SYNTHESIS, PROPERTIES, AND BIOACCUMULATION OF CHLORINATED PARAFFINS

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INTRODUCTION

Chlorinated paraffins (CPs) are a class of C_{10}-C_{30} chlorinated *n*-alkanes which have numerous industrial uses including use as high pressure lubricants and cutting oils, fire retardants,and plasticizers. Little is known about the physical or chemical properties or of the environmental behaviour of chlorinated paraffins. Most of the available information is compounded by the fact that a very complex technical mixture is usually used to report the behaviour or properties of CPs. The objective of our research with CPs was to synthesize a number of chloroparaffin congeners, and then to use the synthesized chemicals to determine key physical properties and bioaccumulation potentials for members of this class of chemical.

SYNTHESIS

Syntheses of individual chlorinated *n*-paraffin congeners were carried out by bubbling chlorine gas directly into solutions of commercially available olefins for 20 minutes at O°C. Nitrogen gas was then bubbled through the reaction mixture, followed by shaking with dilute sodium hydroxide (0.25M). The organic phase was then removed and dried over magnesium sulfate.

The congeners formed in this type of reaction arise from; *(i)* direct addition of chlorine atoms across the double bonds, and *(ii)* free radical substitution of hydrogen atoms by chlorine atoms. Once formed, individual congeners are isolated from the reaction mixture using preparatory gas chromatography (GC). This technique employs a cold trap attached to the end of a megabore GC column, allowing congeners which have been separated chromatographically to condense *in-situ*. The trap is then washed

repeatedly with solvent to remove the isolated material. The molecular weights and the degree of chlorination of the CPs synthesized by this procedure (summarized in Table 1) have been confirmed by positive electron ionization (EI) and electron capture negative ion (ECNI) mass spectroscopy with methane used as the moderating gas.

TABLE 1. Summary of Chlorinated Paraffins Synthesized.

Carbon Chain Length	Chlorinated n-paraffins synthesized				
C-10	$C_{10}H_{20}Cl_2$* 33%	$C_{10}H_{18}Cl_4$ 50%	$C_{10}H_{17}Cl_5$* 56%	$C_{10}H_{16}Cl_6$ 61%	$C_{10}H_{15}Cl_7$* 64%
C-11	$C_{11}H_{22}Cl_2$ 31%	$C_{11}H_{20}Cl_4$ 48%	$C_{11}H_{19}Cl_5$* 54%	$C_{11}H_{18}Cl_6$* 58%	
C-12	$C_{12}H_{24}Cl_2$ 30%	$C_{12}H_{22}Cl_4$ 46%	$C_{12}H_{21}Cl_5$* 51%	$C_{12}H_{20}Cl_6$* 56%	
C-14		$C_{14}H_{26}Cl_4$* 42%	$C_{14}H_{25}Cl_5$* 48%	$C_{14}H_{24}Cl_6$* 52%	

*Indicates two or more congeners with the same %Cl, but with Cl residing in different positions.

PHYSICAL PROPERTIES

In this study, a synthesized polychlorinated decane[1] containing resolvable tetra-, penta- and hexachlorodecane congeners was utilized to determine several key physical properties of individual short chain CPs. Water solubilities of these congeners and of 1,10-dichlorodecane (Aldrich Chemical Co.) were determined at 25°C using the generator column technique[2]. The generator column, consisting of an HPLC column packed with glass beads (60-80 mesh) coated with ~25 mg of the synthesized polychlorinated decane, was temperature controlled using an HPLC column oven. The column was eluted with HPLC grade water at flow rates ranging from 0.2 to 0.5 mL/min. Saturated water was extracted with an in-line C_{18} Sepak. Sepaks were extracted with 40 mL of hexane, spiked with lindane (as internal standard) and concentrated to 100-200 μL. Analyses were performed by GC-ECD using a 30 m x 0.25 mm SPB-5 capillary column.

Table 2 summarizes the solubilities of di-, tetra-, penta- and hexachlorodecane congeners at 25°C. Significant ($p<0.001$; ANOVA, Table 2) decreases in solubilities with increasing chlorine content were observed. Van't Hoff plots, describing the temperature dependence of solubilities, show strong linear relationships (r^2 ranging from 0.93-0.98) over the temperature range of 6 to 25°C for all CPs tested. Enthalpies of solution (ΔH_S) of 46.8, 43.4 and 50.2 kJ/mol were calculated from van't Hoff plots for the tetra-, penta- and hexachlorodecane congeners, respectively. The hydrophobic nature of chlorinated paraffins, as evidenced by the low aqueous solubilities, are supported by relatively high octanol-water partition coefficients[3].

TABLE 2. Solubilities of Polychlorinated Decanes at 25°C

Chemical	Formula	S_W (μg/L)	SE	n
Dichlorodecane	$C_{10}H_{20}Cl_2$	236.0	6.7	5
Tetrachlorodecane	$C_{10}H_{18}Cl_4$	140.6	4.6	4
Pentachlorodecane a	$C_{10}H_{17}Cl_5$	27.9	1.2	4
Pentachlorodecane b	$C_{10}H_{17}Cl_5$	30.6	1.3	4
Pentachlorodecane c	$C_{10}H_{17}Cl_5$	27.7	1.2	4
Pentachlorodecane d	$C_{10}H_{17}Cl_5$	27.8	1.2	4
Hexachlorodecane a	$C_{10}H_{16}Cl_6$	1.6	0.1	4
Hexachlorodecane b	$C_{10}H_{16}Cl_6$	4.0	0.2	4

The gas sparging technique[4] was employed to determine Henry's Law Constants of the polychlorinated decanes. The sparging column, measuring 60 cm x 15 mm i.d., was equipped with a fritted disk to disperse the gas into fine bubbles. Pre-wetted N_2 gas was passed through the column (flow rate 400 mL/min) containing 500 mL of an aqueous solution of the CP. The gas exiting from the sparger was extracted by bubbling through hexane or by passing over a solid phase tenax resin trap. The depletion kinetics of CPs purged from water were utilized to calculate HLCs based on equations described by Yin and Hassett[5].

HLCs were determined for tetra- and pentachlorodecane from a number of sparging runs ranging in duration from 48 to 110 h. HLCs for tetrachlorodecane from four experiments ranged from 1.8 to 12.7 $Pa \cdot m^3 \cdot mol^{-1}$ with a mean of 5.4 ± 2.1 $Pa \cdot m^3 \cdot mol^{-1}$. The pentachlorodecane sparging data yielded more precise results with HLCs from five experiments ranging from 1.8 to 4.6 $Pa \cdot m^3 \cdot mol^{-1}$ with a mean of 3.4 ± 0.6 $Pa \cdot m^3 \cdot mol^{-1}$. HLCs for the tetra and pentachlorodecanes are similar, despite large differences in their water solubilities. The results suggest that vapour pressures and water solubilities of CPs vary in a similar manner with the degree of chlorination.

BIOACCUMULATION

The bioaccumulation potential of CPs has been reported to increase with increasing chlorine content and decreasing carbon chain length. Bioaccumulation factors (BAF) of short chain CPs (C_{10-13}) in rainbow trout (*Onchorhynchus mykiss*) and bleak (*Alburnus alburnus*), fed spiked food, ranged from 2 to 41[6,7], implying a potential to biomagnify in aquatic food chains. In this study juvenile rainbow trout (*O. mykiss*) were exposed to four concentrations of a radiolabelled chlorinated hexadecane (^{14}C-$C_{16}H_{21}Cl_{13}$) to determine an elimination rate, half-life, assimilation efficiency and biomagnification factor. Furthermore, mixed function oxygenase (MO) enzymes were measured on the last day of uptake, and growth parameters were compared between treatments.

Four concentrations (0, 21, 198, 2003 ng/g) of the ^{14}C-labelled $C_{16}H_{21}Cl_{13}$ (specific activity = 82.3 dpm/ng) were established in trout food. Trout were exposed to the spiked food for 40 days followed by 173 days of depuration at a rate of 1.5 grams of food per gram of fish (adjusted after sampling interval). On days 5, 10, 20, 30 and 40 of uptake, and days 5, 10, 20, 40, 80 and 173 of depuration, three fish were sampled from each treatment. Liver, intestine and carcass (whole fish minus liver and intestine) were separated, weighed and analyzed. Analysis of samples, EROD analysis and bioaccumulation parameter calculations were carried out following established procedures[8].

No growth effects, mortality, disease, behavioral changes, or feeding changes were observed in any of the treatments. Bioaccumulation parameters (summarized in Table 3) indicate low elimination rates and half-lives greater than 3 weeks. These results are comparable to values previously reported for 2,3,7,8-tetrachlorodibenzofuran[8]. However, biomagnification did not occur for $C_{16}H_{21}Cl_{13}$ because of low assimilation efficiencies. Further work is underway on four additional ^{14}C-labelled CPs ($C_{16}H_{31}Cl_3$, $C_{12}H_{14}Cl_{12}$, $C_{12}H_{20}Cl_6$) using identical experimental protocol.

TABLE 3: Bioaccumulation Parameters for the Dietary Accumulation of $C_{16}H_{21}Cl_{13}$ in Juvenile Rainbow Trout.

Conc. in food ($ng \cdot g^{-1}$)	Duration		Depuration[a] rate constant ($day^{-1} \times 10^{-3}$)	$t_{1/2}$[b] (days)	BMF[c]	Assimilation[d] efficiency (%)
	Uptake (days)	Depuration (days)				
Ctrl	40	120[e]	-	-	-	-
21	40	120	11.9 ± 2.4 (0.61)	58	0.65	14.4 ± 3.3
198	40	120	15.5 ± 2.5 (0.71)	46	0.27	8.2 ± 1.0
2003	40	120	28.1 ± 7.8 (0.45)	25	0.11	6.5 ± 0.7

[a] Depuration rate constants (k_d) 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 parenthesis). Day 173 of depuration was excluded in the calculations.

[b] Half-life was calculated from the equation $t_{1/2} = 0.693/k_d$.

[c] Biomagnification factor was calculated from the equation $BMF = \alpha \cdot F/k_d$, where α is the assimilation efficiency and F is the feeding rate on a lipid basis.

[d] Assimilation efficiency was calculated by fitting the data to the integrated form of the kinetic rate equation for dietary exposure using iterative nonlinear regression: $C_{fish} = (\alpha F C_{food}/k_d)[1-\exp(-k_d t)]$, where C_{fish} is the concentration in the fish (lipid basis), C_{food} is the concentration in the food (lipid basis), and t is the time (days) of uptake.

[e] Concentrations at day 173 were not used in calculation of bioaccumulation parameters.

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