or greater. Fisk et al. (1998), using rainbow trout of similar size, found that non-metabolized organochlorine compounds (PCBs, mirex, hexachlorobenzene) with log K_{ow}'s between 6 and 7 had a $t_{1/2}$'s between 40 and 60 d. Only the highly chlorinated-PCAs and C_{14}-PCAs had $t_{1/2}$'s between this range. Therefore, PCAs with shorter carbon chain and lower chlorination appear to be susceptible to biotransformation by rainbow trout. We did not directly measure biotransformation products. Decreasing metabolism of PCAs has been associated with increasing Cl content and carbon chain length in birds and fish (Biessmann et al., 1982; Fisk et al., 1996a).

The influence of adjacent and terminal chlorine substitution (1,2,9,10-C_{10}, 1,2,10,11-C_{11} and 1,2,13,14-C_{14}), which was common to all PCAs used here, on PCA $t_{1/2}$, is difficult to interpret. These chlorine atoms could affect bioaccumulation parameters through differences in chemical-physical properties and/or susceptibility to metabolism. Unfortunately, there are no data on the influence of Cl position on physical-chemical properties of PCAs. The metabolism of organochlorines by fishes can vary with chlorine position and content (Sijm and Opperhuizen, 1989). For example, polychlorinated biphenyls that do not have chlorine substituted at the meta- and para- positions are more easily metabolized (Safe, 1990). Darnerud and Brandt (1982) reported that β-oxidation was one step in the

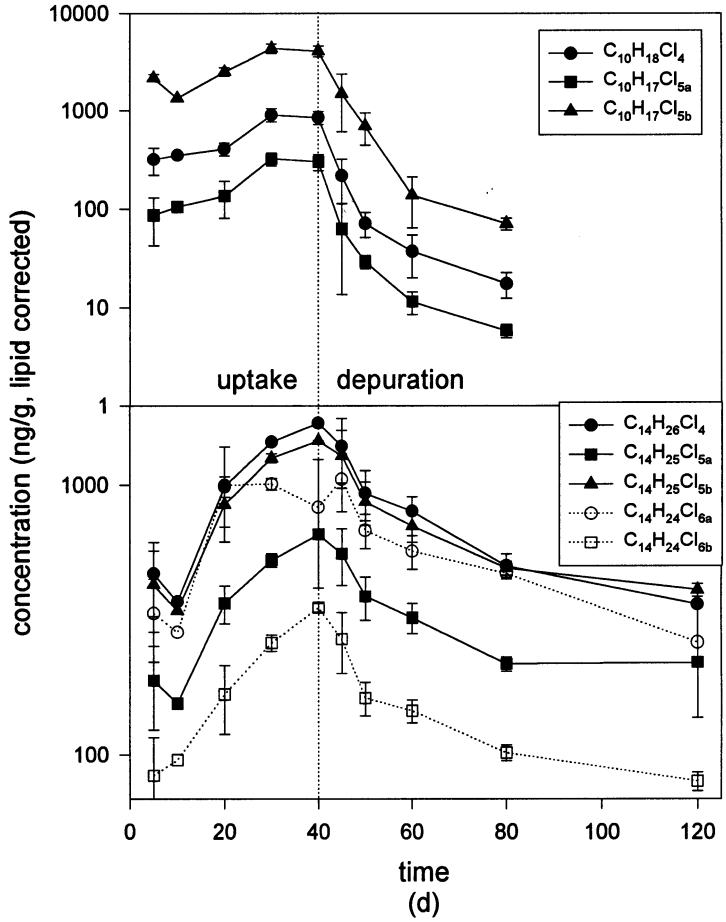

Fig. 3. Accumulation and depuration of C_{10}- and C_{14}-PCAs through dietary exposure to juvenile rainbow trout. Each point is the mean $\pm$ 1 S.E. of three fish. Concentrations are whole fish minus liver and GI tract, corrected for growth dilution and lipid content.

metabolism of PCAs by mice. The initial step in β-oxidation involves a terminal carbon. Therefore, because the capacity for de-chlorination of organochlorine compounds in fish is low (Sijm and Opperhuizen, 1989), a Cl atom substituted on the terminal carbon of a PCA molecule would likely inhibit β-oxidation.

There was a large range in PCA assimilation efficiencies, and for a number of PCAs the assimilation efficiencies were calculated to be greater than 100% (with standard errors of approximately 30%) (Table 1). For other PCAs, e.g., $C_{10}H_{18}Cl_4$ and $C_{10}H_{17}Cl_{5a}$, assimilation efficiencies were lower than expected based on their K_{ow}, which is probably due to metabolism (Fisk et al., 1998). For a majority of the PCAs with assimilation efficiencies $>100\%$, the BMF_{SS} are in agreement with BMF_{CALC} (Table 1). However, assimilation efficiencies of persistent organochlorines of similar molecular size and hydrophobicity as PCAs are believed to be approximately 50% (Gobas et al., 1989; Fisk et al., 1998). One possible explanation for these results is that the depuration rates used to calculate assimilation efficiencies may overestimate the actual rates because they are calculated using depuration phase data (Eq. (1)). This could occur if depuration rates changed due to a gradual induction of metabolic capacity for PCAs. Regardless, these results suggest that these PCAs were assimilated very efficiently (50–100%).

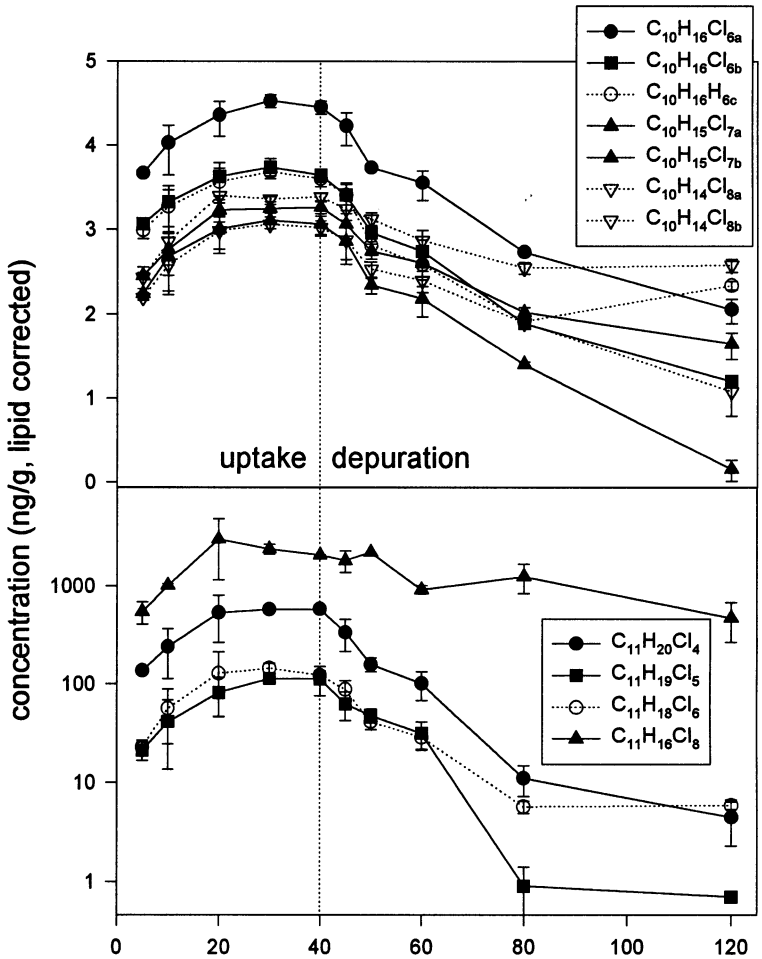

Fig. 4. Accumulation and depuration of C_{10}- and C_{11}-PCAs through dietary exposure to juvenile rainbow trout. Each point is the mean $\pm$ 1 S.E. of three fish. Concentrations are whole fish minus liver and GI tract, corrected for growth dilution and lipid content.

BMF_{EQUIL} were calculated using assimilation efficiencies, and therefore were also confounded by the problems discussed above. A second and third set of BMFs were determined assuming an assimilation efficiency of 50% (BMF_{CALC}) and steady state between PCA concentrations in the fish and food (BMF_{SS}) (Table 1). There was good agreement between the three BMFs for C_{10}- and C_{11}-PCAs with assimilation efficiencies $< 100\%$. The C_{14}-PCAs did not achieve steady state, and therefore BMF_{SS} were less than calculated- or estimated-BMFs. A number of the PCAs, including $C_{10}H_{18}Cl_{6c}$, $C_{10}H_{16}Cl_{8a}$, $C_{11}H_{16}Cl_8$, and all the C_{14}-PCAs, had $BMF_{EQUIL} > 1$ (Table 1), which implies a potential to biomagnify in aquatic food chains.

Half-lives of PCAs were correlated with K_{ow} and carbon chain length but not chlorine number based on simple linear regression (Table 3; Fig. 5A,B,C). It should be noted that the PCAs used to develop these relationships, and those for BMF, represent a small subset of all possible PCAs, and results should be interpreted with caution. Multiple regression using carbon chain length and chlorine number explained more variation in $t_{1/2}$ than either variable alone (Table 3). Therefore, $t_{1/2}$'s of PCA increase with increasing carbon chain length and chlorine content. Carbon

Table 3
Results of linear and multiple regressions between PCA $t_{1/2}$ and BMFs with K_{ow}, carbon number, and chlorine number

Dependent variable	Independent variable(s)	Intercept	Slope	r^2	P-value
$t_{1/2}$	log K_{ow}	−246.1	41.5	0.64	<0.001
$t_{1/2}$	# C	−52.9	7.0	0.42	<0.001
$t_{1/2}$	# Cl	2.8	4.2	0.17	0.05
$t_{1/2}$	log K_{ow}	−228.2	36.0	0.65	<0.001
	# C		1.6		
$t_{1/2}$	log K_{ow}	−259.0	44.3	0.65	<0.001
	# C		−1.0		
$t_{1/2}$	# C	−73.6	6.7	0.56	<0.001
	# Cl		3.8		
$t_{1/2}$	log K_{ow}	−232.9	37.2	0.65	<0.001
	# C		1.4		
	# Cl		−0.2		
BMF	log K_{ow}	−5.7	1.0	0.31	<0.006
BMF	# C	−1.0	0.2	0.22	0.02
BMF	# Cl	1.3	−0.01	0.002	0.86
BMF	log K_{ow}	−5.0	0.8	0.32	0.021
	# C		0.1		
BMF	log K_{ow}	−8.5	1.6	0.52	0.001
	# Cl		−0.2		
BMF	# C	−0.8	0.2	0.23	0.07
	# Cl		−0.03		
BMF	log K_{ow}	−12.0	2.6	0.59	<0.001
	# C		−0.2		
	# Cl		−0.3		

chain length and chlorine number did not improve the variation in $t_{1/2}$ explained by K_{ow} alone (Table 3). This is not unexpected because K_{ow}'s were calculated using a formula that was based on the total number of carbon and chlorine atoms (Sijm and Sinnige, 1995).

A significant linear relationship was also found between BMF_{CALC} for PCAs and log K_{ow} and carbon chain length (Fig. 6A,B; Table 3). BMF_{CALC} was not significantly correlated with chlorine number (Fig. 6C; Table 3). BMFs of recalcitrant organochlorines, such as PCBs, have been observed to increase with K_{ow} up to a log K_{ow} of approximately 7 (Oliver and Niimi, 1988; Thomann, 1989; Fisk et al., 1998). Multiple regression using carbon chain length and chlorine number did not improve the variation in BMF explained by carbon chain length alone (Table 3). Carbon chain length did not improve the variation in BMF explained by K_{ow}; however chlorine number did improve the relationship between BMF and K_{ow} (Table 3). Further, a multiple regression using all three independent variables (log K_{ow}, carbon chain length and chlorine number) explained more variation in BMF than log K_{ow} and chlorine number alone (Table 3). These results suggest that the K_{ow}, carbon chain length and chlorine number all play a role in the bioaccumulation, or biomagnification, of PCAs.

4. Conclusions

These results represent the first data on the dietary accumulation of PCAs of known carbon chain length, chlorine content and chlorine position. All of these PCAs had chlorine substituted at adjacent and terminal carbons (1,2,9,10-C_{10}, 1,2,10,11-C_{11} and 1,2,13,14-C_{14}), but the positions of other Cl atoms were unknown. All of the PCAs were rapidly accumulated and had high assimilation efficiencies from food. PCA $t_{1/2}$'s in rainbow trout ranged from 7 to 53 d, and

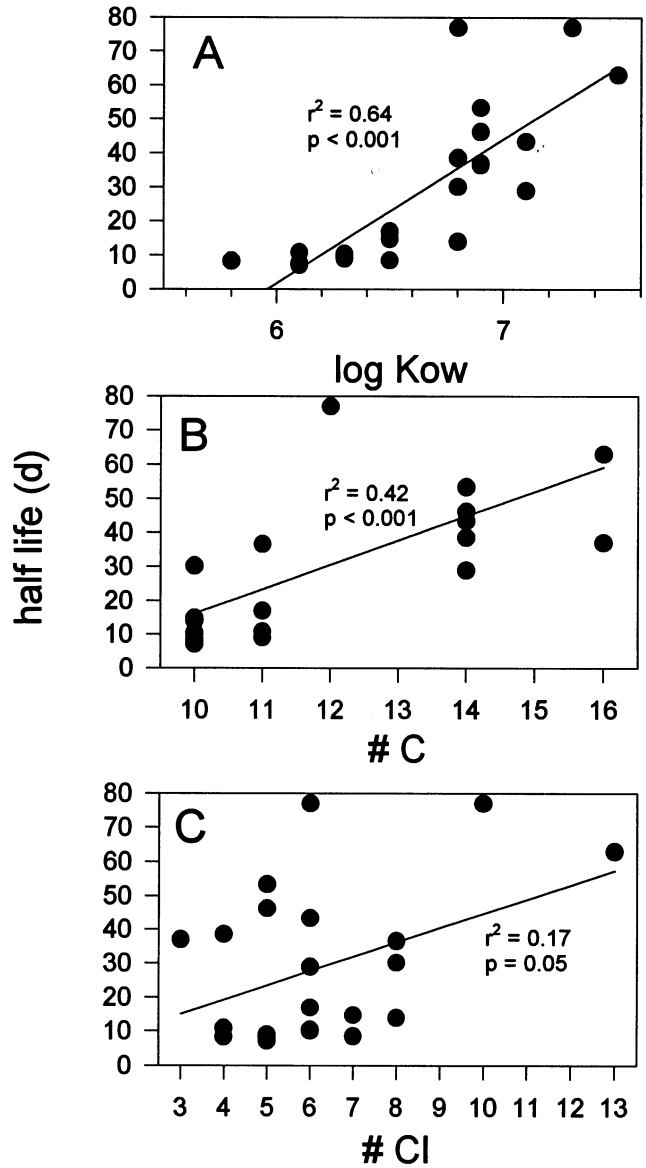

Fig. 5. Relationships between PCA $t_{1/2}$ with K_{ow} (A), carbon number (B) and chlorine number (C). Solid lines are linear regressions (Table 3). Data includes all data from this work and from other free-radically chlorinated C_{12}- (6 and 10 Cl) and C_{16}- (3 and 13 Cl) PCAs (Fisk et al., 1996a).

increased with increasing K_{ow}, carbon chain length and chlorine content. There was indirect evidence for metabolism based on the fact that $t_{1/2}$'s were shorter than for persistent organochlorines of similar log K_{ow}. However, the susceptibility to metabolism decreased with greater carbon chain length and chlorine content. Based on BMFs > 1, higher chlorinated C_{10}- and C_{11}-PCAs, and all C_{14}-PCAs, would biomagnify from food to fish in aquatic food chains. BMFs of PCAs increased with increasing K_{ow}, but were also influenced by carbon chain length and chlorine number.

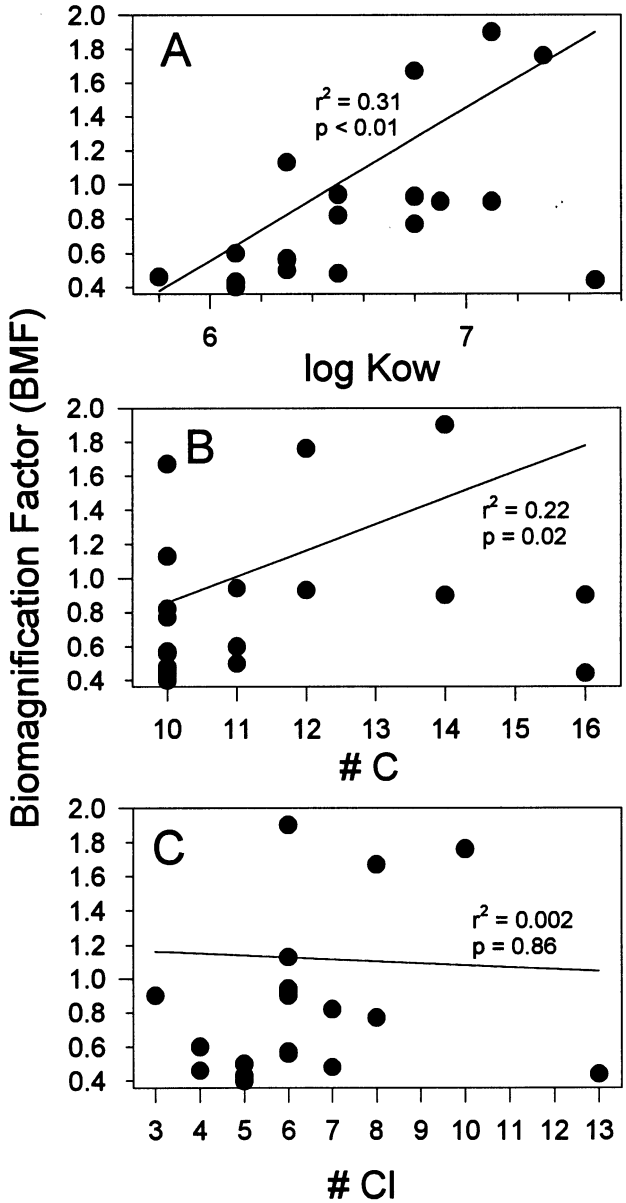

Fig. 6. Relationships between PCA BMF_{calc} with K_{ow} (A), carbon number (B) and chlorine number (C). Solid lines are linear regressions (Table 3). Data includes all data from this work and from other free-radically chlorinated C_{12}- (6 and 10 Cl) and C_{16}- (3 and 13 Cl) PCAs (Fisk et al., 1996a).

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References

Bengtsson, B., Svenberg, O., Linden, E., Lunde, G., Ofstad, E.B., 1979. Structure related uptake of chlorinated paraffins in bleaks (*Alburnus alburnus* L). Ambio 8, 121–122.

Biessmann, A., Brandt, I., Darnerud, P.O., 1982. Comparative distribution and metabolism of two ^{14}C-labelled chlorinated paraffins in Japanese quail *Coturnix coturnix japonica*. Environ. Pollut. 28A, 109–120.

Bruggeman, W.A., Martron, L.B.J.M., Kooiman, D., Hutzinger, O., 1981. Accumulation and elimination kinetics of di-, tri- and tetra chlorobiphenyls by goldfish after dietary and aqueous exposure. Chemosphere 10, 811–832.

Campbell, I., McConnell, G., 1980. Chlorinated paraffins and the environment. 1. Environmental occurrence. Environ. Sci. Technol. 14, 1209–1214.

Canadian Environmental Protection Act, 1993. Priority Substances List Assessment Report: Chlorinated Paraffins. Government of Canada, Ottawa, Canada, 32 pp.

Darnerud, P.O., Brandt, I., 1982. Studies on the distribution and metabolism of a ^{14}C-labelled chlorinated alkane in mice. Environ. Pollut. 27A, 45–56.

Drouillard, K.G., 1996. Physico-chemical property determinations on chlorinated *n*-alkanes (C_{10} to C_{12}). Parameters for estimation of the environmental fate of chlorinated *n*-paraffins, M.Sc. Thesis. University of Manitoba, Winnipeg, MB, Canada.

Fisk, A.T., Cymbalisty, C.D., Bergman, A., Muir, D.C.G., 1996a. Dietary accumulation of C_{12}- and C_{16}-chlorinated alkanes by juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 15, 1775–1782.

Fisk, A.T., Cymbalisty, C., Tomy, G.T., Stern, G.A., Muir, D.C.G., Haffner, G.D., 1996. Chlorinated *n*-alkanes in sediments, mussels, and fishes of the Detroit River. 39th Annual Conference on Great Lakes Research, Toronto, Canada, May 26–30, 1996.

Fisk, A.T., Norstrom, R.J., Cymbalisty, C.D., Muir, D.C.G., 1998. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with K_{ow}. Environ. Toxicol. Chem. 17, 951–961.

Gobas, F.A.P.C., Clark, K.E., Shiu, W.Y., Mackay, D., 1989. Bioconcentration of polybrominated benzenes and biphenyls and related superhydrophobic chemicals in fish: role of bioavailability and elimination into feces. Environ. Toxicol. Chem. 8, 231–245.

Jansson, B., Andersson, R., Asplund, L., Litzen, K., Nylund, K., Sellstrom, U., Uvemo, U., Wahlberg, C., Wideqvist, U., Odsjo, T., Olsson, M., 1992. Chlorinated and brominated persistent organic compounds in biological samples from the environment. Environ. Toxicol. Chem. 12, 1163–1174.

Kato, Y., Kenne, K., 1996. Inhibition of cell-cell communication by commercial chlorinated paraffins in rat liver epithelial IAR 20 cells. Pharmacol. Toxicol. 79, 23–28.

Oliver, B.G., Niimi, A.J., 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environ. Sci. Technol. 22, 388–397.

Rieger, R., Ballschmiter, K., 1995. Semivolatile organic compounds — polychlorinated dibenzo-*p*-dioxins (PCDD), debenzofurans (PCDF), biphenyls (PCB), hexachlorobenzene (HCB), 4,4′-DDE and chlorinated paraffins (CP) — as markers in sewer films. Fres. J. Anal. Chem. 352, 715–724.

Safe, S., 1990. Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. 21, 51–88.

Sijm, D.T.H.M., Sinnige, T.L., 1995. Experimental octanol/water partition coefficients of chlorinated paraffins. Chemosphere 31, 4427–4435.

Sijm, D.T.H.M., Opperhuizen, A., 1989. Biotransformation of organic chemicals by fish: enzyme activities and reactions. In: Handbook of Environmental Chemistry, Vol. 2E. Springer Verlag, Berlin, pp. 163–235.

Stern, G.A., Tomy, G.T., Muir, D.C.G., Westmore, J.B., Dewailly, E., Rosenberg, B., 1997. Polychlorinated *n*-alkanes in aquatic biota and human milk. Presented at: 45th ASMS Conference on Mass Spectrometry and Allied Topics, Palm Springs, CA, June 1997.

Thomann, R.V., 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. Environ. Sci. Technol. 23, 699–707.

Tomy, G.T., Stern, G.A., Muir, D.C.G., Fisk, A.T., Cymbalisty, C.D., Westmore, J.B., 1997. Quantifying C_{10}–C_{13} polychloroalkanes in environmental samples by high resolution gas chromatography/electron capture negative ion high resolution mass spectrometry. Anal. Chem. 69, 2762–2771.