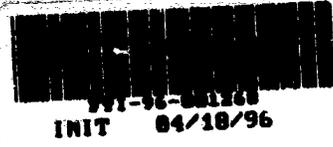


**CHLORINATED PARAFFINS
INDUSTRY ASSOCIATION**



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April 11, 1996



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Dear Sir/Madam:

Enclosed for the Agency's information are two to-be published articles from the University of Manitoba. The first article reports on dietary accumulation of four specific, synthetically derived chlorinated alkanes in laboratory trout, the second reports on analyses of two environmental samples for certain chlorinated paraffin homologs. We submit these articles given EPA's long-term interest in chlorinated compounds.

It is important to note that although the first article discusses chlorinated paraffins (CPs), the compounds tested were four ¹⁴C-polychlorinated alkanes (C₁₂H₂₀Cl₆ (56% CL by weight), C₁₂H₁₆Cl₁₀ (69% CL), C₁₆H₃₁Cl₃ (35% CL) and C₁₆H₂₁Cl₁₃ (69% CL)). While these chlorinated alkanes can be found in industrial chlorinated paraffins products, they do not represent commercial CP products and their method of synthesis is different.

We intend to review these studies more closely and will submit supplemental information to EPA shortly.

Sincerely,

Robert J. Fensterheim
Executive Director

96 APR 23 AM 8:52

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Preliminary report on Concentrations of Chlorinated *n*-Paraffins in Sediment and Fish from the Detroit River.

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Introduction

Chlorinated paraffins are a class of chlorinated *n*-alkanes with the general formula $C_nH_{2n-2}Cl_x$, ranging from C_{10} - C_{30} and with a chlorine content of 30 to 70%. In Canada, CPs are classified as priority toxic substances (group 3) and in the US they have been placed on the Environmental Protection Agency (EPA) Risk Reduction List because of concerns about their environmental persistence, lack of environmental measurements, possible adverse effects on terrestrial and aquatic organisms, and potential carcinogenicity to humans.

Environmental data on CP concentrations are lacking, mainly because the determination of CPs in environmental samples is considered to be very difficult. However, a recent Swedish study found CPs to be among the most prominent chlorinated organic contaminants in 11 representative biological samples, at concentrations ranging from 130 to 4400 ng/g lipid weight. A study of river water and sediments in Germany (Ballschmiter 1994) found CPs present at ng/L concentrations in river water and ng/g in sediments. There have been no reports of CPs in environmental samples in North America except at sites near manufacturing plants (Murray *et al.*, 1988). A study of the only Canadian manufacturing facility found non-detectable CPs in water and sediments of the St. Lawrence River downstream of the plant (Metcalf-Smith *et al.* 1995).

This project was designed as a preliminary survey to analyse selected environmental samples for CPs using detection by gas chromatography (GC) with high-resolution electron-capture negative ion mass spectrometry (ECNIMS). GC-ECNIMS is the most sensitive technique for determining CPs and other highly chlorinated organic compounds in environmental samples (Muller and Schmid 1984). Previous work in Canada, the US and Europe, had used low resolution ECNIMS which may be less sensitive and is potentially subject to many more interferences from chlorinated compounds of similar molecular weight.

The specific objectives are (1) to measure CPs in samples of sediments, benthic invertebrates, and benthic and pelagic fish species in the Detroit River near Windsor ON., and (2) to measure CPs in extracts of selected samples of filtered particles and gas phase (polyurethane foam plug) from an International Air Deposition Monitoring (IADN) site at Pte. Petre on Lake Ontario east of Toronto.

Methods

Sample Collection

Six sediment samples were collected from the Detroit River in August, 1995, three each were from the Trenton Channel and the mouth of the Detroit River at Lake Erie (Figure 2). At each location three ponar grabs of sediment were homogenized in a stainless steel bucket and sub-sampled three times into hexane rinsed brown glass jars. Samples were then frozen until analyzed.

Three yellow perch and three channel catfish were gill netted in August, 1995 at the same location as the sediment collection at the mouth of the Detroit River at Lake Erie. Fish are not normally found in the Trenton Channel due to high pollution levels. Fish were wrapped whole in hexane rinsed aluminium foil and frozen until analyzed.

Three samples of zebra mussel (*Dreissena polymorpha*) were collected over the summer of 1995 at one location in the Detroit River (same location as sediment and fish samples) and three locations in Lake Erie (West Sister Island, East Sister Island and Sandusky Bay I)(Figure 1). Mussels, including shells, were wrapped in hexane-rinsed aluminium foil and frozen until analyzed.

Air sample extracts were obtained from F. Froude (Atmospheric Environment Service, Egbert Ont.) from the IADN site at Pte. Petre on Lake Ontario east of Toronto. Air samples (~300 m³) were collected on polyurethane foam plugs with glass fiber filters using a General Metal Works PS-1 high volume air sampler. PUFs and filters were stored in glass jars and frozen (-20 °C) until analysis.

Sample Extraction and Clean-Up

Extraction procedures were those used in our lab for determining organochlorines in sediment and biota (Muir *et al.* 1995; Muir *et al.* 1990) with small modifications. Sediment samples were freeze-dried and Soxhlet extracted with dichloromethane (DCM) as outlined in Figure 2. Organic carbon content of the sediments is being determined by the Freshwater Institute geochemistry lab. Soxhlet extracts were treated with activated copper to remove sulphur. Samples were then treated with sulfuric acid/fuming nitric acid. The acid treatment served two functions; removal of pigments and organics associated with the sediment and secondly removal of aromatic compounds (PCBs, PAHs) which could interfere with mass spectrometry work and are known to be high in sediment from this region. Florisil column fractionation provided further clean-up of the samples following acid treatment.

Frozen fish and zebra mussels (shells removed) were ground whole in a meat grinder, and subsamples were further homogenized by mixing with (heated treated) sodium sulfate. Following Soxhlet extraction, extracts were split (1:1) for analysis of PCBs and other organochlorines and CPs. Lipids and pigments were removed using gel permeation chromatography (GPC), and the CP fraction was acid treated to remove interfering aromatic organochlorine compounds (PCBs)(Figure 2). Lipids were determined gravimetrically, prior to GPC and acid treatment.

PUFs Soxhlet extracted with hexane and filters were Soxhlet extracted with DCM. Extracts were taken up in hexane. Hexane extracts were split (1:1) for analysis of PAHs and PCBs + CPs. The CPs were separated from most PCBs on a Florisil column.

An internal standard of $^{13}\text{C}_8$ - mirex was added to the final extract prior to GC-MS analysis.

Gas Chromatography-Mass Spectrometry

GC separations were performed with a Hewlett Packard 5890 Series II gas chromatograph using a 30 m x 0.25 mm DB5 high resolution column, with He as the carrier gas. The initial column temperature was set at 80°C, held for 1 minute, ramped to 260°C at a rate of 7°C/minute, held there for 8:18 minutes, then ramped to 280°C at a rate of 10°C/minute and held there for 13 minutes. The injector temperature and transfer interface were set at 200°C and 260°C, respectively.

Electron capture negative ion high resolution mass spectrometry (ECNI/HRMS) was performed on a Kratos Concept high resolution mass spectrometer (EBE geometry) controlled using a Mach 3 data system. Selected ion ECNI/HRMS was performed at a spectrometer resolving power ~12000. Methane was used as the moderating gas and PFK as the mass calibrant. Optimum sensitivity was achieved at a gas pressure of $\sim 2 \times 10^{-4}$ torr, an electron of ~ 180 eV, an accelerating voltage of 5.3 kV; and an ion source temperature of 120°C.

Results

Quantifying Chlorinated *n*-paraffins in environmental matrices:

Due to the inherent complexity of commercially available chlorinated *n*-paraffin (CP) mixtures, it was necessary to characterize the behaviour of the different individual compounds (*i.e.*, $\text{C}_{10}\text{H}_{22-x}\text{Cl}_x$, $\text{C}_{11}\text{H}_{24-x}\text{Cl}_x$ etc.) of known chain length and chlorine number, expected to be present in these mixtures. Specific compounds for study were synthesized by chlorination of selected pure *n*-alkenes; the chlorinated alkanes obtained have electron capture negative ion (ECNI) mass spectra essentially identical to those of chlorinated *n*-paraffins having the same carbon and chlorine numbers (Tomy *et al.*, 1995). Based on full scan mass spectra, under our ECNI conditions, we found $[\text{M}-\text{Cl}]^-$ to be the most prominent ion.

Three commercial products were used as standards for quantitation; the first has a C_{10} - C_{13} carbon chain length, and ~60% chlorine (*Paroil 1160*), the second has a C_{10} - C_{13} carbon chain length, but with ~70% chlorine (*Chlorowax 70-200*), while the third mixture, *Paroil 152*, has a C_{14} - C_{17} carbon chain length, and 50-60% chlorine. The relation between the composition of an industrial mixture and the molecular formulae, $\text{C}_x\text{H}_{2x-2}\text{Cl}_z$, of compounds in the mixture is:

$$z = c(14x+2)/(35.5-34.5c)$$

where *c* is the mass fraction of chlorine (Muller and Schmid 1984). The composition of *Paroil 1160*, along with the characteristic ions (*i.e.*, $[\text{M}-\text{Cl}]^-$) used in our method and the mass of one of its isotopes (^{35}Cl), is shown in Table 1.

High resolution gas chromatography elution profiles for all of the components in each of the mixtures were established, then time windows for each mixture, containing the ions necessary for monitoring environmental samples, were created. In the ion chromatograms the area corresponding to these ions in the standard were summed, and compared to the area of the $[\text{M}-4\text{Cl}]^-$ signal for $^{13}\text{C}_8$ -mirex, which was used as an internal standard: quantification of CP residues in test samples was achieved by comparison of their signals to that of mirex in the same manner.

Unfortunately, the levels CPs in environmental samples for each mixture had to be monitored separately, because of the vast number of components characteristic to each of the mixtures. The time of analysis for Paroil 1160, Chlorowax 70-200 and Paroil 152 are 37, 46 and 52 minutes, respectively. Figure 1 shows some of the components found in the Paroil 1160 mixture.

Preliminary results for environmental samples

The method outlined above has established the presence, and determined the levels, of CPs in fish and sediment taken from the Detroit River. The CPs have been quantified using GC-high resolution ECNIMS in one sediment and one yellow perch sample from the mouth of the Detroit River at Lake Erie (Table 2). The quantification was performed for individual homologs using the industrial CP products Paroil 1160 (C₁₀₋₁₃ 50-60% Cl) and Chlorowax 70-200 (C₁₀₋₁₃ 70% Cl). Paroil 152 (C₁₄₋₁₇ 50-60% Cl) was undetected in both samples. Figure 3 and 4 show some of the components of Paroil 1160 that were found in the yellow perch and sediment, respectively. Organic carbon levels are not available on the sediment sample at this time. Lipid percentage in the yellow perch is 3.4%.

All other samples have been extracted and await analysis. Analysis has been slowed by instrumental problems over the past 2 months as well as by the need to quantify CPs using three injections per sample to enable each group of homologs in each commercial product to be quantified separately.

Table 1. Composition and the characteristic ions used in the quantitation of Paroil 1160 in environmental matrices.

Chain Length	Molecular Formula	Characteristic Ion	M(³⁵ Cl)
10	C ₁₀ H ₁₈ Cl ₄	C ₁₀ H ₁₈ Cl ₃	243.0474
	C ₁₀ H ₁₇ Cl ₃	C ₁₀ H ₁₇ Cl ₄	277.0084
	C ₁₀ H ₁₆ Cl ₆	C ₁₀ H ₁₆ Cl ₅	312.9665
	C ₁₀ H ₁₅ Cl ₇	C ₁₀ H ₁₅ Cl ₆	346.9275
11	C ₁₁ H ₁₉ Cl ₅	C ₁₁ H ₁₉ Cl ₄	291.0241
	C ₁₁ H ₁₈ Cl ₆	C ₁₁ H ₁₈ Cl ₅	325.9822
	C ₁₁ H ₁₇ Cl ₇	C ₁₁ H ₁₇ Cl ₆	360.9432
12	C ₁₂ H ₂₀ Cl ₆	C ₁₂ H ₂₀ Cl ₅	340.9978
	C ₁₂ H ₁₉ Cl ₇	C ₁₂ H ₁₉ Cl ₆	374.9588
	C ₁₂ H ₁₈ Cl ₈	C ₁₂ H ₁₈ Cl ₇	408.9199
13	C ₁₃ H ₂₂ Cl ₆	C ₁₃ H ₂₂ Cl ₅	355.0135
	C ₁₃ H ₂₁ Cl ₇	C ₁₃ H ₂₁ Cl ₆	388.9745
	C ₁₃ H ₂₀ Cl ₈	C ₁₃ H ₂₀ Cl ₇	422.9355

Table 2: CP concentrations in sediment (ng/g dry wt) and yellow perch (ng/g wet wt) collected at the mouth of the Detroit River at Lake Erie (August, 1995).

Sample	C ₁₀	C ₁₁	C ₁₂	C ₁₃	C ₁₄ -C ₁₇	Total CPs
1. Short chain, 60% Cl (based on Paroil 1160)						
Sediment	105	270	748	240	-	1,360
Yellow Perch	76	83	75	17	-	251
2. Short chain, 70% Cl (based on Chlorowax 70-200)						
Sediment	9	45	9	5	-	68
Yellow Perch	30	128	25	9	-	193
3. Medium chain, 50-60% Cl (based on Paroil 152)						
Sediment	-	-	-	-	-	-
Yellow Perch	-	-	-	-	-	-
4. Total (sum of Paroil 1160, Chlorowax 70-200 and Paroil 152)						
Sediment	114	315	757	245	-	1,428
Yellow Perch	106	211	100	26	-	444

¹ C₁₄-C₁₇ chain lengths determined only with Paroil 152 as standard

Discussion

To the best of our knowledge, these results represent the first data on CPs levels using high resolution mass spectrometry, and the first which reports CP concentrations according to homologue groups. Apart from studies near a manufacturing plant (Murray *et al.*, 1988) this is the first report of CPs in environmental samples in the Great Lakes. Metcalfe-Smith *et al.*, (1995) were unable to detect CPs, using low resolution mass spectrometry, in sediment and biota from the St. Lawrence River near a manufacturing plant in Canada. However, CPs have been found in terrestrial and aquatic biota in Sweden (Jansson *et al.*, 1993) and in water and sediment from Germany (Ballschmiter 1988) using techniques similar to Metcalfe-Smith *et al.*

Because high resolution ECNIMS was used we believe these data are more accurate than previous measurements which used low resolution MS. There are a lot of potential interferences including higher chlorinated PCBs, toxaphene and chlordane compounds which all have similar molecular weights (i.e. in the range 360- 500 daltons). Some procedures have used less characteristic ions, i.e. *m/z* 70-73 (e.g. Jansson *et al.* 1993), rather than specific individual M-Cl ions, which could result in overestimation of CP concentrations.

Results are too limited to make detailed comparison with other organochlorine contaminants in Detroit River sediment and biota. Concentration of total PCBs (sum of 42 congeners) in sediment and silver bass muscle (similar trophic level as yellow perch) from the western basin of Lake Erie have been reported as 47.5 ng/g (dry wt) and 88.0 to 1190 ng/g (wet wt), respectively (Koslowski *et al.*, 1994). Therefore it would appear that CP concentrations may be higher than the other major organochlorine contaminant in the Detroit River (G. D. Haffner 1995, personal communication). The biological significance of these residues are difficult to assess because toxicological data on CPs is limited. But CPs are not thought to be as toxic as PCBs to aquatic life (for e.g. they do not induce cytochrome P450 1A1 activity in rainbow trout

(Fisk et al. 1996, manuscript submitted).

Although our method of quantifying CPs in environmental matrices is unique, it is still not without problems. The complex nature of these mixtures poses inherent difficulties associated with the ion source of the mass spectrometer. In addition, the long analysis time needed for each mixture, puts a limitation on the number of samples that can be run in a day. As a result, we plan to continue work on our high resolution ECNIMS work but also conduct similar analyses using low resolution ECNIMS with a single CP commercial mixture as a standard to complete the backlog of samples by March 31, 1996.

References

- Bollschmiter, K. 1994. *Unpublished data.*
- Environment Canada, Priority Substances Program, CEPA Assessment Report, Chlorinated Paraffins. 1993. Commercial Chemicals Branch, Hull QU K1A 0H2
- Fisk, A., C. Cymbalisty, A. Bergman, and D.C.G. Muir 1996. Manuscript accepted for publication in *Environ. Toxicol. Chem.*, Feb. 1996
- Jansson, B., R. Andersson, L. Asplund, K. Litzen, K. Nylund, U. Sellstrom, U. Uvemo, C. Wahllberg, U. Wideqvist, T. Odsjo and M. Olsson. 1993. *Environ. Toxicol. Chem.* 12: 1163-1174.
- Koslowski, S. E., C. D. Metcalfe, R. Lazar and G. D. Haffner. 1994. *J. Great Lakes Res.* 20(1): 260-270.
- Muir, D.C.G., N. P. Grift, W.L. Lockhart, P. Wilkinson, B. N. Billeck and G. J. Brunskill. 1995. *Sci. Total Environ.* 160/161:447-457.
- Muir, D.C.G., C.A. Ford, N.P. Grift, D.A. Metner and W.L. Lockhart. 1990. *Arch. Environ. Contam. Toxicol.* 19: 530-542.
- Muller, M. D.; Schmid, P.P. *J. High Resol. Chromatogr. Chromatogr. Comm.* 1984, 7, 33-37.
- Murray, T. M., D. H. Frankenberry, D. H. Steele and R. G. Health. 1988. Chlorinated Paraffins: A report on the findings from two field studies, Sugar Creek, Ohio and Tinkers Creek, Ohio., Vol. 1, Technical Report, U.S. Environmental Protection Agency, EPA/560/5-87/012, 150 pp.
- Tomy, G.T.; Muir, D.C.G.; Westmore, J.B.; Stern, G.A. *Synthesis and mass spectrometric studies of chlorinated n-paraffins.* 42nd Annual Conference on Mass Spectrometry and Allied Topics, Chicago, 1993, p263.

$C_{13}H_{20}Cl_8$
m/z 422.9355

$C_{12}H_{18}Cl_8$
m/z 408.9199

$C_{11}H_{17}Cl_7$
m/z 360.9432

$C_{10}H_{16}Cl_6$
m/z 312.9665

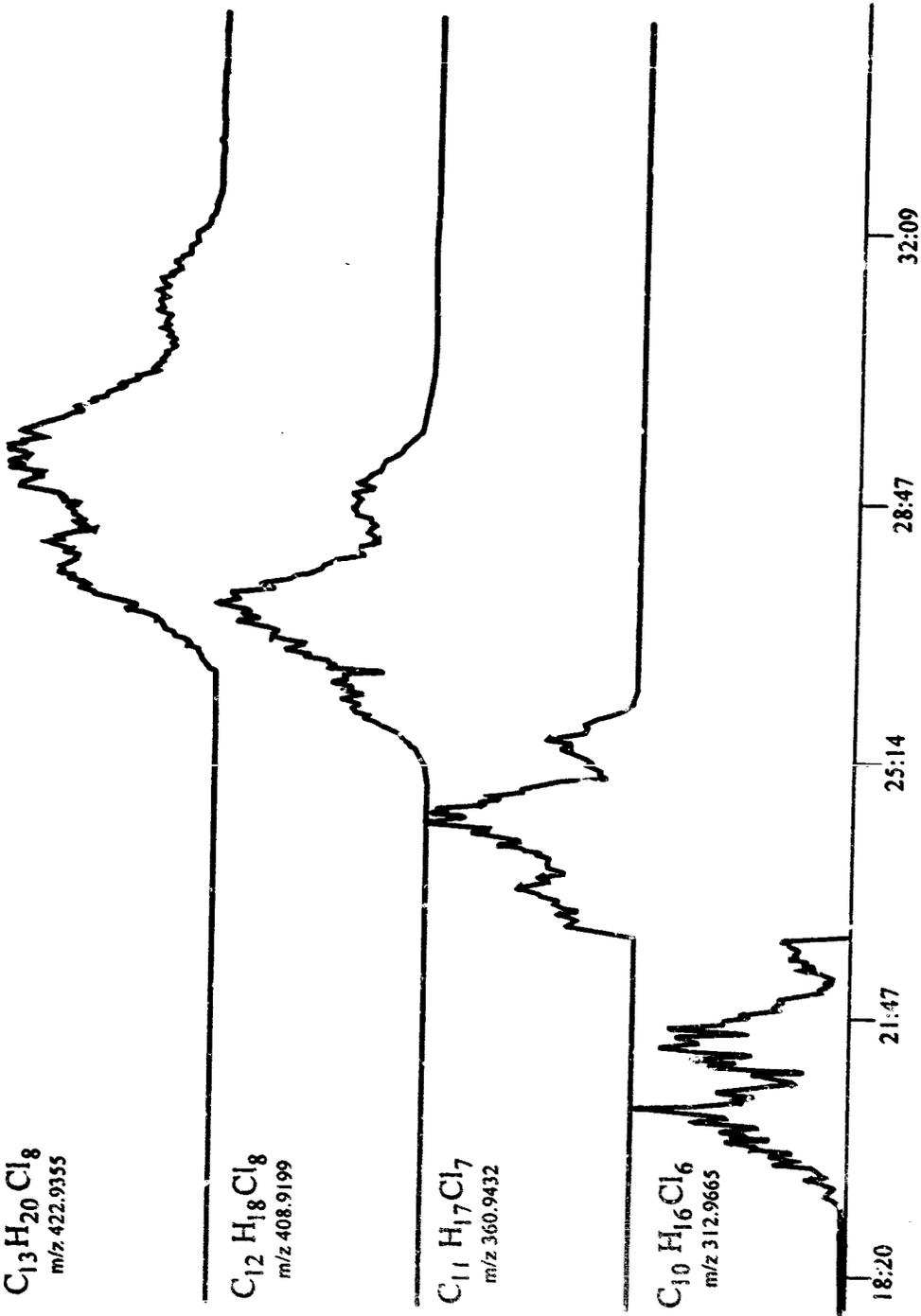


Figure 1: ECNIMS selected ion chromatograms of some of the components found in the chlorinated paraffin industrial mixture Paroil 1160.

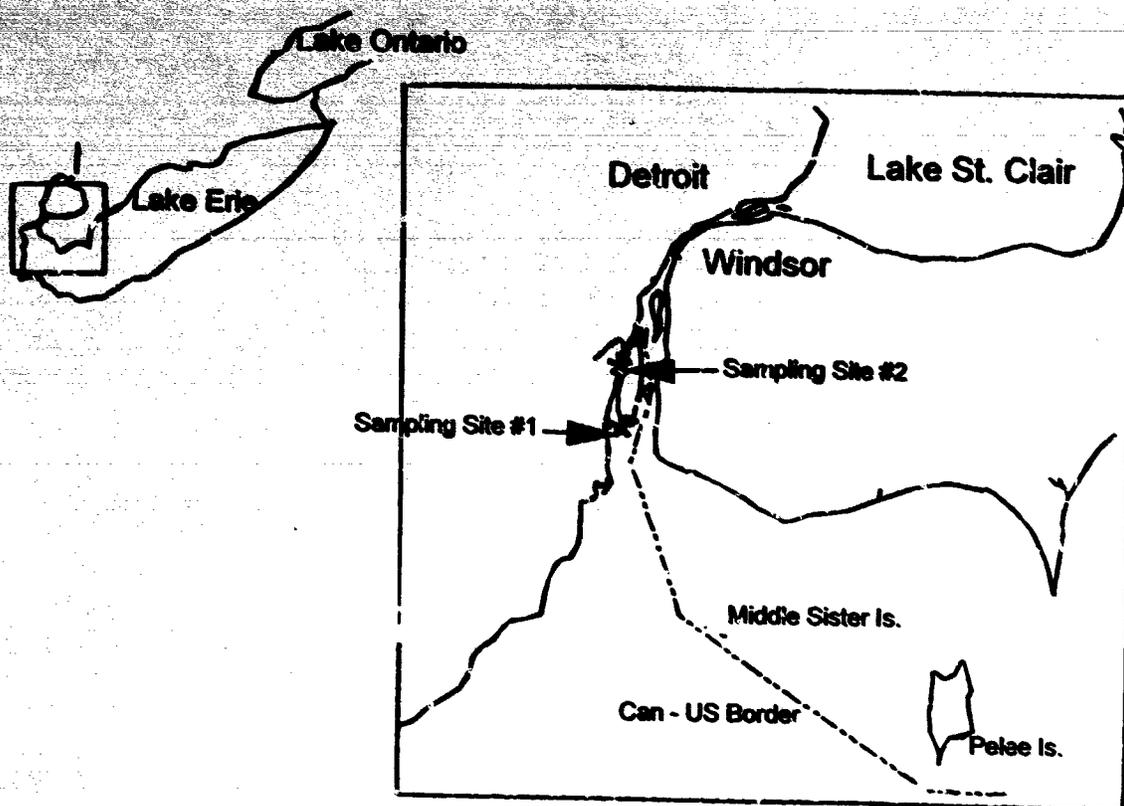


Figure 2: Location of sampling sites on the Detroit River. Sediment, yellow perch, channel catfish and zebra mussels were collected at sampling site #1. Sediment from the Trenton Channel was collected at sampling site #2.

C₁₃H₂₀Cl₈
m/z 422.9355

C₁₂H₁₈Cl₈
m/z 408.9199

C₁₁H₁₇Cl₇
m/z 360.9432

C₁₀H₁₆Cl₆
m/z 312.9665

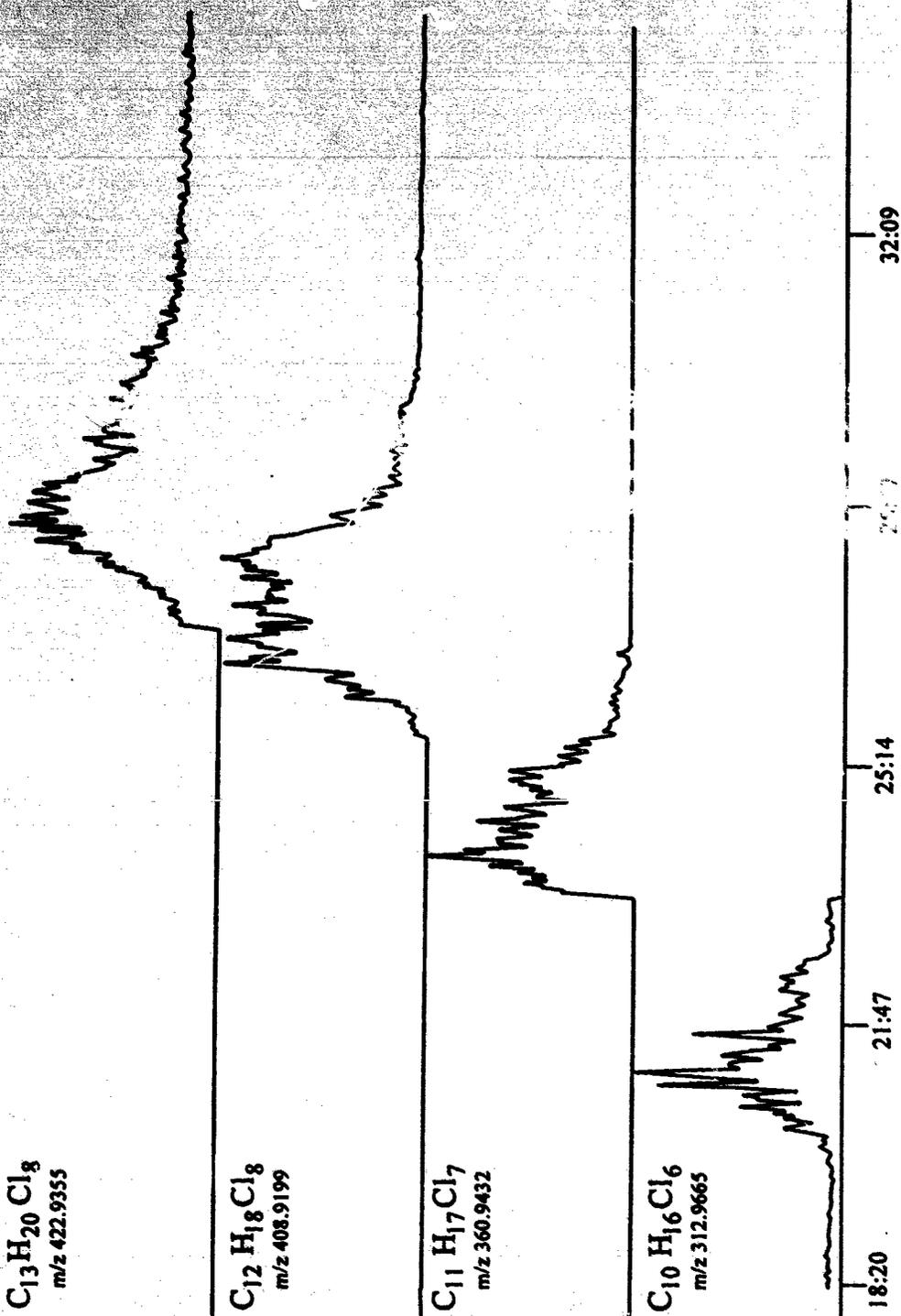


Figure 3: ECNIMS selected ion chromatograms of short chain chlorinated paraffins found in yellow perch collected from the Detroit River.

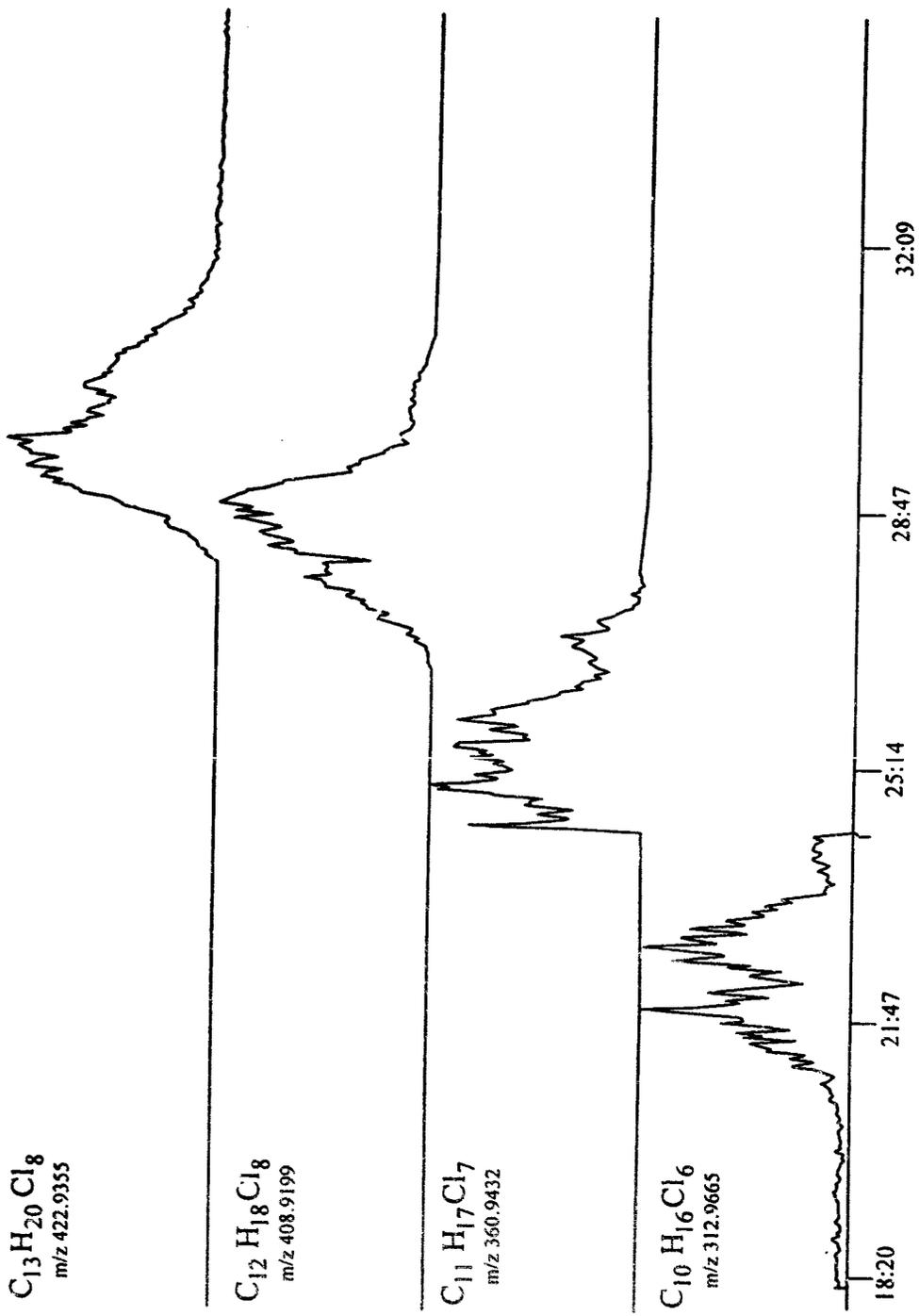


Figure 4: ECNIMS selected ion chromatograms of short chain chlorinated paraffins found in sediment collected from the Detroit River.

Dietary accumulation of C_{12} and C_{18} 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 ($\text{C}_{12}\text{H}_{21}\text{Cl}_4$ (56% Cl by weight), $\text{C}_{12}\text{H}_{16}\text{Cl}_{10}$ (69% Cl), $\text{C}_{16}\text{H}_{21}\text{Cl}_3$ (35% Cl) and $\text{C}_{16}\text{H}_{21}\text{Cl}_9$ (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 CPs. Trout were exposed for 40 days to nominal concentrations of 20 and 200 $\text{ng}\cdot\text{g}^{-1}$ of each chlorinated alkane, as well 2,000 $\text{ng}\cdot\text{g}^{-1}$ for $\text{C}_{16}\text{H}_{21}\text{Cl}_3$, followed by up to a 173 day elimination period. Whole body half lives in the rainbow trout ranged from 37 ± 2 days for $\text{C}_{16}\text{H}_{21}\text{Cl}_3$ to 87 ± 11 days for $\text{C}_{12}\text{H}_{16}\text{Cl}_{10}$, and assimilation efficiencies of $\text{C}_{16}\text{H}_{21}\text{Cl}_3$ (33 to 35%) and $\text{C}_{12}\text{H}_{16}\text{Cl}_{10}$ (34 to 38%) were highest among the four alkanes. Bioamplification factors ranged from 0.44 for $\text{C}_{16}\text{H}_{21}\text{Cl}_3$ to 2.15 for $\text{C}_{12}\text{H}_{16}\text{Cl}_{10}$. Accumulation of $\text{C}_{16}\text{H}_{21}\text{Cl}_3$ (MW = 674) may be sterically hindered due to its large molecular size. Low chlorinated alkanes, e.g. $\text{C}_{16}\text{H}_{21}\text{Cl}_3$, had shorter half lives than higher chlorinated alkanes, probably due to increased metabolism. HPLC- ^{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_{14-16}) 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]. CPs vary in both carbon chain length (10 to 30 carbons) and chlorine content (35% to 69% chlorine by weight), and consist of 1000's of possible structural isomers. Despite relatively large global production of CPs (300 kilotonnes per annum) [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 is needed for a complete ecological risk assessment [5], however this type of data for CPs is quite limited. Bengtsson and Distad [6] exposed bleak (*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. Further, 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 CPs (MW > 600) have been found to have low, or non-existent, 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.

1 Chlorinated alkanes have low water solubilities [10] and high K_{ow} 's [11] that vary with
 2 carbon chain length and chlorine content. Chemicals with similar physical-chemical properties to
 3 chlorinated alkanes, such as PCBs [12], have been found to bioaccumulate [13], and biomagnify
 4 [14,15], in aquatic food chains. The main objective of this study was the determination of
 5 bioaccumulation parameters (depuration rate, half life, biomagnification factor (BMF) and
 6 assimilation efficiency) of four ^{14}C -polychlorinated alkanes in juvenile rainbow trout
 7 (*Oncorhynchus mykiss*) through dietary exposure. The four chlorinated alkanes used include two
 8 C_{12} ($C_{12}H_{20}Cl_6$ (56% Cl by weight) and $C_{12}H_{16}Cl_{10}$ (69% Cl)) and two C_{16} ($C_{16}H_{31}Cl_5$ (35% Cl) and
 9 $C_{16}H_{27}Cl_{11}$ (69% Cl)) compounds, and are found in CP commercial products. We hypothesize that,
 10 like PCBs [16] and chlorinated dioxins and furans (PCDD/Fs) [17], certain chlorinated alkanes
 11 may be more persistent as a result of the number of Cl and the length of the carbon chain. These
 12 results provide the first bioaccumulation parameters for chlorinated alkanes of known carbon chain
 13 length and percentage Cl.

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

19
 20 **MATERIALS AND METHODS**

21 *Chemicals and Food Preparation*

22 All ^{14}C -polychlorinated alkanes were synthesized and purified using techniques outlined previously
 23 [19]. The [^{14}C] dodecanes contained 55.9 and 68.5% chlorine (mean of 5.9 and 9.8 chlorine atoms
 24 per molecule, respectively). The [^{14}C] hexadecane had 34.1% chlorine (3.3 chlorine atoms per
 25 molecule), and the [^{14}C] hexadecane had 69% chlorine (13.4 chlorines per molecule). For
 26 simplicity, the number of chlorine atoms in each compound have been rounded to the nearest
 27 integer.

28 Food was spiked by suspending a known quantity of each chlorinated alkane standard in
 29 150 ml of hexane and 100 g of commercial fish food (Martin's Feed Mills Ltd., Elmira, ON,
 30 Canada) and slowly evaporating to dryness. Food was air-dried for 24 hours and stored at 10°C.
 31 The fish food consisted of 41% protein, 14% lipid and 3% fiber. Concentrations in the food were
 32 determined by the same analytical techniques used to determine levels in the rainbow trout tissue
 33 (see below), and are found in Table 1. Control food was treated in an identical manner, but without
 34 the addition of a chlorinated alkane compound.

35
 36 *Experiment*

37 Juvenile rainbow trout (*Oncorhynchus mykiss*) (initial weights 2 - 7 g) were exposed to the spiked
 38 food for 40 days followed by 160 ($C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$ and $C_{16}H_{31}Cl_5$) to 173 days ($C_{16}H_{27}Cl_{11}$) of
 39 depuration. The daily rate of feeding was equal to 1.5% of the mean weight of the rainbow trout,
 40 corrected after each sampling period. Fifty fish were used in control tank 1 and the three $C_{16}H_{27}Cl_{11}$
 41 treatments, and 36 fish were used in the remaining treatments. Three fish were sampled from each
 42 treatment for ^{14}C determination on days 5, 10, 20, 30 and 40 of the uptake period, and days 5, 10,
 43 20, 40, 80 and 160 (or 173 for $C_{16}H_{27}Cl_{11}$ treatments) of the depuration period. Six additional fish in
 44 the $C_{16}H_{27}Cl_{11}$ high concentration treatment were exposed to the chlorinated alkane spiked food for

1 80 days, to follow the uptake for an extended period. Sampled fish were separated into liver, GI
2 tract (includes stomach, pyloric caeca, spleen, intestines, and adipose fat associated with these
3 organs; as well as gut contents), and carcass (whole fish minus liver and GI tract). Each tissue
4 (including the carcass) was weighed and analyzed separately for ^{14}C -radioactivity. At day 40 of
5 uptake, three additional fish were sacrificed for EROD measurements from each of the high
6 concentration treatments for each chlorinated alkane, and from the two control treatments. Liver
7 samples were weighed (0.05-0.3 g) and homogenised in 0.5 to 2.0 ml HEPES-KCl (0.02 M
8 HEPES, 0.15 M KCl, pH 7.5), depending on sample size, and homogenates were centrifuged for
9 20 min (15,600 x g). All preparative steps were done in a coldroom at 2 °C. The supernatants were
10 frozen at minus 80°C until analysed.

11 ^{14}C Analysis

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

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

24 Lipids were first removed from the samples using gel permeation chromatography (GPC)
25 followed by elution through a Florisil column. The GPC columns (i.d. 29.5 mm, length 400 mm,
26 500 mL reservoir) were packed with 60 grams (dry weight) of 200-400 mesh Bio-Beads® S-X3
27 beads (Bio-Rad Laboratories, Hercules, CA, USA), which had been soaked in DCM:hexane (1:1)
28 overnight. The column was eluted with 300 ml of DCM:hexane; the first 125 ml contained lipids
29 and were discarded. The remaining eluate containing the chlorinated alkanes, was evaporated to
30 1 mL for Florisil clean-up. After adding the GPC eluate to the Florisil column (8 grams of 1.2%
31 deactivated Florisil), the chlorinated alkanes were recovered by successive elution with 42 mL of
32 hexane, 38 mL of 85% hexane:15% DCM and 52 mL of 50% hexane:50% DCM. The Florisil
33 elutions were combined and then evaporated to near dryness under a gentle N_2 stream and made up
34 in either methanol (C_{12} -chlorinated alkanes) or acetonitrile (C_{16} -chlorinated alkanes) for HPLC
35 analysis. Due to the more hydrophobic nature of the C_{16} -chlorinated alkanes, a less polar solvent,
36 acetonitrile, was substituted for methanol to insure that the C_{16} -chlorinated alkanes completely
37 dissolved. Samples were injected on a Varian 5000 liquid chromatograph (Varian Canada Inc.,
38 Mississauga, ON, Canada) equipped with a Prep Nova pak HR C-18 column (Waters Division of
39 Millipore, Milford, MA, USA), an autosampler and an automated fraction collector. The mobile
40 phase used for the C_{12} -chlorinated alkane samples consisted of 90% methanol and 10% water; 3
41 minute fractions were collected over a 60 minute period. For the C_{16} -chlorinated alkane samples a
42 mobile phase of 90% acetonitrile and 10% water was used, and 4 minute fractions over 80 minutes
43 were collected. Fractions were counted using LSC.

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

5
 6 **MFO assays**

7 Analysis of liver samples for MO enzyme activity was carried out with post mitochondrial
 8 supernatants as described previously [20]. The small size of the livers precluded preparation of
 9 microsomal fractions. EROD activity was measured using the method of Pohl and Fouts [21] with
 10 several modifications [20]. The reaction was started by the addition of 10 µl of ethoxyresorufin in
 11 dimethylsulphoxide (0.04 mg.ml⁻¹). The samples were incubated for precisely 2 min in a water bath
 12 at 25°C and then the reaction was stopped by addition of 2.5 ml of methanol. The samples were
 13 centrifuged at 24,000 x g to pellet the precipitated protein, and the amount of resorufin in the
 14 supernatant was determined spectrofluorometrically using an excitation wavelength of 530 nm and
 15 an emission wavelength of 585 nm. Protein was determined using the Lowry method as modified
 16 by Markwell *et al.* [22].

17
 18 **Data analysis**

19 Growth rates were determined by fitting all fish and liver weight data to an exponential model (ln
 20 fish weight = a + b time (days); where a is a constant and b is the growth rate) [20]. Chlorinated
 21 alkane concentrations were corrected for growth dilution and lipid normalized for all
 22 bioaccumulation parameters. Assimilation efficiencies (α) were calculated by fitting the
 23 concentration data to the integrated form of the kinetic rate equation for constant dietary exposure
 24 [23] using iterative nonlinear regression:

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

25 where F is the feeding rate (lipid corrected), C_{fish} is the concentration in the fish (lipid basis and growth
 26 corrected), C_{food} is the concentration in the food (on a lipid basis), and t is the time of uptake (days).
 27 Feeding rate (F) is assumed to be 1.5% of the body weight of the fish, corrected for the lipid percentage
 28 of the food (14% - determined in the same manner as the lipid percentage in the fish) and the fish.
 29 Depuration rates (k_d) were calculated by fitting the depuration phase data to a first-order decay curve (ln
 30 conc. = a + b time (days); where a is a constant and b is the depuration rate). Equilibrium biomagnification
 31 factor (BMF) was predicted from the equation BMF = α F / k_d.

32 Differences between growth rate constants among treatments, and depuration rates among
 33 treatments, were examined by testing the homogeneity of slopes in an analysis of covariance. The
 34 Student t test was used to compare pairs of elimination rate and growth rate constants at the p <
 35 0.05 level of significance.

36
 37
 38 **RESULTS**

39 **Effects**

40 The growth rates of both C₁₂H₁₈Cl₁₆ treatments, the low concentration C₁₂H₂₂Cl₁ treatment, and the
 41 low concentration C₁₆H₃₄Cl₃ treatment, were found to be significantly higher than the first control
 42 group, the high concentration C₁₆H₃₄Cl₃ treatment and the low and medium concentration C₁₆
 43 treatments (t-test, p < 0.05) (Table 1). Based on the growth rates, it is unlikely that the chlorinated
 44 alkanes had any negative effect on the growth of juvenile rainbow trout. The same pattern holds for

1 the liver growth rates, with the controls having the slowest growth rates (Table 1). A fin rot disease
2 spread through the control 2, low concentration $C_{12}H_{20}Cl_4$ and both $C_{16}H_{31}Cl_3$ treatment tanks,
3 causing a number of mortalities. However, none of the infected fish were used for data analysis, and
4 since there is no pattern to the mortalities with respect to treatment, and the affected tanks were side
5 by side, it is unlikely that the disease was a result of the chlorinated alkane exposures. Liver
6 somatic index (LSI = [liver weight/whole fish weight * 100]) and lipid percentages did not vary
7 between treatments (Table 1), although the percentage lipid increased throughout the experiment.

8 EROD levels in chlorinated alkane exposed rainbow trout were not higher than non-
9 exposed rainbow trout on the last collection day (day 40) of uptake, corresponding to wet weight
10 liver concentrations ($ng \cdot g^{-1}$) on day 40 of: 16.2 ± 1.0 (mean \pm 1 S.E.) for $C_{12}H_{20}Cl_4$; 23.4 ± 0.3 for
11 $C_{12}H_{16}Cl_{10}$; 27.9 ± 3.1 for $C_{16}H_{31}Cl_3$; and 75.6 ± 5.0 for $C_{16}H_{21}Cl_{13}$.

13 *Bioaccumulation parameters*

14 Accumulation of all four chlorinated alkanes from food by juvenile rainbow trout was observed
15 (Fig. 1) by day 5 of the uptake phase. None of the four compounds reached steady state after 40
16 days of exposure (Fig. 1), and the $C_{16}H_{21}Cl_{13}$ did not reach steady state after 80 days of exposure
17 (*data not shown*). The depuration rate in rainbow trout exposed to $C_{16}H_{31}Cl_3$ was significantly more
18 rapid than the depuration rates of rainbow trout exposed to $C_{12}H_{20}Cl_4$, $C_{12}H_{16}Cl_{10}$ and $C_{16}H_{21}Cl_{13}$
19 (Table 2). With the exception of the $C_{16}H_{31}Cl_3$, depuration rates for each chlorinated alkane did not
20 differ significantly between concentrations ($p < 0.05$) (Table 2), although no comparison was made
21 between the two $C_{12}H_{21}Cl_4$ treatments because data was not available for exactly the same time
22 period of depuration for the lower concentration exposure ($26.2 \text{ ng} \cdot g^{-1}$). Trends of depuration rates
23 in whole fish were consistent with depuration rates determined using only concentration data from
24 carcass tissue. Whole body half-lives varied from 37 ± 2 days in the $C_{16}H_{31}Cl_3$ high concentration
25 treatment to 87 ± 11 days in the $C_{12}H_{21}Cl_{10}$ low concentration treatment (Table 2).

26 Assimilation efficiencies (α) based on whole body concentrations ranged from 9.4 ± 1.1 %
27 in the medium concentration $C_{16}H_{21}Cl_{13}$ treatment to 37.6 ± 1.1 % in the low $C_{12}H_{16}Cl_{10}$ treatment
28 (Table 2). Whole body BMFs varied from 0.44 in the medium concentration $C_{16}H_{21}Cl_{13}$ treatment
29 to 2.15 in the low concentration $C_{12}H_{21}Cl_{10}$ treatment. Assimilation efficiency and BMF values
30 calculated with whole body and carcass tissue only concentrations were similar and followed the
31 same trends for all four chlorinated alkanes.

33 *Tissue distribution and metabolic transformation*

34 The carcass contained the greatest percentage of ^{14}C (including extractable and non-extractable)
35 throughout the experiment for all four chlorinated alkanes, ranging from 50% to greater than 70%
36 (Table 3). The relative proportion of ^{14}C increased in the carcass overtime, due mainly to increasing
37 amounts of non-extractable ^{14}C . There was a slight drop in relative amounts of extractable ^{14}C in the
38 GI tract from the beginning until the end of the uptake phase, which is probably due to the greater
39 proportion of ^{14}C in the GI tract resulting from the undigested spiked-food in the gut. Non-
40 extractable ^{14}C decreased in the liver GI tract over time, which could be explained by the high
41 turnover of the liver and GI tract lining. Relative amounts in the liver were low because the liver
42 accounted for only about 1.5% of the total fish weight. Both extractable and non-extractable ^{14}C
43 decreased in the liver throughout the experiment, providing evidence that metabolic transformation
44 of chlorinated alkanes may occur in the liver.

1 HPLC chromatograms of the toluene extracts differed from the analytical standards on day
 2 40 of uptake for all four chlorinated alkanes (Fig. 2). This difference is most pronounced in the
 3 $C_{12}H_{20}Cl_4$ treatment, where a number of larger peaks in the fish extracts are minor in the analytical
 4 standard. After 20 days of depuration (no exposure to treated food), all four of the chlorinated
 5 alkanes show chromatographic profiles markedly different from the analytical standards (Fig. 2).
 6 Toluene non-extractable residues are assumed to represent chlorinated alkanes which have been
 7 metabolically transformed and have become more polar, and therefore unextractable with toluene.
 8 The higher chlorinated C_{12} - and C_{16} -alkanes had a greater proportion of toluene extractable ^{14}C , or
 9 parent compound (Table 3), implying less metabolic transformation of these compounds.

10
11 DISCUSSION

12 Chlorinated alkanes with 12 and 16 carbons and 35 - 69% Cl content (by weight) are
 13 accumulated through dietary exposure by juvenile rainbow trout despite relatively large molecular
 14 weight. As other authors have observed with CPs, accumulation of chlorinated alkanes is dependent
 15 on the carbon chain length [6], number of chlorines [3], and molecular size of the molecule [9]. The
 16 assimilation efficiency of the C_{12} -chlorinated alkanes in this experiment increased from a mean of
 17 23 to 36% (whole fish) with the addition of four chlorine atoms, corresponding to an increase in
 18 K_{ow} ($\log K_{ow}$'s of $C_{12}H_{20}Cl_4$ and $C_{12}H_{16}Cl_8$ are approximately 5.2 and 6.8, respectively; G. R. B.
 19 Webster, personal communication). However, the assimilation efficiency of the C_{16} -chlorinated
 20 alkanes decreased from a mean 34 to 11% when the number of chlorine atoms increased from 3 to
 21 13, despite an increase in K_{ow} ($\log K_{ow}$'s of $C_{16}H_{31}Cl_3$ and $C_{16}H_{21}Cl_{13}$ are approximately 6.9 and 7.4,
 22 respectively; G. R. B Webster, personal communication). The $C_{16}H_{21}Cl_{13}$ was found to have the
 23 highest K_{ow} of the four chlorinated alkanes but the lowest accumulation and assimilation efficiency.
 24 The reduced uptake of the $C_{16}H_{21}Cl_{13}$ may be due to its' large size (MW = 674) [24-26]. Low
 25 uptake of long chain CPs has also been observed by Zitko [9], who found that two industrial CP
 26 products with high molecular weights (MW = 579-922) showed little bioaccumulation in juvenile
 27 Atlantic salmon.

28 The assimilation efficiencies calculated for these chlorinated alkanes are higher than
 29 assimilation efficiencies reported for commercial CP products using breams (*Abramis alburnus*)
 30 [6]. An industrial CP mixture consisting of C_{10-12} CPs with 71% Cl had a "mean effectiveness in
 31 uptake" from food of only 6%, which was half the assimilation found for a less chlorinated (49%
 32 Cl) C_{10-12} CP commercial product [6]. We found that an increase in chlorination of C_{12} -chlorinated
 33 alkanes resulted in an increase in assimilation efficiency. The assimilation efficiency of the
 34 chlorinated alkanes used in this experiment are comparable to tetra and pentachlorodibenzofurans
 35 under similar experimental conditions, using juvenile rainbow trout [20,27], and to a hexa and
 36 octachlorobiphenyl in guppies (*Poecilia reticulata*) [28].

37 Half lives of the chlorinated alkanes in this experiment are different than half lives reported
 38 for other CPs [6,29]. Madeley and Maddock [29] reported a half life range from 16.5 days in dorsal
 39 muscle to 23.9 days in the viscera of rainbow trout exposed to water borne short chain (C_{10-12}) 58%
 40 chlorinated paraffin, about half the value we calculated for the $C_{12}H_{20}Cl_4$. Bengtsson *et al.* [6]
 41 reported very rapid elimination ($t_{1/2} < 7$ days) of a 49% Cl short chain (C_{10-12}) CP mixture. However,
 42 Bengtsson *et al.* [6] reported that there was no elimination of a short chain (C_{10-12}) CP with 71% Cl
 43 after 316 day elimination period, much longer than the 77 to 87 day half life calculated for the
 44 $C_{12}H_{16}Cl_8$ used in this experiment. It should be noted that the concentrations of CPs reported by

1 Bengtsson *et al.* [6] were determined by measuring chlorine levels in the fish and not CPs. It may
2 well be that the CPs in Bengtsson *et al.* [6] work were metabolically transformed, but the Cl
3 remained in the fish.

4 The $C_{12}H_{14}Cl_2$ would be expected to biomagnify in aquatic food webs based on
5 equilibrium biomagnification factors (BMF) of 1.76 and 2.15. A BMF value greater than one
6 implies increasing concentrations along aquatic food webs, or biomagnification [15,27]. The low
7 concentration $C_{12}H_{14}Cl_2$ treatment was also found to have a BMF above 1 (BMF = 1.07), however
8 the high concentration treatment had a BMF of only 0.90. The addition of one or two chlorines to
9 $C_{12}H_{14}Cl_2$ could reduce the depuration rate, by decreasing metabolism, and increase the assimilation
10 efficiency, due to a higher K_{ow} , sufficiently to result in a BMF greater than one. The $C_{16}H_{22}Cl_2$ and
11 the $C_{12}H_{14}Cl_4$ would not biomagnify in aquatic food webs based on a predicted equilibrium BMF of
12 0.44 to 0.50 and 0.60 to 0.93, respectively.

13 From the results of these experiments, it appears that the relationship of bioaccumulation
14 and carbon chain length and chlorine content of chlorinated alkanes is complex. Although
15 metabolism of short chain CPs (C_{10-13}) with low chlorination may reduce accumulation, low
16 chlorinated medium (C_{14-18}) and long (C_{19-30}) chain length CPs may be less susceptible to
17 metabolism because of the carbon chain length [30]. High chlorination of short chain chlorinated
18 alkanes results in sufficient accumulation for biomagnification, but makes medium and long chain,
19 highly chlorinated alkanes too large to diffuse through biological membranes without hindrance.
20 The results suggest that, despite having some characteristics of bioaccumulative chemicals (i.e.
21 high K_{ow} , low biotransformation rate), highly chlorinated (>60%), medium (C_{14-18}) and long (C_{19-30})
22 carbon chain alkanes are not likely to biomagnify in aquatic food webs. A further confounding
23 factor, to which we could not address with these ^{14}C -labelled chlorinated alkanes, is the position of
24 the chlorines on the carbon chain. The HPLC chromatogram of the fish extracts showed that certain
25 chlorinated alkanes within the standards were accumulating to a greater extent than others. Chlorine
26 positioning on the alkane chain could explain the persistence of some alkanes, and low persistence
27 of other chlorinated alkanes, with the same molecular formula. This differential bioaccumulation,
28 which is observed in invertebrates, fish and mammals with other chlorinated compounds such as
29 chlordane [31], toxaphene [32] and PCBs [33], needs further study.

30 These experiments provide the first dietary bioaccumulation parameters for chlorinated
31 alkanes with a single carbon chain length and known amount of chlorine, although the positions
32 and exact number of chlorine atoms are not known. From Bergman *et al.* [19], and our own HPLC
33 work, it appears that the number of chlorine atoms per compound in each standard is close to the
34 integer value assigned in this paper, although due to non-selective synthesis procedures for these
35 ^{14}C compounds, these standards are likely composed of numerous compounds and positional
36 isomers. Confounding this problem is the identical electron capture negative ion (ECNI) mass
37 spectra of CP congeners containing similar carbon and chlorine atoms (G. T. Tomy, personal
38 communication), making it difficult to identify separate chlorinated alkanes based on chlorine
39 substitution patterns.

40 There is evidence that CPs are oxidized in fish [30,34], with short carbon chain (C_{10-13}) CPs
41 being more susceptible to metabolism than medium (C_{14-18}) and long chain (C_{19-30}) CPs [30].
42 Ahlman *et al.* [35] have found sulphur-containing metabolites of CPs in rats, implying that CPs
43 may covalently bind to biological macromolecules. Lower Cl substitution resulted in greater
44 relative amounts of non-toluene extractable ^{14}C for the C_{12} and C_{16} chlorinated alkanes. Because the

1 ¹⁴C was extractable from the tissue with toluene, it is considered to represent a more polar
 2 compound than the standard (such as a hydroxylated or carboxylic acid substituted product) or has
 3 been incorporated with biological macromolecules.

4 The C₁₂H₂₀Cl₄ showed greater change from than analytical standard than the higher
 5 chlorinated C₁₂H₁₈Cl₆, but this relationship is not as evident for the C₁₄-chlorinated alkanes.
 6 Madley and Birtley [30] reported that short chain (< C₁₁) CPs with low chlorination (< 60%) are
 7 the most readily oxidized in microorganisms. However, it is obvious from the HPLC-¹⁴C
 8 chromatogram of day 20 depuration, that all of the chlorinated alkanes used in this experiment are
 9 susceptible to metabolism. Therefore, the rate of metabolism of chlorinated alkanes is dependent on
 10 both chlorine substitution and carbon chain length, but may also be dependent on chlorine position.

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

20 In summary, bioaccumulation parameters of chlorinated alkanes reported here differ from
 21 previous results on bioaccumulation of CPs in fish. These chlorinated alkanes are found in
 22 commercial CP products, although they are synthesized in a different manner, and this represents
 23 the first work that has used chlorinated alkanes of known carbon chain length and chlorine content
 24 for dietary bioaccumulation studies. The elimination rates, half lives and assimilation efficiencies of
 25 the chlorinated alkanes are similar to PCDD/Fs and PCBs. Reduced accumulation of lower
 26 chlorinated C₁₂-alkane could be attributed to metabolism while uptake of highly chlorinated C₁₄-
 27 alkane may have been hindered because of its large molecular size. The C₁₂H₁₈Cl₆ would likely
 28 biomagnify in aquatic food chains based on a BMF of greater than 1 (1.76 and 2.15). Highly
 29 chlorinated short chain (C₁₀₋₁₁) CPs and lower chlorinated medium chain (C₁₄₋₁₈) CPs have the
 30 greatest potential for bioaccumulation by aquatic organisms.

31
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39
 40 **REFERENCES**

- 41 1. Canadian Environmental Protection Act. 1993. Priority Substances List Assessment
 42 Report: Chlorinated Paraffins. Government of Canada, Ottawa, Canada, 32 pp.
 43 2. Campbell, I. and G. McConnell. 1980. Chlorinated paraffins and the environment. 1.
 44 Environmental occurrence. *Environ. Sci. Technol.* 14: 1209-1214.

- 10
- 1 3. Darnerud, P. O., A. Bergman, B. O. Lind and I. Brandt. 1989. Selective accumulation of
2 chlorinated paraffins (C₁₂ and C₁₄) in the olfactory organ of rainbow trout. *Chemosphere* 13:
3 1821-1827.
 - 4 4. Jansson, B., R. Andersson, L. Asplund, K. Litzen, K. Nyland, U. Sellstrom, U. Uvemo,
5 C. Wahlberg, U. Wideqvist, T. Odsjo and M. Olsson. 1992. Chlorinated and brominated
6 persistent organic compounds in biological samples from the environment. *Environ.*
7 *Toxicol. Chem.* 12: 1163-1174.
 - 8 5. Franka, C., G. Studinger, G. Berger, S. Bohling, U. Bruchmann, D. Cohors-
9 Fresseberg and U. Johncke. 1994. The assessment of bioaccumulation. *Chemosphere* 29:
10 1501-1514.
 - 11 6. Bengtsson, B. and E. B. Ofstad. 1982. Long-term studies of uptake and elimination of
12 some chlorinated paraffins in the bleak, *Alburnus alburnus*. *Ambio* 11: 38-40.
 - 13 7. Bengtsson, B., O. Svenberg, E. Linden, G. Lunde and E. B. Ofstad. 1979. Structure
14 related uptake of chlorinated paraffins in bleaks (*Alburnus alburnus* L.). *Ambio* 8: 121-122.
 - 15 8. Lombardo, P., J. L. Dennison and W. W. Johnson. 1975. Bioaccumulation of chlorinated
16 paraffin residues in fish fed Chlorowax 500C. *Journal of the Association of Official*
17 *Analytical Chemists* 58: 707-710.
 - 18 9. Zitko, V. 1974. Uptake of chlorinated paraffins and PCB from suspended solids and food
19 by juvenile Atlantic salmon. *Bull. Environ. Contam. Toxicol.* 12: 406-412.
 - 20 10. Drouillard, K. G., T. Hiebert, D. C. G. Muir and K. J. Friesen. 1995. Water solubility
21 and Henry's Law constants of short chain chlorinated paraffins. *Proceedings, 38th*
22 *Conference on Great Lakes Research, East Lansing, MI, USA. May 28 - June 1, 1995, pp.*
23 *118-119.*
 - 24 11. Sijm, D. T. H. M. and T. L. Sinnige. 1995. Experimental octanol/water partition
25 coefficients of chlorinated paraffins. *Chemosphere* 31: 4427-4435.
 - 26 12. Svanberg, O., B-F. Bengtsson, E. Linden, G. Lunde and E. B. Ofstad. 1978. Chlorinated
27 paraffins - A case of accumulation and toxicity to fish. *Ambio* 7: 64-65.
 - 28 13. Oliver, R. G. and A. J. Nijmi. 1988. Trophodynamic analysis of polychlorinated biphenyl
29 congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environ. Sci.*
30 *Technol.* 22: 388-397.
 - 31 14. Evans, M. S., G. E. Noguchi and C. P. Rice. 1991. The biomagnification of
32 polychlorinated biphenyls, toxaphene, and DDT compounds in a Lake Michigan offshore
33 food web. *Arch. Environ. Contam. Toxicol.* 20: 87-93.
 - 34 15. Rasmussen, J. B., D. J. Rowan, D. R. S. Lean and J. H. Carey. 1990. Food chain
35 structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and
36 other pelagic fish. *Can. J. Fish. Aquat. Sci.* 47: 2030-2038.
 - 37 16. McFarland, V. A. and J. U. Clarke. 1989. Environmental occurrence, abundance, and
38 potential toxicity of polychlorinated biphenyl congeners: Considerations for a congener-
39 specific analysis. *Environ. Health Perspect.* 81: 225-239.
 - 40 17. Opperhuizen, A. and D. T. H. M. Sijm. 1990. Bioaccumulation and biotransformation of
41 polychlorinated dibenzo-*p*-dioxins and dibenzofurans in fish. *Environ. Toxicol. Chem.* 9:
42 175-186.

1 18. Haas, C., A. Larsson, U. Lidman, L. Furita, T. Hansson and M. Johansson-Sjöbeck. 11
 2 1982. Sublethal physiological effects of chlorinated paraffins on the flounder, *Platichthys*
 3 *fasciatus*. *L. Ecotoxicol. Environ. Safety* 6: 49-59.
 4 19. Bergman, A., I. Leonardsson and C. A. Wachmeister. 1981. Synthesis of polychlorinated
 5 [¹⁴C] alkanes (PCA) of high specific activity. *Chemosphere* 10(8): 857-863.
 6 20. Muir, D. C. G., A. L. Yarechewski, D. A. Metner, W. L. Lockhart, G. R. B. Webster
 7 and K. J. Frisosa. 1990. Dietary accumulation and unsaturated hepatic mixed function
 8 oxidase enzyme induction by 2,3,4,7,8-pentachlorodibenzofuran in rainbow trout. *Environ.*
 9 *Toxicol. Chem.* 9:1463-1472.
 10 21. Fahl, R. J. and J. R. Fouts. 1980. A rapid method for assaying the metabolism of 7
 11 ethoxycoumarin by microsomal cellular fractions. *Anal. Biochem.* 107: 150-155.
 12 22. Markwell, M. A. K., S. M. Haas, N. E. Tolbert and L. L. Bieber. 1981. Protein
 13 determination in membrane and lipoprotein samples: Manual and automated procedures.
 14 *Methods Enzymol.* 72: 296-393.
 15 23. Bruggeman, W. A., A. Opperhuizen, A. Wijkema and O. Hutzinger. 1984.
 16 Bioaccumulation of super-lipophilic chemicals in fish. *Toxicol. Environ. Chem.* 7: 173-189.
 17 24. Gobas, F. A. F. C., D. C. G. Muir and D. Mackay. 1988. Dynamics of dietary
 18 bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish.
 19 *Chemosphere* 17: 943-962.
 20 25. Nihal, A. J. and B. G. Oliver. 1987. Influence of molecular weight and molecular volume
 21 on dietary absorption efficiency of chemicals by fishes. *Can. J. Fish. Aquat. Sci.* 45: 222-
 22 227.
 23 26. Opperhuizen, A., E. W. v. d. Velde, F. A. P. C. Gobas, D. A. K. Liem and J. M. D. v. d.
 24 Steen. 1985. Relationship between bioconcentration in fish and steric factors of
 25 hydrophobic chemicals. *Chemosphere* 14: 1871-1896.
 26 27. Muir, D. C. G., A. L. Yarechewski, D. A. Metner and W. L. Lockhart. 1992. Dietary
 27 2,3,7,8-tetrachlorodibenzofuran in rainbow trout: Accumulation, disposition, and hepatic
 28 mixed-function oxidase enzyme induction. *Toxicol. Appl. Pharmacol.* 117:65-74.
 29 28. Opperhuizen, A. and S. M. Schrap. 1988. Uptake efficiencies of two polychlorobiphenyls
 30 in fish after dietary exposure to five different concentrations. *Chemosphere* 17(2): 253-262.
 31 29. Madley, J. R. and B. G. Maddock. 1983. The bioconcentration of chlorinated paraffin in
 32 the tissues and organs of rainbow trout (*Salmo gairdneri*). ICI Brixham Laboratory Report
 33 BL/B/2310, Brixham, UK.
 34 30. Madley, J. R. and R. D. N. Britly. 1980. Chlorinated paraffins and the environment. 2.
 35 Aquatic and avian toxicology. *Environ. Sci. Technol.* 14: 1215-1221.
 36 31. Wilcock, R. J., T. J. Smith, R. D. Pridmore, S. F. Thrush, V. J. Cummings and J. E.
 37 Hewitt. 1993. Bioaccumulation and elimination of chlordane by selected intertidal benthic
 38 fauna. *Environ. Toxicol. Chem.* 12: 733-742.
 39 32. Billman, T. F., M. D. Wafa, D. C. G. Muir and G. A. Stern. 1993. Selective
 40 accumulation of polychlorocamphenes in aquatic biota from the Canadian arctic. *Environ.*
 41 *Toxicol. Chem.* 12: 701-709.
 42 33. Koslowski, S. E., C. D. Metcalfe, R. Lazar and G. D. Haffner. 1994. The distribution of
 43 42 PCBs, including three coplanar congeners, in the food web of the western basin of Lake
 44 Erie. *J. Gr Lakes Res.* 20: 260-270.

- 1 34. Darnerud, P. O., B. -E. Bengtsson, A. Bergman and I. Brandt. 1983. Chlorinated 12
2 paraffins: Disposition of a polychloro-[1-¹⁴C]-hexadecane in carp (*Cyprinus carpio*) and
3 bleak (*Alburnus alburnus*). *Toxicol. Letters* 19: 345-351.
- 4 35. Ahlman, M., A. Bergman, P. O. Darnerud, B. Egestad and J. Sjövall. 1986. Chlorinated
5 paraffins: formation of sulphur-containing metabolites of polychlorohexadecane in rats.
6 *Xenobiotica* 16: 225-232.
- 7 36. Linden, E., B-E. Bengtsson, O. Svanberg and G. Sandstrom. 1979. The acute toxicity of
8 78 chemicals and pesticide formulations against two brackish water organisms, the bleak
9 (*Alburnus alburnus*) and the harpacticoid (*Nitocra spinipes*). *Chemosphere* 11/12: 843-851.
- 10 37. Safe, S. 1992. Toxicology, structure-function relationships, and human and environmental
11 health impacts of polychlorinated biphenyls: Progress and problems. *Environ. Health*
12 *Perspect* 100: 259-268.
- 13 38. Svanberg, O. and E. Linden. 1973. Chlorinated paraffins an environmental Hazard?
14 *Ambio* 8: 206-209.

1 Table 1: Growth parameters (mean \pm 1 standard error) of juvenile rainbow trout exposed to four ^{14}C -chlorinated alkane compounds.
 2 Significant differences (t-test, $p < 0.05$) in body and liver growth rates for all treatments are indicated by italics.

Chemical	Conc. in food ($\mu\text{g g}^{-1}$)	uptake period		growth rates ²		LSD ³	% mean
		(days)	(days)	body ($\text{day}^{-1} \times 10^3$)	liver ($\text{day}^{-1} \times 10^3$)		
control 1	-	40	173	10.4 \pm 1.1 (0.74) ^d	8.6 \pm 1.4 (0.56) ^e	1.47 \pm 0.33	0
control 2	-	40	80	10.4 \pm 2.5 (0.39) ^{bd}	7.9 \pm 2.9 (0.22) ^{bc}	1.37 \pm 0.21	15.8
$\text{C}_{17}\text{H}_{34}\text{Cl}_2$	26	40	80	21.5 \pm 3.1 (0.65) ^a	16.5 \pm 2.8 (0.57) ^a	5.7 \pm 0.2	18.4
	242	40	120	14.0 \pm 1.9 (0.63) ^{bd}	11.8 \pm 1.7 (0.61) ^{bc}	1.23 \pm 0.07	0
$\text{C}_{17}\text{H}_{34}\text{Cl}_4$	21	40	120	16.2 \pm 1.8 (0.71) ^{ab}	12.8 \pm 1.6 (0.68) ^{abc}	1.29 \pm 0.03	0
	222	40	120	15.3 \pm 1.6 (0.76) ^{abc}	12.6 \pm 1.3 (0.75) ^{abc}	1.10 \pm 0.07	0
$\text{C}_{18}\text{H}_{36}\text{Cl}_2$	29	40	120	15.8 \pm 1.9 (0.72) ^{abc}	12.6 \pm 1.4 (0.72) ^{abcd}	1.23 \pm 0.18	7.9
	286	40	120	10.1 \pm 2.6 (0.59) ^d	8.3 \pm 1.6 (0.49) ^e	1.12 \pm 0.08	5.3
$\text{C}_{18}\text{H}_{36}\text{Cl}_4$	21	40	173	1.6 \pm 1.2 (0.72) ^d	8.7 \pm 1.3 (0.59) ^e	1.27 \pm 0.15	0
	198	40	173	1.4 \pm 1.1 (0.77) ^d	9.2 \pm 1.8 (0.46) ^e	1.20 \pm 0.10	0
	2000	40	173	11.6 \pm 1.2 (0.74) ^{dc}	9.9 \pm 1.2 (0.70) ^{dc}	1.46 \pm 0.10	0

3 The growth rates (\pm 1 standard error) were calculated using the equation $\ln \text{weight} = a + b \text{ time (days)}$, where b is the growth rate (coefficient of determination for the model is shown in parentheses).

4 The percent lipid is an average (\pm 1 standard error) of all fish in a treatment from day 5 until the end of the experiment.

5 Liver somatic index (LSI) (\pm 1 standard error) calculated at day 40 of the uptake phase.

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Table 2: Bioaccumulation parameters of four ¹⁴C-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 days of depuration data are indicated with italics.

Chemical	Conc. in food ¹ (ng/g)	Length of Depuration (days)	Depuration rate constant ² (10 ⁻² · day ⁻¹)	t _{1/2} ³ (days)	BMF ⁴	Assimilation efficiency ⁵ (%)
C ₁₂ H ₂₀ Cl ₄	26.2 ^y	80	1.8 ± 0.2 (0.83)	39 ± 4	0.60	25.3 ± 2.5
	241.6	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
	221.7	160	0.9 ± 0.1 (0.75) ^c	77 ± 9	1.76	34.1 ± 1.3
C ₁₆ H ₃₁ Cl ₅	28.9	160	1.4 ± 0.2 (0.73) ^b	50 ± 7	1.07	33.1 ± 2.0
	295.9	160	1.9 ± 0.1 (0.93) ^a	37 ± 2	0.90	35.1 ± 2.0
C ₁₆ H ₂₁ Cl ₁₃	20.8 ^z	80	1.2 ± 0.2 (0.62)	58 ± 10	0.72	30.0 ± 7.2
	198.0	173	1.1 ± 0.2 (0.69) ^c	63 ± 11	0.44	9.4 ± 1.1
	2003.1	173	0.9 ± 0.1 (0.68) ^c	77 ± 9	0.50	11.7 ± 1.3

1 - Concentration of food is given in wet weight.
 2 - 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 solvent-extractable radioactivity for 120 days of depuration (coefficient of determination for the model is shown in parentheses).
 3 - Half life (± 1 standard error) is calculated from the equation t_{1/2} = 0.693/k_d.
 4 - Biomagnification factor (BMF) is calculated from the equation BMF = αFF₀, where F is the feeding rate on a lipid basis.
 5 - 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} = (αFC_{food}/k_d) [1 - exp(-k_dt)] 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 (days).
 6 - Due to mortalities, no fish from this treatment were available for day 160 analysis.
 7 - Depuration data of the low concentration C₁₆H₂₁Cl₁₃ treatment did not significantly fit a linear relationship when day 160 was included and therefore, depuration rate was only calculated with 80 days of depuration data.

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1 **Table 3: Extractable and non-extractable radioactivity, as a percentage of the total fish**
 2 **radioactivity, in the liver, GI tract, carcass and total fish, of juvenile rainbow trout exposed to**
 3 **four ¹⁴C-polychlorinated alkane compounds (U refers to the uptake phase and D the depuration**
 4 **phase).**

tissue	day	C ₁₂ H ₂₀ Cl ₂		C ₁₂ H ₁₈ Cl ₂		C ₁₆ H ₂₂ Cl ₂		C ₁₆ H ₂₀ Cl ₂	
		ext	non-ext	ext	non-ext	ext	non-ext	ext	non-ext
liver	10 U	1.2	1.5	1.9	1.4	1.7	1.7	16.0 ^y	3.1 ^y
	20 U	1.1	1.2	1.4	1.1	0.8	1.0	6.6 ^z	2.6 ^z
	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 ^y	16.3 ^y
	20 U	32.0	18.6	24.8	11.9	22.0	9.8	30.5 ^z	15.0 ^z
	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 ^y	11.3 ^y
	20 U	31.9	15.3	51.0	9.7	46.0	20.4	35.7 ^z	9.6 ^z
	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 ^y	30.7 ^y
	20 U	65.1	34.9	77.2	22.8	68.8	31.2	72.8 ^z	27.2 ^z
	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

5 ^y Day 10 C₁₆H₂₀Cl₂ liver samples were lost, percentages represent day 5 liver samples.

6 ^z Day 20 C₁₆H₂₀Cl₂ liver samples were lost, percentages represent day 30 liver samples.

- 16
- 1 **Figure 1: Accumulation and depuration of four ^{14}C -polyhalogenated alkane compounds through**
2 **dietary exposure to juvenile rainbow trout. Each point is the mean \pm one standard error of three**
3 **fish. Concentrations are for whole fish, corrected for growth dilution and lipid content. Exposure**
4 **concentrations (lipid corrected) are provided in the legend.**
5
- 6 **Figure 2: HPLC chromatograms of the ^{14}C -polyhalogenated alkane standards and fish carcass**
7 **toluene extracts from day 40 of uptake and day 20 of depuration. Each bar in the C_{12} -chlorinated**
8 **alkane and C_{14} -chlorinated alkane chromatograms represent the radioactivity in a three or four**
9 **minute fraction, respectively, as a percentage of the total radioactivity.**

