DIETARY ACCUMULATION, SEDIMENT BIOAVAILABILITY AND TOXICITY OF POLYCHLORINATED *n*-ALKANES

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

DOCTORATE OF PHILOSPHY

Department of Soil Science
University of Manitoba
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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Fisk, Aaron Thomas. Ph.D., The University of Manitoba, March, 1998. Dietary Accumulation, Sediment Bioavailability and Toxicity of Polychlorinated *n*-Alkanes. Major Professor; Derek C. G. Muir. Polychlorinated *n*-alkanes (PCAs), also known as chlorinated paraffins, are a class of high molecular weight organochlorines (OCs) used as high pressure lubricants, flame retardants and a number of other industrial applications. Reviews of information available on PCAs have concluded that there is a lack of data to assess their fate and effects in the environment. This work has examined the dietary accumulation, sediment bioavailability and toxicity of a range of PCAs which vary in carbon chain length and chlorine content. PCAs (C_{10}-, C_{11}-, C_{12}-, C_{14}- and C_{16}) were accumulated from food by juvenile rainbow trout (*Oncorhynchus mykiss*). PCA half lives ($t_{1/2}$, range 7.1 - 87 d) and biomagnification factors (BMFs) (0.14 - 2.2) increased with carbon chain length and chlorine content. PCAs were biotransformed but the biotransformation rate was inversely related to chlorine content. Highly chlorinated (>60% Cl) short chain (C_{10-13}) and medium and lower chlorinated (<60% Cl) medium chain (C_{14-18}) PCAs appear to have the greatest potential for bioaccumulation. Dietary accumulation experiments using juvenile rainbow trout and 23 hydrophobic recalcitrant OCs revealed curvilinear relationships between K_{ow} and assimilation efficiency, $t_{1/2}$, and BMF, peaking at a log K_{ow} of approximately 7.0. Decreasing $t_{1/2}$ above log K_{ow} of 7 was unexpected and may have been due to an insufficient accumulation period (30 d). C_{12} (56 and 69% Cl)-

and C_{16} (35 and 69% Cl)-PCAs were accumulated from sediments by oligochaetes (*Lumbriculus variegatus*) suggesting that sediment-sorbed PCAs should be available to benthic biota. Half lives of the C_{12}-PCAs (11-13 d) were lower than C_{16}-PCAs (24-43 d). Biota sediment accumulation factors (BSAF) were > 1 for the C_{12}-PCAs and the lower chlorinated C_{16}-PCA (35% Cl), but < 1 for the high chlorinated C_{16}-PCA (69% Cl). Toxicity tests on 6 PCAs of a single carbon chain length and known chlorine content using Japanese medaka (*Oryzias latipes*) embryos and juvenile rainbow trout suggested that the toxic mode-of-action is narcosis. Some evidence of histopathological lesions in the liver were observed in rainbow trout exposed to high dietary concentrations of PCAs, suggesting that PCA may cause sub-lethal effects in fish at very high exposure concentrations.

For Melissa, your love and support made all of this possible.

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FOREWORD

This thesis has been written in a manuscript style as outlined in the *A Guide to Thesis Preparation for Graduate Students in the Department of Soil Science* (November 1996). Chapters 2 through 8 have been published, submitted or will be submitted for publication. Chapter 2 is part of a review paper to Rev. Environ. Contam. Toxicol. which has Gregg T. Tomy as the lead author. My contribution to this review paper were the sections Toxicokinetics, Toxicity, and Exposure and Risk Assessment, which make up a large percentage of my literature review chapter. Sections which I did not write in this paper were not included in this chapter.

Chapter 2: Tomy, G.T., A.T. Fisk, J.B. Westmore and D.C.G. Muir. 1997. Environmental chemistry and toxicology of polychlorinated *n*-alkanes. Rev. Environ. Contam. Toxicol. (*submitted Dec. 5/1997)*

Chapter 3: Fisk, A.T., C.D. Cymbalisty, A. Bergman and D.C.G. Muir. 1996. Dietary accumulation of C_{12}- and C_{16}-chlorinated alkanes by juvenile rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. (15: 1775-1782).

Chapter 4: Fisk, A.T., R.J. Norstrom, C.D. Cymbalisty and D.C.G. Muir. 1997. Dietary accumulation and depuration of hydrophobic organochlorines: Bioaccumulation parameters and their relationship with K_{ow}. Environ. Toxicol. Chem. (*accepted 12/9/97*).

Chapter 5: Fisk, A.T., C.D. Cymbalisty, G.T. Tomy and D.C.G. Muir. 1997. Dietary accumulation and depuration of C_{10}-, C_{11}- and C_{14}-polychlorinated alkanes by juvenile rainbow trout (*Oncorhynchus mykiss*). Aquat. Toxicol. (*accepted 10/11/1997*).

Chapter 6: Fisk, A.T., S.C. Wiens, G.R.B. Webster, A. Bergman and D.C.G. Muir. 1997. Accumulation and depuration of sediment-sorbed C_{12}- and C_{16}-polychlorinated alkanes by oligochaetes (*Lumbriculus variegatus*). Environ. Toxicol. Chem. (*accepted for publicatiosn with revisions January 4, 1998*).

Chapter 7: Fisk, A.T., G.T. Tomy and D.C.G. Muir. 1998. The Toxicity of C_{10}-, C_{11}-, C_{12}- and C_{14}-polychlorinated alkanes to Japanese medaka (*Oryzias latipes*) eggs. Environ. Toxicol. Chem. (*to be submitted*).

Chapter 8: Fisk, A.T., M. Cooley, S.C. Wiens, G.T. Tomy and D.C.G. Muir. 1998. Examination of the behavior and the liver, kidney and thyroid histology of juvenile rainbow trout (*Oncorhynchus mykiss*) exposed to high dietary concentrations of C_{10}-, C_{11}-, C_{12}- and C_{14}-polychlorinated *n*-alkanes. Journal Undecided. (*to be submitted*).

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1. INTRODUCTION

Polychlorinated *n*-alkanes (PCAs), also known as chlorinated paraffins, are one the last high molecular weight organochlorines (OCs) produced and used in North America and western Europe (Swedish National Chemicals Inspectorate 1991). These industrial compounds are used for a variety of purposes, including high pressure lubricants and flame retardants (Environment Canada 1993), and are currently being released into, and found in, the environment. For example, short chain (C_{10-13}) PCAs were among the most prominent OCs in aquatic and terrestrial biota from Sweden (Jansson et al. 1993) and sediment from mid-latitude and arctic lakes (Tomy et al. 1997a).

PCAs have physical chemical properties, e.g. low water solubility and vapor pressures, which are similar to other high molecular weight OCs, such as polychlorinated biphenyls and DDT, which bioaccumulate and have been demonstrated to have biological effects in wildlife. PCAs have been found in sediments of remote arctic lakes (Tomy et al. 1997a) and in ringed seals from the arctic (Jansson et al. 1993) which suggest that they are a global contaminant. Data also suggests that they are persistent in the environment. For example, PCAs are found in sediment core slices dating back to the 1940s and 50s (Tomy et al. 1997a).

Regulation of short chain (C_{10-13}) PCAs in Canada, or short chain chlorinated paraffins as they are referred to by Environment Canada, is currently under debate. In 1989, short chain (C_{10-13}) PCAs were placed on the first Priority Substances List (PSL 1)

(Canada Gazette, February 1989), and were later deemed toxic under the Canadian Environmental Protection Act (CEPA) section IIc (Government of Canada 1993). They also concluded that there were insufficient data on medium (C_{14-17}) and long (C_{18-30}) carbon chain PCAs to make a regulatory decision on these compounds at that time. In 1997, Environment Canada proposed short chain PCAs for management under Track 1 of the Toxic Substances Management Policy (Government of Canada 1997). Track 1 compounds are those that are toxic (as defined by CEPA) and considered persistent, bioaccumulative and enter the environment primarily as a result of human activity. Track 1 compounds must be virtually eliminated from the environment based on strategies to prevent the measurable release of the substance into the environment. In areas where no measurable release limits cannot be satisfied, generation or use of a substance will not be acceptable (Government of Canada 1995). In 1997, the Chlorinated Paraffins Industrial Association (CPIA) contested the placement of short chain PCAs on Track 1 and a decision on the regulatory fate of short chain PCAs is pending.

Regulation of PCAs has been a difficult process, mainly because there is a lack of good data on PCAs for virtually all aspects of environmental exposure and risk assessment. Information is lacking on the bioaccumulation, sediment bioavailability, persistence and toxicity of PCAs. Of the data that are available, a majority has been generated using industrial formulations, which consist of literally thousands of different compounds with a large range of physical-chemical properties and unknown additives (Tomy et al. 1998). Therefore, the general objective of this work was to generate data on the dietary accumulation, sediment bioavailability and toxicity for exposure and risk

assessment using a range of PCAs with single carbon chain lengths and known chlorine contents.

Specifically, the objectives of this work were:

1) To examine the dietary bioaccumulation (assimilation efficiency, half life, biomagnification factor) of a range of PCAs with single carbon chain lengths (C_{10}, C_{11}, C_{12}, C_{14} and C_{16}) and chlorine contents using juvenile rainbow trout (*Oncorhynchus mykiss*).
2) To develop relationships between dietary bioaccumulation parameters (assimilation efficiency, half life and biomagnification factor) and K_{ow} for a range of recalcitrant hydrophobic OCs which are known to bioaccumulate and biomagnify in aquatic food chains using juvenile rainbow trout. These relationships can then be used to assess the PCA dietary accumulation results.
3) To examine the bioavailability, and generate bioaccumulation parameters (uptake rate, half life, biota sediment accumulation factors), of sediment-sorbed PCAs using the oligochaete (*Lumbriculus variegatus*).
4) To examine the acute toxicity of a range of PCAs using the Japanese medaka embryo toxicity assay.
5) To examine the behavior and liver, thyroid and kidney histology of rainbow trout exposed to high concentrations of PCAs.

2. LITERATURE REVIEW

2.1 Introduction

Commercially produced polychlorinated n-alkanes (PCAs), also known as chlorinated paraffins (CPs), are used for a variety of industrial applications including lubricating additives, flame retardants, adhesives, sealants and a number of other miscellaneous applications (Windrath and Stevenson 1985; Government of Canada 1993; Willis et al. 1994). The carbon chain lengths of commercially produced PCAs range from 10 to 30, with chlorine contents of 35 to 70% by weight. PCAs are categorized as short (C_{10-13}), medium (C_{14-17}) or long (C_{18-30}) carbon chain compounds, and subcategorized by chlorine content (e.g. 45% Cl). PCAs have been produced since 1930 (Scheer 1994 in Mukherjee 1990), with current world consumption levels greater than 300 kilotonnes per annum (Government of Canada 1993).

PCAs are produced by chlorination of n-alkane feedstocks at elevated temperatures (50-150°C) and elevated pressures and/or in the presence of UV light (Zitko 1980). Due to this free radical chlorination method and the range of carbon chain lengths and chlorine contents, PCA formulations are very complex and consist of thousands of different compounds (Figure 2.1). For example, in one commercial PCA product (C_{10-13}, 60% Cl) there are 4200 theoretically possible congeners, assuming 1 chlorine per carbon, and not including enantiomers. Individual compounds cannot be resolved from these complex formulations using high resolution gas chromatography or liquid chromatography

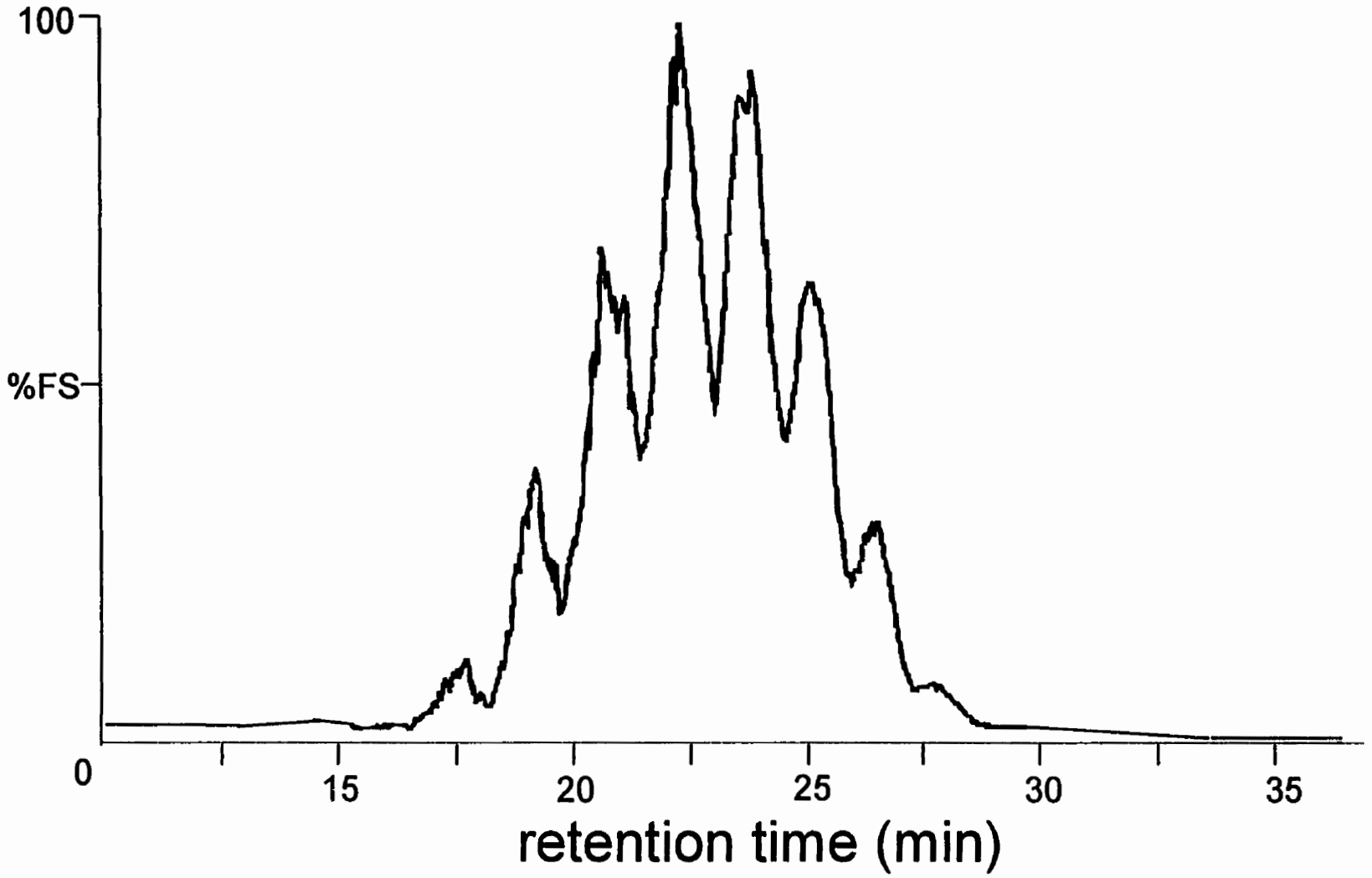

Figure 2.1 High resolution GC-electron capture negative ion high resolution MS total ion chromatogram of a commercial, short chain PCA (C_{10-13}, 60% Cl) formulation (from Tomy et al. 1998).

techniques (Tomy et al. 1993; Tomy 1997).

The release of PCAs into the environment could occur during (i) production, (ii) storage, (iii) transportation, (iv) industrial use and carry-off on manufactured products, (v) release from plastics, paints and sealants in which they are incorporated, (vi) leaching, runoff or volatilization from landfills, sewage sludge amended soils, or other waste disposal sites. Of these, however, the major releases are thought to be from production and from industrial usage (Government of Canada 1993; Swedish National Chemicals Inspectorate 1991, Environmental Protection Agency 1991). Of particular interest are the C_{10}-C_{13} PCAs, which have the greatest potential for environmental release (Government of Canada 1993), and the highest toxicity of PCA products (Willis et al. 1994, Mukerjee 1990, Serrone et al. 1987).

In Canada, PCAs are classified as Priority Toxic Substances under the Canadian Environmental Protection Act (CEPA). In the United States they have been placed on the Environmental Protection Agency (EPA) Toxic Release Inventory (TRI). Presently, PCAs represent the largest group of chlorinated hydrocarbons produced in Western Europe and in North America, and are among the last industrially produced high molecular weight organochlorine compounds (Swedish National Chemicals Inspectorate 1991).

2.2 Physical-chemical properties of PCAs

Physical-chemical property data of environmental pollutants are important parameters because they provide insight into fate of the pollutant in the environment and are essential to all environmental fate models. Unfortunately, a majority of the PCA physical-chemical property data have been produced for commercial CP products and not

individual PCA compounds. This presents problems because commercial CP products consist of thousands of individual compounds (Tomy 1997) with a large range of physical-chemical properties (Drouillard 1996). In general, PCAs have low water solubilities (WS), vapour pressures (VP) and Henry's law constants (HLC), and high K_{ow} and K_{oc} values (Table 2.1). These physical-chemical properties are similar to other organochlorine pollutants, such as polychlorinated biphenyls (PCBs), DDT, polychlorinated dibenzo-*p*-dioxins, and polychlorinated dibenzofurans (Mackay et al. 1991a; 1991b). Increasing carbon chain length and chlorine content has been associated with decreasing WS, VP and HLCs, and increasing K_{ow} and K_{oc} (Government of Canada 1993; Tomy et al. 1998).

2.3 Degradation of PCAs in the Environment

The rate at which a chemical is degraded upon entering the environment plays a key role in its fate and dynamics. A number of processes can degrade or transform a chemical in the environment, including photolysis, hydrolysis, oxidation and biodegradation.

2.3.1 Photolysis, Hydrolysis and Oxidation

Photolysis reactions occur by direct absorption of UV radiation, or indirectly by the transfer of energy from some other excited species to the chemical (sensitized photolysis) (Lyman et al. 1982; Schwarzenbach et al. 1993). Hydrolysis is a reaction in which a chemical reacts with hydroxide ions in water creating a new carbon-oxygen bond with concomitant loss of a functional group from the chemical (Lyman et al. 1992). Oxidations occur by the reaction of a chemical with oxygen, or by loss of hydrogen from the molecule.

Table 2.1 Selected physical chemical properties of PCAs. See Tomy et al. (1998) for a more complete list of PCA physical-chemical property data.

Physical-chemical property (units)	PCA	Value	Reference
water solubility ($mg \cdot L^{-1}$)	$C_{10}H_{18}Cl_4$	0.67	1
	$C_{11}H_{17}Cl_4$	0.58	1
	$C_{12}H_{20}Cl_6$	0.037	1
	$C_{14}H_{23}Cl_7$	0.014	2
	$C_{17}H_{27}Cl_9$	6.6×10^{-4}	2
	$C_{26}H_{44}Cl_{10}$	5.3×10^{-6}	2
vapor pressure (Pa)	$C_{10}H_{18}Cl_4$	0.066	1
	$C_{11}H_{17}Cl_4$	0.01	1
	$C_{12}H_{20}Cl_6$	0.00014-0.00052	1
	$C_{14}H_{23}Cl_7$	1.1×10^{-5}	2
	$C_{17}H_{27}Cl_9$	1.7×10^{-8}	2
	$C_{26}H_{44}Cl_{10}$	6.3×10^{-15}	2
Henry's Law Constant ($Pa \cdot m^{-3} \cdot mol^{-1}$)	$C_{10}H_{18}Cl_4$	17.7	1
	$C_{11}H_{17}Cl_4$	6.3	1
	$C_{12}H_{19}Cl_5$	1.4	1
	$C_{14}H_{23}Cl_7$	0.36	2
	$C_{17}H_{27}Cl_9$	0.01	2
	$C_{26}H_{44}Cl_{10}$	0.003	2
$\log K_{ow}$ [A]	$C_{10}H_{18}Cl_4$	5.9	3
	$C_{11}H_{17}Cl_4$	5.9	3
	$C_{12}H_{19}Cl_5$	6.4-6.8	3
	C_{14-17} (37-70% Cl)	6.8-9.0	3
	C_{18-26} (34-54% Cl)	8.7-12.7	3
$\log K_{oc}$ [B]	$C_{12}H_{20}Cl_6$	4.8-4.9	1

[A] K_{ow} is the *n*-octanol water partition coefficient.

[B] K_{oc} is the organic carbon water partition coefficient.

1 – Drouillard 1996

2 – Government of Canada 1993.

3 – Sijm and Sinnige 1995.

Direct photolysis of PCAs has been observed to be negligible (Friedman and Lombardo 1975), which is not surprising because PCAs are normally manufactured in the presence of UV light. It has been suggested that PCAs are vulnerable to indirect photolysis by oxidizing radicals in the troposphere (Government of Canada 1993). Based on Atkinson's OH radical reaction model, theoretical half-lives of PCAs in the atmosphere range from 1.2 to 1.8 days for C_{10}-C_{13} (no information given on Cl content), 0.85 to 1.1 days for C_{14}-C_{17} and 0.5 to 0.8 days for C_{18}-C_{30} (Atkinson 1986). Similarly, Willis et al. (1994) concluded that photochemical degradation rates would be inversely related to carbon chain length.

In the aqueous phase, rates of hydrolysis and oxidation of PCAs are considered negligible at ambient temperatures (Willis et al. 1994, Government of Canada 1993, Mukherjee 1990). However, catalysts, known to be present in the aquatic environment, might induce hydrolysis or oxidation reactions, although no studies have been done to demonstrate this.

2.3.2 Biodegradation

There are a few studies on the biodegradation (microbial transformation) of PCAs, the results of which suggest that biodegradation is inhibited by greater chlorine content and longer carbon chains. Hildebrecht (1972, as reported in Madeley and Birtley 1980) reported partial microbial degradation of two PCAs (C_{10}-C_{13} (60% Cl) and C_{20}-C_{30} (40% Cl)) in a biochemical oxygen demand (BOD) test. However, the results were confounded by the presence of a surfactant and unspecified nutrients. Zitko and Arsenault (1974) examined the aerobic and anaerobic biodegradation of two long carbon chain (C_{20}-C_{30}) PCAs (42 and

70% Cl) in a suspension of sea water and decomposing organic matter at room temperature (19-22°C). They found that the rate of biodegradation was higher under anaerobic than aerobic conditions, and that the higher chlorinated PCAs (70% Cl) were degraded to a greater extent than the lower chlorinated PCAs (42% Cl). TLC with infra-red spectrophotometry was used to measure the PCAs, but only trace amounts were detected (Zitko and Arsenault 1974). In the most extensive examination of the biodegradation of PCAs, Madeley and Birtley (1980) used BOD tests to examine the biodegradation of a range of PCAs with different carbon chain lengths and chlorine contents. They concluded that (i) acclimatized microorganisms showed a greater ability to degrade PCAs than organisms normally used for treating domestic sewage, (ii) increasing chlorination inhibited biodegradation, (iii) short carbon chain PCAs (< 60% Cl) appeared to be rapidly and completely degraded, and (iv) medium and long carbon chain PCAs with up to 45% Cl degraded more slowly than shorter carbon chain PCAs. No significant oxygen uptake was observed in tests using the highly chlorinated PCAs, which included two short carbon chain (60 and 70% Cl) PCAs and one medium carbon chain (58% Cl) PCA. Madeley and Birtley (1980) also examined the breakdown of a ^{14}C-labelled 42% Cl pentacosane (C_{25}) and found that after 8 weeks with nonacclimatized microorganisms, 11% of the original ^{14}C could be collected as CO_2. Omori et al. (1987) studied the PCA dechlorination potential of a series of soil bacterial strains. Although they could not isolate a bacterial strain which could use PCAs as a sole carbon source, they did find that different strains pretreated with *n*-hexadecane had different dechlorination abilities. A mixed culture (4 bacterial strains) released 15 to 57% of the Cl of five PCA products. Less Cl was released from products with longer carbon chain length and higher chlorine content. Activated sludge from a

sewage treatment plant, acclimated to *n*-hexadecane for 60 days, dechlorinated only 2% of a medium chain PCA ($C_{15.4}Cl_{5.6}$). Omori et al. (1987) concluded that PCAs with 2 or more Cl and adjacent Cl groups were less dechlorinated by bacterial strains.

2.4 Environmental Levels

The amount of data on levels of PCAs in the environment is limited due, at least until recently, to a lack of appropriate standards and analytical techniques. Extraction and cleanup techniques are similar to methods used for the determination of other persistent organochlorines (Tomy et al. 1998). Early analytical methods employed thin-layer chromatography (Hollies et al. 1979; Campbell and McConnell 1980) or neutron activation (Svanberg et al. 1978), which had obvious limitations. More recent methods have used low resolution mass spectrometry (MS) (Gjos and Gustavsen 1982; Muller and Schmid 1984; Murray et al. 1988; Jansson et al. 1991; Jansson et al. 1993; Junk and Meisch 1993; Rieger and Ballschmiter 1995; and Metcalfe-Smith et al. 1995). However, low resolution MS methods lack the specificity and sensitivity to measure trace amounts of PCAs because these methods monitor ions at nominal mass and there are interferences from PCBs, toxaphene and other high molecular weight organochlorines (Tomy et al. 1997b; Tomy et al. 1998). Recently, Tomy et al. (1997b) reported a method for high resolution MS that eliminates many of the problems associated with earlier methods.

Selected examples of published environmental concentrations of PCAs in various matrices are shown in Table 2.2. A more complete list is available in Tomy et al. (1998). PCA concentrations have been found to be similar to concentrations of other common

Table 2.2 Published environmental concentrations of PCAs.

Matrix (units)	PCA	Location	Concentration	Ref.
Air ($pg \cdot m^{-3}$)				
	C_{10-30}	manufacturing plant, FRG	30×10^9	1
	C_{10-13}, 60-70% Cl	southern Ontario, Can.	543	2
Water ($\mu g \cdot L^{-1}$)				
	C_{10-30}, 45-52% Cl	North Sea, UK	< 0.5	3
	C_{10-13}, 60% Cl	Sugar Creek, OH	0.20 – 0.30	4
	C_{14-17}, 52% Cl	"	0.16 – 0.24	4
	C_{20-30}, 42% Cl	"	0.35 – 0.62	4
	C_{14-17}, 52% Cl	St. Lawrence River, Can.	< 1	5
	C_{10-13}, 50-70% Cl	Red River, Selkirk, MB, Can.	0.02 – 0.05	2
Sediments ($\mu g \cdot kg^{-1}$, dry wt)				
	C_{10-30}, 45-52% Cl	North Sea, UK	< 0.0005 – 350	3
	C_{10-13}, 60% Cl	Sugar Creek, OH	8 – 11	4
	C_{14-17}, 52% Cl	"	<1.5 – 7.3	4
	C_{20-30}, 42% Cl	"	<1.5 – 8.2	4
	C_{14-17}, 52% Cl	St. Lawrence River, Can.	< 3500	5
	C_{10-13}, 50-70% Cl	Lake Winnipeg, MB, Can.	21 – 135	2
	C_{10-13}, 50-70% Cl	Hazen Lake, NWT, Can.	4 - 10	2
Aquatic organisms ($\mu g \cdot kg^{-1}$, wet wt)				
zebra mussel	C_{10-30}, 45-52% Cl	North Sea, UK	200	3
"	C_{10-13}, 60% Cl	Sugar Creek, OH	<7 – 280	4
"	C_{14-17}, 52% Cl	"	<7 – 170	4
"	C_{20-30}, 42% Cl	"	<7 – 180	4
"	C_{10-13}, 60-70% Cl	Detroit River, MI	1205	2
yellow perch (whole fish)	"	"	1148	2
beluga (blubber)	"	St. Lawrence River, Can.	370 - 1363	2
"	"	N.W. Greenland	106 - 253	2

Terrestrial organisms ($\mu g \cdot kg^{-1}$, wet wt)				
cormorant eggs	C_{10-30}, 45-52% Cl	Mochrum Loch, UK	100 - 2000	3
osprey (muscle)	C_{10-13}, 60% Cl	Sweden	530	6
rabbit (muscle)	"	"	2900	6
moose (muscle)	"	"	4400	6
Human & foodstuffs ($\mu g \cdot kg^{-1}$, wet wt)				
dairy products	C_{10-30}, 45-52% Cl	UK	300	3
human liver	"	UK	<50 - 1500	3
human adipose tissue	C_{14-18}, 52% Cl	SWZ	200	7
human breast milk	C_{10-13}, 52% Cl	Can	11 - 17	2

References:

1 - Mukherjee 1990
2 - Tomy 1997
3 - Campbell and McConnell 1980
4 - Murray et al. 1988
5 - Metcalfe-Smith et al. 1995
6 - Jansson et al. 1993
7 - Schmid and Muller 1985

organochlorines, such as PCBs in the Detroit River (Fisk et al. 1996) and PCBs in aquatic and terrestrial biota from Sweden (Jansson et al. 1993).

2.5 Bioaccumulation

Understanding the fate and dynamics of contaminants in the environment requires knowledge of their bioaccumulation and persistence in biota. Unfortunately, the amount of bioaccumulation data on PCAs is limited due, in part, to a lack of appropriate analytical techniques and standards. The hydrophobic nature of PCAs (log K_{ow}s > 5.0), and results from a limited number of studies, suggest that these compounds bioconcentrate in aquatic organisms and have the potential to biomagnify through aquatic food webs.

Bioconcentration (uptake from the dissolved phase in water) of PCAs has been found to vary with carbon chain length and chlorine content of PCAs and species of organism (Table 2.3). Bengtsson et al. (1979) found that the rate of uptake of commercial PCA products from water by fish increased with decreasing carbon chain length and chlorine content. The greater uptake rates of the short chain PCAs were probably due to their greater water solubility. Highly chlorinated short chain PCAs are predicted to have the greatest bioconcentration factor (BCF) because they are more hydrophobic and appear to be more resistant to biotransformation than lower chlorinated PCAs, and their accumulation does not appear to be hindered by large molecular size or high K_{ow} as observed for intermediate and long carbon chain PCAs (Zitko 1974). The high BCF of 140,000 for a highly chlorinated C_{12} PCA in mussels reported by Renberg et al. (1986), is significantly greater than that found for lower chlorinated, short chain PCAs, and for intermediate and long chain PCAs (Table 2.3). BCFs also vary among species of organism.

Table 2.3 Bioconcentration factors (BCF) of PCAs.

PCA	Organism	BCF_{wet}	Comments	Reference
C_{11} (58% Cl)	freshwater alga	1.5 - 7.6	-14 d BCF -short exposure, rapid cell growth, and changing water conc. probably significantly reduced BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	1
C_{11} (58% Cl)	marine alga	<1 - 3.5	-14 d BCF -short exposure, rapid cell growth, and changing water conc. probably significantly reduced BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	2
C_{11} (58% Cl)	common mussel	24,800 - 40,900	-147 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	3
C_{11} (58% Cl)	common mussel	5,785 - 25,292	-60 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	1
C_{11} (58% Cl)	rainbow trout	3,600 - 5,300	-168 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	4
C_{11} (58% Cl)	rainbow trout	1181 - 7816	-60 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	5
C_{12} (69% Cl)	common mussel	140,000	-28 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	6
C_{16} (35% Cl)	common mussel	6,920	-28 d BCF -conc. used for water may overestimate true bioavailable conc.	6

			reducing BCF	
C_{15} (52% Cl)	common mussel	429 - 2856	-60d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	2
C_{15} (52% Cl)	rainbow trout	45 - 67	-60 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	7
$C_{18\text{-}26}$ 43% Cl	rainbow trout	17.9 - 37.6	-60 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	8
$C_{18\text{-}26}$ 70% Cl	rainbow trout	5.7 - 53.8	-60 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	9
C_{25} (43% Cl)	common mussel	261 - 1158	-60 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF	10
C_{25} (70% Cl)	common mussel	223 - 341	-60 d BCF -conc. used for water may overestimate true bioavailable conc. reducing BCF -impurities in ^{14}C material	11

References:

1 - Thompson and Madeley 1983a
2 - Thompson and Madeley 1983b
3 - Madeley et al. 1983
4 - Madeley and Maddock 1983a
5 - Madeley and Maddock 1983b
6 - Renberg et al. 1986
7 - Madeley and Maddock 1983c
8 - Madeley and Maddock 1983d
9 - Madeley and Maddock 1983e

10 - Madeley and Thompson 1983c
11 - Madeley and Thompson 1983d

The BCF (wet weight concentrations) of a $^{14}C_{11}$ PCA (58% Cl) was approximately 7 times greater in mussels than in rainbow trout, a result probably due to greater biotransformation and/or lipid content in the trout. The BCF of the C_{11} PCA (58% Cl) in rainbow trout is well below what would be expected for a compound of this K_{ow} (log K_{ow} ~ 6.5) based on relationships between BCF and K_{ow} for persistent organochlorines (Veith et al. 1979; Mackay 1982). Thus, BCFs of PCAs cannot be readily estimated from K_{ow} values because of differential biotransformation of PCAs with varying carbon chain length and chlorine content.

Owing to their hydrophobic nature, PCAs should be bioaccumulated predominantly through food chain transfer as opposed to water (Thomann 1989). PCAs are readily accumulated from food by fish in laboratory experiments, although, as with bioconcentration, dietary accumulation is influenced by carbon chain length and chlorine content. Lombardo et al. (1975) found a concentration of a short chain PCA (60% Cl) of 1.1 $\mu g \cdot g^{-1}$ in juvenile rainbow trout which were fed a diet containing high levels (10 $\mu g \cdot g^{-1}$) of this PCA. Bengtsson and Ofstad (1982) found that the accumulation of two short chain PCA products with different Cl contents (49 and 71%) by a brackish water fish (bleak (*Alburnus alburnus*)) was similar, but much greater than that of a long chain PCA (C_{18}-C_{26}, 49% Cl). Zitko (1974) observed very low accumulation of two long carbon chain PCAs (40 and 70% Cl) by juvenile Atlantic salmon fed a diet which had high concentrations (100 $\mu g \cdot g^{-1}$) of the PCAs. To date, there has not been any effort to examine food chain transfer of PCAs in the environment, and laboratory results have not been confirmed.

There are limited data on the half lives for PCAs in biota. Bengtsson and Ofstad (1982) found rapid elimination of lower chlorinated PCA products (C_{10}-C_{13} and C_{18}-C_{26}, 49% Cl) but virtually no elimination of a higher chlorinated product (C_{10}-C_{13}, 70% Cl) despite a depuration period of 316 days. However, no half life ($t_{1/2}$) was reported, and only organic Cl and not PCAs was measured, with no effort to verify that the Cl originated from the original PCA products. The half lives of a ^{14}C-C_{11} PCA (59% Cl) were similar in the common mussel ($t_{1/2}$ = 9.2 - 19.8 d) (Madeley et al. 1983) and rainbow trout ($t_{1/2}$ = 9.9 – 23.9 d) (Madeley and Maddock 1983a), and were found to vary with water exposure concentrations.

2.6 Biotransformation and Enzyme Induction

A number of studies using ^{14}C labelled PCAs have found that PCAs are degraded to $^{14}CO_2$ by mammals, birds and fish; degradation was found to be influenced by the degree of chlorination, exposure method and species. Darnerud and Brandt (1982) reported that within 8 h of exposure in mice, 44 and 33% of a C_{16} PCA (34.1% Cl) was exhaled as CO_2 when administered intravenously (tail vein) or by gavage, respectively. In a similar study Darnerud (1984) found that the amount of CO_2 exhaled by mice exposed by gavage to C_{12} PCAs decreased with increasing chlorination. Within 6 h of exposure, 6.3, 25, 33 and 47% of the $C_{12}H_{16.2}Cl_{9.8}$, $C_{12}H_{20.1}Cl_{5.9}$, $C_{12}H_{25}Cl$ and $C_{12}H_{26}$ had been exhaled as CO_2, respectively. Japanese quail (*Coturinix coturnix*) exhaled 40 and 20% of $C_{16}H_{30.7}Cl_{3.3}$ and $C_{12}H_{20.1}Cl_{5.9}$ as CO_2, respectively, within 8 h of exposure by gavage (Bliessman et al. 1982). Fish do not appear to be as proficient at degrading PCAs to CO_2 as mice and quail, as only 6% of $C_{16}H_{30.7}Cl_{3.3}$, injected intra-arterially in carp (*Cyprinus*

carpio), was exhaled as CO_2 after 96 h (Darnerud et al. 1983).

Darnerud and Brandt (1982) and Bliessman et al. (1982) concluded that lower chlorinated PCAs are biotransformed to short fragments and utilised by intermediary metabolic routes in mice and quail, but that other metabolic pathways and routes of excretion probably also occur. Madeley and Birtley (1980) found that only 27-33% of a ^{14}C labelled C_{25} PCA (42% Cl) in rainbow trout was extractable by hexane 49 days after exposure *via* food, suggesting extensive biotransformation of this long chain PCA. In the same study, TLC analysis of the extracts of the rainbow trout suggested that there was splitting of the PCA molecule before all chlorine atoms had been removed. Using the same evidence, the authors concluded that biotransformation was not a significant elimination method for this PCA in the common mussel (*Mytilus edulis*). Darnerud and Brandt (1982) suggested that the metabolic pathway involved in PCA biotransformation may involve oxidation of the alkane, based on evidence that the tissue distribution of a ^{14}C labelled C_{16} PCA (34.1% Cl) in mice exposed *via* gavage was similar to that of a ^{14}C-palmitic acid with the same chain length and terminal labelling as the C_{16} PCA. This is supported by Omori et al. (1987), who concluded that bacterial strains used β-oxidation because they produced chlorinated fatty acids after exposure to PCAs. Darnerud (1984) examined the biodegradation of a series of $^{14}C_{12}$ PCAs (0, 1, 6 and 10 Cl) in rats with a series of cytochrome P450 monooxygenaze enzyme inducers (phenobarbital, 3-methylchlolanthrene and Aroclor 1254), inhibitors (piperonyl butoxide and metyxapone), and three PCA commercial products (2 short chain (49 and 70% Cl) and one medium chain (52% Cl)). The CYP450 inducers and PCAs did not cause a statistically significant change in the amount of CO_2 produced by the rats, although this does not rule out increased

biotransformation of the PCAs by CYP450. The CYP450 inhibitors, however, caused a statistically significant decrease in exhaled CO_2, with the degree of CO_2 exhalation by piperonyl butoxide positively correlated with the degree of PCA chlorination. Rats were found to extensively metabolize a higher chlorinated ^{14}C PCA (C_{16} 65% Cl), with a major portion of the radioactivity found as a PCA mercapturic acid metabolite (Åhlman *et al.* 1986). It appears, therefore, that lower chlorinated PCAs are mineralised in biota but higher chlorinated PCAs are more recalcitrant, although a percentage may be mineralised, and are biotransformed by oxidation and conjugation reactions.

A small amount of data suggests that PCAs can induce phase I enzymes (mixed-function-oxygenase enyzmes, e.g. CYP450) and phase II enzymes (conjugation reactions, e.g. mercapturic acid synthesis) in fish. Haux et al. (1982) found a statistically significant increase in benzo(*a*)pyrene hydroxylase activity in female flounder held in brackish water, after 27 days exposure to high concentrations of a C_{10}-C_{13}, (49% Cl) PCA (one gavage treatment at 1000 mg·kg-bw^{-1}), although the activity was only twice as high as in the control fish. No induction was observed in males held in brackish or sea water, or in females held in sea water, or any fish exposed to a C_{10}-C_{13}, (70% Cl) PCA. In addition, there was no increase in PNA-*O*-demethylase activity in any fish exposed to either PCA.

Increases in concentrations of CYP450 ($P450_{50}$ and $P450_{54}$ but not $P450_{55}$), a phase I enzyme, have been observed in rats exposed intraperitoneally for 4 days with 1000 mg·kg^{-1}·d^{-1} of two short chain PCAs (49 and 71% Cl) (Nilsen and Toftgard 1981). Total P450 was found to increase by 8-18% and 25-29% with the 49 and 71% Cl PCAs, respectively. Nilsen et al. (1981) examined the CYP450 induction potential of 5 PCA formulations in rats and found that the higher chlorinated short chain PCAs (59 and 71%

Cl) caused a statistically significant increase in P450 (nmol·mg microsomal protein^{-1}) and EROD metabolism after 4 days of gavage doses of 1,000 mg·kg^{-1}. At the same exposure concentrations, the lower chlorinated short chain (49% Cl), medium chain (50% Cl) and long chain (49% Cl) PCAs did not cause a statistically significant increase in P450 or EROD metabolism. Poon et al. (1995) observed no alterations in aniline hydroxylase (phase I enzyme) or EROD activity in male and female rats exposed to a C_{14-17} (52% Cl) PCA 363 and 419 mg·kg^{-1}-body weight·d^{-1}, respectively. Meijer et al. (1981) found increases in microsomal CYP450, epoxide-hydrolase and glutathione-S-transferase (phase II enzyme) activities in rats exposed intraperitoneally for 5 days to 1000 mg·kg^{-1}·d^{-1} of a C_{14}-C_{17} (58% Cl), C_{23} (70% Cl) and C_{10}-C_{23} (70% Cl) PCA, with the C_{10}-C_{23} (70% Cl) PCA causing the strongest inductions. None of these activities increased in rats exposed to a C_{22}-C_{26} (42% Cl) PCA at the same exposure concentrations. The authors concluded that the hydrolyase and transferase were not involved in the metabolism of the PCA, but were likely due to the induction of CYP450 enzymes. Poon et al. (1995) observed increases in UDP-glucuronosyltransferase (phase II enzyme) in male and female rats exposed to a C_{14-17} (52% Cl) PCA at doses of 363 and 419 mg·kg^{-1}-body weight·d^{-1}, respectively, but not at lower exposure concentrations. These female rats were also observed to have increases in aminopyrine *N*-demethylase activities (phase II enzyme).

Therefore, there is evidence that PCAs can cause an increase in enzyme activities, with short chain, highly chlorinated PCAs having the greatest induction potential. However, the high levels required for induction, and the use of industrial PCA formulations, suggests that impurities in the PCA mixture may play a role in these enzymatic inductions.

2.7 Sediment Bioavailability

The hydrophobic nature of PCAs suggests that a large fraction will be associated with suspended and bottom sediments in aquatic systems. Knowledge of the bioavailability of sediment-sorbed PCAs is important for understanding their fate, and exposure of benthic biota. To date, there has been no published data on the bioavailability of sediment-sorbed PCAs.

2.8 Toxicity

In comparison to other types of environmental data for PCAs, there is a fairly large data base on the toxicity of PCAs, which has been summarised in a number of recent risk assessment documents (World Health Organization 1996; Willis et al. 1994; Government of Canada 1993). Most toxicity data have been generated from experiments using commercial PCA products, which presents a number of problems. First, because PCA products consist of thousands of compounds, differences in the toxicity of individual components cannot easily be identified. Second, due to the "weathering" of these products in the environment, the relative abundance of individual PCA compounds to which an organism is exposed may vary from the original PCA product. Third, the lack of appropriate analytical techniques has resulted in uncertain estimates of exposure and body burdens. Finally, stabilisers, which are added to commercial PCA products, and impurities may cause false positives in toxicity tests. Although stabilisers and impurities may occur at very low concentrations in PCA products, the high exposure concentrations used in many of the PCA toxicity tests could result in significant concentrations of these compounds.

2.8.1 Microbial Toxicity

From the limited data available, it would appear that microorganisms are fairly resistant to the toxic effects of PCAs. Hildebrecht (1972, in Willis et al. 1994) concluded that four PCAs (C_{10}-C_{13} 59% Cl, C_{18}-C_{30} 43 and 70% Cl, and Exchlor 5C (composition unknown)) at concentrations between 1 and 200 $mg \cdot L^{-1}$ did not affect the oxygen utilisation by a sewage sludge bacteria (species not reported). Birtley et al. (1980) reported that there was no indication that PCAs (C_{10}-C_{13} 49% Cl, C_{14}-C_{17} 52% Cl, and C_{20}-C_{30} 42% Cl) were toxic to four strains of *Salmonella typhimurium* at concentrations as high as 2,500 $ug \cdot plate^{-1}$. Madeley et al. (1983, in Willis et al. 1994) found that a short chain PCA (58% Cl) caused significant inhibition (> 10%) of gas production by an anaerobic microorganism at concentrations of 3.2, 5.6 and 10% on digester volatile suspended solids. Effects were only observed for the first 3-4 days of the experiments and, by day 10, gas production had returned to normal levels.

2.8.2 Aquatic Toxicity

A small body of work on the toxicity of PCAs to aquatic organisms exists with the majority of work having been performed by the Brixham Laboratory (formerly of Imperial Chemical Industries, UK)(Table 2.4). Based on these data, PCAs have low acute and chronic toxicity to aquatic organisms. However, it should be stressed that most of the studies examined gross toxicological effects such as mortality and growth, and there has been very little, or no work done on sub-lethal effects such as histology, enzyme function, population dynamics or reproduction. Other investigators have since observed a number of sub-lethal effects of PCAs in mammals (Section 2.8.5). The toxicity of PCAs appears

Table 2.4 PCA toxicity statistics for aquatic biota.

Species	PCA formula	Statistic	Number	Comment	Reference
Plants					
freshwater alga	$C_{10\text{-}13}$ 58% Cl	10 d EC_{50} - 50% reduction in cell density	1310 $\mu g \cdot l^{-1}$	-EC_{50} conc. Exceed highest water conc. measured -^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	1
marine alga	$C_{10\text{-}13}$ 58% Cl	4 d EC_{50} - 50% reduction in cell density	42-56 $\mu g \cdot l^{-1}$	-no significant reduction in cell density after 10 days (highest water conc. 70 $\mu g \cdot l^{-1}$) -^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	2
Invertebrates					
Daphnia magna	$C_{10\text{-}13}$ 58% Cl	48 h EC_{50} 6 d LC_{50} NOEC	530 $\mu g \cdot l^{-1}$ 12 $\mu g \cdot l^{-1}$ 5.0 $\mu g \cdot l^{-1}$	-EC_{50} conc. Exceed highest water conc. Measured -^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	3
Daphnia magna	Chlorowax 45LV	48 h LC_{50} NOEC	120 $\mu g \cdot l^{-1}$ < 75 $\mu g \cdot l^{-1}$		4
marine shrimp	$C_{10\text{-}13}$ 58% Cl	96 h LC_{50} NOEC	14-16 $\mu g \cdot l^{-1}$ 7.3 $\mu g \cdot l^{-1}$	-^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	5
midges	$C_{10\text{-}13}$ 58% Cl	MATC	78 < MATC < 121 $\mu g \cdot l^{-1}$	-MATC based on no adult emergence in long term study at conc. of 121 and 394 $\mu g \cdot l^{-1}$ -^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	6
common mussel	$C_{10\text{-}13}$ 58% Cl	60 d LC_{50} NOEC	74 $\mu g \cdot l^{-1}$ < 13 $\mu g \cdot l^{-1}$	-NOEC based on observational data -^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	7
common mussel	$C_{10\text{-}13}$ 58% Cl	84 d EC_{50} NOEC	9.3 $\mu g \cdot l^{-1}$ 2.3 $\mu g \cdot l^{-1}$	-EC_{50} and NOEC based on growth rate (shell length and soft tissue)	8

				-^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	
	common mussel	C_{14-18} 52% Cl	60 d NOEC	220 <> 3800 $\mu g \cdot l^{-1}$	-no mortalities reported, only effect observed was qualitative at 3800 $\mu g \cdot l^{-1}$ (reduce filtering) -high exposure conc. was cloudy and probably above water solubility -^{14}C-C_{15} (52% Cl) used to estimate total conc. of PCAs	9
	common mussel	C_{18-26} 43% Cl	60 d NOEC	120 <> 2180 $\mu g \cdot l^{-1}$	-no mortalities reported, only effect observed was qualitative at 2180 $\mu g \cdot l^{-1}$ (reduce filtering) -high exposure conc. was cloudy and probably above water solubility -^{14}C-C_{25} (43% Cl) used to estimate total conc. of PCAs	10
	common mussel	C_{18-26} 70% Cl	60 dNOEC	460 <> 1330 $\mu g \cdot l^{-1}$	-no mortalities reported, only effect observed was qualitative at 1330 $\mu g \cdot l^{-1}$ (reduce filtering) -some deposition of PCA in high exposure conc. and probably above water solubility -^{14}C-C_{25} (70% Cl) used to estimate total conc. of PCAs -impurities in ^{14}C material	11
Fish						
	rainbow trout	C_{10-13} 58% Cl	growth effects	> 17.2 $\mu g \cdot l^{-1}$	-no significant mortality to fish populations -a marginal significant increase in growth for population exposed to the highest concentrations (17.2 $\mu \cdot l^{-1}$) -^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	12
	rainbow trout	C_{10-13} 58% Cl	60 d LC_{50} 1070 $\mu g \cdot l^{-1}$	340 $\mu g \cdot l^{-1}$ 31 d	-at all exposure conc. (350, 1070 and 3050 $\mu g \cdot l^{-1}$) fish populations developed symptoms of abnormal	13

		LT_{50}		behaviour -^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	
rainbow trout	C_{14-17} 52% Cl	60 d LC_{50}	>4500 $\mu g \cdot l^{-1}$	-no toxic effects were observed at any water conc. (1050 and 4500 $\mu g \cdot l^{-1}$) -water conc. probably exceeded water solubility -^{14}C-C_{15} (52% Cl) used to estimate total conc. of PCAs	14
rainbow trout	C_{18-26} 43% Cl	60 d LC_{50}	>4000 $\mu g \cdot l^{-1}$	-no toxic effects were observed at any water conc. (970 and 4000 $\mu g \cdot l^{-1}$) -water conc. probably exceeded water solubility -^{14}C-C_{25} (43% Cl) used to estimate total conc. of PCAs	15
rainbow trout	C_{18-26} 70% Cl	60 d LC_{50}	>3800 $\mu g \cdot l^{-1}$	-no toxic effects were observed at any water conc. (1000 and 3800 $\mu g \cdot l^{-1}$) -water conc. probably exceeded water solubility -^{14}C-C_{25} (70% Cl) used to estimate total conc. of PCAs	16
rainbow trout	Chlorowax 45 LV (C_{10-13})	96 h LC_{50} NOEC	>5000 $\mu g \cdot l^{-1}$ 600 $\mu g \cdot l^{-1}$	-water concentrations of PCAs were not measured -non-lethal effects (1200, 2500 and 5000 $\mu g \cdot l^{-1}$) include abnormal surfacing, loss of equilibrium and fish on the bottom of aquarium -surface film observed on aquarium water of PCA spiked treatments	17
bluegill sunfish	Chlorowax 45 LV	96 h LC_{50} NOEC	> 10000 $\mu g \cdot l^{-1}$ 5000 $\mu g \cdot l^{-1}$	-water concentrations of PCAs were not measured -non-lethal effects (1200, 2500 and 5000 $\mu g \cdot l^{-1}$) include abnormal surfacing, loss of equilibrium and fish on the bottom of aquarium -surface film observed on aquarium water of PCA spiked treatments	18

sheepshead minnow embryos and larvae	C_{10-13} 58% Cl	reduced growth NOEC	621 $\mu g \cdot l^{-1}$ 280 $\mu g \cdot l^{-1}$	-no significant effect on hatchability or survival at all but highest conc. (highest conc 621 $\mu g \cdot l^{-1}$) -significantly higher growth in larvae exposed to 36 and 71 $\mu g \cdot l^{-1}$ -^{14}C-C_{11} (58% Cl) used to estimate total conc. of PCAs	19
bleak	C_{10-13} 49% Cl	96 h LC_{50}	> 5 $g \cdot l^{-1}$	-water concentrations of PCAs were not measured -water conc. far exceed water solubility	20
"	C_{10-13} 56% Cl		> 10 $g \cdot l^{-1}$		
"	C_{10-13} 63% Cl		> 5 $g \cdot l^{-1}$		
"	C_{10-13} 70% Cl		> 10 $g \cdot l^{-1}$		
"	C_{10-13} 71% Cl		> 5 $g \cdot l^{-1}$		
"	C_{14-17} 40% Cl		> 5 $g \cdot l^{-1}$		
"	C_{14-17} 50% Cl		> 10 $g \cdot l^{-1}$		
"	C_{14-17} 52% Cl		> 5 $g \cdot l^{-1}$		
"	C_{22-26} 42% Cl		> 5 $g \cdot l^{-1}$		
bleak	C_{10-13} 49% Cl	OEC	~50 $\mu g \cdot g^{-1}$ body weight	-sluggish movements, absence of shoaling behavior and abnormal vertical postures (OEC effects) most pronounced in the C_{10-13} (49 and 59% Cl) exposed fish and less in the C_{10-13} (71% Cl) exposed fish -behavior became normal again after a few days in clean water -no behavior abnormalities were observed in the long chain PCAs	21
"	C_{10-13} 59% Cl	OEC	~50 $\mu g \cdot g^{-1}$ body weight		
"	C_{10-13} 71% Cl	OEC	~10 $\mu g \cdot g^{-1}$ body weight		
"	C_{14-17} 50% Cl	OEC	> ~1 $\mu g \cdot g^{-1}$ body weight		
"	C_{18-26} 49% Cl	OEC	> ~1 $\mu g \cdot g^{-1}$ body weight		
bleaks	C_{10-13} 49% Cl	OEC	~30 - 50 $\mu g \cdot g^{-1}$ body	-bleaks exposed via spiked food -sluggish swimming near the bottom of the	22

			weight	aquarium	
"	C_{10-13} 71% Cl	OEC	~70 $\mu g \cdot g^{-1}$ body weight	(OEC effect) -behavior became normal again after a few days in clean	
"	C_{18-26} 49% Cl	OEC		water -no behavior abnormalities were observed in the long chain PCAs	
bleaks	C_{10-13} 70% Cl	28d OEC	20 - 33 $\mu g \cdot g^{-1}$	-bleaks exposed via spiked food -sluggish swimming, abnormal orientation and tetanic spasms (OEC effect)	23

References:

1 - Thompson and Madeley 1983a
2 - Thompson and Madeley 1983b
3 - Thompson and Madeley 1983c
4 - Forbis et al. 1986
5 - Thompson and Madeley 1983d
6 - E and G Bionomics 1983
7 - Madeley and Thompson 1983a
8 - Thompson and Shillabeer 1983
9 - Madeley and Thompson 1983b
10 - Madeley and Thompson 1983c
11 - Madeley and Thompson 1983d
12 - Madeley and Maddock 1983f
13 - Madeley and Maddock 1983b
14 - Madeley and Maddock 1983c
15 - Madeley and Maddock 1983d
16 - Madeley and Maddock 1983e
17 - Swigert and Bowman 1986a
18 - Swigert and Bwoman 1986b
19 - Hill and Maddock 1983a and 1983b

20 - Linden et al. 1979
21 - Bengtsson et al. 1979
22 - Bengtsson et al. 1982
23 - Svanberg et al. 1978

to be inversely related to carbon chain length. However, because most of these studies have used water as an exposure route and body burdens were not measured, the relatively higher water solubility of the shorter carbon chain PCAs probably resulted in greater exposure for these PCAs.

A majority of PCA effects concentrations (e.g. lowest observable effects concentration (LOEC) or no observable effects (NOEC), and LC_{50}) for aquatic organisms are above 100 $\mu g \cdot L^{-1}$, and in many cases are above 1,000 $\mu g \cdot L^{-1}$. However, there are a number of exceptions with invertebrates (Table 2.4). A NOEC for a short chain PCA (C_{10}-C_{13}, 58% Cl) of 5.0 $\mu g \cdot L^{-1}$ in *Daphnia magna* was the lowest reported no effect concentration (Table 2.4). However, the researchers reported that many of the *D. magna* were found floating at the top of the aquarium and the toxic effects may have been a mechanical effect (*i.e.*, oil slick effect), and not a toxic effect. The same researchers reported a NOEC of 7.3 $\mu g \cdot L^{-1}$ for the same PCA using a marine shrimp, but none of the shrimp were observed to float at the surface. Other low effects concentration data in invertebrates are observational, such as reduced filtering rates in mussels, and should be interpreted with caution.

There is some evidence that high concentrations of PCAs, those approaching water solubility, may inhibit the growth of aquatic plants. Thompson and Madeley (1983a) reported significant inhibition of growth of freshwater alga (*Selenastrum capricornutum*) when exposed to a short chain PCA (58% Cl) at water concentrations of 570 $\mu g \cdot L^{-1}$. EC_{50}'s for cell density over 4, 7 and 10 days were reported as 3,690, 1,550 and 1,310 $\mu g \cdot L^{-1}$, respectively. Even at the lowest exposure used (110 $\mu g \cdot L^{-1}$), concentrations were probably greater than the water solubility of this PCA. Using the same PCA, Thompson

Table 2.5 PCA toxicity data for birds and mammals.

Species	PCA formula	Statistic	Number	Comment	Reference
Birds					
mallard duck	C_{10-13} 58% Cl	NOEC	166 ug·kg^{-1}	-ducks exposed via spiked food -some egg shell thinning in ducks exposed to 1 mg·g^{-1} -10% loss of viability in hatchlings exposed to 1 mg·g^{-1} for 14 days	1
ring-necked pheasant	C_{14-17} 52% Cl	oral LD_{50}	>10.3 mg·g^{-1} body weight	-neither oral dose or spiked food produced any mortality or toxicological effects -depressed food intake in mallard ducks exposed to 24 mg·g^{-1} in food	2
		dietary subacute LC_{50}	> 24 mg·g^{-1}		
mallard duck	C_{14-17} 52% Cl	oral LD_{50}	>24.6 mg·g^{-1} body weight		
		dietary subacute LC_{50}	> 24 mg·g^{-1}		
mammals					
rat	C_{10-13} 59% Cl	oral LD_{50}	> 29 mg·g^{-1}	-no mortalities observed in rat or guinea pigs after doses of 50 and 25 g·kg body weight^{-1}, respectively	3
rat	C_{10-13} 41-50% Cl C_{10-13} 51-60% Cl C_{10-13} 61-70% Cl C_{14-17} 51-60% Cl C_{20-30} 41-50% Cl C_{20-30} 51-60% Cl	oral LD_{50}	all >4 mg·g^{-1} (body weight)	-although authors report oral LD_{50} there was no mention of death, only non-specific toxicity (piloerection, muscular incoordination and urinary and fecal incontinence) -beagles feed the same diets showed no toxic effects (highest dose 10 mg·g^{-1} body weight)	4

	C_{20-30} 61-70% Cl C_{14-17} 52% Cl	90 d NOAEL	250 $\mu g \cdot g^{-1}$	-no deaths in any treatment over 90 days (highest conc. 5,000 $mg \cdot kg^{-1}$ body weight) -reduction in body weight at dose of => 500 $mg \cdot kg^{-1}$ body weight which may be due to reduce palatability -increased liver and kidney weight at exposures of 500 $mg \cdot kg^{-1}$ and greater	
	C_{14-17} 52% Cl	90 d NOAEL	30 $\mu g \cdot g^{-1}$ body $weight \cdot d^{-1}$	-no deaths in any treatment over 90 days (highest conc. 100 mg·kg b.w. $^{-1}$)	
rat	C_{14-17} 47% Cl	LD_{50}	26.1 $mg \cdot g^{-1}$ body weight		5
mice	C_{14-17} 47% Cl	LD_{50}	21.9 $mg \cdot g^{-1}$ body weight		
rat	C_{10-13} 58% Cl	90 d NOEL	10 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$	-diet or gavage exposure of C_{10-13} PCA produced similar toxicity statistics -NOEL based on increase in liver and kidney weight	6
	C_{14-17} 52% Cl	90 d NOEL	10 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$	-NOEL based on increase in liver and kidney weight	
	C_{20-30} 43% Cl	90 d NOEL	> 3750 (males), < 100 (females) $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$	-no effects observed in any male rat (highest dose 3750 mg·kg b.w. $^{-1} \cdot d^{-1}$) -female NOEL based on increase in liver and kidney weight	
	C_{20-30} 70% Cl	90 d NOEL	900 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$	-NOEL based on increase in liver and kidney weight and reduced weight gain	

rat	C_{10-12} 60% Cl			-no mortalities after single dose (highest doses 13,600 mg·kg b.w.$^{-1}$·d^{-1}) -1/5 and 2/5 male and female rats, respectively, died at exposure concentrations of 7,500 mg·kg b.w.$^{-1}$·d^{-1} in 16 day exposures and no deaths were reported in 13 week exposures (high conc. 5,000 mg·kg b.w.$^{-1}$·d^{-1}) -liver weights were increased in rats exposed at 938 mg·kg b.w.$^{-1}$·d^{-1} and greater in 16 d exposures, at 313 mg·kg b.w.$^{-1}$·d^{-1} and greater in 13 week exposures, at 312 mg·kg b.w.$^{-1}$·d^{-1} and greater in 2 year exposures -evidence of kidney nephrosis at concentrations of 5,000 mg/kg b.w./d in 13 week exposures	7
	C_{22-26} 43% Cl	16 d NOEC	> 3,750 mg·kg^{-1} body weight·d^{-1}	-no mortalities after single dose (highest doses 11,700 mg·kg b.w.$^{-1}$·d^{-1}) or in 16 d, 13 week or 2 year exposures (highest dose 3,750 mg·kg b.w.$^{-1}$·d^{-1}) -evidence of granulomatous inflammation in liver of female rats in 13 week study (300 and greater mg·kg b.w.$^{-1}$·d^{-1}) and 2 year study (312 mg·kg b.w.$^{-1}$·d^{-1} and greaeter)	
mice	C_{10-12} 60% Cl			-no mortalities after single dose (highest doses 23,400 mg·kg b.w.$^{-1}$·d^{-1}) -4/5 and 2/5 male and female mice, respectively, died at exposure concentrations of 1,875 mg·kg b.w.$^{-1}$·d^{-1}, all mice died at conc. of 3,750 mg·kg b.w.$^{-1}$·d^{-1} and greater in 16 day exposures, no mice died in 13 week exposures (high conc. 7,500 mg·kg b.w.$^{-1}$·d^{-1}) and 2 year studies (highest conc. 250 mg·kg b.w.$^{-1}$·d^{-1})	

				-liver weights were increased in mice exposed at 938 mg·kg b.w.$^{-1}$·d^{-1} and greater in 16 d exposures, at 500 mg·kg b.w.$^{-1}$·d^{-1} and greater in 13 week exposures, at 125 mg·kg b.w.$^{-1}$·d^{-1} and greater in 2 year exposures	
	C_{22-26} 43% Cl	16 d NOEC	> 7,500 mg·kg^{-1}·d^{-1}	-no mortalities after single dose (highest doses 23,400 mg·kg^{-1}·d^{-1}), in 16 d and 13 week exposures (highest dose 7,500 mg·kg b.w^{-1}·d^{-1}), or in 2-year study (highest dose 5,000 mg·kg b.w.$^{-1}$·d^{-1})	
rat	C_{14-17} 52% Cl	91 d NOEL	4.2 mg·kg^{-1} body wt·d^{-1}	-authors did not report a NOEL -NEOL based on histopathological changes in liver, increased urinary excretion of ascorbic acid and decreased hepatic vitamin A	8
rat	C_{10-13} 58% Cl		184 mg·kg^{-1}	-PCAs administered by gavage	9
	C_{10-13} 56% Cl	14 d NOEC	600 mg·kg^{-1}	-NOEC based on hepatic peroxisome proliferation	
	C_{14-17} 40% Cl		473 mg·kg^{-1}		
	C_{10-13} 58% Cl		74 mg·kg^{-1}	-PCAs administered by gavage	
	C_{10-13} 56% Cl	14 d NOEC	51 mg·kg^{-1}	-NOEC based increase in liver weight	
	C_{14-17} 40% Cl		31 mg·kg^{-1}		
mice	C_{10-13} 58% Cl		180 mg·kg^{-1}	-PCAs administered by gavage	
	C_{10-13} 56% Cl	14 d NOEC	120 mg·kg^{-1}	-NOEC based on hepatic peroxisome proliferation	
	C_{14-17} 40% Cl		252 mg·kg^{-1}		
	C_{10-13} 58% Cl		215 mg·kg^{-1}	-PCAs administered by gavage	
	C_{10-13} 56% Cl	14 d NOEC	70 mg·kg^{-1}	-NOEC based increase in liver weight	
	C_{14-17} 40% Cl		426 mg·kg^{-1}		

References:

1 - SDS Biotech 1984, as reported in Willis et al. 1994

2 - Madeley and Birtley 1980

3 - Howard et al. 1975

4 - Birtley et al. 1980

5 - Abasov (1970), as reported in Willis et al. 1994
6 - Serrone et al. 1987
7 - Bucher et al. 1987
8 - Poon et al. 1995
9 - Wyatt et al. 1983

and Madeley (1983b) found that a water concentration of 19.6 $\mu g \cdot L^{-1}$ caused significant growth inhibition in a marine alga (*Skeletonema costatum*). EC_{50}'s for cell density were calculated to be 42.3 and 55.6 $\mu g \cdot L^{-1}$ after 4 days exposures, and EC_{50} for growth rate was calculated to be 31.6 $\mu g \cdot L^{-1}$ after 2 days exposure. However, both cell density and growth rate returned to normal by day 10 of the exposure. The authors proposed that the observed effects were due to the effects of the PCA on the lag phase of the algae population growth curves. It was also suggested that lower concentrations of the PCA in the water by the end of the experiment may have contributed to recovery of cell densities and growth rates.

Fish appear to be quite resistant to PCAs, and in most cases, the reported effects concentrations and the water concentrations are well beyond the water solubility of the PCA (Table 2.4). Unfortunately, body burdens in these fish were not reported and therefore exposure is unknown. Due to the low water solubility of these chemicals, exposure may have been low, particularly with the intermediate and longer chain formulations. Madeley and Maddock (1983a) found no significant mortality or behaviour abnormalities during a long term exposure (169 days) of rainbow trout to short chain PCA (58%) at concentrations of 3.1 and 14.3 $\mu g \cdot L^{-1}$, or during the first 63 days of depuration (clean water). However, starting on day 63 of the depuration phase, rainbow trout, previously exposed to 14.3 $\mu g \cdot L^{-1}$ PCA, were reluctant to feed, and by day 70 all had died (8 fish total). Similar effects were observed in rainbow trout previously exposed to 3.1 $\mu g \cdot L^{-1}$, but only 5 of 8 fish died. Behaviour returned to normal in surviving fish, and no effects were observed in control or acetone control fish. Infectious disease was eliminated as a cause and the authors could find no explanations for the effects and deaths observed.

2.8.3 Avian and Mammalian Toxicity

There are only two published reports on the toxicity of PCAs to birds, and only gross toxicological effects were reported. Madeley and Birtley (1980) exposed mallard duck (*Anas platyrhynchos*) and ring-necked pheasant (*Phasanius colchius*) to a medium chain PCA (52% Cl) by gavage and via spiked food. Despite high concentrations (24 $mg \cdot g^{-1}$), no significant toxicological effects were observed in either species, although ducks showed "inferior" food intake at the highest concentration (24 $mg \cdot g^{-1}$). Mallard ducks were also used in a one generation reproduction study to assess the toxicity of a short chain PCA (58% Cl) (SDS Biotech 1984, in Willis et al. 1994). Ducks were exposed at nominal dietary concentrations of 0, 28, 166 and 1,000 $\mu g \cdot g^{-1}$. No abnormal survival, condition, body weight or food consumption were observed in the adult ducks. Some egg laid by ducks exposed at the highest concentration were noted to have thinner shells, however the authors described this as being of "questionable biological significance". Hatchlings were fed the same PCA spiked diets for 14 days. A 10% loss of viability was observed in the 1,000 $\mu g \cdot g^{-1}$ group.

Based on the limited data available, the acute and chronic toxicity of PCAs to mammals is low (Table 2.5). Although a number of studies determined LD_{50} concentrations, death was rarely observed, and most effects concentrations were based on sub-lethal effects. The most common effect observed in mammals exposed to PCAs was an increase in absolute and/or relative liver weight at very high doses of PCA. This effect appears to be inversely related to carbon chain length (Nilsen et al. 1981; Nilsen and Toftgard 1981; Bucher et al. 1987; Wyatt et al. 1993; Elcombe et al. 1994), but the relationship to chlorine content is not obvious. In many studies, an increase in liver weight

was the only toxicological effect observed (Table 2.5). Wyatt et al. (1993) attributed the increase in liver weights to CYP450 enzyme induction, peroxisomal proliferation, smooth endoplasmic reticulum proliferation, and increase in cell proliferation.

2.8.4 Reproductive and Embryo Toxicity and Teratogenicity

Only one study has examined the toxic effects of PCAs on reproduction. Rats were exposed to high concentrations (0, 100, 1000 and 6250 $mg \cdot kg^{-1}$ feed) of a medium chain PCA (C_{14-17}, 52% Cl) 28d prior to mating, during mating and 21 d postnatal (IRDC 1985, in World Health Organization 1996). Pups were also exposed to the same concentrations for 70 days following weaning. No effects were observed in the mating rats or in the pups prior to lactation day 7. However, no pups from the highest exposure group survived to weaning, and all pups from the mid to high exposures experienced decreased activity, laboured breathing, pale discoloration and/or blood around the orifices. LOELs, based on decreased pup weight, were set at 5.7 and 7.2 $mg \cdot kg\text{-}bw^{-1} \cdot d^{-1}$ for males and females, respectively.

The teratogenic potential of a short chain PCA (C_{10-13}, 58% Cl) was examined in pregnant rats and rabbits (IRDC 1982a and IRDC 1982b, in World Health Organization 1996). Female rats were exposed daily by gavage from days 6 through 19 of gestation at concentrations of 0, 100, 500 and 2000 $mg \cdot kg\text{-}bw^{-1} \cdot d^{-1}$. There was increased mortality (32%) and decreased body weight in dams exposed at the highest concentrations. Other clinical effects were observed at lower concentrations, including matting and staining of the genital fur, soft stool, decreased activity, oily fur and excessive salivation. Treatment at the highest concentration resulted in fetal malformations, an increase incidence of post-

implantation loss and fewer viable fetuses. The NOEL for teratogenic effects was set at 500 mg·kg-bw^{-1}·d^{-1}. The rabbits were exposed at lower concentrations (0, 10, 30 and 100 mg·kg-bw^{-1}·d^{-1}) on gestation days 6-27. No significant effects were observed in dams or fetuses. The NOAEL was set at 100 mg·kg-bw^{-1}·d^{-1}.

The teratogenic potential of a medium chain (C_{14-17}, 52% Cl) and two long chain (C_{22-26} 49 and 70% Cl) PCAs was examined in pregnant rats and rabbits (IRDC 1984a, IRDC 1983a, IRDC 1983b, IRDC 1984b, IRDC 1982c and IRDC 1983c, in World Health Organization 1996). Female rats were exposed by gavage to the three PCAs at concentrations of 0, 500, 200 and 5000 mg·kg-bw^{-1}·d^{-1} on gestation days 6-19. No changes in mortality, body weight or uterus weight were observed in any of the treatments and no adverse effects were found in the fetuses. Female rabbits were exposed at lower concentrations to the medium chain (0, 10, 30 and 100 mg·kg-bw^{-1}·d^{-1}) and higher chlorinated long chain (of 0, 100, 300 and 1000 mg·kg-bw^{-1}·d^{-1}) PCAs but the same concentration as the rats for the lower chlorinated long chain PCA. No effects on mortality, body weight or uterus weight were found for the dams and no adverse effects were observed in the fetuses. However, there was some congestion of the lobes of the lungs in dams exposed to the medium chain PCA, but it was not dose-related. Darnerud and Lundkvist (1987, in World Health Organization 1996) observed no effect on implanation and embryonic survival in pregnant mice exposed to a single intraperitoneal injection of 100 mg·kg-bw^{-1} of a medium chain PCA (C_{14-17} 70% Cl).

2.8.5 Mechanisms and Sub-lethal Indicators of Toxicity

The results of a number of studies using the brackish water fish the bleak, suggest that the mechanism of acute toxicity is narcosis. In these studies, bleak exposed to high levels of PCAs in food had sluggish movements, swam near the bottom of the aquarium, and had abnormal shoaling behaviour and positioning (Svanberg et al. 1978, Bengtsson et al. 1979). These responses are consistent with the early stages of narcosis (McKim et al. 1987), and the PCA concentrations in the fish (~0.2 $mol \cdot g^{-1}$) were about ten times less than that considered to elicit narcosis (McCarty et al. 1986). Furthermore, the extremely high PCA concentrations required to elicit toxic effects in invertebrates, fish and mammals provide additional evidence that the mode of acute toxic action for PCAs is non specific (i.e., narcosis).

There are, however, some data that suggest that PCAs have specific toxic actions, with evidence that the liver, kidney and thyroid are target organs (Serrone et al. 1987, Elcombe et al. 1994). Elcombe et al. (1994) concluded that PCAs act via three non genotoxic mechanisms: (i) hepatic peroxisome proliferation/cell proliferation, (ii) perturbation of the thyroid homeostasis, and (iii) a male rat specific protein mediated nephropathy.

Peroxisomal proliferation has also been observed in the liver of mammals exposed to high doses of PCAs. Peroxisomal fatty acid oxidation, which is a marker for peroxisomal proliferation, showed a significant increase in rats and mice exposed to two short chain PCAs (56 and 58% Cl) and one medium chain PCA (40% Cl) (Wyatt et al. 1993) (Table 2.5). The short chain PCAs elicited a greater response in fatty acid oxidase activity than the medium chain PCAs in both rats and mice. Rats exposed to the highest

dose (1,000 mg·kg^{-1}·d^{-1}) of the short chain PCAs (58% Cl) had an almost 3 fold increase in fatty acid oxidase activity. Fatty acid oxidase activity in the mice was even greater, with statistically significant increases at doses of 250 mg·kg^{-1}·d^{-1} for the 58 and 56% Cl PCAs. The authors suggested that the differences in fatty acid oxidase activity in the two species might be due to differing rates of metabolism of the PCAs to proximate peroxisome proliferating metabolites. Mice exposed to the highest dose (1,000 mg·kg^{-1}·d^{-1}) of the 58 and 56% Cl PCAs had a 7 and 10 fold increase in fatty acid oxidase activity, respectively. In a separate experiment using the same short chain PCAs, Elcombe et al. (1994) confirmed peroxisome proliferation in rats exposed to PCAs by microscopic examination and morphometric analysis of liver samples. Male guinea pigs showed no treatment related changes in peroxisome proliferation when exposed to similar concentrations of two short carbon chain PCAs; however, guinea pigs are known to be insensitive to the effects of peroxisomal proliferating agents.

Female rats exposed to a long chain PCA (43% Cl) at concentrations of 300 and 900 mg·kg-bw^{-1}·d^{-1} were found to have granulomatous inflammation in their livers after 13 weeks exposure (Bucher et al. 1987). Granulomatous inflammation of the liver, which was characterised by multiple, randomly distributed accumulations of histiocytes within the liver sinusoids, was also observed in male and female rats exposed for 2 years to the same long chain PCA (exposure conc. 1 to 3,750 mg·kg^{-1}-body weight·d^{-1}) but was not observed in mice (highest conc. 5,000 mg·kg^{-1}-body weight·d^{-1}), or in rats or mice exposed to a short chain PCA (60% Cl) at concentrations of 625 and 250 mg·kg^{-1}·body weight·d^{-1}, respectively. However, hepatocyte hypertrophy and significantly fewer hepatocytes counted per given microscopic field were observed in livers of rats exposed for 2 years to

the short chain PCA at a concentration of 625 mg·kg-bw^{-1}·d^{-1}. Poon et al. (1995) observed slight histopathological changes in the liver of rats exposed to a medium chain PCA (52% Cl) for 13 weeks at 500 and 5,000 mg·kg^{-1} in their diet. These changes included minimal to mild anisokaryosis and vesiculation of the nuclei, increased perivenous homogeneity, and single cell necrosis in the hepatic lobes.

Bucher et al. (1987) reported a dose-related increase in absolute and relative kidney weights after 6 and 12 month exposure to a short chain PCA (60% Cl). Incidence and severity of lesions of the kidney tubules and of interstitial inflammation were also dose related. There was microscopic evidence of nephrosis in the kidney of male and female rats exposed to 5,000 mg·kg^{-1}·d^{-1} of a long chain PCA (43% Cl). No effects of these PCAs on the kidney of mice were noted. Poon et al. (1995) noted cytoplasmic inclusions resembling hyaline droplets and cytoplasmic shedding in the outer cortex tubules in a dose-related fashion in kidneys of male rats exposed to a medium chain PCA (52% Cl). Female rats had dose-related focal dilation of the tubules of the inner medulla with focal pyknosis of epithelial nuclei of the kidney. With both sexes, the changes were minimal to mild in nature.

Wyatt et al. (1993) also examined the effects of two short chain PCAs (56 and 58% Cl) and one medium chain PCA (40% Cl) on the thyroid of rats exposed at high concentrations (1,000 mg·kg^{-1}-body weight). All three PCAs caused a two-fold increase in liver uridine diphosphate glucuronosyl (UDPG) transferase activity and levels of plasma thyroid stimulating hormone (TSH), and a 30-40% decrease in total and free T_4. No changes in T_3 levels were found. Elcombe et al. (1994) observed similar changes in UDPG transferase activity and TSH and T_4 levels in male and female rats exposed to similar

concentrations of a short chain PCA (58% Cl). Thyroid follicullar cell hypertrophy and replicative DNA synthesis in thyroid cells were also noted at days 56 and 91, respectively, in rats exposed at concentrations of 313 $mg \cdot kg^{-1} \cdot d^{-1}$ and above. The UDPG transferase, which is produced in the liver, decreases the plasma T_4 levels and causes an increase in TSH release by the pituitary. TSH causes the thyroid to produce and release T_4. This continued stimulation and release of T_4 leads to hypertrophy and hyperplasia in the thyroid, and consequently a tendency to develop thyroid neoplasia (Wyatt et al. 1993). Poon et al. (1995) found dose-related morphological changes in the thyroid glands of rats exposed to a medium chain PCA (53% Cl) beginning at concentrations of 4.2 $mg \cdot kg\text{-}bw^{-1} \cdot d^{-1}$. These changes, which were minimal to mild, affected both the architecture of the thyroid follicles (reduced follicle sizes and collapsed angularity) and the epithelium of the thyroid (increased cell height, cytoplasmic vacuolation and nuclear vesiculation).

Besides effects observed in the liver, kidney and thyroid, Poon et al. (1995) also observed a number of other effects in rats exposed to a medium chain PCA (52% Cl). These included: increases in serum cholesterol in females starting at 4.2 $mg \cdot kg\text{-}bw^{-1} \cdot d^{-1}$; decreases in corpuscular volume in female rats at 419 $mg \cdot kg\text{-}bw^{-1} \cdot d^{-1}$; increases in N-acetylglucosaminidase (urinary enzyme) and ascorbic acid levels in female rats exposed to 419 $mg \cdot kg\text{-}bw^{-1} \cdot d^{-1}$; and decreases in vitamin A in the liver of male and female rats exposed to 363 and 42 $mg \cdot kg\text{-}bw^{-1} \cdot d^{-1}$, respectively.

Eriksson and Kihlstrom (1985) examined motor performance and thermoregulation in mice exposed to high concentrations (30 to 300 $mg \cdot kg\text{-}bw^{-1}$) of two short chain PCAs (49 and 70% Cl) via intravenous injection of the tail vein. There was a statistically significant decrease in motor performance in mice 15 minutes after exposure to

the highest concentration (300 mg·kg-bw^{-1}) of the lower chlorinated PCA. A statistically significant decrease in rectal temperature was observed at the highest concentration (300 mg·kg-bw^{-1}) for both PCAs 60 minutes after exposure. Decreasing motor capacity and rectal temperature with increasing dose was observed for both PCAs.

Kato and Kenne (1996) found that short (50 and 60% Cl) and intermediate (45 and 52% Cl) carbon chain length PCAs are potent inhibitors of gap junction intercellular communication in rat epithelial cells and may act as tumour promoters. Short carbon chain PCAs were more potent inhibitors (complete inhibition at 15-20 μg·mL^{-1}) than the intermediate carbon chain length PCAs (complete inhibition at 35-80 μg·mL^{-1}), and long carbon chain length PCAs (42 and 48% Cl) did not cause any inhibition despite *in vitro* concentrations as high as 200 μg·mL^{-1}.

2.8.6 Mutagenicity and Carcinogenicity

Meijer et al. (1981) used the Ames test to examine the mutagenic and carcinogenic potential of a C_{10}-C_{23} (70% Cl) PCA on three different strains of *Salmonella typhimurium*. No toxic effects were observed. The authors did not report the concentrations used in the test.

Bucher et al. (1987) examined the carcinogenicity of two PCA commercial products (C_{10}-C_{13} 60% Cl and C_{22}-C_{26} 43% Cl), and concluded that "the short chain, heavily chlorinated paraffin appears to have a greater potential for chronic toxicity and carcinogenicity than the longer chain, lightly chlorinated paraffin". The authors based this statement on the results of a repeated-dose, 2 year gavage study, using mice and rats with high exposure concentrations (1 to 5,000 mg·kg^{-1}·d^{-1}). Female rats exposed to the long

chain PCA (43% Cl) for 2 years showed slight increases in adrenal medullary pheochromocytomas at exposure concentrations of 100 to 900 mg·kg-bw^{-1}·d^{-1}. The long chain PCA also caused a marginal increase in heptacellular neoplasms in female mice at 5000 mg·kg-bw^{-1}·d^{-1}, and distinct increases in malignant lymphomas in male mice. Carcinogenicity was observed in rats and mice exposed to the short chain PCA (60% Cl) at much lower doses (312 and 625 mg·kg-bw^{-1}·d^{-1} in rats and 125 and 250 mg·kg-bw^{-1}·d^{-1} in mice) than the long chain PCA. The short chain PCA caused hepatocellular neoplasms in both sexes of rats and mice, kidney tubular cell adenomas and adenocarcinomas and mononuclear cell leukemia in male rats, and thyroid follicular cell neoplasms in female rats and mice.

3. DIETARY ACCUMULATION OF C_{12}- AND C_{16}-POLYCHLORINATED ALKANES BY JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

3.1 Abstract

Dietary exposures, using juvenile rainbow trout (*Oncorhynchus mykiss*), were conducted with four ^{14}C-polychlorinated alkanes (PCAs) ($C_{12}H_{20}Cl_6$ (56% Cl by weight), $C_{12}H_{16}Cl_{10}$ (69% Cl), $C_{16}H_{31}Cl_3$ (35% Cl) and $C_{16}H_{21}Cl_{13}$ (69% Cl)) in order to measure bioaccumulation parameters, metabolism and tissue distributions. These PCAs are found in industrial chlorinated paraffin (CP) products, although their method of synthesis is different from those for CPs. Trout were exposed for 40 days to nominal concentrations of 20 and 200 ng·g^{-1} of each PCA, as well as 2,000 ng·g^{-1} for $C_{16}H_{21}Cl_{13}$, followed by up to a 173 day elimination period. Whole body half lives in the rainbow trout ranged from 37 ± 2 days for $C_{16}H_{31}Cl_3$ to 87 ± 11 days for $C_{12}H_{16}Cl_{10}$, and assimilation efficiencies of $C_{16}H_{31}Cl_3$ (33 to 35%) and $C_{12}H_{16}Cl_{10}$ (34 to 38%) were highest among the four PCAs. Biomagnification factors ranged from 0.44 for $C_{16}H_{21}Cl_{13}$ to 2.15 for $C_{12}H_{16}Cl_{10}$. Accumulation of $C_{16}H_{21}Cl_{13}$ (MW = 674) may be sterically hindered due to its large molecular size. Low chlorinated PCAs, e.g. $C_{16}H_{31}Cl_3$, had shorter half lives than higher chlorinated PCAs, probably due to increased metabolism. HPLC-^{14}C analysis of fish tissue extracts revealed that the PCA mixtures were selectively biotransformed with certain unknown components persisting in tissues. Lower chlorinated PCAs had greater proportions of polar ^{14}C, which implies greater metabolism of these compounds. Highly chlorinated, short carbon chain

(C_{10-13}) PCAs and lower chlorinated, medium carbon chain (C_{14-18}) PCAs appear to have the greatest potential for biomagnification among CP components. No reduced growth rates or hepatic monoxygenase enzyme induction were seen in any of the PCA exposures when compared with controls.

3.2 Introduction

Chlorinated paraffins (CPs) are a class of polychlorinated alkanes (PCAs), that are used as plasticizers, flame retardants, high pressure lubricants and in a number of other industrial applications (Government of Canada 1993; Campbell and McConnell 1980). 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) (Darnerud et al. 1989) 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 (Jansson *et al.* 1993).

Bioaccumulation data are needed for a complete ecological risk assessment (Franke et al. 1994); however, this type of data for CPs is quite limited. Bengtsson and Ofstad (1982) exposed bleaks (*Alburnus alburnus*) to three formulations of CPs differing in carbon chain length and degree of chlorination, and found that they had different uptake and elimination rates. Further, Bengtsson et al. (1979) 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 (Zitko 1974; Lombardo et al.

1975; Bengtsson et al. 1979). 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 PCAs of known carbon chain length or Cl content.

PCAs have low water solubilities (Drouillard et al. 1995) and high K_{ow}s (Sijm and Sinnige 1995) that vary with carbon chain length and chlorine content. Chemicals with similar physical-chemical properties to PCAs, such as PCBs (Svanberg et al. 1978), have been found to bioaccumulate (Oliver and Niimi 1988), and biomagnify (Rasmussen et al. 1990; Evans et al. 1991), in aquatic food chains. The main objective of this study was the determination of bioaccumulation parameters (depuration rate, half life, biomagnification factor (BMF) and assimilation efficiency) of four ^{14}C-PCAs in juvenile rainbow trout (*Onchorhynchus mykiss*) through dietary exposure. The four PCAs used include two 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} ($C_{16}H_{31}Cl_3$ (35% Cl) and $C_{16}H_{21}Cl_{13}$ (69% Cl)) compounds, and are found in CP commercial products. We hypothesize that, like PCBs (McFarland and Clarke 1989) and chlorinated dioxins and furans (PCDD/Fs) (Opperhuizen and Sijm 1990), certain PCAs may be more persistent as a result of the number of Cl and the length of the carbon chain. These results provide the first bioaccumulation parameters for PCAs of known carbon chain length and percentage Cl.

A second objective of this work was to evaluate the toxicity of PCAs by monitoring growth rates, condition and histopathology of the rainbow trout, and to measure CYP1A1 mixed function oxygenase enzyme activity by measuring ethoxyresorufin-*O*-deethylase (EROD) activity in liver. There is one report of elevated EROD levels in female flounder (*Platichthys flesus*) exposed to high dietary concentrations of an industrial CP product (Haux et al. 1982).

3.3. Materials and Methods

3.3.1 Chemicals and Food Preparation

All ^{14}C-PCAs were synthesized and purified using techniques outlined previously (Bergman et al. 1981). The [1-^{14}C] dodecanes contained 55.9 and 68.5% chlorine (mean of 5.9 and 9.8 chlorine atoms per molecule, respectively). The [1-^{14}C] hexadecane had 34.1% chlorine (3.3 chlorine atoms per molecule), and the [U-^{14}C] hexadecane had 69% chlorine (13.4 chlorines per molecule). For simplicity, the number of chlorine atoms in each compound have been rounded to the nearest integer.

Food was spiked by suspending a known quantity of each PCA standard in 150 mL of hexane and 100 g of commercial fish food (Martin's Feed Mills Ltd., Elmira, ON, Canada) and slowly evaporating to dryness. Food was air-dried for 24 hours and stored at 10°C. The fish food consisted of 41% protein, 14% lipid and 3% fiber. Concentrations in the food were determined by the same analytical techniques used to determine levels in the rainbow trout tissue (*see below*), and are found in Table 3.1. Control food was treated in an identical manner, but without the addition of a PCA compound.

3.3.2 Experiment

Juvenile rainbow trout (*Oncorhynchus mykiss*)(initial weights 2 - 7 g) were exposed to the spiked food for 40 days followed by 160 ($C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$ and $C_{16}H_{31}Cl_3$) to 173 days ($C_{16}H_{21}Cl_{13}$) of depuration. The daily rate of feeding was equal to 1.5% of the mean weight of the rainbow trout, corrected after each sampling period. Fifty fish were used in control tank 1 and the three $C_{16}H_{21}Cl_{13}$ treatments, and 36 fish were used in the remaining treatments. Three fish were sampled from each treatment for ^{14}C determination on days 5, 10, 20, 30 and 40 of

the uptake period, and days 5, 10, 20, 40, 80 and 160 (or 173 for $C_{16}H_{21}Cl_{13}$ treatments) of the depuration period. Six additional fish in the $C_{16}H_{21}Cl_{13}$ high concentration treatment were exposed to the PCA-spiked food for 80 days, to follow the uptake for an extended 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). Each tissue (including the carcass) was weighed and analyzed separately for ^{14}C-radioactivity. At day 40 of uptake, three additional fish were sacrificed for EROD measurements from each of the high concentration treatments for each PCA, and from the two control treatments. Liver samples were weighed (0.05-0.3 g) and homogenised in 0.5 to 2.0 ml HEPES-KCl (0.02 M HEPES, 0.15 M KCl, pH 7.5), depending on sample size, and homogenates were centrifuged for 20 min (15,600 X *g*). All preparative steps were done in a coldroom at 2 °C. The supernatants were frozen at -80°C until analysed.

3.3.3 ^{14}C Analysis

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

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

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

the C_{16}-PCA samples a mobile phase of 90% acetontrile and 10% water was used, and 4 minute fractions over 80 minutes were collected. Fractions were counted using LSC.

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

3.3.4 MFO assays

Analysis of liver samples for MO enzyme activity was carried out with post mitochondrial supernatants as described previously (Muir et al. 1990). The small size of the livers precluded preparation of microsomal fractions. EROD activity was measured using the method of Pohl and Fouts (Pohl and Fouts 1980) with several modifications (Muir et al. 1990). The reaction was started by the addition of 10 µL of ethoxyresorufin in dimethylsulphoxide (0.04 $mg \cdot mL^{-1}$). The samples were incubated for precisely 2 min in a water bath at 25°C and then the reaction was stopped by addition of 2.5 mL of methanol. The samples were centrifuged at 24,000 X *g* to pellet the precipitated protein, and the amount of resorufin in the supernatant was determined spectrofluorometrically using an excitation wavelength of 530 nm and an emission wavelength of 585 nm. Protein was determined using the Lowry method as modified by Markwell et al. (1981).

3.3.5 Data analysis

Growth rates were determined by fitting all fish and liver weight data to an exponential model (ln fish weight = $a + b$ time (days); where a is a constant and b is the growth rate) (Muir et al. 1990). PCA concentrations were corrected for growth dilution and lipid normalized for all bioaccumulation parameters. Assimilation efficiencies (α) were calculated by fitting the concentration data to the integrated form of the kinetic rate equation for constant dietary exposure (Bruggeman et al. 1984) using iterative nonlinear regression:

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

where F is the feeding rate (lipid corrected), C_{fish} is the concentration in the fish (lipid basis and growth corrected), C_{food} is the concentration in the food (on a lipid basis), and t is the time of uptake (days). Feeding rate (F) is assumed to be 1.5% of the body weight of the fish, corrected for the lipid percentage of the food (14% - determined in the same manner as the lipid percentage in the fish) and the fish. Depuration rates (k_d) were calculated by fitting the depuration phase data to a first-order decay curve (ln conc. = $a + b$ time (days); where a is a constant and b is the depuration rate). Equilibrium biomagnification factor (BMF) was predicted from the equation $BMF = \alpha \cdot F/k_d$. Differences between growth rate constants among treatments, and depuration rates among treatments, were examined by testing the homogeneity of slopes in an analysis of covariance. The Student t test was used to compare pairs of elimination rate and growth rate constants at the $p < 0.05$ level of significance.

3.4 Results

3.4.1 Effects

The growth rates of both $C_{12}H_{16}Cl_{10}$ treatments, the low concentration $C_{12}H_{20}Cl_6$ treatment, and the low concentration $C_{16}H_{31}Cl_3$ treatment, were found to be significantly higher than the first control group, the high concentration $C_{16}H_{31}Cl_3$ treatment and the low and medium concentration C_{16} treatments (t-test, $p < 0.05$) (Table 3.1). Based on the growth rates, it is unlikely that the PCAs had any negative effect on the growth of juvenile rainbow trout. The same pattern holds for the liver growth rates, with the controls having the slowest growth rates (Table 3.1). A fin rot disease spread through the control 2, low concentration $C_{12}H_{20}Cl_6$ and both $C_{16}H_{31}Cl_3$ treatment tanks, causing a number of mortalities. However, none of the infected fish were used for data analysis, and since there is no pattern to the mortalities with respect to treatment, and the affected tanks were side by side, it is unlikely that the disease was a result of the PCA exposures. Liver somatic index (LSI = [liver weight/whole fish weight * 100]) and lipid percentages did not vary between treatments (Table 3.1), although the percentage lipid increased throughout the experiment.

EROD levels in PCA exposed rainbow trout were not higher than non-exposed rainbow trout on the last collection day (day 40) of uptake, corresponding to wet weight liver concentrations ($ng \cdot g^{-1}$) on day 40 of: 16.2 ± 1.0 (mean ± 1 S.E.) for $C_{12}H_{20}Cl_6$; 23.4 ± 0.3 for $C_{12}H_{16}Cl_{10}$; 27.9 ± 3.1 for $C_{16}H_{31}Cl_3$; and 75.6 ± 5.0 for $C_{16}H_{21}Cl_{13}$.

3.4.2 Bioaccumulation parameters

Accumulation of all four PCAs from food by juvenile rainbow trout was observed (Figure 3.1) by day 5 of the uptake phase. None of the four compounds reached steady state

Table 3.1 Growth parameters (mean ± 1 standard error) of juvenile rainbow trout exposed to four ^{14}C-PCA compounds. Significant differences (t-test, $p < 0.05$) in body and liver growth rates for all treatments are indicated by italics.

Chemical	Conc. in food ($ng \cdot g^{-1}$)	Uptake period (days)	depurat. period (days)	Growth rates[x] body ($day^{-1} \times 10^{-3}$)	Growth rates[x] liver ($day^{-1} \times 10^{-3}$)	% lipid[y]	LSI[z] %	% mort.
control 1	-	40	173	10.4 ± 1.1 (0.74)^{d}	8.6 ± 1.4 (0.56)^{e}	6.1 ± 0.4	1.47 ± 0.35	0
control 2	-	40	80	10.4 ± 2.5 (0.39)^{bd}	7.9 ± 2.9 (0.22)^{be}	6.4 ± 0.3	1.37 ± 0.21	15.8
$C_{12}H_{20}Cl_6$	26	40	80	21.5 ± 3.1 (0.65)^{a}	16.5 ± 2.8 (0.57)^{a}	5.7 ± 0.2	1.16 ± 0.07	18.4
	242	40	120	14.0 ± 1.9 (0.63)^{bd}	11.8 ± 1.7 (0.61)^{ce}	6.2 ± 0.3	1.23 ± 0.07	0
$C_{12}H_{16}Cl_{10}$	21	40	120	16.2 ± 1.8 (0.71)^{ab}	12.8 ± 1.6 (0.68)^{abc}	5.9 ± 0.3	1.29 ± 0.03	0
	222	40	120	15.3 ± 1.6 (0.76)^{abc}	12.6 ± 1.3 (0.75)^{abc}	5.7 ± 0.2	1.10 ± 0.07	0
$C_{16}H_{31}Cl_3$	29	40	120	15.8 ± 1.9 (0.72)^{abc}	12.6 ± 1.4 (0.72)^{abcd}	5.6 ± 0.3	1.23 ± 0.18	7.9
	296	40	120	10.1 ± 2.6 (0.59)^{d}	8.3 ± 1.6 (0.49)^{e}	5.7 ± 0.3	1.12 ± 0.08	5.3
$C_{16}H_{21}Cl_{13}$	21	40	173	11.6 ± 1.2 (0.72)^{d}	8.7 ± 1.3 (0.59)^{e}	5.9 ± 0.4	1.27 ± 0.15	0
	198	40	173	11.4 ± 1.1 (0.77)^{d}	9.2 ± 1.8 (0.46)^{e}	5.5 ± 0.3	1.20 ± 0.10	0
	2000	40	173	11.6 ± 1.2 (0.74)^{dc}	9.9 ± 1.2 (0.70)^{de}	6.9 ± 0.8	1.46 ± 0.10	0

[x] The growth rates (± 1 standard error) were calculated using the equation ln weight = $a + b$ time (days), where b is the growth rate (coefficient of determination for the model is shown in parentheses).

[y] The percent lipid is an average (± 1 standard error) of all fish in a treatment from day 5 until the end of the experiment.

[z] Liver somatic index (LSI) (± 1 standard error) calculated at day 40 of the uptake phase.

after 40 days of exposure (Figure 3.1), and the $C_{16}H_{21}Cl_{13}$ did not reach steady state after 80 days of exposure (*data not shown*). The depuration rate in rainbow trout exposed to $C_{16}H_{31}Cl_3$ was significantly more rapid than the depuration rates of rainbow trout exposed to $C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$ and $C_{16}H_{21}Cl_{13}$ (Table 3.2). With the exception of the $C_{16}H_{31}Cl_3$, depuration rates for each PCA did not differ significantly between concentrations ($p < 0.05$) (Table 3.2), although no comparison was made between the two $C_{12}H_{21}Cl_6$ treatments because data were not available for exactly the same time period of depuration for the treatment with the lower concentration (26.2 $ng \cdot g^{-1}$). Trends of depuration rates in whole fish were consistent with depuration rates determined using only concentration data from carcass tissue. Whole body half-lives varied from 37 ● 2 days in the $C_{16}H_{31}Cl_3$ high concentration treatment to 87 ● 11 days in the $C_{12}H_{21}Cl_{10}$ low concentration treatment (Table 3.2).

Assimilation efficiencies (α) based on whole body concentrations ranged from 9.4 ± 1.1 % in the medium concentration $C_{16}H_{21}Cl_{13}$ treatment to 37.6 ± 1.1 % in the low $C_{12}H_{16}Cl_{10}$ treatment (Table 3.2). Whole body BMFs varied from 0.44 in the medium concentration $C_{16}H_{21}Cl_{13}$ treatment to 2.15 in the low concentration $C_{12}H_{21}Cl_{10}$ treatment. Assimilation efficiency and BMF values calculated with whole body and carcass tissue only concentrations were similar and followed the same trends for all four PCAs.

3.4.3 Tissue distribution and metabolic transformation

The carcass contained the greatest percentage of ^{14}C (including extractable and non-extractable) throughout the experiment for all four PCAs, ranging from 50% to greater than

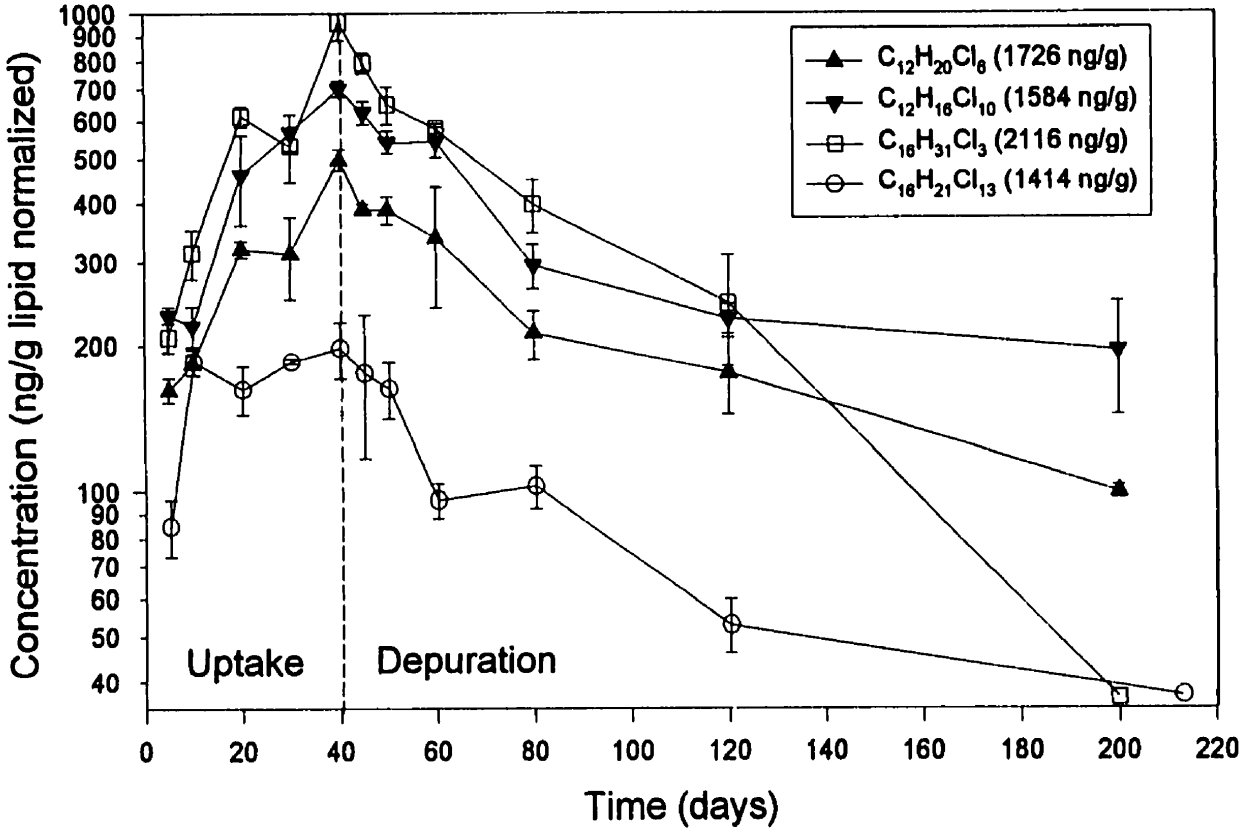

Figure 3.1 Accumulation and depuration of four ^{14}C-PCAs through dietary exposure to juvenile rainbow trout. Each point is the mean ± one standard error of three fish. Concentrations are for whole fish, corrected for growth dilution and lipid content. Exposure concentrations (lipid corrected) are provided in the legend.

Table 3.2 Bioaccumulation parameters of four ^{14}C-PCAs 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[t] (ng/g)	Length of Depuration (days)	Depuration rate constant[u] ($10^{-2} \cdot day^{-1}$)	$t_{1/2}$[v] (days)	BMF[w]	Assimilation efficiency[x] (%)
$C_{12}H_{20}Cl_6$	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
$C_{16}H_{31}Cl_3$	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

[t] - Concentration of food is given in wet weight.

[u] - 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).

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

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

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

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

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

70% (Table 3.3). The relative proportion of ^{14}C increased in the carcass over time, due mainly to increasing amounts of non-extractable ^{14}C. There was a slight drop in relative amounts of extractable ^{14}C in the GI tract from the beginning until the end of the uptake phase, which is probably due to the greater proportion of ^{14}C in the GI tract resulting from the undigested spiked-food in the gut. Non-extractable ^{14}C decreased in the liver GI tract over time, which could be explained by the high turnover of the liver and GI tract lining. Relative amounts in the liver were low because the liver accounted for only about 1.5% of the total fish weight. Both extractable and non-extractable ^{14}C decreased in the liver throughout the experiment, providing evidence that metabolic transformation of PCAs may occur in the liver.

HPLC chromatograms of the toluene extracts differed from the analytical standards on day 40 of uptake for all four PCAs (Figure 3.2). This difference is most pronounced in the $C_{12}H_{20}Cl_6$ treatment, where a number of larger peaks in the fish extracts are minor in the analytical standard. After 20 days of depuration (no exposure to treated food), all four of the PCAs show chromatographic profiles markedly different from the analytical standards (Figure 3.2). Toluene non-extractable residue is assumed to represent PCAs that have been metabolically transformed and have become more polar, and therefore unextractable with toluene. The higher chlorinated C_{12}- and C_{16}-PCAs had a greater proportion of toluene extractable ^{14}C, or parent compound (Table 3.3), implying less metabolic transformation of these compounds.

Table 3.3 Extractable and non-extractable radioactivity, as a percentage of the total fish radioactivity, in the liver, GI tract, carcass and total fish, of juvenile rainbow trout exposed to four ^{14}C-PCA compounds (U refers to the uptake phase and D the depuration phase).

		$C_{12}H_{20}Cl_6$		$C_{12}H_{16}Cl_{10}$		$C_{16}H_{31}Cl_3$		$C_{16}H_{21}Cl_{13}$	
tissue	day	ext	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

[y] Day 10 $C_{16}H_{21}Cl_{13}$ liver samples were lost, percentages represent day 5 liver samples.

[z] Day 20 $C_{16}H_{21}Cl_{13}$ liver samples were lost, percentages represent day 30 liver samples.

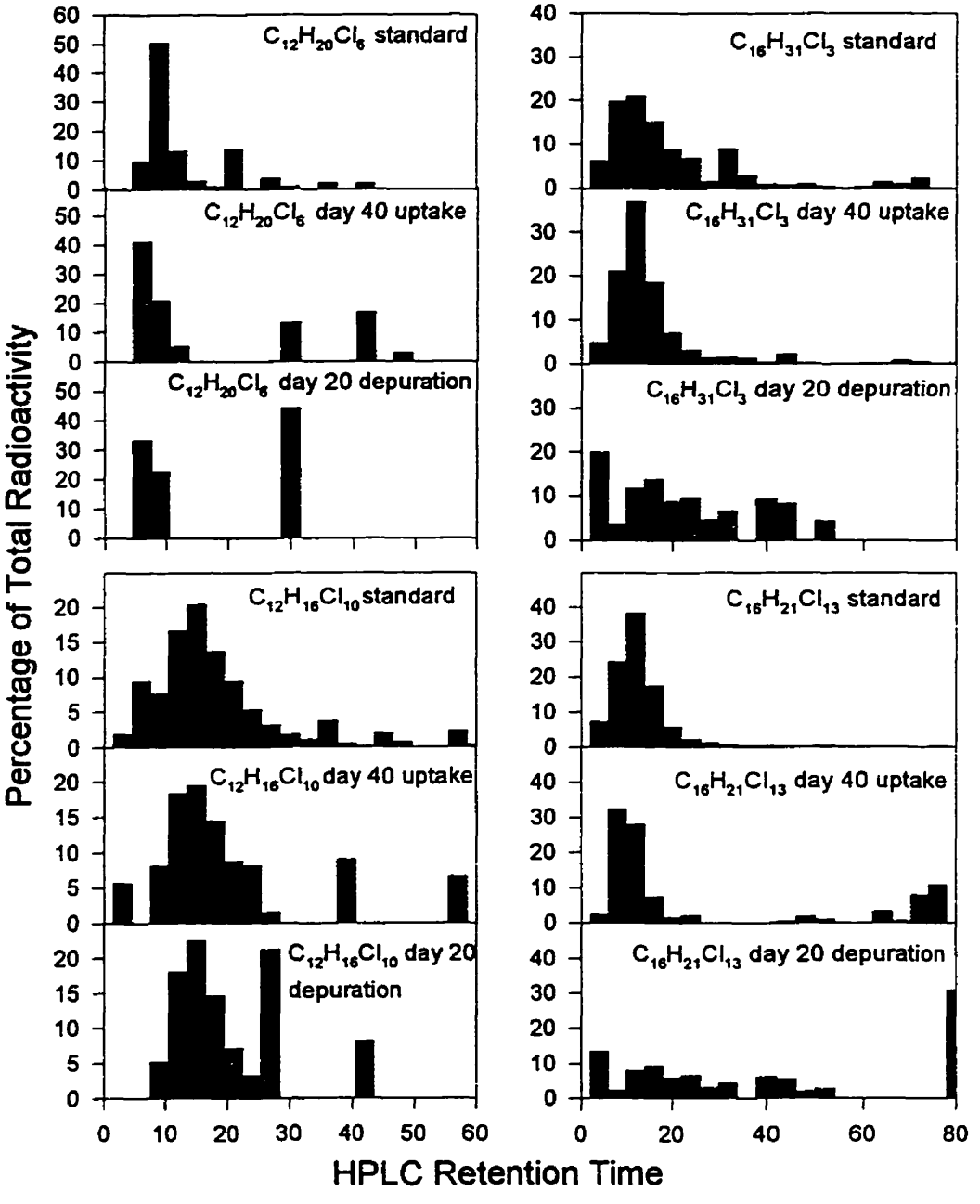

Figure 3.2 HPLC chromatograms of the 14C-PCA standards and fish carcass toluene extracts from day 40 of uptake and day 20 of depuration. Each bar in the C_{12}-PCA and C_{16}-PCA chromatograms represent the radioactivity in a three or four minute fraction, respectively, as a percentage of the total radioactivity.

3.5 Discussion

PCAs with 12 and 16 carbons and 35 - 69% Cl content (by weight) are accumulated through dietary exposure by juvenile rainbow trout despite relatively large molecular weight. As other authors have observed with CPs, accumulation of PCAs is dependent on the carbon chain length (Bengtsson and Ofstad 1982), number of chlorines (Darnerud et al. 1989), and molecular size of the molecule (Zitko 1974). The assimilation efficiency of the C_{12}-PCAs in this experiment increased from a mean of 23 to 36% (whole fish) with the addition of four chlorine atoms, corresponding to an increase in K_{ow} (log K_{ow}s of $C_{12}H_{20}Cl_6$ and $C_{12}H_{16}Cl_{10}$ are approximately 5.2 and 6.8, respectively; G. R. B. Webster, personal communication). However, the assimilation efficiency of the C_{16}-PCAs decreased from a mean 34 to 11% when the number of chlorine atoms increased from 3 to 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, respectively; G. R. B Webster, personal communication). The $C_{16}H_{21}Cl_{13}$ was found to have the highest K_{ow} of the four PCAs but the lowest accumulation and assimilation efficiency. The reduced uptake of the $C_{16}H_{21}Cl_{13}$ may be due to its large size (MW = 674) (Opperhuizen et al. 1985; Niimi and Oliver 1987; Gobas et al. 1988). Low uptake of long chain CPs has also been observed by Zitko (1974), who found that two industrial CP products with high molecular weights (MW = 579-922) showed little bioaccumulation in juvenile Atlantic salmon.

The assimilation efficiencies calculated for these PCAs are higher than assimilation efficiencies reported for commercial CP products using bleaks (*Alburnus alburnus*) (Bengtsson and Ofstad 1982). An industrial CP mixture consisting of $C_{10\text{-}12}$ CPs with 71% Cl had a "mean effectiveness in uptake" from food of only 6%, which was half the assimilation found for a less chlorinated (49% Cl) $C_{10\text{-}12}$ CP commercial product (Bengtsson and Ofstad 1982). We found

that an increase in chlorination of C_{12}-PCAs resulted in an increase in assimilation efficiency. The assimilation efficiency of the PCAs used in this experiment are comparable to tetra and pentachlorodibenzofurans under similar experimental conditions using juvenile rainbow trout (Muir et al. 1990; Muir et al. 1992), and to a hexa and octachlorobiphenyl in guppies (*Poecilia reticulata*) (Opperhuizen and Schrap 1988).

Half lives of the PCAs in this experiment are different from half lives reported for other CPs (Bengtsson and Ofstad 1982; Madeley and Maddock 1983). Madeley and Maddock (1983) reported a half life range from 16.5 days in dorsal muscle to 23.9 days in the viscera of rainbow trout exposed to waterborne short chain (C_{10-12}) 58% chlorinated paraffin, about half the value we calculated for the $C_{12}H_{20}Cl_6$. Bengtsson and Ofstad (1982) reported very rapid elimination ($t_{1/2}$ < 7 days) of a 49% Cl short chain (C_{10-13}) CP mixture. However, Bengtsson and Ofstad (1982) reported that there was no elimination of a short chain (C_{10-13}) CP with 71% Cl after 316 day elimination period, much longer than the 77 to 87 day half life calculated for the $C_{12}H_{16}Cl_{10}$ used in this experiment. It should be noted that the concentrations of CPs reported by Bengtsson and Ofstad (1982) were determined by measuring chlorine levels in the fish and not CPs. It may well be that the CPs in Bengtsson and Ofstad (1982) work were metabolically transformed, but the Cl remained in the fish.

The $C_{12}H_{16}Cl_{10}$ would be expected to biomagnifiy in aquatic food webs based on equilibrium biomagnification factors (BMF) of 1.76 and 2.15. A BMF value greater than one implies increasing concentrations along aquatic food webs, or biomagnification (Rasmussen et al. 1990; Muir et al. 1992). The low concentration $C_{16}H_{31}Cl_3$ treatment was also found to have a BMF above 1 (BMF = 1.07), however the high concentration treatment had a BMF of only 0.90. The addition of one or two chlorines to $C_{16}H_{31}Cl_3$ could reduce the depuration rate by

decreasing metabolism, and increase the assimilation efficiency owing to a higher K_{ow}, sufficiently to result in a BMF greater than one. The $C_{16}H_{21}Cl_{13}$ and the $C_{12}H_{20}Cl_6$ would not biomagnify in aquatic food webs based on a predicted equilibrium BMF of 0.44 to 0.50 and 0.60 to 0.93, respectively.

From the results of these experiments, it appears that the relationship of bioaccumulation and carbon chain length and chlorine content of PCAs is complex. Although metabolism of short chain CPs (C_{10-13}) with low chlorination may reduce accumulation, low chlorinated medium (C_{14-18}) and long (C_{19-30}) chain length CPs may be less susceptible to metabolism because of the carbon chain length (Madeley and Birtley 1980). High chlorination of short chain PCAs results in sufficient accumulation for biomagnification, but makes medium and long chain, highly chlorinated PCAs too large to diffuse through biological membranes without hindrance. The results suggest that, despite having some characteristics of bioaccumulative chemicals (i.e., high K_{ow}, low biotransformation rate), highly chlorinated (>60%), medium (C_{14-18}) and long (C_{19-38}) carbon chain PCAs are not likely to biomagnify in aquatic food webs. A further confounding factor, which we could not address with these ^{14}C-labelled PCAs, is the position of the chlorines on the carbon chain. The HPLC chromatogram of the fish extracts showed that certain PCAs within the standards were accumulating to a greater extent than others. Chlorine positioning on the alkane chain could explain the persistence of some PCAs, and low persistence of other PCAs, with the same molecular formula. This differential bioaccumulation, which is observed in invertebrates, fish and mammals with other chlorinated compounds such as chlordane (Wilcock et al. 1993), toxaphene (Bidleman et al. 1993) and PCBs (Koslowski et al. 1994), needs further study.

These experiments provide the first dietary bioaccumulation parameters for PCAs with a single carbon chain length and known amount of chlorine, although the positions and exact number of chlorine atoms are not known. From Bergman et al. (1981), and our own HPLC work, it appears that the number of chlorine atoms per compound in each standard is close to the integer value assigned in this paper, although due to non-selective synthesis procedures for these ^{14}C compounds, these standards are likely composed of numerous compounds and positional isomers. Confounding this problem is the identical electron capture negative ion (ECNI) mass spectra of CP congeners containing similar carbon and chlorine atoms (G. T. Tomy, personal communication), making it difficult to identify separate PCAs based on chlorine substitution patterns.

There is evidence that CPs are oxidized in fish (Madeley and Birtley 1980; Darnerud et al. 1983), with short carbon chain (C_{10-13}) CPs being more susceptible to metabolism than medium (C_{14-18}) and long chain (C_{19-30}) CPs (Madeley and Birtley 1980). Åhlman et al. (1986) found sulphur-containing metabolites of CPs in rats, implying that CPs may covalently bind to biological macromolecules. Lower Cl substitution resulted in greater relative amounts of non-toluene extractable ^{14}C for the C_{12}- and C_{16}-PCAs. Because the ^{14}C was unextractable from the tissue with toluene, it is considered to represent a more polar compound than the standard (such as a hydroxlated or carboxylic acid substituted product) or a PCA which has been incorporated into or with biological macromolecules.

The $C_{12}H_{20}Cl_6$ showed greater change from the analytical standard than the higher chlorinated $C_{12}H_{16}Cl_{10}$, but this relationship is not as evident for the C_{16}-PCAs. Madeley and Birtley (1980) reported that short chain ($< C_{13}$) CPs with low chlorination ($< 60\%$) are the most readily oxidized in microorganisms. However, it is obvious from the HPLC-^{14}C

chromatogram of day 20 depuration, that all of the PCAs used in this experiment are susceptible to metabolism. Therefore, the rate of metabolism of PCAs is dependent on both chlorine substitution and carbon chain length, but may also be dependent on chlorine position.

No toxic effects were observed in any of the treatments. Past toxicity studies on CPs have found that large doses are required for toxic effects in fish (Linden et al. 1979; Madeley and Maddock 1983). No EROD induction was observed for any of the treatments on day 40 of uptake when burdens in the fish were at their highest. This is not surprising, because PCAs do not have the same planar ringed structure normally found in EROD inducing organochlorines such as PCDD/Fs and co-planar PCBs (McFarland and Clarke 1989; Safe 1992). However, in the only other work involving EROD induction and CPs, female flounder exposed to extremely high doses of an industrial CP mixture were found to have elevated EROD levels (Haux et al. 1982). The EROD induction found in the flounder may be a result of impurities, such as chlorinated aromatics, which have been found within industrial CP mixtures (Svanberg and Linden 1979).

3.6 Summary and Conclusions

In summary, bioaccumulation parameters of PCAs reported here differ from previous results on bioaccumulation of CPs in fish. These PCAs are found in commercial CP products, although they are synthesized in a different manner, and this represents the first work that has used PCAs of known carbon chain length and chlorine content for dietary bioaccumulation studies. The elimination rates, half lives and assimilation efficiencies of the PCAs are similar to PCDD/Fs and PCBs. Reduced accumulation of the lower chlorinated C_{12}-PCA could be attributed to metabolism while uptake of highly chlorinated C_{16}-PCA may have been hindered

because of its large molecular size. The $C_{12}H_{16}Cl_{10}$ would likely biomagnify in aquatic food chains based on a BMF of greater than 1 (1.76 and 2.15). Highly chlorinated short chain (C_{10-13}) CPs and lower chlorinated medium chain (C_{14-18}) CPs have the greatest potential for bioaccumulation by aquatic organisms.

4. DIETARY ACCUMULATION AND DEPURATION OF HYDROPHOBIC ORGANOCHLORINES: BIOACCUMULATION PARAMETERS AND THEIR RELATIONSHIP WITH K_{ow}

4.1 Abstract

Dietary accumulation of 24 hydrophobic organochlorines (OCs) by juvenile rainbow trout (*Oncorhynchus mykiss*) was studied with the objective of obtaining relationships between bioaccumulation parameters and K_{ow}. A wide range of OCs were used including 16 polychlorinated biphenyls (CBs 18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 187, 189, 195, 206 and 206), hexachlorobenzene, mirex, tris(4-chlorophenyl)methane (TCPMe), tris(4-chlorophenyl)methanol (TCPMeOH) and three toxaphene congeners (Cl_7-CHB (Hp-sed), Cl_8-CHB (T2) and Cl_9-CHB (T12)). TCPMe ($t_{1/2}$ = 65 d) was more persistent than TCPMeOH (half life ($t_{1/2}$) = 20 d), and TCPMe was not biotransformed to TCPMeOH by rainbow trout. Cl_7-CHB ($t_{1/2}$ = 32 d) was more rapidly eliminated, and appears to be more readily metabolized, than Cl_8-CHB ($t_{1/2}$ = 43 d) and Cl_9-CHB ($t_{1/2}$ = 42 d). With the exception of TCPMeOH, Cl_7-CHB and CB 18, all of the OCs had biomagnification factors (BMFs) > 1, implying a potential to biomagnify. Half lives had a significant curvilinear relationship with K_{ow} ($R^2 = 0.85$, $p < 0.001$), with a maximum $t_{1/2}$ for OCs with log K_{ow} ~ 7.0. Decreasing $t_{1/2}$ for OCs of log K_{ow} > 7.0 may be related to slow

kinetics of these super hydrophobic OCs and the short exposure phase, which results in insufficient time for the super hydrophobic OCs to reach slower clearing compartments of the rainbow trout. Assimilation efficiency was not as well described by K_{ow} as $t_{1/2}$ and biomagnification factor (BMF), although a significant curvilinear relationship was observed ($R^2 = 0.53$, $p = 0.004$). BMF had a significant curvilinear relationship with log K_{ow} ($R^2 = 0.84$, $p < 0.001$). Recalcitrant OC's with a log K_{ow} of ~ 7.0 would appear to have the greatest potential for food chain biomagnification in fish.

4.2 Introduction

Fish, and many aquatic invertebrates, accumulate organochlorine compounds (OCs) from water (bioconcentration) and from food (biomagnification). The relative importance of these pathways varies with the water solubility of the OC and the trophic position of the organism. With decreasing water solubility (Bruggeman et al. 1984; Oliver and Niimi 1985; Connolly and Pedersen 1988) and increasing trophic position (Bruggeman et al.1984; Oliver and Niimi 1988; Rasmussen et al. 1990) there is greater accumulation from food. Greater than 99% of the polychlorinated biphenyls (PCBs) (log $K_{ow} > 5.0$) in Lake Michigan lake trout have been estimated to be accumulated through the food chain (Thomann and Connolly 1984). As well, Thomann et al. (1992) concluded that OCs of log $K_{ow} > 5$ in Lake Ontario sculpin are almost entirely derived from the food chain. Food has also been identified as the most important exposure route for PCBs in the aquatic food chain from the western basin of Lake Erie (Koslowski et al. 1994).

Bioaccumulation parameters, such as bioconcentration factor (BCF) or biomagnification factor (BMF), are important for the prediction of exposure to OCs in the aquatic environment. The relationship between the bioconcentration (BCF) of hydrophobic OCs and K_{ow} has been extensively studied (Veith et al. 1979; Mackay 1982; Connell and Hawker 1988; Isnard and Lambert 1988; Bintein et al. 1993; Fox et al. 1994; Devillers et al. 1996). However, application of BCF-K_{ow} relationships may not provide adequate data for fate and exposure assessment of recalcitrant hydrophobic OCs (log K_{ow} > 5) in the environment. Laboratory derived BCF-K_{ow} comparison were only useful in predicting field-derived BCF's for Lake Ontario rainbow trout of OCs of short half-lives, but not for OCs of longer half life (Oliver and Niimi 1985). Swackhamer and Hites (1988) concluded that laboratory-derived BCF-K_{ow} models gave a poor approximation of the bioaccumulation of hydrophobic OCs in lake trout and whitefish. Further, Thomann and Connolly (1984) determined that PCB levels in Lake Michigan lake trout were not predicted by the BCF-K_{ow} relationship. Surprisingly, there have been few attempts at developing relationships between dietary accumulation of hydrophobic OCs and K_{ow}.

The lack of dietary accumulation-K_{ow} relationships is probably due to the scarcity and variability of kinetic data (Sijm et al. 1992). Dietary accumulation data obtained for hydrophobic OCs in laboratory-derived experiments is more variable compared with BCF experiments (Bruggeman et al. 1981). This variability is a function of the differences in food, the logistics of feeding fish and differences in experimental methodology (Gobas et al. 1988). Dietary composition may also influence adsorption efficiency of organochlorines. Efficiency may be less in low digestible diets which are low in fat and

protein, and high in fibre (Parkerton 1993). Fish of different species, age, size and sex will feed and digest food at different rates, which could influence assimilation efficiency. Although size and species differences also affect uptake of OCs from water, their influence on dietary accumulation parameters may be greater. Feeding rates (i.e., g food·d^{-1}) established by the investigator can influence parameters. An increase in the rate of feeding was found to decrease the absorption efficiency of OCs (Clark and Mackay 1991). As well, high concentrations in the food may cause fish to stop feeding through avoidance or because of a toxic response in the fish. In either case the fish are no longer being exposed, whereas fish exposed to high water concentrations cannot avoid the OCs.

Our objective was to develop physical-chemical property-dietary bioaccumulation relationships using a wider range of hydrophobic OCs than has been studied previously. We exposed juvenile rainbow trout to dietary concentrations of 24 OCs, including 16 PCB congeners, hexachlorobenzene (HCBz), mirex, 3 toxaphene congeners (Cl_7-chlorobornanes(CHB), Cl_8-CHB and Cl_9-CHB), tris(4-chlorophenyl)methane (TCPMe) and tris(4-chlorophenyl)methanol (TCPMeOH), to determine bioaccumulation parameters. We chose PCB congeners with 3 through 10 chlorines to provide a range of K_{ow}'s that include the most hydrophobic of the PCBs. To reduce the influence of metabolic transformation, PCB congeners with meta and para Cl substitution were chosen. Toxaphene, TCPMe and TCPMeOH are global contaminants (Jarman et al. 1992; Bidleman et al. 1993; de Boer et al. 1996), but have not been studied in laboratory dietary bioaccumulation experiments. Toxaphene, and in particular Cl_8-CHB and Cl_9-CHB (Glassmeyer et al. 1997), have been found to bioaccumulate and biomagnify in aquatic

food chains (Evans et al. 1991; Kidd et al. 1995). The PCB congeners, mirex and HCBz have well established K_{ow}s, and for many of these OCs, data is available on dietary accumulation by fish. By exposing the rainbow trout to a mixture of all the OCs we have sufficient data to develop dietary accumulation parameter-K_{ow} relationships without many of the confounding problems of compiling data from different experiments and exposures.

4.3 METHODS AND MATERIALS

4.3.1 Chemicals and Food Preparation

HCBz, mirex and the 16 PCB congeners (CB 18, 28, 44, 52, 66, 101, 105, 118, 128, 138, 153, 187, 189, 195, 206 and 209) were purchased from Ultra Scientific (North Kingstown, RI, USA). TCPMe and TCPMeOH were synthesized at Carleton University (Ottawa, ON, Canada). The three toxaphene congeners [Cl_7-CHB (2-exo,3-endo,5-exo,6-endo,8c,9b(or 8b,9c),10a-heptachlorobornane), Cl_8-CHB (2-exo,3-endo,5-exo,6-endo,8,8,10,10-octachlorobornane) and Cl_9-CHB (2-exo,3-endo,5-exo,6-endo,8,8,9,10,10-nonachlorobornane)] were isolated and purified from arctic mammal blubber or sediments using methods outlined previously (Stern et al. 1992; 1996).

Food was spiked by mixing a known quantity of each of the 24 organochlorines (listed above), dissolved in 150 mL of hexane, with 60 g of commercial fish food (Martin's Feed Mills, Elmira, ON, Canada) and slowly evaporating to dryness. Control food was treated in an identical manner but without the addition of the organochlorine compounds. Food was air-dried for 24 hours and stored at 10°C. The fish food consisted of 41% protein, 14% lipid and 3% fiber. Concentrations of each OC were determined in control

and spiked food using the same analytical techniques used to determine concentrations in the rainbow trout tissue (Table 4.1).

4.3.2 Experiments

Juvenile rainbow trout (*Oncorhynchus mykiss*)(initial weights 2 - 4 g) were exposed to the spiked food (containing all OCs) for 30 days followed by 160 days of depuration. Rainbow trout were maintained in fiberglass aquaria (40 L) with flow-through, UV and carbon dechlorinated, City of Winnipeg water (~10°C) with a 12 h light: 12 h dark schedule. The daily rate of feeding was equal to 1.5% of the mean weight of the rainbow trout, corrected after each sampling period. All food was consumed in < 1 minute after being offered to the fish. Three fish were sampled from each treatment for organochlorine analysis on days 5, 10, 20 and 30 of the uptake period, and days 5, 10, 20, 40, 80 and 160 of the depuration period. A volume of 1 L water samples were also taken 1 hour and 24 hours after feeding on day 30 of the uptake phase. 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). Only results from the carcass samples were used for calculation of bioaccumulation parameters.

In a second experiment, juvenile rainbow trout were exposed to trout food spiked with high concentrations of TCPMe to determine if TCPMe is biotransformed to TCPMeOH. After 21 d of feeding, three rainbow trout were sacrificed and analyzed for TCPMe and TCPMeOH.

Table 4.1 Concentrations ($ng \cdot g^{-1}$, wet weight) of organochlorines (mean ± 1 S.E., n = 3) in the control, low and high treatment food.

Compound	control	low	high
Misc. Organochlorines			
TCPMe	54 ± 28*	55 ± 22	190 ± 3.2
TCPMeOH	< 0.01	34 ± 4.7	280 ± 4.9
mirex	1.1 ± 0.3	21 ± 0.3	140 ± 4.4
HCBz	1.3 ± 0.1	14 ± 0.3	100 ± 5.6
Toxaphene Congeners			
Cl_7-CHB	0.2 ± 0.0	21 ± 0.7	140 ± 2.0
Cl_8-CHB	2.6 ± 0.1	18 ± 0.1	120 ± 3.4
Cl_9-CHB	3.8 ± 0.3	17 ± 0.1	130 ± 2.4
Polychlorinated Biphenyls			
CB 18	< 0.01	29 ± 0.9	180 ± 11
CB 28	< 0.01	16 ± 0.5	110 ± 8.8
CB 44	0.9 ± 0.0	18 ± 0.4	130 ± 6.7
CB 52	3.4 ± 0.2	22 ± 0.7	130 ± 6.9
CB 66	1.0 ± 0.0	21.1 ± 0.2	160 ± 7.8
CB 101	4.3 ± 0.3	20 ± 0.4	150 ± 14
CB 105	< 0.01	17 ± 0.6	130 ± 3.4
CB 118	2.0 ± 0.1	20 ± 0.2	130 ± 9.1
CB 128	< 0.01	8.3 ± 1.1	99 ± 2.7
CB 138	8.8 ± 0.3	31 ± 1.4	180 ± 7.0
CB 153	7.1 ± 0.3	22 ± 0.3	120 ± 5.7
CB 187	3.2 ± 0.1	19 ± 0.1	120 ± 5.1
CB 189	0.1 ± 0.0	19 ± 0.2	140 ± 2.5
CB 195	0.2 ± 0.0	24 ± 0.3	180 ± 3.1
CB 206	0.4 ± 0.0	20 ± 0.3	140 ± 3.0
CB 209	0.2 ± 0.0	62 ± 0.8	700 ± 56

* TCPMe was not quantifiable in one control food sub-sample but had high levels in the other two sub-samples. Control fish did not accumulate TCPMe to extent expected from a concentration this high and suggests that the high concentration reported for the control food is not accurate.

4.3.3 OC Analysis

Tissue samples were weighed, frozen, freeze dried and homogenized in toluene. CB 30 was added to samples before the extraction step as a surrogate recovery standard. Samples were centrifuged, the toluene supernatant was decanted, and the toluene extraction was repeated and combined with the first extraction. The toluene was exchanged for 2 mL of hexane, and 250 µL was used to determine lipids gravimetrically. Lipids were removed from the sample by gel permeation chromatography (GPC). The GPC columns (inner diameter, 29.5 mm; length, 400 mm, reservoir, 500 mL) were packed with 60 g (dry weight) of 200- to 400-mesh Bio-Beads® S-X3 beads (Bio-Rad Laboratories, Hercules, CA, USA). The column was eluted with 300 mL of DCM:hexane, the first 125 mL contained lipids and were discarded. The lipid-free eluate, containing the OCs, was evaporated to 1 mL and applied to a Florisil column (8 g, 1.2% deactivated). The OCs were recovered by consecutive elution with 35 mL hexane (F1), 38 mL of 85% hexane: 15% DCM (F2), and 52 mL of 50% hexane: 50% DCM (F3). Fraction 1 (F1) contained 5% of the Cl_7-CHB; 90% of the HCBz, mirex, Cl_8-CHB, and Cl_9-CHB; and 100% of all the PCBs. Fraction 2 (F2) contained 10% of the HCBz, mirex, Cl_8-CHB, and Cl_9-CHB; 95% of the Cl_7-CHB; and 100% of the TCPMe. Fraction 3 (F3) contained 100% of the TCPMeOH. All fractions were evaporated, transferred to 2,2,4-trimethyl pentane and evaporated to approximately 100 µL. Aldrin was added as a volume corrector.

Water samples (1 L) were extracted with 300 mL of DCM:hexane in a 2 L glass separatory funnel. The solvent was decanted, evaporated to 1 mL and applied to a Florisil column using the same methods that were used for the fish tissue.

Samples were analyzed on a Varian 3600 gas chromatograph (GC) equipped with a 60 m DB-5 column and an ^{63}Ni-electron capture detector (ECD)(Muir et al. 1996). The carrier gas was H_2 and N_2 was used as the make-up gas for the ECD.

4.3.4 K_{ow}

K_{ow}s were obtained for PCB congeners from Hawker and Connell (1988), for HCBz from Mackay et al. (1992), and for mirex from Suntio et al. (1988). There are no published data on the K_{ow}s of TCPMe, TCPMeOH or any toxaphene congeners, and therefore fragment constants were used to estimate their K_{ow}s (Lyman et al. 1982). The K_{ow}s of TCPMe and TCPMeOH were determined by adding and subtracting the appropriate fragments from DDT. The K_{ow}s of the toxaphene congeners were determined by adding all the fragments and correcting by appropriate rules (Lyman et al. 1982).

4.3.5 Data Analysis

Growth rates were determined by fitting all fish and liver weight data to an exponential model (ln fish weight = $a + b \cdot$ time (d); where a is a constant and b is the growth rate)(Neely 1980). All concentrations were corrected for growth dilution by multiplying the fish concentrations by a factor of $(1 + b \cdot \text{time})$. Many of the compounds used (TCPMe, Cl_8-CHB, Cl_9-CHB, mirex, HCBz, and CBs 52, 101, 118, 128, 138, 153 and 187) were found to have significant concentrations in the non-spiked food (Table 1) and control rainbow trout. For chemicals which were at steady state between food and control fish, and did not significantly increase in the control fish over the course of the experiment, a mean concentration was determined in the control fish and subtracted from the exposed fish

concentration. For OCs which showed a significant increase in concentration in the control fish over the duration of the experiment, concentrations in the exposed fish were corrected by subtracting the mean concentration of the control fish for the same collection day.

Depuration rates (k_d) were determined by fitting the data to a first order decay curve (ln conc = $a + b \cdot$ time (d), were a is a constant and b is the k_d). $T_{1/2}$ is = ln 2 / k_d. Assimilation efficiency (α) was determined by fitting the concentration data to the integrated from of the kinetic rate equation for constant dietary exposure using iterative non-linear regression (Bruggeman et al. 1981):

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

where F is the feeding rate (F = 0.015 g food · g of fish^{-1} · d^{-1}, 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 BMFs were predicted from the equation BMF = $\alpha \cdot F/k_d$.

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

4.4 Results and Discussion

4.4.1 Effects

No significant differences in whole fish or liver growth rates were found between control and exposure juvenile rainbow trout populations (Table 4.2). Liver somatic indices (LSI)

were similar between treatment populations on days 30 and 190, although they decreased between days 30 and 190 (Table 4.2). Three out of 39 rainbow trout died in the low and high exposure treatments; no fish died in the control population. Lipid percentages in the fish increased with time and were similar between treatment populations on all sampling days except day 190, when lipid levels in the control were lower (Table 2).

4.4.2 Bioaccumulation Parameters

All OCs were detected in the carcass after 5 days of exposure to spiked food (Figures 4.1 and 4.2). None of the compounds reached steady state after 30 days (Figure 4.1 and 4.2), and uptake curves and assimilation efficiencies were similar for most compounds (Figures 4.1 and 4.2; Table 4.3). Unfortunately, for OCs which had concentrations in the non-spiked food exceeding 1.0 $ng \cdot g^{-1}$ (wet weight)(TCPMe, mirex, Cl_8-CHB, Cl_9-CHB, CBs 52, 101, 118, 138, 153, and 187), the results for the lower concentration exposures could not be used. Because significant quantities of OCs were present in the non-spiked food used during the depuration phase, the rainbow trout continued to accumulate OCs during this phase. This resulted in an apparent, slower depuration rate and consequently higher assimilation efficiencies and BMFs which are incorrect. The source of the OCs in the food is probably from fish oils used by the manufacturer in the food preparation

Concentrations of OCs in the non-spiked food were 17 to 300 times lower than those in the high exposure food concentrations, and therefore had only a minor influence on the depuration rates from the high exposure. For OCs not confounded by the presence

Table 4.2 Growth parameters (mean ± 1 standard error) of juvenile rainbow trout exposed to 25 organochlorines (significant differences [t-test, $p < 0.05$] in body and liver growth rates between treatments are indicated by capital letters).

	Growth rate[y]						
treat.	body (10^{-3}/d)	liver (10^{-3}/d)	% lipid day 30	% lipid day 190	LSI day 30	LSI day 190	% mortality
control	12.9 ± 1.3 (0.75)^{A}	9.8 ± 1.4 (0.61)^{B}	3.4 ± 0.5	2.5 ± 0.4[z]	1.6 ± 0.2	1.0 ± 0.1	0
low	12.6 ± 1.4 (0.73)^{A}	9.3 ± 1.3 (0.62)^{B}	3.4 ± 0.3	5.9 ± 0.3	1.5 ± 0.1	0.9 ± 0.1	7.7
high	10.4 ± 1.5 (0.60)^{A}	8.0 ± 1.5 (0.47)^{B}	4.1 ± 0.6	6.3 ± 2.7	1.5 ± 0.1	1.1 ± 0.2	7.7

[y] The growth rates were calculated using the equation ln weight = $a + b$ time (days), where b is the growth rate (coefficient of determination for the model is shown in parentheses).

[z] Lipid percentages were similar between the three treatments throughout the experiment with the exception of day 190.

of concentrations in the non-spiked food, bioaccumulation parameters were in good agreement between the two exposure treatments. CB 30 recoveries were 75.6 ± 5.9 % (mean ± 1 SE), and no corrections were made for these recoveries. No OCs were detectable in the water (< 1 $ng \cdot L^{-1}$), 1 and 24 h after feeding.

4.4.3 PCBs

All of the PCBs used in this study were selected based on the criteria of maximal, or near maximal, meta-/para- chlorine substitution. These PCBs represent a subset which should have the slowest elimination and greatest bioaccumulation potential (Bruggeman et al. 1981; Niimi and Oliver 1983; Sijm et al. 1992). With the exception of CB 18, all PCBs in this experiment had BMFs > 1. PCBs with similar meta-/para- Cl substitution have been found to biomagnifiy in laboratory experiments using fish (Bruggeman et al. 1981; Clark and Mackay 1991; Sijm et al. 1992), and in aquatic food chains (Oliver and Niimi 1988; Porte and Albaiges 1993). However, PCB BMFs from this study are as much as a factor of 5 lower than BMFs determined for adult Lake Ontario lake trout (4.1 - 5.2 kg) (Niimi 1996). The difference in PCB BMFs could be due to the small fish used in our study. As discussed below, PCB depuration rates are greater in smaller fish which would result in lower BMF.

Assimilation efficiencies of PCBs (31-49%), and HCBz (34%), are similar to those reported by Gobas et al. (1993) for goldfish and Sijm et al. (1992) for guppies, but are much lower than those reported for rainbow trout (PCBs: 63-85% (Niimi and Oliver 1983); HCBz: 73 to 88% (Niimi and Oliver 1988)). In both of Niimi and Oliver's (1983;

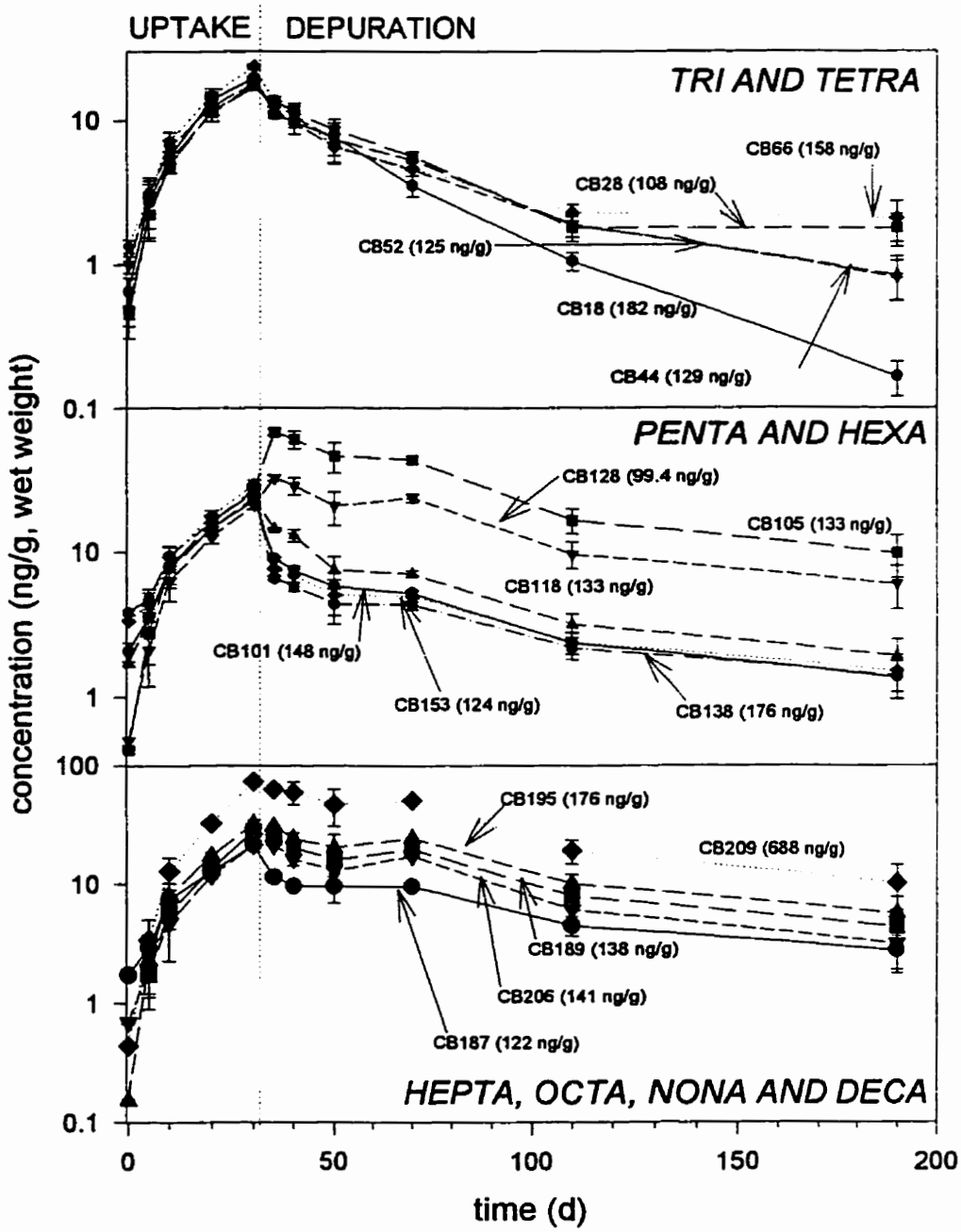

Figure 4.1 Accumulation and depuration of 16 PCB congeners through dietary exposure to juvenile rainbow trout. Each point is the mean concentration ± 1 standard error of three fish carcass (minus GI tract and liver). Exposure concentrations (wet weight) are provided beside each OC.

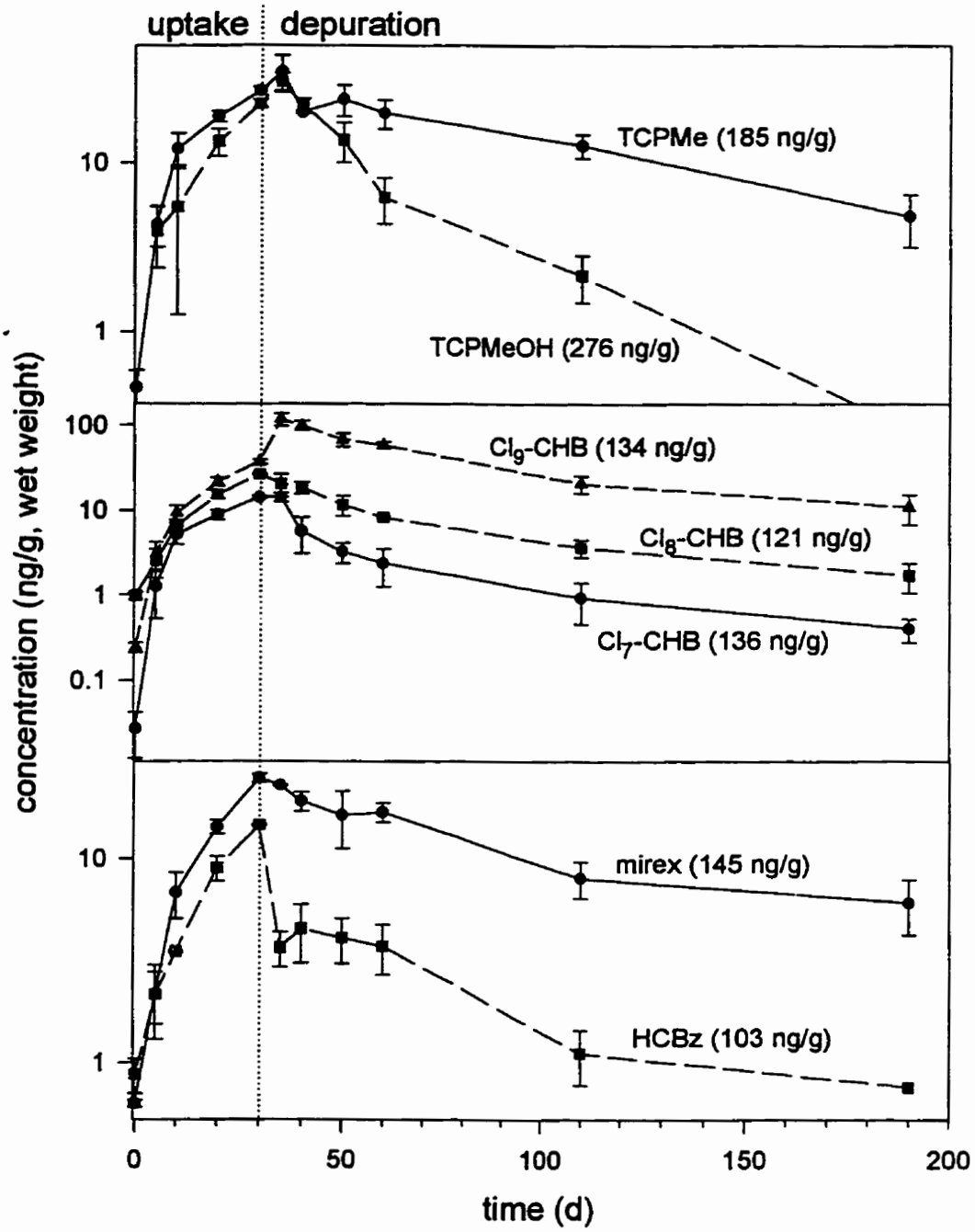

Figure 4.2 Accumulation and depuration of TCPMe, TCPMeOH, Cl_7-CHB, Cl_8-CHB, Cl_9-CHB, HCBz and mirex through dietary exposure to juvenile rainbow trout. Each point is the mean concentration ± 1 standard error of three fish carcass (minus GI tract and liver). Exposure concentrations (wet weight) are provided beside each OC.

Table 4.3 Bioaccumulation parameters for 25 organochlorines for dietary exposures using juvenile rainbow trout data for carcass concentrations.

compound	food conc.[t] ($ng \cdot g^{-1}$)	depuration rate[u] ($10^{-2} d^{-1}$)	$t_{1/2}$[v] (d)	BMF[w]	α[x] (%)
Misc. Organochlorines					
TCPMe	55	0.4 ± 0.1 (0.45)	178 ± 45	4.6	25 ± 2
	185	1.1 ± 0.1 (0.77)	65 ± 5.9	2.3	36 ± 2
TCPMeOH	34	3.6 ± 0.5 (0.81)	19 ± 2.6	0.6	32 ± 1
	276	3.3 ± 0.4 (0.85)	21 ± 2.5	0.5	23 ± 2
mirex	21	1.7 ± 0.3 (0.77)	42 ± 7.4	1.8	41 ± 3
	145	0.9 ± 0.1 (0.70)	78 ± 8.7	2.9	37 ± 2
HCBz	14	1.6 ± 0.2 (0.77)	43 ± 5.4	2.3	50 ± 7
	103	1.7 ± 0.3 (0.60)	42 ± 7.4	1.4	34 ± 2
Toxaphene Congeners					
Cl_7-CHB	21	1.6 ± 0.3 (0.61)	43 ± 8.1	0.8	18 ± 2
	136	2.2 ± 0.4 (0.63)	32 ± 5.8	0.9	28 ± 2
Cl_8-CHB	18	0.7 ± 0.1 (0.84)	95 ± 14	4.9	49 ± 5
	121	1.6 ± 0.2 (0.85)	43 ± 5.4	2.1	51 ± 3
Cl_9-CHB	17	0.8 ± 0.1 (0.86)	83 ± 10	4.6	53 ± 3
	134	1.7 ± 0.2 (0.87)	42 ± 4.9	2.6	63 ± 4
Polychlorinated Biphenyls					
CB 18	29	2.0 ± 0.2 (0.79)	36 ± 3.6	1.2	32 ± 2
	182	2.9 ± 0.1 (0.96)	24 ± 0.8	0.7	31 ± 2
CB 28	16	1.6 ± 0.2 (0.85)	44 ± 5.5	2.1	45 ± 4
	108	1.5 ± 0.2 (0.77)	46 ± 6.1	1.8	39 ± 2
CB 44	18	1.4 ± 0.1 (0.87)	49 ± 3.5	2.1	42 ± 7
	129	1.8 ± 0.1 (0.90)	38 ± 2.1	1.3	34 ± 2
CB 52	22	1.1 ± 0.2 (0.74)	65 ± 12	2.9	43 ± 5
	125	1.8 ± 0.1 (0.91)	39 ± 2.2	1.5	38 ± 2
CB 66	21	0.9 ± 0.1 (0.83)	82 ± 9.1	4.0	47 ± 6
	158	1.3 ± 0.2 (0.74)	55 ± 8.5	1.9	35 ± 2
CB 101	20	0.5 ± 0.1 (0.62)	131 ± 26		
	148	1.2 ± 0.1 (0.86)	56 ± 4.7	2.0	37 ± 2
CB 105	17	1.4 ± 0.2 (0.77)	48 ± 6.9	2.8	55 ± 5
	133	1.4 ± 0.1 (0.85)	50 ± 3.6	2.3	47 ± 3
CB 118	20	0.7 ± 0.1(0.65)	103 ± 15	6.0	55 ± 8
	133	1.3 ± 0.1(0.84)	53 ± 4.1	2.2	42 ± 2
CB 128	8	0.9 ± 0.1 (0.79)	75 ± 8.3	5.8	75 ± 8
	99	1.2 ± 0.1 (0.79)	59 ± 4.9	2.8	49 ± 3
CB 138	31	0.5 ± 0.1 (0.59)	139 ± 28	7.1	49 ± 10

	176	1.1 ± 0.1 (0.81)	64 ± 5.8	2.4	38 ± 2
CB 153	22	0.3 ± 0.1 (0.40)	224 ± 75	16	68 ± 15
	124	1.0 ± 0.1 (0.80)	69 ± 6.9	3.3	48 ± 3
CB 187	19	0.5 ± 0.1 (0.68)	131 ± 26	6.1	45 ± 8
	122	1.0 ± 0.1 (0.78)	71 ± 7.1	2.8	40 ± 2
CB 189	19	1.1 ± 0.1 (0.82)	64 ± 5.8	2.0	30 ± 2
	138	1.2 ± 0.2 (0.76)	58 ± 9.7	2.4	42 ± 3
CB 195	24	1.0 ± 0.1 (0.81)	67 ± 6.7	2.4	34 ± 2
	176	1.1 ± 0.2 (0.76)	61 ± 11	2.4	40 ± 3
CB 206	20	1.6 ± 0.2 (0.79)	45 ± 5.6	1.6	34 ± 4
	141	1.3 ± 0.2 (0.72)	53 ± 8.2	1.8	34 ± 2
CB 209	62	1.3 ± 0.2 (0.81)	52 ± 8.0	1.1	21 ± 1
	688	1.3 ± 0.2 (0.74)	52 ± 8.0	1.1	22 ± 2

[t] - Food concentration is wet weight.

[u] - 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 parentheses).

[v] - $t_{1/2}$ is calculated from the equation $t_{1/2} = 0.693/k_d$.

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

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

1988) studies, trout were exposed to PCBs and HCBz in herring oil by gavage, which suggests that the high lipid content may have resulted in greater assimilation. In a gavage exposure, all lipid and most of the chemical is absorbed because there is no fecal elimination. Gobas et al. (1993) found that increasing the lipid content of the food used for exposure resulted in a decrease in assimilation efficiencies of HCBz, and PCBs, which they attributed to greater digestibility of the lower lipid food. Based on the results of Gobas et al. (1993), the high lipid diet used in this experiment (14%) would underestimate the assimilation efficiency of these compounds in the environment. The digestibility of the food used to administer the contaminant to the fish must therefore be considered, particularly when comparisons are made to wild fish.

Generally, PCB $t_{1/2}$s increased with Cl number up to a maximum $t_{1/2}$ for PCBs with 7 or 8 Cl, but decreased for congeners with 9 and 10 Cl. Niimi and Oliver (1983) concluded that the $t_{1/2}$s of PCB congeners in whole rainbow trout were positively correlated with chlorine number, but also found that $t_{1/2}$s in muscle tissue were not as strongly correlated with Cl number. In fact, Niimi and Oliver (1983) found $t_{1/2}$s to decrease at high Cl number (> 6 Cl) which they attributed to redistribution of the PCB congeners to other tissues and reduced lipid in the muscle. K_{ow}, which is influenced by both Cl number and substitution pattern, was also correlated with the $t_{1/2}$ of the PCB congeners in this study. Coristine et al. (1996) concluded that the elimination of PCB congeners in rainbow trout is influenced by hydrophobicity and Cl position.

Comparing the results reported by Oliver and Niimi (1983) and Coristine et al. (1996) with this work showed that PCB $t_{1/2}$s in rainbow trout are positively related to

rainbow trout size (Figure 4.3). Half lives of similar PCB congeners were consistently highest in Oliver and Niimi (1983) who used 900 g rainbow trout, and lowest in the 10 g rainbow trout used in this work. Sijm and van der Linde (1995) have also observed a positive correlation between PCB $t_{1/2}$ and fish size.

4.4.4 Mirex and HCBz

Mirex had a greater $t_{1/2}$ in juvenile rainbow trout than HCBz, which agrees with similar studies on HCBz and mirex in rainbow trout (Niimi and Palazzo 1985). Based on BMFs >1 (Table 4.3), both HCBz and mirex would biomagnify in aquatic food chains. Clark and Mackay (1991) concluded that mirex would biomagnify but that significant biomagnification of HCBz is unlikely based on dietary uptake studies using guppies. Mirex has been found to biomagnify in aquatic food chains (Kiriluk et al. 1995).

4.4.5 TCPMe and TCPMeOH

The depuration rate of TCPMeOH was significantly greater than TCPMe; TCPMe was much more persistent in juvenile rainbow trout (Table 4.3). TCPMeOH differs from TCPMe by a single hydroxyl group on the methane carbon, which may explain the significant differences in their bioaccumulation parameters. The assimilation efficiency of TCPMeOH (α = 23 to 32%) was slightly less than the TCPMe (α = 36%), although this could be an artifact of the higher depuration rate of TCPMeOH. TCPMe had a BMF > 1, implying that it may biomagnify in aquatic food chains. The BMFs of TCPMeOH were 0.5

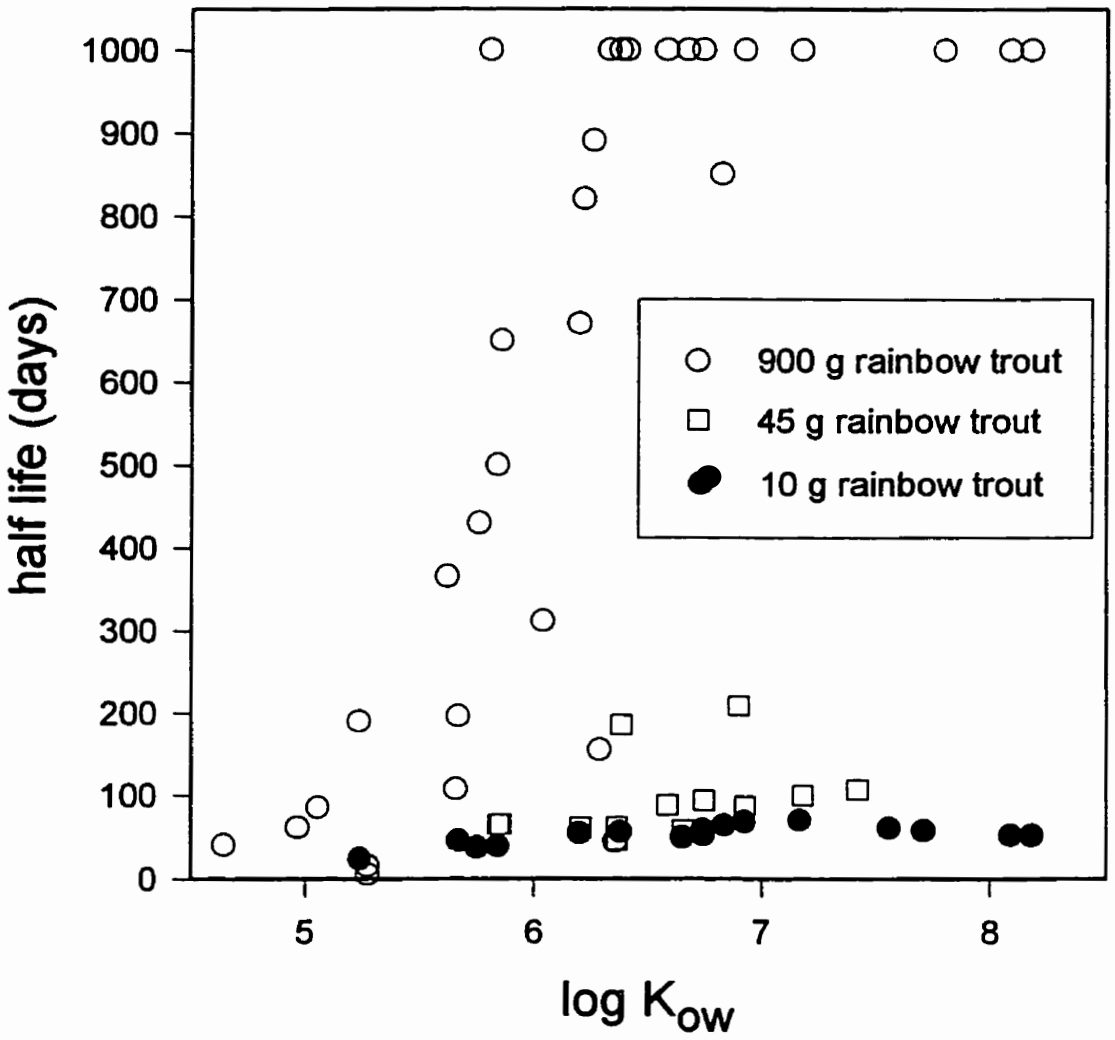

Figure 4.3 Half life ($t_{1/2}$s) of PCB congeners in rainbow trout versus log K_{ow} for three sizes of rainbow trout [O - 900 g from Oliver and Niimi (1985); □ - 100 g Coristine et al. (1996); ● - 10 g (this work)]. PCB K_{ow}s are from Hawker and Connell (1988).

and 0.6, which suggests that this compound will not biomagnify but food chain transfer may still play an important role in the accumulation of TCPMeOH by aquatic organisms. This contrasts with the results of de Boer et al. (1996), who concluded that TCPMeOH biomagnifies, based on concentrations in mussels, cod liver and marine mammals.

In a separate experiment, TCPMeOH was not detectable in juvenile rainbow trout (liver and GI tract included) which had been exposed to TCPMe for 21 days and had accumulated TCPMe to high concentrations (~1,000 $ng \cdot g^{-1}$). Jarman et al. (1992) hypothesized that TCPMeOH found in birds and mammals could be a derivative of either TCPMe or a chemically related compound not identified. Although our work does not rule-out the biotransformation of TCPMe to TCPMeOH in birds and mammals, it suggests that salmonids cannot perform this biotransformation.

4.4.6 Toxaphene congeners

Toxaphene has been observed to biomagnify in aquatic food webs (Evans et al. 1991; Kidd et al. 1995), however there are less data on the bioaccumulation of individual toxaphene congeners. The data of Glassmeyer et al. (1997) suggest that higher chlorinated toxaphene homologues (8 and 9 Cl) biomagnify in Great Lakes lake trout, but that lower chlorinated homologue groups do not. Bidleman et al. (1993) reported that more highly chlorinated (octa- and nonachloro) toxaphene congeners were selectively accumulated by arctic biota. This is in agreement with the BMFs calculated for Cl_8-CHB (octachloro) and Cl_9-CHB (nonachloro) in rainbow trout (BMFs = 2.1 and 2.6, respectively) in this study. It is also in general agreement with the BMF of 0.8-0.9 for Cl_7-CHB which suggests that

toxaphene congeners with less than 8 Cl are not likely to biomagnify in aquatic food webs, although food will still play an important in the accumulation of TCPMeOH by aquatic organisms. Other heptachloro-toxaphene congeners with different Cl substitution patterns may have greater accumulation than this Cl_7-CHB. Cl_8-CHB and Cl_9-CHB have BMFs similar to PCBs with 5 Cl, but have $t_{1/2}$'s closer to PCBs with only 4 Cl.

4.4.7 Bioaccumulation parameter - K_{ow} relationships

Bioaccumulation parameter-K_{ow} relationships were developed from the high concentration treatment data only due to problems with the lower concentration treatment (discussed above). Bioaccumulation parameters ($t_{1/2}$, BMF and assimilation efficiency) were log transformed for comparisons with log K_{ow}.

The OCs used to develop these relationships are slowly, if at all, metabolized in fish. OCs with $t_{1/2}$s and assimilation efficiencies which were lower than predicted are probably biotransformed by the fish (Gobas et al. 1986; de Wolf et al. 1992). For example, Fisk et al. (Chapter 3), using nearly identical experimental protocol to this work, reported a $t_{1/2}$ of approximately 45 d for a chlorinated *n*-alkane ($C_{16}H_{31}Cl_3$) in juvenile rainbow trout despite a log K_{ow} of 6.9. Based on our $t_{1/2}$ -K_{ow} relationship, $C_{16}H_{31}Cl_3$ has a $t_{1/2}$ approximately half what is expected for a non-metabolized OC of this K_{ow}. However, caution should exercised when using data from laboratory bioaccumulation studies. Fish species, fish size and experimental protocol may all influence laboratory derived bioaccumulation parameters (Sijm and van der Linde 1995; Fisk et al. 1997).

4.4.8 Depuration rate-K_{ow} relationships

Depuration rates (k_d) of the OCs were strongly related to the K_{ow}. This is exemplified by the curvilinear relationship between log $t_{1/2}$ ($t_{1/2} = 0.693/k_d$) and log K_{ow} ($R^2 = 0.85$, $p < 0.001$) (Figure 4.4), with a maximum $t_{1/2}$ for OCs of log K_{ow} 7.2. More rapid depuration above log $K_{ow} > 7$ is difficult to rationalize because greater K_{ow} implies less partitioning out of the fish lipids, and therefore greater retention in the tissue and longer $t_{1/2}$'s. The tissue used to determine concentrations and bioaccumulation parameters did not include the GI tract, therefore super-hydrophobic OCs accumulated in lipid or sorbed to the epithelial cells of the GI tract and eliminated through subsequent cell turnover cannot explain this phenomena. Fox et al. (1994) also observed a similar curvilinear relationship between K_{ow} and depuration rates of PCBs by zebrafish (*Brachydanio rerio*), but made no attempt to explain this relationship. Gobas et al. (1989) observed a linear relationship between log depuration rate and log K_{ow} for OCs with log K_{ow}s between 4 and 7, and a leveling-off of elimination rates for OCs of log K_{ow} > than 7.0. Although Gobas et al. (1989) discuss the importance of fecal elimination as an additional elimination pathway of super hydrophobic OCs (log $K_{ow} > 7.0$) and its affect on BCF, no explanation was offered for the leveling-off of depuration rates.

There are a number of possible explanations for the curvilinear relationship observed between depuration rate and K_{ow}.

4.4.8.1 Insufficient data. Only 4 compounds were used that had log K_{ow} greater than 7.2, and the curve could be interpreted as leveling-off and not decreasing. However, CBs 206 and 209 (log K_{ow} 8.09 and 8.18, respectively) had significantly more rapid depuration rates

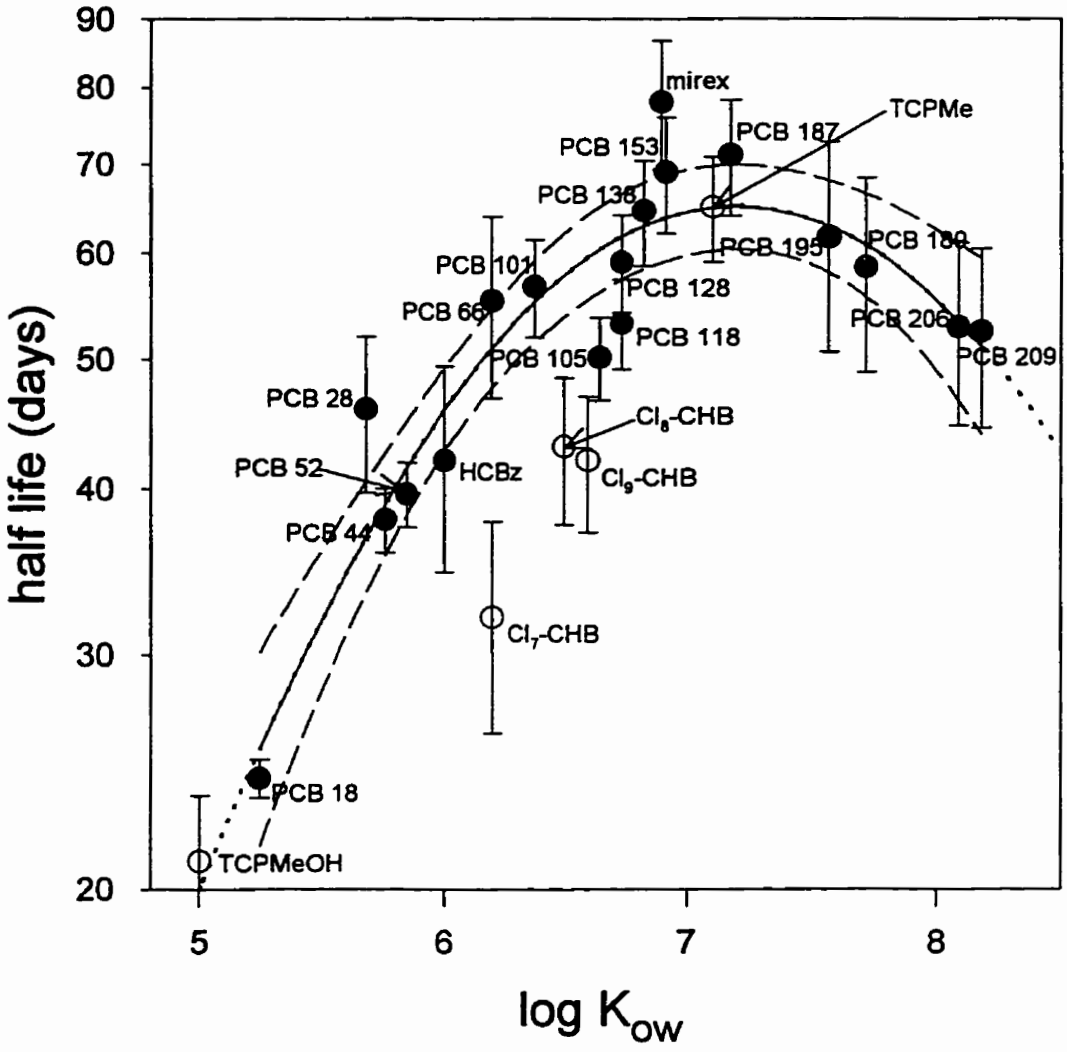

Figure 4.4 Log $t_{1/2}$ of hydrophobic OCs in juvenile rainbow trout versus log K_{ow}. Solid circles represent OCs with published K_{ow} values. Open circles represent OCs that do not have published K_{ow} values but were estimated using the K_{ow} fragment constant method (Lyman et al. 1982). The quadratic regression (solid line) was derived from the OCs that have published K_{ow} values (closed circles) (log $t_{1/2}$ = -3.7 + (1.5 ·log K_{ow}) - (0.1 ·log K_{ow}^2), $r^2 = 0.85$, $p < 0.001$). The dashed line represents the 95% confidence intervals, and the dotted line carries the data past the last points to the axis. K_{ow}'s of PCBs were taken from Hawker and Connell (1988), HCBz from Mackay et al. (1992), and mirex from Suntio et al. (1988).

than mirex, CBs 153 and 187 (log K_{ow}s 6.9, 6.2 and 7.2, respectively) (ANCOVA, $p < 0.05$). The depuration rates of OCDD and OCDF, compounds of very high K_{ow} (8.2 and 8.0, respectively (Mackay et al. 1992)), in juvenile rainbow trout were also much more rapid than the maximum rates observed in this study (Muir et al. 1986; Muir et al. 1992). Therefore, it seems unlikely that insufficient data is the cause of the curvilinear relationship between depuration rate and K_{ow}, although it may influence the magnitude of equation coefficients.

4.4.8.2 Inaccurate K_{ow}. Two problems arise for the K_{ow} of super-hydrophobic compounds. It is difficult to measure an accurate K_{ow} of very hydrophobic compounds due to their extremely low water solubility (Chiou 1985), and estimates of high K_{ow}s are considered less accurate for compounds with higher K_{ow} (Schüürmann and Klein 1988). Secondly, at high K_{ow} there is a loss of linearity between K_{ow} and lipid-water partition coefficients (Gobas et al. 1986). Chessells et al. (1992) concluded that lower than expected BCFs of super-hydrophobic OCs was partially due to relatively lower lipid solubility that is not reflected in K_{ow}. Because our relationship was developed mainly from PCB data it is unlikely that inaccurate K_{ow}s could account completely for the curvilinear relationship between $t_{1/2}$ and K_{ow}, but it may have influenced the magnitude of equation coefficients.

4.4.8.3 Dis-equilibrium between fish compartments (experiment length). The time to reach steady state concentrations between compartments in the rainbow trout should be negatively correlated with water solubility. Therefore, by the end of the uptake phase, super-hydrophobic OCs (log $K_{ow} > 7$, eg., CBs 206 and 209) would be further from steady

state between compartments of the fish than moderately hydrophobic OCs (log K_{ow} 5-7). These super-hydrophobic OCs may not have reached slower clearing compartments during the uptake phase (30 d), and may therefore be in faster clearing compartments when compared with moderately hydrophobic OCs. Faster clearing compartments could include lipids and proteins of the blood system or even cell membranes. With a greater percentage of total burden in faster clearing compartments, super-hydrophobic OCs are more available for elimination during the depuration phase compared to moderately hydrophobic OCs (log K_{ow} 5-7). Conversely, OCs with intermediate K_{ow}'s may have a greater percentage of their total body burden in slower clearing compartments and therefore are less available for elimination than super-hydrophobic OCs. Sijm et al. (1992) observed a negative linear relationship between PCB elimination rates, including CB 209, and K_{ow} in guppies which had been exposed for 210 days. As well, de Boer et al. (1996) observed no apparent elimination of higher chlorinated PCB congeners in wild populations of eels (*Anguilla anguilla*). Therefore, the length of exposure in bioaccumulation experiments using super hydrophobic OCs can be an experimental artifact, and may influence their depuration rates and $t_{1/2}$'s. As well, storage in various lipid compartments may vary depending on the method of exposure (feeding, gavage or intraperitoneal injection) and should be considered when comparing results of different experiments.

4.4.9 Assimilation efficiency-K_{ow} Relationships

Assimilation efficiency had a significant curvilinear relationship with K_{ow} (R^2 = 0.56, p = 0.04) (Figure 4.5), but it was not as strong as the $t_{1/2}$-K_{ow} and BMF-K_{ow}

relationships. This result suggests that hydrophobicity does not have as strong an influence on uptake and assimilation as it does on $t_{1/2}$ and BMF. Bruggeman et al. (1981) observed that the uptake rates of di-, tri- and tetra-PCBs from food by goldfish were similar. As well, Gobas et al. (1988) reported that absorption efficiencies for a range of hydrophobic OCs were constant up to a log K_{ow} of 7, but decreased for OCs of greater K_{ow}. Therefore, the bioaccumulation of OCs with log K_{ow}s < 7 are not as strongly influenced by the uptake rate or assimilation efficiency as they are from the depuration rate. However, at very high K_{ow}s (> 7) there is a reduction in assimilation efficiency, perhaps due to reduced bioavailability in the food and/or steric hindrance in crossing biological membranes.

4.4.10 BMF-K_{ow} Relationships

A significant curvilinear relationship was found between log BMF and log K_{ow} (R^2 = 0.84, $p < 0.001$) (Figure 4.6), which is more easily rationalized than the $t_{1/2}$-Kow relationship. At steady state the BMF is equal to the uptake rate divided by the depuration rate. Because the uptake is essentially constant, and the elimination rate decreases, for OCs with log K_{ow}s < 7, the BMF should increase with K_{ow} up to a log K_{ow} of 7. Oliver and Niimi (1988) reported such a relationship for OCs, including PCBs, in Lake Ontario salmonids. For OCs with log K_{ow}s above 7.0, lower assimilation efficiencies and/or lower uptake rates give rise to lower BMFs. As well, the depuration rates increase above log K_{ow} of 7, further reducing BMFs.

A curvilinear relationship has been observed numerous times with BCF-K_{ow} relationships, where BCFs for compounds with log K_{ow} > 7 fall below the expected 1:1

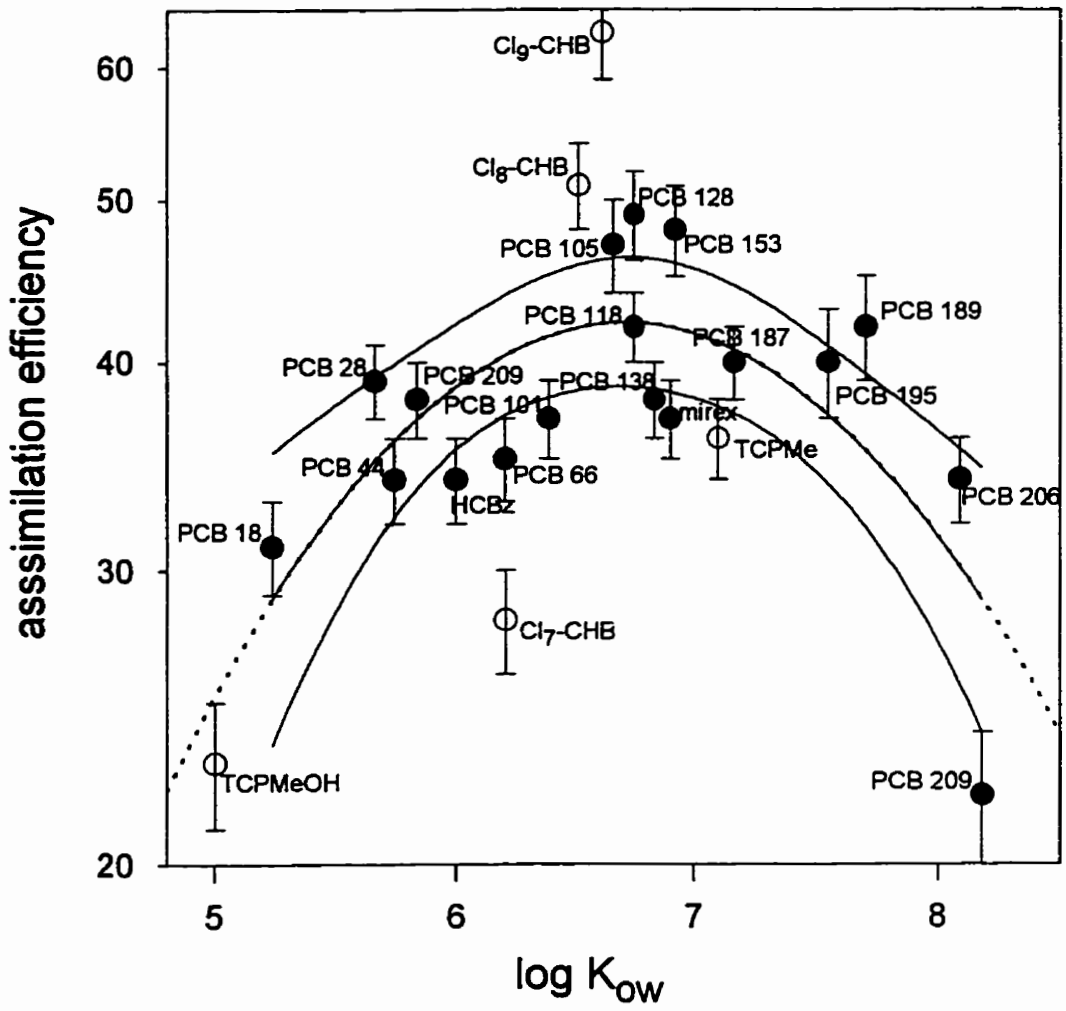

Figure 4.5 Log assimilation efficiency of hydrophobic OCs by juvenile rainbow trout versus log K_{ow}. Solid circles represent OCs with published K_{ow} values. Open circles represent OCs that do not have published K_{ow} values but were estimated using the K_{ow} fragment constant method Lyman et al. 1982). The quadratic regression (solid line) was derived from the OCs that have published K_{ow} values (closed circles) (log assimilation efficiency = -1.8 + (1.0 · log K_{ow}) - (0.08 · log K_{ow}^2), r^2 = 0.53, p = 0.004). The dashed line represents the 95% confidence intervals, and the dotted line carries the data past the last points to the axis. K_{ow}'s of PCBs were taken from Hawker and Connell (1988), HCBz from Mackay et al. (1982), and mirex from Suntio et al. (1988).

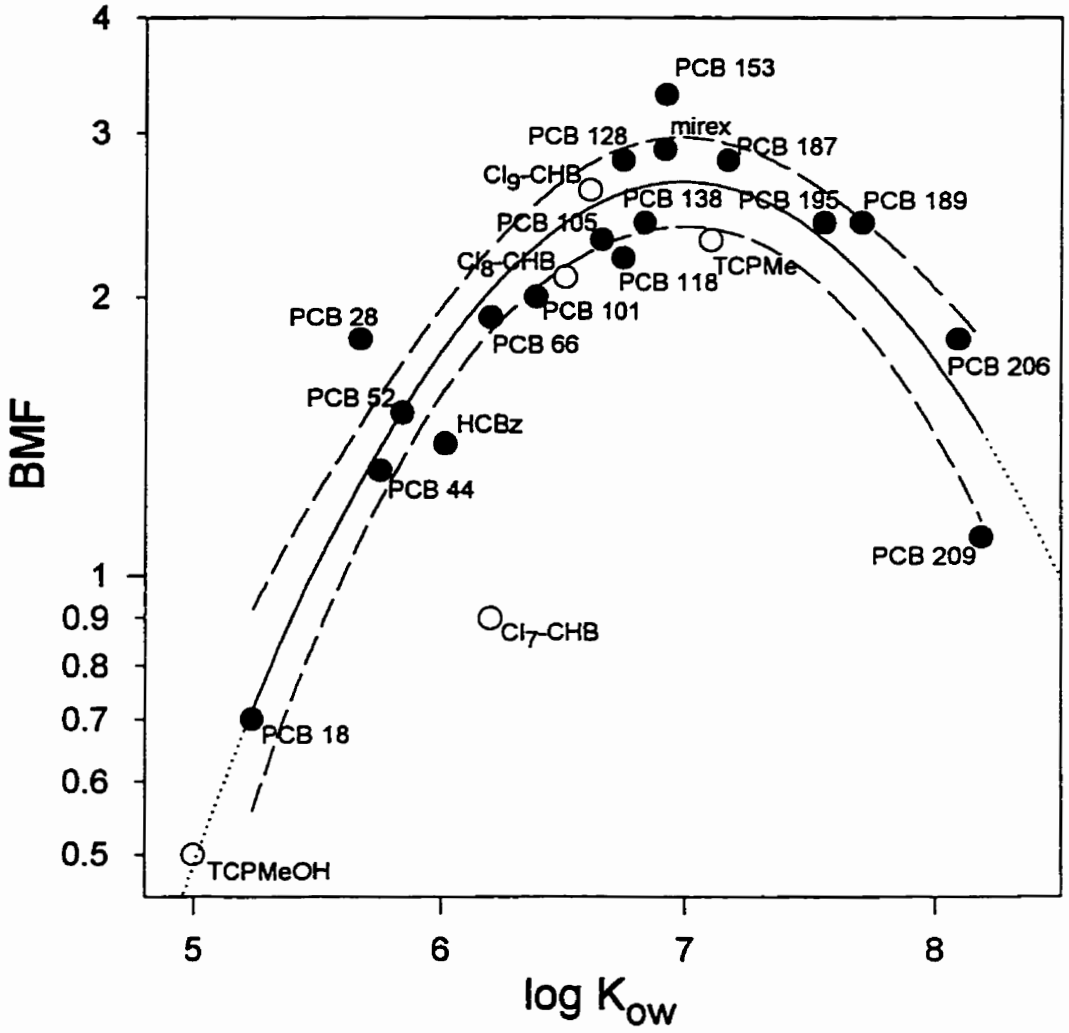

Figure 4.6 Log BMF of hydrophobic OCs in juvenile rainbow trout versus log K_{ow}. Solid circles represent OCs with published K_{ow} values. Open circles represent OCs that do not have published K_{ow} values but were estimated using the K_{ow} fragment constant method Lyman et al. (1982). The quadratic regression (solid line) was derived from the OCs that have published K_{ow} values (closed circles) (log BMF = -8.7 + (2.6 ·· log K_{ow}) - (0.2 ·· log K_{ow}^2), $r^2 = 0.84$, $p < 0.001$). The dashed line represents the 95% confidence intervals, and the dotted line carries the data past the last points to the axis. K_{ow}'s of PCBs were taken from Hawker and Connell (1988), HCBz from Mackay et al. (1982), and mirex from Suntio et al. (1988).

relationship (Bruggeman et al. 1984; Muir et al. 1985; Gobas et al. 1989; Fox et al. 1994; Bremle et al. 1995). The curvilinear BCF-K_{ow} phenomena has been attributed to a number of factors including overestimation of bioavailable water concentrations (or reduced bioavailability) (Bruggeman et al. 1984; Gobas et al. 1989), reduced membrane passage of the larger super-hydrophobic chemicals (Bruggeman et al. 1984; Opperhuizen et al. 1985), inaccurate K_{ow}s (Chessells et al. 1992) and elimination into feces (Gobas et al. 1989). By exposing fish to dietary concentrations we have eliminated any problems associated with water uptake pathways, but the issues of disequilibrium, reduced membrane passage, inaccurate K_{ow}s and elimination into feces remain.

4.4.11 Comparison of estimated K_{ow}'s: Fragment constant vs. bioaccumulation parameter-K_{ow} relationships

Half life-K_{ow} relationships were used to predict the K_{ow} of TCPMe, TCPMEOH, Cl_7-CHB, Cl_8-CHB and Cl_9-CHB. K_{ow}s for TCPMe and TCPMeOH predicted by this relationship agreed with K_{ow}s determined by the fragment constant method (Table 4.4). This suggests that there is little, or no, metabolism of TCPMe or TCPMeOH by the juvenile rainbow trout. K_{ow}s, determined by the fragment constant method for Cl_7-CHB, Cl_8-CHB and Cl_9-CHB are lower than those predicted by the $t_{1/2}$-K_{ow} relationship. It is possible that the fragment constant method overestimated the K_{ow}s of the toxaphene congeners. There is greater uncertainty with fragment constant method for highly hydrophobic compounds (Lyman et al. 1982). Alternatively, these toxaphene congeners are metabolized by the rainbow trout, and therefore the $t_{1/2}$-K_{ow} relationship cannot

Table 4.4: Estimated log K_{ow}'s of TCPMe, TCPMeOH, CL_7-CHB, Cl_8-CHB and Cl_9-CHB using the fragment constant method (Lyman et al. 1982) and bioaccumulation parameter-K_{ow} relationships (Figures 4, 5 and 6). Because the bioaccumulation-K_{ow} relationships are curvilinear 2 estimates have been given when appropriate.

OC	fragment constant method	$t_{1/2}$-K_{ow} relationship	α-K_{ow} relationship	BMF-K_{ow} relationship
TCPMe	7.1	6.8 or 7.6	6.4 or 7.7	6.4 or 7.5
TCPMeOH	5.0	5.0	4.9	5.0
Cl_7-CHB	6.2	5.4	5.4	5.2
Cl_8-CHB	6.5	5.8	6.7[a]	6.9 or 7.1
Cl_9-CHB	6.6	5.8	6.7[a]	6.3 or 7.7

[a] - The assimilation efficiencies of Cl_8-CHB and Cl_9-CHB are > the α-K_{ow} equation and therefore the K_{ow} estimate represents the greatest α.

accurately predict their K_{ow}. Based on a greater distance from the $t_{1/2}$-K_{ow} curve, Cl_7-CHB is more readily metabolized than Cl_8-CHB and Cl_9-CHB.

4.5 Summary and conclusions

We have reported the first data on laboratory-derived bioaccumulation parameters of TCPMe, TCPMeOH, and three toxaphene congeners in juvenile rainbow trout. These compounds have high BMFs confirming observations from field data. Dietary bioaccumulation parameter-K_{ow} relationships derived from 16 recalcitrant OCs revealed that assimilation efficiency, $t_{1/2}$, and BMF all have curvilinear relationships with K_{ow}. Organochlorine compounds that have a log K_{ow} of approximately 7, and are not biotransformed, had the greatest persistence and biomagnification in fish. The curvilinear relationship of $t_{1/2}$ and K_{ow} suggests that internal kinetics of contaminants in fish play a significant role in their fate, and that the length of exposure of super hydrophobic OCs (log $K_{ow} > 7$) will affect their depuration rates and $t_{1/2}$s. Variation in the $t_{1/2}$s of PCB congeners in different size rainbow trout suggests that additional research is needed on the influence of fish size on bioaccumulation parameters. Based on comparison of these data with past studies, the composition of food affects the assimilation of PCB congeners in the digestive tract of fish, but relationships are not clear and require further study.

5.DIETARY ACCUMULATION AND DEPURATION OF INDIVIDUAL C_{10}-, C_{11}- AND C_{14}-POLYCHLORINATED ALKANES BY JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*)

5.1 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 paraffins (CPs) 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, it is reasonable to assume that 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.

5.2 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 a number of other industrial applications (Campbell and McConnell 1980; Government of Canada 1993). Annual global production of CPs is approximately 300 kt, 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 the Canadian Environmental Protection Act (CEPA) 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; Chapter 3). 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. 1993), sewer films from Germany (Reiger and Ballschmiter 1995), biota, sediment and water from Lakes Erie and Winnipeg,

Canada (Fisk et al. 1996; Tomy 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 fully resolved by high resolution, capillary gas chromatography (GC) columns (Tomy 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.

5.3 Methods and Materials

5.3.1 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 (Figure 1). The dominant product of each synthesis was an alkane derived by Cl addition to the double bonds (Figure 1). As well,

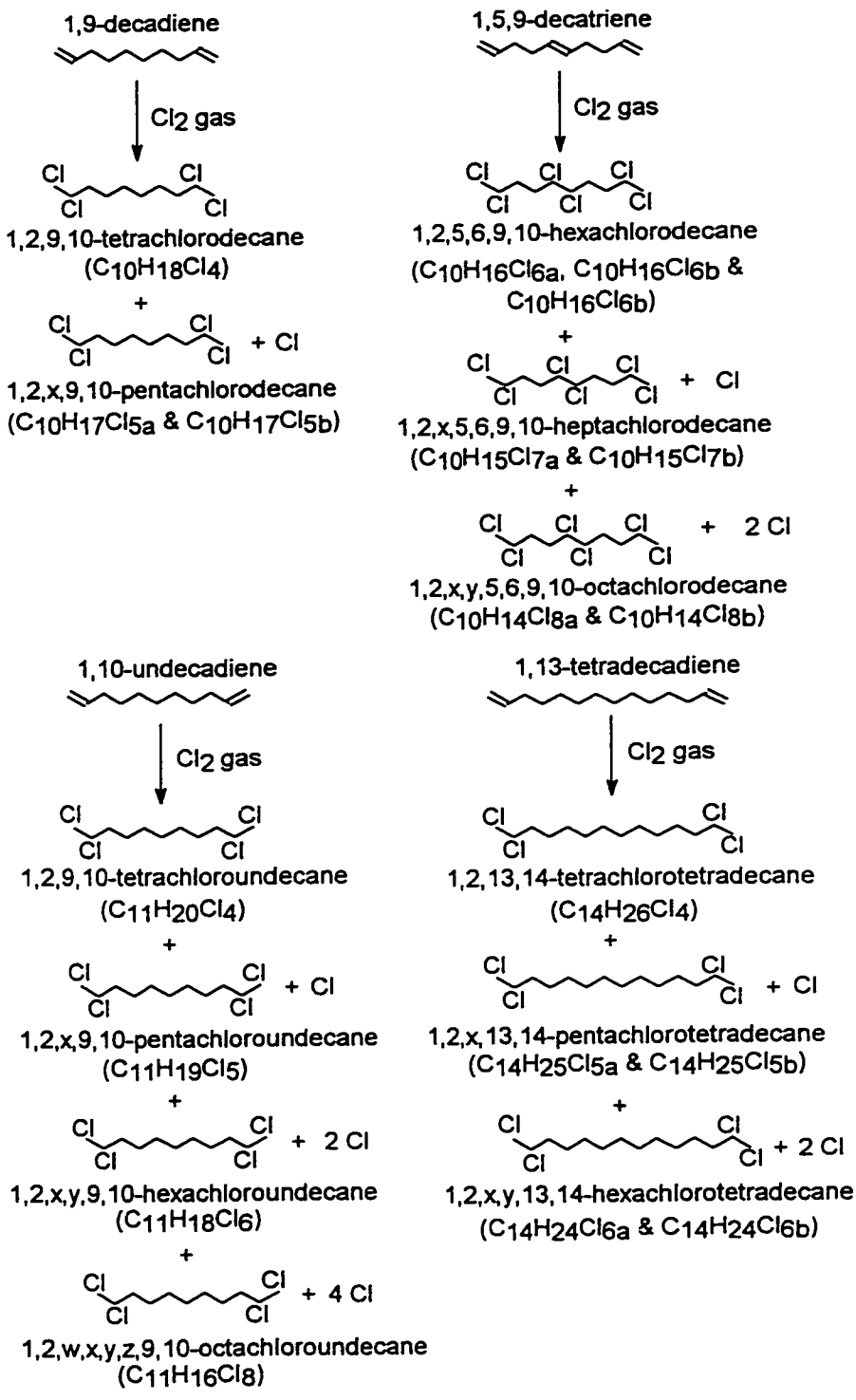

Figure 5.1 Polychlorinated alkanes (PCAs), and their starting material, used for dietary accumulation experiments using juvenile rainbow trout. Letters in the chemical name represents a Cl atom whose position on the alkane is unknown. PCAs with the same molecular formula but different arrangements of Cl atoms are differentiated with a letter.

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 (Figures 5.1 and 5.2). PCAs with the same molecular formula but different Cl substitution patterns have been differentiated with a letter at the end of the chemical formula.

5.3.2 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 are referred to as standard DT (Figure 5.2). The combined products of the 1,5,9-decatriene and the 1,10- undecadiene chlorination are 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 (42% carbohydrates, 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 rainbow trout tissue (Table 5.1). 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, 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

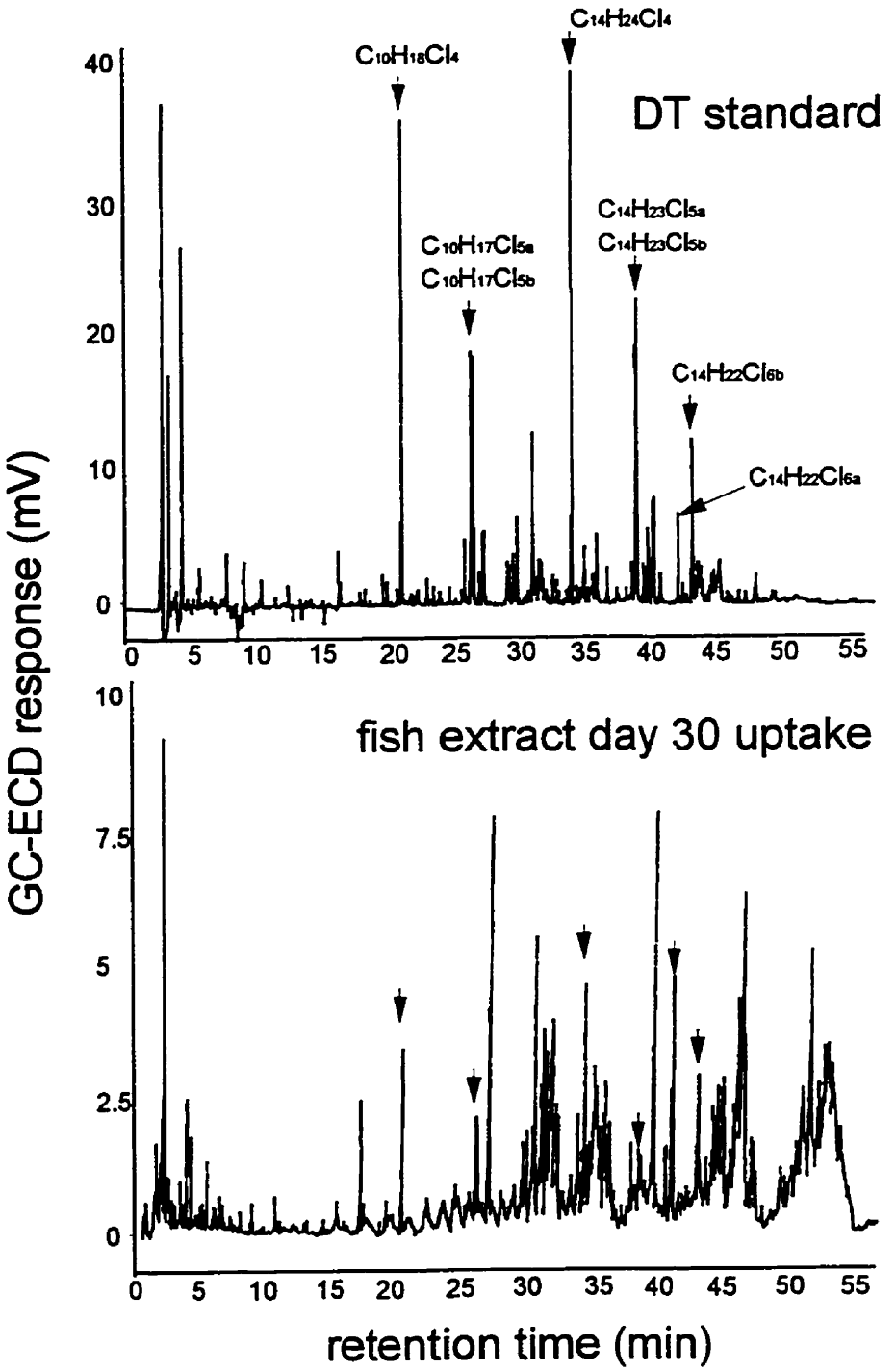

Figure 5.2 GC-ECD chromatograms of the DT standard and fish extract after 30 days exposure to the DT standard in food. Chlorination of the alkene compounds produced PCAs with the same molecular formula but different Cl substitution patterns. These PCAs have been identified with a letter at the end of the chemical formula.

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.

5.3.3 Extraction and Analysis

Extraction of PCAs was identical to the methods used in Chapter 4. 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 μL 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 with an electron capture detector (ECD). Samples were analyzed on a Varian 3600-GC equipped with a 60 m x 0.25 mm DB-5 column and an ^{63}Ni-ECD. The carrier gas was H_2. Responses of individual

PCA compounds were monitored by analyzing DU and DT standards after every 4 samples. The limits of detection were approximately 1 $ng \cdot g^{-1}$.

5.3.4 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):

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

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 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. The Student t test was used to compare pairs of growth rate constants at the $p < 0.05$ level of significance.

5.4 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 in high proportions in commercial CP mixtures because they contain Cl on terminal and adjacent carbons which is chemically and energetically unfavorable under free radical mechanisms used in the production of commercial CP products. Unfortunately, PCA standards with known chlorine content and chlorine position that do not have terminal chlorine substitution, or synthesis methods for such compounds, 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 5.2). Lipid percentages and liver somatic indices were also similar between treatments (Table 5.2). No deaths occurred in any of the treatments.

All of the PCAs were detected in the trout after five days of exposure (Figures 5.3 and 5.4). 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 (Figure 5.3 and 5.4). 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

Table 5.1 Bioaccumulation parameters of chlorinated alkanes from dietary exposures using juvenile rainbow trout. Parameters were calculated from carcass concentrations (3 trout / sampling day), and were corrected for growth dilution and lipid normalized.

compound[q]	log K_{ow}[r]	conc. in food[s] ($ng \cdot g^{-1}$)	dep. length[t] (days)	depuration rate[u] (10^{-2})	half life[v] (days)	assimilation efficiency[w] (%)	BMF_{CALC}[x]	BMF_{EQUIL}[y]	BMF_{SS}[z]
$C_{10}H_{18}Cl_4$	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

[q] - 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.

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

[s] - Concentration in food is given as wet weight.

[t] - 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.

[u] - 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).

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

[w] - The assimilation efficiency (α) (± 1 standard error) is determined by fitting the data to the integrated form of the kinetic rate equation for constant dietary exposure using iterative nonlinear regression: $C_{fish} = (\alpha F C_{food}/k_d)*[1 - \exp(-k_d t)]$ 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.

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

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

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

Table 5.2 Growth parameters of juvenile rainbow trout exposed to mixtures of polychlorinated alkanes (significant differences in growth rates are indicated by capital letters [ANCOVA, $p < 0.05$]).

Treat.	grow rate[a] (10^{-3}/d)	% lipid[b]	LSI[c] day 40	LSI[c] day 120	% mortality
control	16.7 ± 1.8 (0.76)[A]	2.6 ± 0.1	1.6 ± 0.3	1.0 ± 0.1	0
DU	16.4 ± 1.9 (0.73)[A]	3.0 ± 0.2	1.6 ± 0.8	0.9 ± 0.1	0
DT	15.4 ± 1.1 (0.87)[A]	2.7 ± 0.1	1.3 ± 0.1	1.0 ± 0.0	0

[a] - The growth rates (± 1 standard error) were calculated using the equation ln weight = $a + b$ time (d), where b is the growth rate (coefficient of determination for the model is shown in parentheses).

[b] - The percent lipid is an average (± 1 standard error) of all fish in a treatment from day 5 until the end of the experiment. Control does not include day 80 or 120 fish.

[c] - Liver somatic index (LSI) (1 ± standard error).

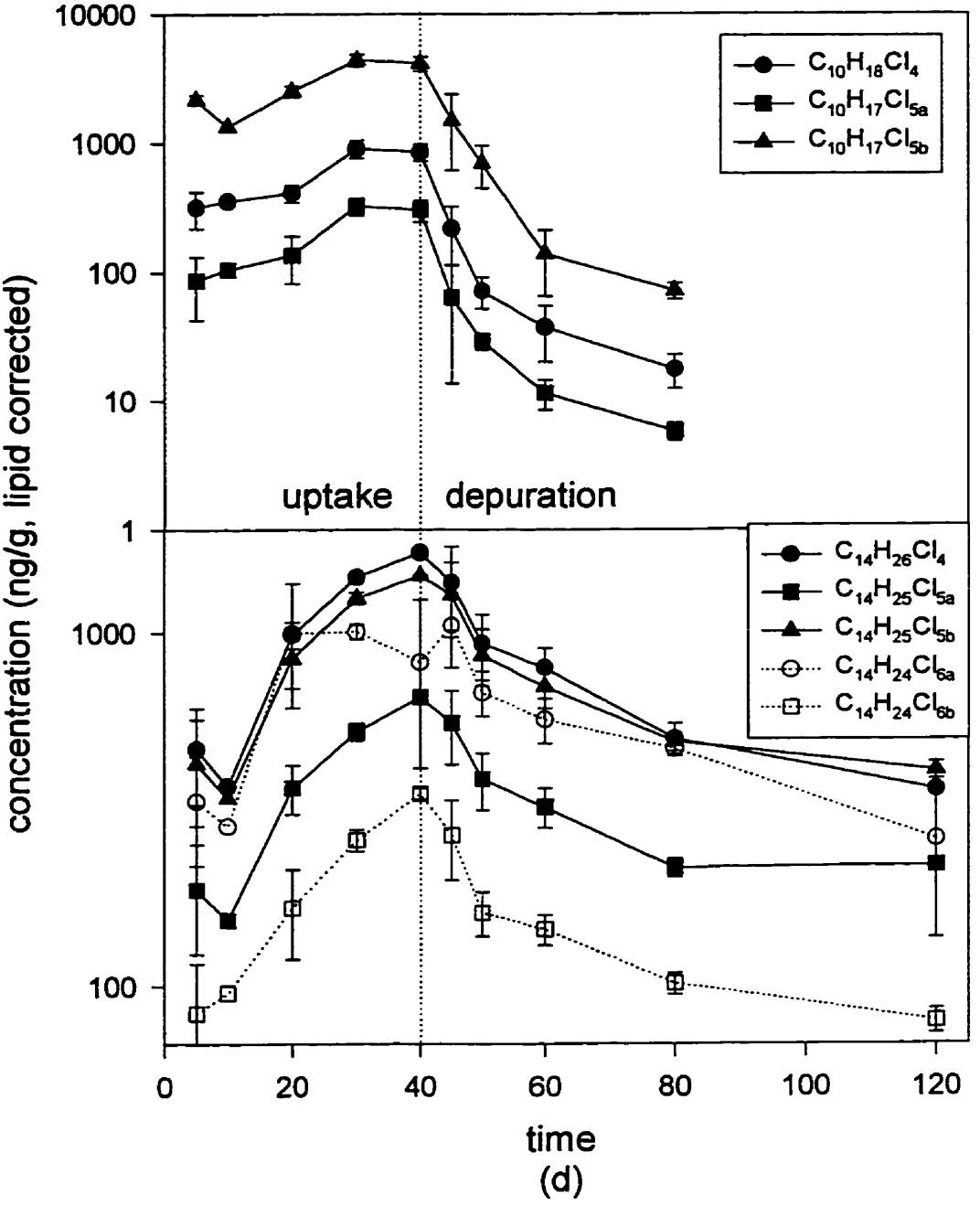

Figure 5.3 Accumulation and depuration of C_{10}- and C_{14}-PCAs through dietary exposure to juvenile rainbow trout. Each point is the mean ± 1 S.E. of three fish. Concentrations are whole fish minus liver and GI tract, corrected for growth dilution and lipid content.

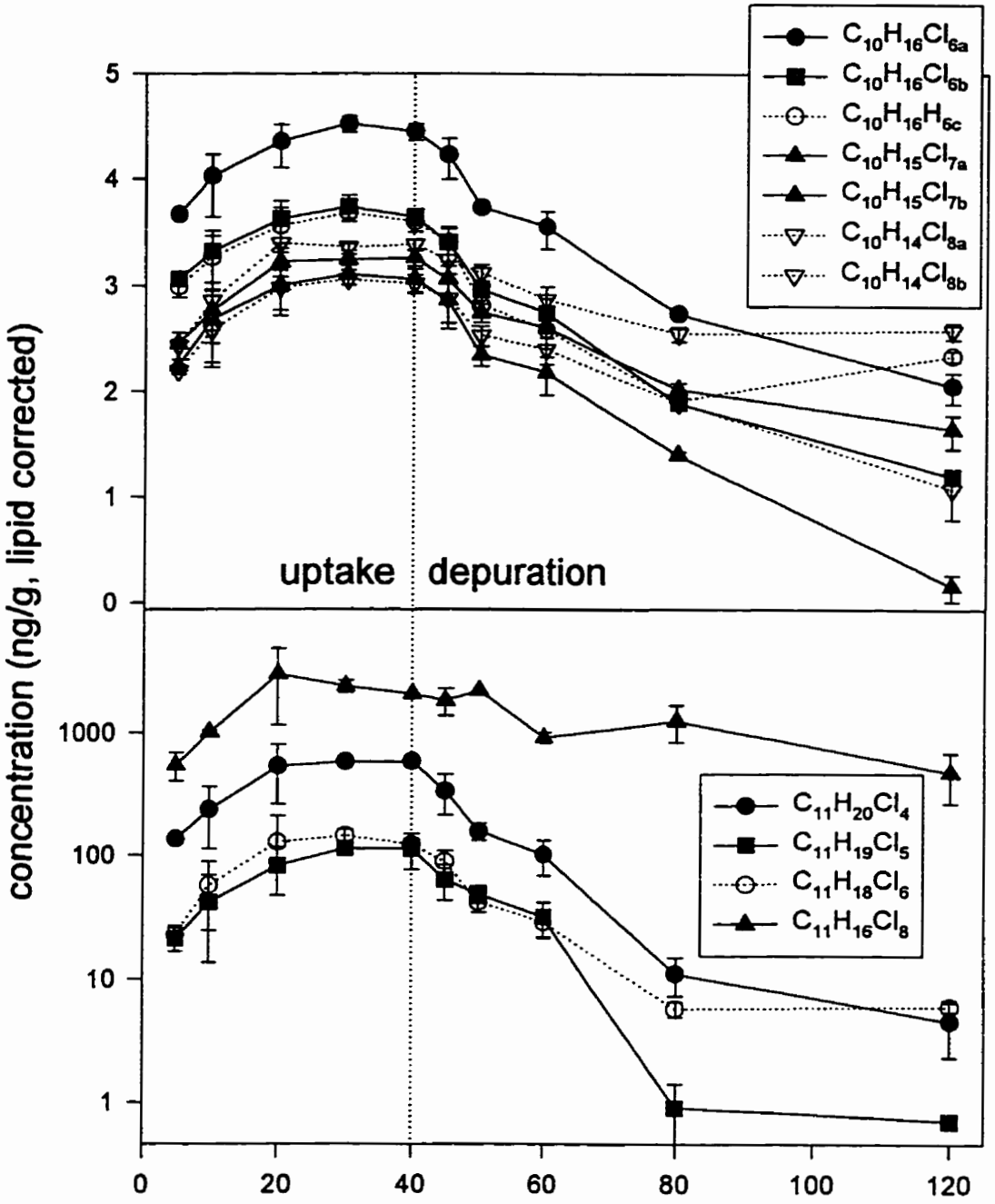

Figure 5.4: Accumulation and depuration of C_{10}- and C_{11}-PCAs through dietary exposure to juvenile rainbow trout. Each point is the mean ± 1 S.E. of three fish. Concentrations are whole fish minus liver and GI tract, corrected for growth dilution and lipid content.

$C_{10}H_{15}Cl_{7b}$) 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 were quite similar because they were synthesized from diene or triene starting material (Figure 5.1). The positions of the free radical substituted Cl are unknown, so that it was not possible 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 in 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 (Chapter 4), 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 in 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; Chapter 3).

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}), 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 physical-chemical properties and/or susceptibility to metabolism. Unfortunately, there are no data on the influence of Cl position of 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 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 5.1). 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 (Chapter 4). For a majority of the PCAs with assimilation efficiencies > 100%, the BMF_{SS} are in agreement with BMF_{CALC} (Table 5.1). However, assimilation efficiencies of persistent organochlorines of similar molecular size and hydrophobicity as PCAs are believed to be approximately 50% (Gobas et al. 1989; chapter 4). 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). Overestimation 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 to 100%).

BMF_{EQUIL} were calculated using assimilation efficiencies, and therefore were also confounded by 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 5.1). 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 5.1), which implies a potential to biomagnify in aquatic food chain.

Half lives of PCAs were correlated with K_{ow} and carbon chain length but not chlorine number based on simple linear regression (Table 5.3; Figure 5.5). 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 5.3). Therefore, $t_{1/2}$s of PCA increase with increasing carbon chain length and chlorine content. Carbon chain length and chlorine number did not improve the variation in $t_{1/2}$ explained by K_{ow} alone (Table 5.3). 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 1996).

A significant linear relationship was also found between BMF_{CALC} for PCAs and log K_{ow} and carbon chain length (Figure 5.6a,b and Table 5.3). BMF_{CALC} was not significantly correlated with chlorine number (Figure 5.6c and Table 5.3). 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; chapter 4). Multiple

Table 5.3 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
	# Cl		-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		

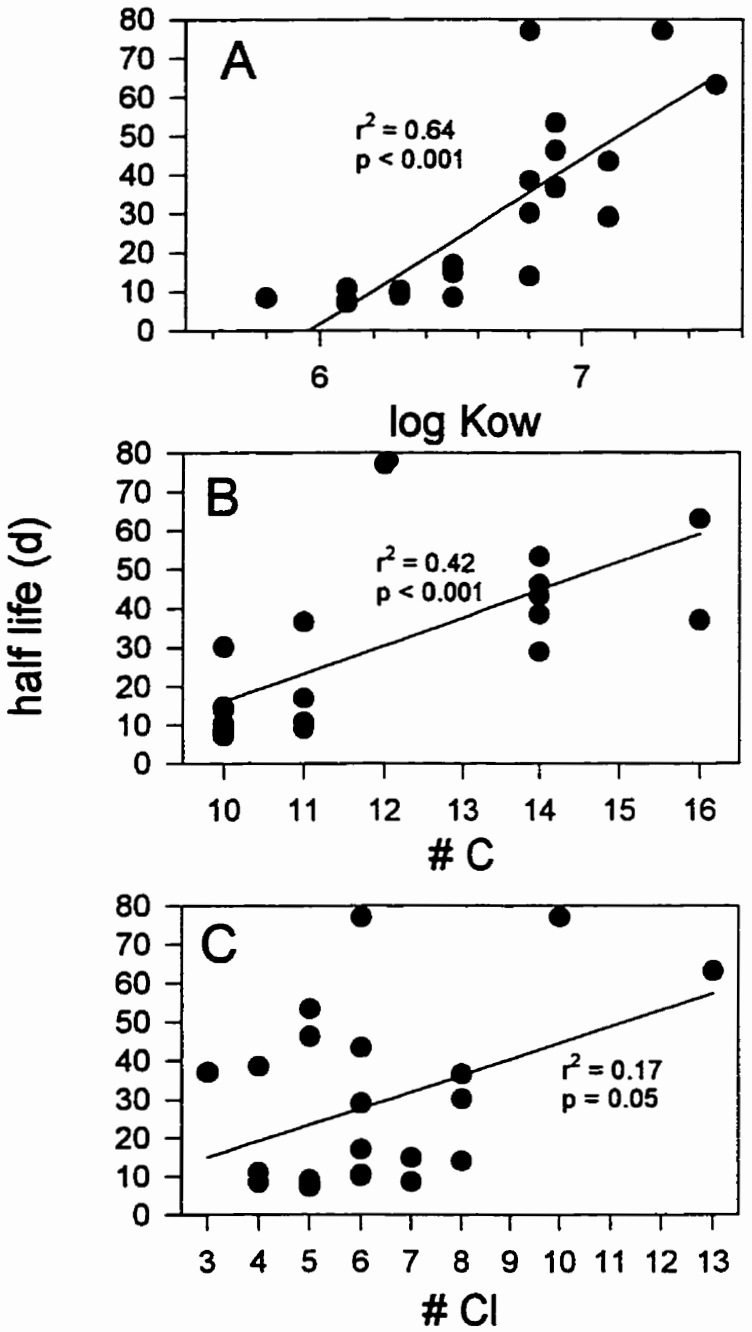

Figure 5.5: Relationships between PCA $t_{1/2}$ with K_{ow} (a), carbon number (b) and chlorine number (c). Solid lines are linear regressions (Table 5.3). Data includes all data from this work and from other free-radically chlorinated C_{12}- (6 and 10 Cl) and C_{16}-(3 and 13 Cl) PCAs (chapter 3).

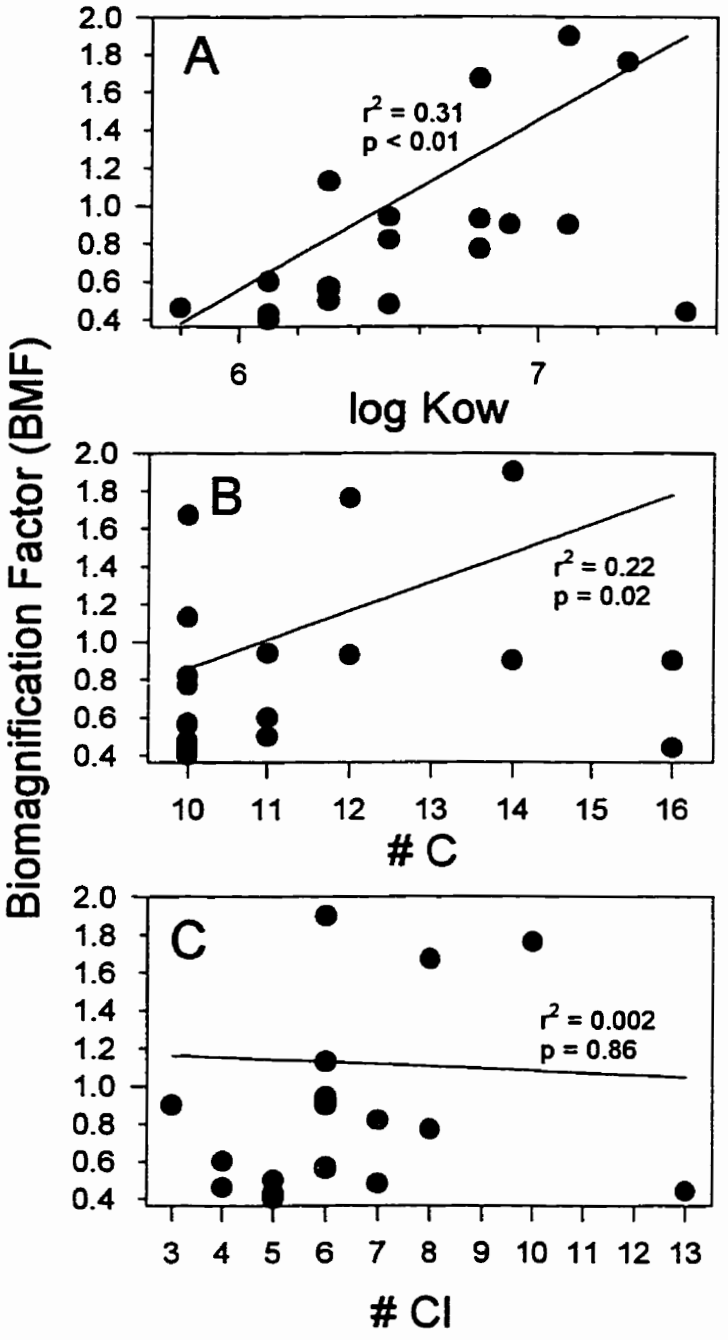

Figure 5.6 Relationships between PCA BMF_{EST} with K_{ow} (a), carbon number (b) and chlorine number (c). Solid lines are linear regressions (Table 5.3). Data includes all data from this work and from other free-radically chlorinated C_{12}- (6 and 10 Cl) and C_{16}-(3 and 13 Cl) PCAs (chapter 3).

regression using carbon chain length and chlorine number did not improve the variation in BMF explained by carbon chain length alone (Table 5.3). 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 5.3). 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 5.3). These results suggest that the K_{ow}, carbon chain length and chlorine number all play a role in the bioaccumulation, or biomagnification, of PCAs.

5.5 Summary and 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 increased with increasing K_{ow} and carbon chain length. Chlorine content did not, as a single variable, have a significant affect on $t_{1/2}$. There was indirect evidence for metabolism based on the fact that $t_{1/2}$s were shorter than for persistent OCs 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. Chlorine content did not, as a single variable, have a significant affect on BMF.

6. ACCUMULATION AND DEPURATION OF SEDIMENT-SORBED C_{12}- AND C_{16}-POLYCHLORINATED ALKANES BY OLIGOCHAETES (*Lumbriculus variegatus*)

6.1 Abstract

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

benthic invertebrates due to differences in the bioaccumulation and degradation of PCAs of varying carbon-chain-length and chlorine content.

6.2 Introduction

Chlorinated paraffins (CPs) are a class of polychlorinated *n*-alkanes (PCAs) that are used as plasticizers, flame retardants, high pressure lubricants and for a number of other industrial applications (Campbell and McConnell 1980; Government of Canada 1993). CPs have carbon chain lengths between 10 and 30 and with chlorine content varying from 35 to 70% by weight. Each commercial CP product has thousands of different compounds and isomers (Tomy et al. 1998). The global production of CPs has been estimated at over 300 kilotonnes/y, with a majority having medium carbon-chain lengths (C_{14-18}) (Government of Canada 1993).

CPs are very hydrophobic (K_{ow} > 5.0) Sijm and Sinnige 1995), and are bioaccumulated from water and food by fish in laboratory experiments (Zitko 1974; Lombardo et al. 1975; Bengtsson et al. 1979; Bengtsson and Ofstad 1982; Chapter 3). Although the amount of data is limited, CPs have been quantified in environmental samples (Tomy et al. 1997b; Jansson et al. 1993; Rieger and Ballschmiter 1995; Fisk et al. 1996). Fisk et al. (1996) found that concentrations of short chain CPs (60-70% Cl) were similar to polychlorinated biphenyls (PCBs) in sediment and zebra mussel collected from the Detroit River and the western basin of Lake Erie. Jansson et al. (1993) reported that CPs (C_{10-13}; 60% Cl) were the predominant organochlorine in a representative set of terrestrial and aquatic organisms from Sweden.

In aquatic systems, compounds of low aqueous solubility partition to a large extent onto suspended and bottom sediment. Once bound to sediment, compounds may not be available for direct uptake by fish and other non-filtering aquatic invertebrates. Sediment-bound compounds can re-enter the food chain through benthic organisms via interstitial water or by consumption of sediments. Therefore, the bioavailability of these sediment-sorbed chemicals controls their fate and exposure to organisms in aquatic ecosystems. For example, concentrations of organochlorines in Lake Ontario sculpin are thought to be derived from sediment via the benthic food chain (Thomann et al. 1992).

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

6.3 MATERIALS AND METHODS

6.3.1 Chemicals, Sediment and Sediment Spiking

The four ^{14}C-polychlorinated alkanes (PCAs) consist of two C_{12}- and two C_{16}-alkanes which were synthesized in a different manner from commercial CPs (Bergman et al. 1981). However, GCMS analysis shows these ^{14}C-products contain a similar range of components to those in commercial products (G.T. Tomy, personal communication). The

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

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

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

6.3.2 Experiment

Oligochaetes (*Lumbriculus variegatus*) were exposed to two concentrations (Table 6.1) of each ^{14}C-PCA in separate experiments (only one concentration was established for the $C_{16}H_{21}Cl_{13}$). One control experiment was performed with untreated sediment.

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

6.3.3 Analysis of ^{14}C in oligochaetes

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

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

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

Sediment samples were collected on days 0 and 14 of the uptake phase for analysis of ^{14}C. Sediment was frozen and freeze dried, and ^{14}C was determined by three methods. The toluene sonication method involved measuring a known quantity of freeze dried sediment (~0.5 g) into a test tube with 3 ml of toluene and sonicating for 30 min. After allowing the sediment and toluene to stand for 24 h, 1 ml of toluene was counted by LSC. The second method used Soxhlet extraction of freeze dried sediment (~ 1.0 g) with 250 ml of dichloromethane (DCM) for 16 h. The 250 ml of DCM was roto-evaporated and transferred to approximately 1 ml of toluene and counted on the LSC. The third method involved direct combustion of a sample of unextracted freeze dried sediment (~ 0.2 g) on the oxidizer. Recovery of PCAs, after spiking freeze dried sediment (n = 3) and using the toluene sonication method, ranged from 82 to 97%. The organic carbon content of the sediments from each of the treatments was determined by high temperature combustion with detection of CO_2 by thermal conductance, corrected for $CaCO_3$ (Stainton et al. 1977). The sediment size fractions of the sediment used for the C_{12}- and C_{16}-PCA exposures was determined by the second reading hydrometer method (Carter 1993).

A portion of the sediment was centrifuged at 10000 rpm for 30 min to collect interstitial water for determination of PCA concentrations. To obtain PCA concentrations in interstitial water, 1 ml of the interstitial water was counted. A second millilitre of interstitial water was eluted through a C_{18} Sep-Pak (Waters Division of Millipore, Milford, MA, USA) to determine freely dissolved and dissolved organic carbon-bound concentrations of the PCAs (Landrum et al. 1984). The Sep-Pak was then eluted with 15 ml of hexane to give the free dissolved concentrations of the PCA.

6.3.5 HPLC analysis of sediment and oligochaete extracts

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

6.3.6 K_{ow} and K_{oc} Determinations

K_{ow}s of the four PCAs were determined experimentally using RP-HPLC. Methods have been described in detail previously (Sarna et al. 1984). HPLC was carried out with a Waters model 6000A pump, a U6-K injector, a model 490 multi-wavelength UV detector, a Whatman Partisil 10 µm ODS-3 C_{18} column (Waters Division of Millipore), using a

methanol-water mixture for the mobile phase. A LKB 2111 Multirac Fraction collector was used to collect radio-labeled fractions of the PCAs.

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

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

$$K_{oc} = K_p / f_{oc},$$

where $K_p = C_{sediment}$ ($ng \cdot g^{-1}$, dry weight) / $C_{interstitial\ water}$ ($ng \cdot ml^{-1}$) and f_{oc} is the fraction of organic carbon in the sediment.

6.3.7 Data Analysis

Growth rates were determined by fitting oligochaete weight data (total wet weight of oligochaetes in one jar) to an exponential model (ln oligochaete weight = $a + b \cdot$ time (d); where a is a constant and b is the growth rate). All concentrations determined for oligochaetes were lipid normalized and corrected for growth dilution for bioaccumulation parameter calculations. Only toluene extractable concentration data was used for bioaccumulation parameter calculation. Depuration rates (k_2) were determined by fitting the depuration phase data to a first-order decay curve (ln conc = $a + b \cdot$ time (d), where a is a constant and b is the depuration rate). Uptake rates (k_1), which represent uptake from

interstitial water and through ingestion of sediment, were calculated by fitting the uptake phase concentrations to the integrated form of a first order uptake model (Bruggeman et al. 1981) for constant exposure to sediment-sorbed contaminant using non-linear regression:

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

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

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

6.4 Results

6.4.1 Organic carbon content of sediments

Although the sediment used for all exposures came from a single source, differences in organic carbon content were found between C_{12}- and C_{16}-PCA exposures (Table 6.1). All spiking procedures and times were kept consistent between treatments; however, the C_{12}-PCA experiments were carried out prior to the C_{16}-PCA experiments. This procedure resulted in a greater percentage of sand in the C_{16}-PCA experiment (76%

sand, 21% silt, 3% clay) than in the C_{12}-PCA experiments (40% sand, 58% silt, 2% clay), and probably explains the lower organic carbon levels in the C_{16}-PCA experiments.

6.4.2 Oligochaete recovery, growth and lipids

Recovery of oligochaete populations ranged from 97 to 120% for all the treatments (mean of all sampling dates). Recoveries greater than 100% suggest that the oligochaetes were reproducing. Growth rates ranged from 0.006 ± 0.003 to 0.014 ± 0.007 d^{-1} (mean ± 1 S.E.), but r^2 were low (0.03 to 0.43) and there was no significant growth in some treatments. Lipid content of the oligochaetes declined throughout the experiments but increased slightly upon transfer to new sediments at the beginning of the depuration phase (Figure 6.1). Lipid percentages were greater in the oligochaetes exposed to C_{12}-PCAs than those exposed to the C_{16}-PCAs (Table 6.1).

6.4.3 Accumulation of ^{14}C-PCA

All PCAs were detectable in oligochaetes after one day of exposure to spiked sediment (Figure 6.1). The uptake rate of $C_{12}H_{20}Cl_6$ was the highest among the PCAs while that of $C_{16}H_{21}Cl_{13}$ was the lowest (Table 6.1). Concentrations of $C_{16}H_{21}Cl_{13}$ in the oligochaetes decreased rapidly during the first day of depuration. This has been observed with other very hydrophobic compounds in similar sediment bioavailability tests (Loonen et al. 1997), and is likely due to high concentrations of the $C_{16}H_{21}Cl_{13}$ bound to sediment in the GI tract of the oligochaetes which are not absorbed and are excreted during the depuration phase. Depuration rates for $C_{16}H_{21}Cl_{13}$ have been calculated with data from day 2 to 56 only. Depuration data for the $C_{16}H_{31}Cl_3$ high exposure did not have a significant

Table 6.1 Bioaccumulation parameters of four sediment-sorbed ^{14}C-PCAs for the oligochaete (*Lumbriculus variegatus*) (significant differences ($p < 0.05$) between depuration rates are indicated with capital letters).

Chemical	conc.[a] (ng·g^{-1})	log K_{ow}[b]	o. c.[c] (%)	lipids[d] (%)	uptake rate[e] (* 10^{-2} g·g^{-1}·d^{-1})	depuration rate[f] (* 10^{-2} d^{-1})	half-life[g] (d)	kinetic[h] BSAF
$C_{12}H_{20}Cl_6$	26.5	6.2	2.3	3.7 ± 0.6	22 ± 2.1 (0.77)	5.0 ± 0.7 (0.75)^{A}	14 ± 2.0	4.4
	106		3.8	2.9 ± 0.3	51 ± 11 (0.38)	5.6 ± 0.9 (0.71)^{A}	12 ± 1.9	9.1
$C_{12}H_{16}Cl_{10}$	124	6.6	3.6	3.6 ± 0.8	9.0 ± 0.8 (0.77)	4.8 ± 1.1 (0.50)^{A}	14 ± 3.2	1.9
	442		3.1	3.4 ± 0.8	11 ± 1.2 (0.69)	5.8 ± 0.8 (0.75)^{A}	12 ± 1.7	1.9
$C_{16}H_{31}Cl_3$	47.1	7.2	1.4	2.5 ± 0.3	9.3 ± 1.0 (0.68)	2.1 ± 0.7 (0.32)^{BC}	33 ± 11	4.4
	135[i]		1.3	2.3 ± 0.2	7.6 ± 0.7 (0.71)	0.73 ± 0.5 (0.10)^{B}	95 ± 65	10
$C_{16}H_{21}Cl_{13}$	264	7.4	1.5	2.0 ± 0.2	1.3 ± 0.1 (0.84)	2.3 ± 0.6 (0.51)^{C}	30 ± 7.8	0.6

[a] Sediment concentrations are dry weight.

[b] K_{ow} values for $C_{12}H_{20}Cl_6$ and $C_{16}H_{31}Cl_3$ are a mean of 2 and 4 values determined by HPLC, respectively.

[c] Organic carbon content are for dry weight of sediment.

[d] Lipids percentage determined as the mean of all samples.

[e] Uptake rate constants (k_1) (± 1 standard error) (coefficient of determination is shown in parentheses) were determined by fitting the data to the integrated form of the kinetic rate equation for constant uptake from sediment using iterative nonlinear regression: $C_{olgio} = (C_{sed}k_1/k_2)\cdot(1-\exp(-k_2t))$, where C_{olgio} is the lipid and growth corrected concentration in the olgiochaetes, C_{sed} is the organic carbon normalized concentration in the sediment corrected for the loss of toluene extractable ^{14}C, k_2 is the depuration rate, and t is the time in days.

[f] Depuration rate constants (k_2) (± 1 standard error) were calculated using the model ln concentration = $a + b$ (time) were b is the depuration rate (coefficient of determination for the model is shown in parentheses). Depuration rates for $C_{16}H_{21}Cl_{13}$ have been calculated from data for day 2 to 56 only.

[g] Half life (± 1 standard error) is calculated from the equation $t_{1/2} = 0.693/k_2$.

[h] The kinetic biota sediment accumulation factor (BSAF) were determined from the equation BSAF = k_1/k_2.

[i] The depuration data for this treatment did not have a significant linear relationship with time.

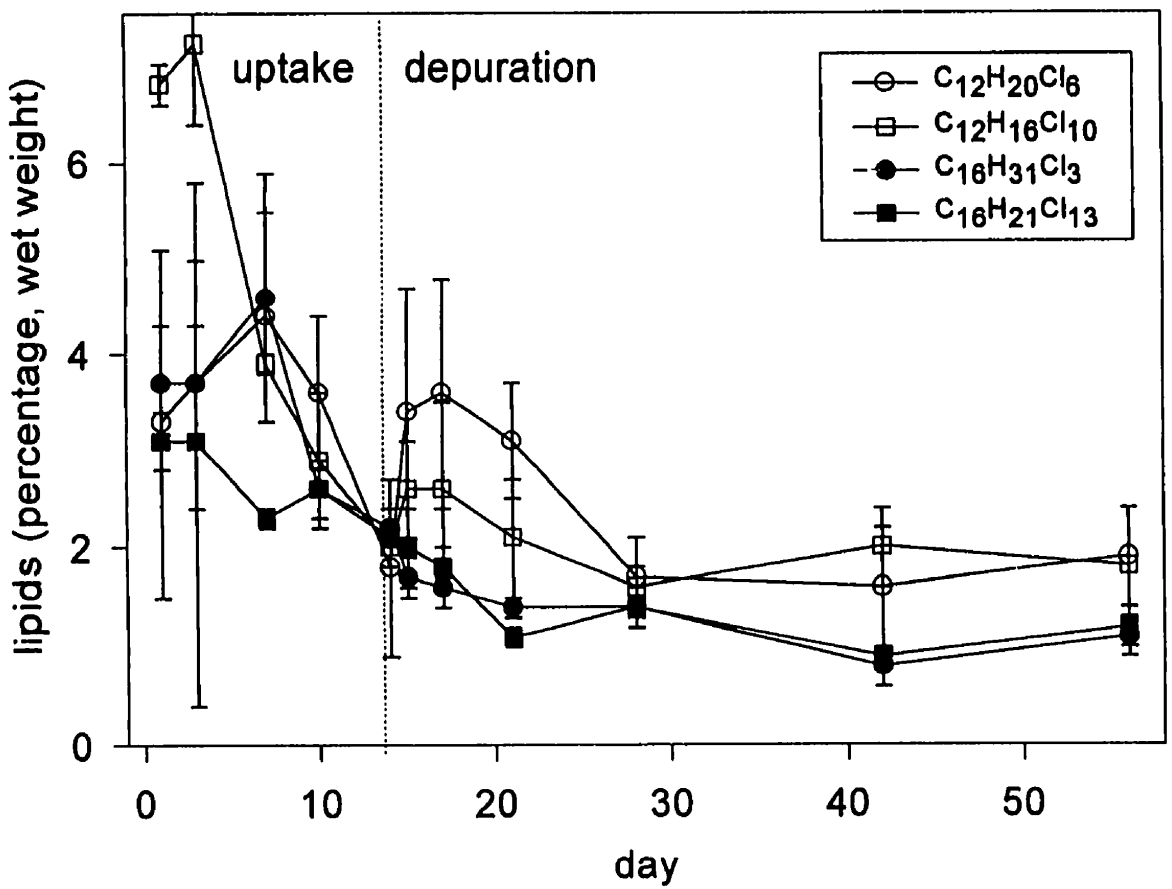

Figure 6.1 Lipid percentages (wet weight) of oligochaetes exposed to four ^{14}C- polychlorinated alkanes. Each point is the mean lipid percentage ± 1 standard error of three samples of oligochaetes (~15 individuals). Data from the high concentration exposures were used.

linear relationship with time, and parameters for this treatment should be viewed with caution. With the exception of the $C_{16}H_{31}Cl_3$ high exposure and the $C_{16}H_{21}Cl_{13}$ exposure, no differences in depuration rates were observed for PCAs of the same carbon chain length; however, the C_{12}-PCAs were found to have statistically significant greater depuration rates than the C_{16}-PCAs (Table 6.1). Accordingly, half lives ($t_{1/2}$) of the C_{12}-PCAs were about half those of the C_{16}-PCAs ($t_{1/2}$ 12-14 and 30-95 d, respectively).

C_{12}-PCAs did not achieve equilibrium between oligochaete and sediment after 14 d of exposure (Figure 6.1), and therefore no steady state $BSAF_{ss}$ could be calculated. The kinetic $BSAF_{kin}$ for $C_1H_{20}Cl_6$ ranged from 4.4 to 12, and for $C_{12}H_{16}Cl_{10}$ was 1.9 (Table 6.1). After 21 days' exposure the C_{16}-PCAs appeared to have achieved steady state between sediment and oligochaete (Figure 6.2). However, the $BSAF_{ss}$ for these compounds were less than half the $BSAF_{kin}$ (Table 6.1). $C_{16}H_{31}Cl_3$ had BSAFs of between 0.7 (steady state) and 4.4-10 (kinetic). $C_{16}H_{21}Cl_{13}$ had BSAF values much less than one (0.2 to 0.6, steady state and kinetic BSAF, respectively).

6.4.4 HPLC of oligochaete and sediment extracts

HPLC chromatograms of toluene extracts of sediment and oligochaetes on day 14 of the uptake phase showed similar patterns of ^{14}C-PCA in oligochaete extracts and the PCA standards (Figure 6.3). The chromatograms of the extracts from oligochaete exposed to $C_{16}H_{21}Cl_{13}$ varied from the sediment and standard. However, concentrations of ^{14}C in these oligochaetes were below detection limits of ^{14}C in most HPLC fractions. These results suggest that toluene extracted the parent PCA compounds but little of the transformed compounds (see discussion).

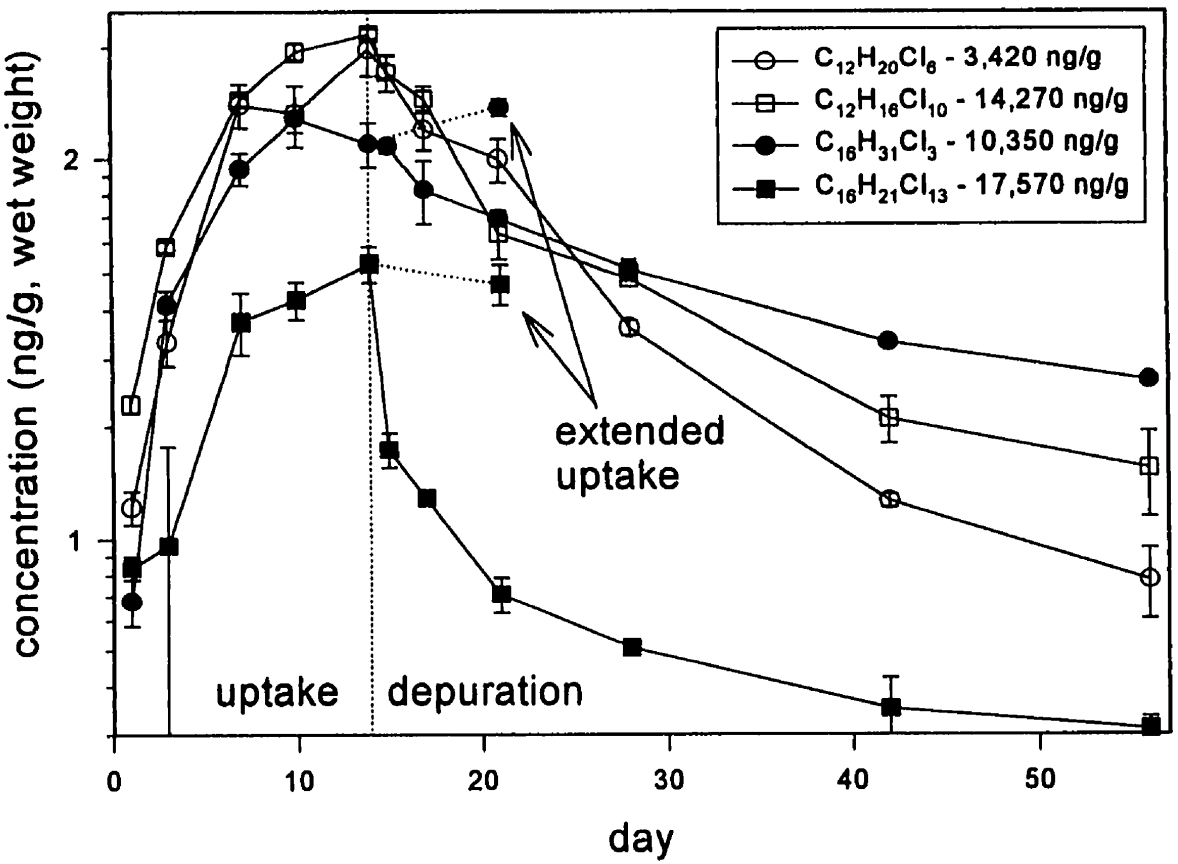

Figure 6.2 Accumulation and depuration of four sediment-sorbed ^{14}C- polychlorinated alkanes from the highest exposure treatments. Concentrations in legend represent the sediment concentrations (organic carbon corrected). Each point is the mean concentration (wet weight) ± 1 standard error of three oligochaetes.

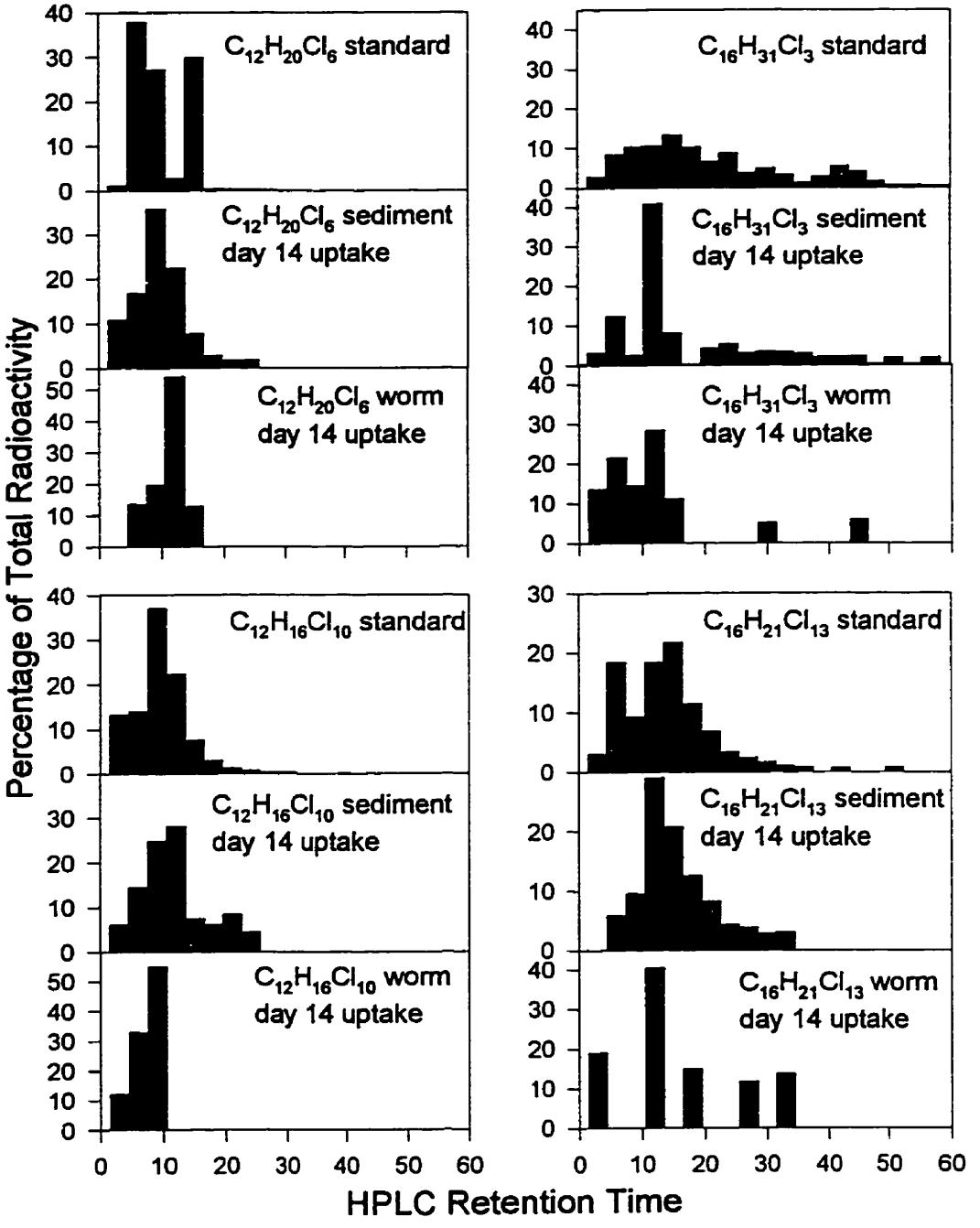

Figure 6.3 High-performance liquid chromatography chromatograms of the ^{14}C-polychlorinated alkane standards and sediment and oligochaete toluene extracts from day 14 of the uptake phase. Each bar represents the radioactivity in a 4-min fraction as a percentage of the total radioactivity.

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

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

$$\log K_{ow} = 3.13 \cdot \log R_t + 2.80.$$

The equation and fit of the line ($r^2 = 0.96$) are comparable to previous K_{ow} determinations by HPLC (Sarna et al. 1984). The PCA standards had broad HPLC peaks because they are synthesized in a manner which does not produce a single compound but a series of compounds with similar chlorine content and numerous positional isomers. Four peaks were resolved for the $C_{16}H_{31}Cl_3$ resulting in a number of K_{ow} estimates (Table 6.2). A majority of the radioactivity for this PCA was found in the second peak which corresponds to a mean K_{ow} value of 7.2 (Table 6.2). Log K_{ow}s of the PCAs determined by this equation ranged from 5.0 to 8.2, increasing with greater carbon-chain-length and chlorine content. K_{ow}s of the PCAs determined using equations developed by Sijm and Sinnige (1995) are in general agreement with the HPLC-derived K_{ow}s of the C_{16}-PCAs, but are higher than the C_{12}-PCA HPLC-derived K_{ow}s (Table 6.2).

Log K_{oc}s of PCAs ranged from 4.1 to 5.2, and as with K_{ow}, increased with greater carbon-chain-length and chlorine content (Table 6.3). Drouillard (1996), using gas sparging methods, reported log K_{oc} values of 4.6 to 6.0 for the same ^{14}C-$C_{12}H_{20}Cl_6$ used in this experiment. This suggests that our methods may have underestimated the true K_{oc} value of these compounds or that measured K_{oc} values are lower at higher sediment to water ratios. There are no other published data on K_{oc}s of PCAs.

Table 6.2 Log K_{ow} values for the ^{14}C-PCA determined experimentally by reverse-phase HPLC and using the equation of Sijm and Sinnige (1995). PCAs produced broad HPLC peaks and estimates of K_{ow}. Mean values represent the mean retention time of the peak. Multiple estimates for $C_{16}H_{31}Cl_3$ represent resolved peaks.

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

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

[b] Estimated-log K_{ow} determined using the equation $\log K_{ow} = -0.386 + 0.600 \cdot N_{tot} - 0.0113 \cdot N_{tot}^2$, where N_{tot} is the total number of carbon and chlorine atoms (Sijm and Sinnige 1995).

Table 6.3 Sediment ($ng \cdot g^{-1}$ dry sediment) and interstitial water ($ng \cdot ml^{-1}$) concentrations (mean ± 1 S.E.), sediment sorption partition coefficients ($K_p = C_{sediment, dw} / C_{water}$) and organic carbon-water partition coefficients ($K_{oc} = K_p / f_{oc}$; f_{oc} is the fraction organic carbon in sediment) of ^{14}C-chlorinated alkanes on day 14 of the uptake phase.

compound	sediment ($ng \cdot g^{-1}$, dw)	total interstitial water[a] ($ng \cdot ml^{-1}$)	DOC and POC[a] ($ng \cdot ml^{-1}$)	freely dissolved[a] ($ng \cdot ml^{-1}$)	K_p	log K_{oc}
$C_{12}H_{20}Cl_6$	107 ± 1.8	3.0 ± 0.1	1.5 ± 0.1	0.2 ± 0.03	535	4.1
$C_{12}H_{16}Cl_{10}$	442 ± 19	1.1 ± 0.03	0.3 ± 0.1	0.3 ± 0.002	1473	4.7
$C_{16}H_{31}Cl_3$	135 ± 4.6	2.4 ± 0.1	1.3 ± 0.1	0.1 ± 0.03	1350	5.0
$C_{16}H_{21}Cl_{13}$	263 ± 5.1	0.8 ± 0.1	0.3 ± 0.04	0.1 ± 0.02	2630	5.2

[a] A portion of the sediment was centrifuged at 10000 rpm for 30 minutes to collect interstitial water for determination of PCA concentrations. To obtain total interstitial water PCA concentrations, 1 ml of the interstitial water was counted. A second mL of interstitial water was eluted through a C_{18} Sep-Pak to determine freely dissolved and dissolved organic carbon concentrations of the chlorinated alkanes (Landrum et al. 1984). The Sep-Pak was then eluted with 15 mL of hexane to give the free dissolved concentrations of the PCA.

6.4.6 Extractable and non-extractable ^{14}C

Concentrations of PCAs in sediment determined by toluene sonication and Soxhlet extraction with DCM showed good agreement for all treatments (Table 6.4). However, concentrations of chlorinated alkanes determined by oxidation were 1.6 to 4.4 times higher than those determined by sonication or Soxhlet extraction (Table 6.4). For the lower chlorinated alkanes, $C_{12}H_{20}Cl_6$ and $C_{16}H_{31}Cl_3$, the extractable ^{14}C accounted for only 23-25 and 32-40% of the total ^{14}C, respectively. For the higher chlorinated alkanes, $C_{12}H_{16}Cl_{10}$ and $C_{16}H_{21}Cl_{13}$, the extractable ^{14}C accounted for 63-64 and 58% of the total ^{14}C, respectively. Extractable/non-extractable ratios in sediment were similar to those observed in the oligochaetes during the uptake phase (Figure 6.4).

6.5 Discussion

Accumulation of C_{12} (56 and 69% Cl, by weight) and C_{16} (35 and 69% Cl) PCAs from sediments by oligochaetes was strongly influenced by carbon length, chlorine content, and K_{ow} (or K_{oc}). Therefore, relative abundance of individual components of CP mixtures in benthic invertebrates would be expected to be different from those observed in sediment or commercial products. Oliver (1984) and Wood et al. (1997) have reported changes in relative abundance of polychlorinated biphenyls (PCBs) congeners and polycyclic aromatic hydrocarbons between sediment and benthic invertebrates due to differences in bioavailability and biotransformation rates of individual compounds.

Lipid content of the oligochaetes varied between treatments and decreased in all treatments over the course of the experiments. Therefore, all data were lipid corrected prior to parameter calculations. Unfortunately this introduced error to all the parameters,

Table 6.4 Sediment concentrations (mean ± 1 S.E., n = 3) of ^{14}C-polychlorinated alkanes determined by toluene sonication, Soxhlet extraction and oxidation. Sediment was collected on day 14 of the uptake phase.

compound	toluene ext. conc. ($ng \cdot g^{-1}$, dry wt.)	Soxhlet ext. conc. ($ng \cdot g^{-1}$, dry wt.)	oxidizer conc. ($ng \cdot g^{-1}$, dry wt.)	% tol. ext. / oxid.
$C_{12}H_{20}Cl_6$	36 ± 3.3	27 ± 1.8	159 ± 3.1	23
	113 ± 4.4	107 ± 1.9	424 ± 2.8	27
$C_{12}H_{16}Cl_{10}$	112 ± 3.5	124 ± 4.1	175 ± 2.1	64
	372 ± 9.1	442 ± 19	590 ± 4.8	63
$C_{16}H_{31}Cl_3$	51 ± 2.1	47 ± 3.9	128 ± 7.6	40
	128 ± 7.6	135 ± 4.6	399 ± 40	32
$C_{16}H_{21}Cl_{13}$	283 ± 6.7	263 ± 5.1	485 ± 69	58

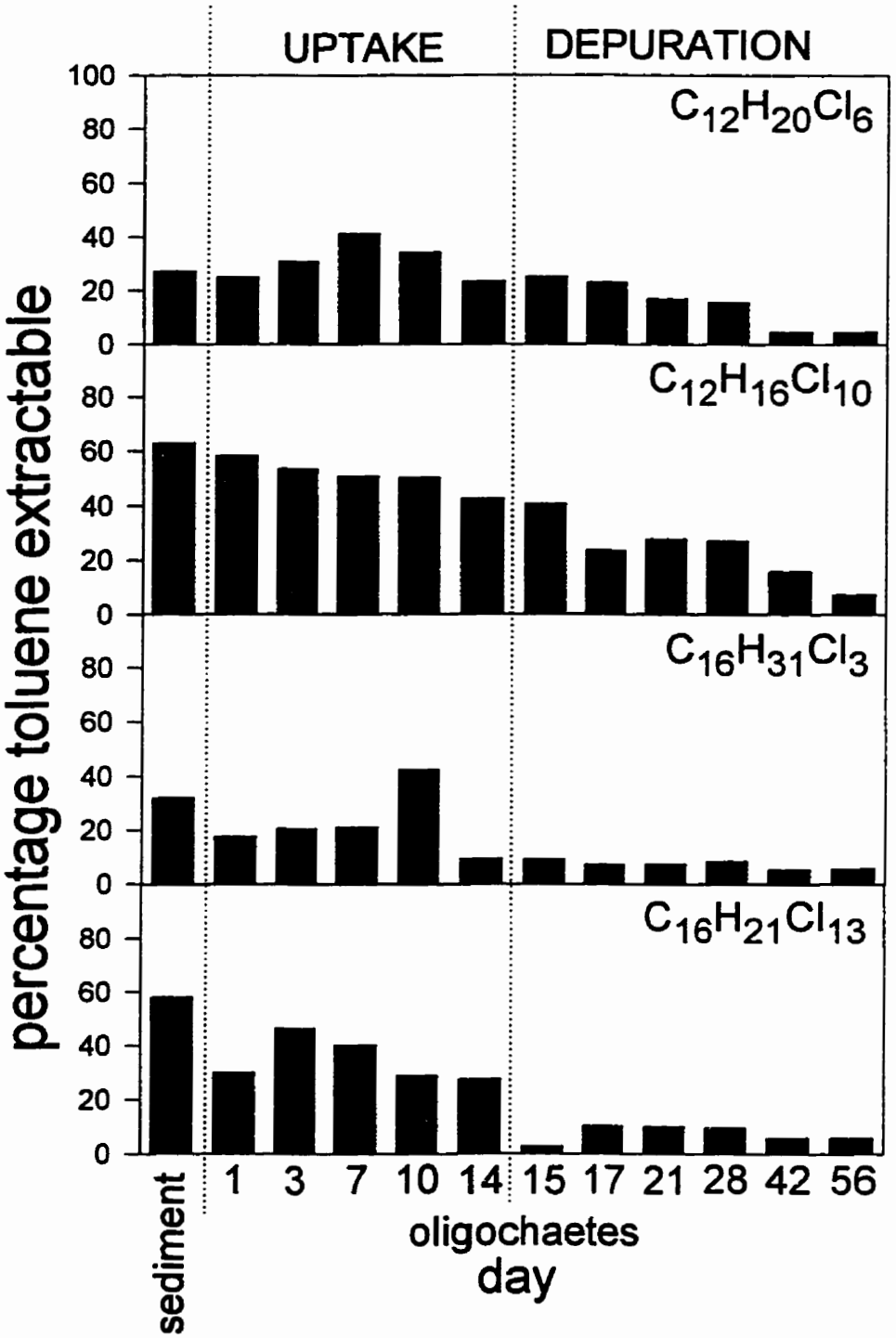

Figure 6.4 Percentage of toluene extractable ^{14}C in ^{14}C-PCA spiked sediment (day 14 uptake) and oligochaetes exposed to the sediment for 14 days followed by 56 days exposed to unspiked sediment.

in particular the depuration rates. r^2 values for PCA depuration rates using data that were not lipid-corrected were > 0.76 (data not shown), and were higher than the r^2 values generated with lipid corrected data (Table 6.1). Although lipid correction had a relatively small impact (generally < 10%) on the parameters (small increases in k_1 and decreases in k_2), it did alter the relative magnitude of the uptake rates. This suggests that proper evaluation of these data required lipid correction. The method used for lipid determination (solvent extraction and gravimetric determination) was not as accurate as methods to determine PCA concentrations because of the small mass and low lipid content of the oligochaetes.

The half-lives of C_{16}-PCAs were significantly greater than those of the C_{12}-PCAs; however, chlorine content did not have a significant effect on the half-life of PCAs (ANCOVA, $p < 0.05$). Half-lives of tri- and tetrachlorobiphenyls in wild-collected oligochaetes (*Tubifex* sp. and *Limnodrilus hoffmeisteri*)($t_{1/2}$s 26 to 43 d) were similar to the C_{16}-PCA half-lives but were greater than those for C_{12}-PCAs (Oliver 1987). West *et al.* (1997) reported that the half-life of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in oligochaetes (*L. variegatus*) ranged from 13 to 21 d, after exposing the oligochaetes via spiked food. These results are greater than the half-lives of the C_{12}-PCAs but less than the C_{16}-PCAs. Loonen *et al.* (1997) reported TCDD and octachlorodibenzo-*p*-dioxin half-lives in *L. variegatus* of 4 and 13 d, respectively, after exposure to spiked sediments. These results suggest that the half-life of C_{12}- and C_{16}-PCAs in oligochaetes is similar to chlorinated aromatic contaminants of comparable K_{ow}. Therefore, benthic invertebrates could provide a pathway for transfer of PCAs, or CPs, from sediment into aquatic food chains.

Half-lives of the C_{16}-PCAs might have been larger if the lipid content of these oligochaetes had been the same as in the C_{12}-PCA exposures. Greater lipid percentage might have resulted in lower depuration rates of hydrophobic compounds in invertebrates (Landrum 1988; Harkley et al. 1994a). However, greater organic carbon content of sediments is also associated with higher elimination rates and shorter half lives of hydrophobic compounds in benthic invertebrates over short periods (50 h) (Lydy et al. 1992). Lydy et al. (1992) did not have a mechanism for the higher elimination rates observed in the higher organic carbon sediment. They did proposed that behavioral changes, feces with higher lipid contents, or disruption of the stationary water layer around the invertebrate, might explain the changes in elimination rates.

The fact that chlorine content had little effect on the $t_{1/2}$ of PCAs was unexpected based on the higher K_{ow} and lower metabolism of the higher chlorinated alkanes (see below). Half-lives have been observed to increase with increasing K_{ow} in aquatic invertebrates (Landrum 1988; Lydy et al. 1992). Fisk (Chapter 3), using the same ^{14}C-PCAs, observed longer half-lives for the more highly chlorinated C_{12}- and C_{16}-PCAs in rainbow trout.

PCA uptake rates decreased with increasing K_{ow}, or K_{oc}. Compounds of high K_{ow}, or K_{oc}, have been reported to have lower interstitial water concentrations and slower rates of desorption from sediment to interstitial water (Landrum 1989). Assimilation of compounds from ingested sediment should also decrease with increasing K_{ow} or K_{oc}. Variations in PCA uptake rates may also be due to differences in the types of sediment particles that the PCAs were sorbed to (Harkley et al. 1994a). It should be noted that the

greater organic carbon content in the sediment, and lipid percentage in the oligochaetes, of the C_{12} exposures may have enhanced the C_{12}-PCA uptake rates (Harkley et al. 1994b).

Uptake of the PCAs from the sediment will occur through accumulation from interstitial water and through gut absorption from ingested sediments. However, bioconcentration factors (BCF) of PCAs (BCFs = 7.4 to 7.9), determined from lipid corrected concentrations in oligochaetes divided by freely dissolved interstitial water concentrations, were greater than their K_{ow} values for all PCAs but $C_{16}H_{21}Cl_{13}$ (BCF = 1), suggesting that additional accumulation had occurred through ingestion of sediments. Ingestion of sediments has been identified as an important route of accumulation of sediment-associated hydrophobic contaminants (Spacie and Hamelink 1982; Belfroid et al. 1996). BCFs decreased with increasing K_{ow} which may be due to lower concentrations of the more hydrophobic PCAs in interstitial water and reduced assimilation in the gut.

The $BSAF_{kin}$s of $C_{12}H_{20}Cl_6$, $C_{12}H_{16}Cl_{10}$ and $C_{16}H_{31}Cl_3$ were > 1, suggesting that the concentrations of PCAs with low and medium chlorination (35-60% Cl) and short- (C_{10-13}) and medium-carbon-chains (C_{14-17}) would magnify between sediments and sediment-ingesting invertebrates. $C_{16}H_{21}Cl_{13}$ had a $BSAF_{kin}$ < 1 suggesting that highly chlorinated medium-carbon-chain PCAs, when associated with sediment, have lower bioavailability to benthic invertebrates than short chain and medium chlorinated medium-carbon chain PCAs. Based on these results, BSAFs of PCAs decrease with increasing K_{ow}. This is consistent with the results of Oliver (1987), who in similar experiments using oligochaetes (*Tubifex* sp. and *L. hoffmeisteri*) and organochlorine compounds, observed decreasing BSAF above log K_{ow} of 6. It should be noted that BSAFs may not be well described by simple relationships with K_{ow} (Landrum 1989).

An unexpected result of this work were the relative amounts of toluene extractable and non-extractable ^{14}C in oligochaetes and sediments (Figure 6.4). ^{14}C-PCAs were efficiently recovered from spiked oligochaetes and sediment. Therefore, it is likely that ^{14}C which is not extracted by toluene, but is measured by complete combustion, is probably associated with more polar compounds, or more tightly bound, than the starting material. The non-extractable ^{14}C in the oligochaetes might be explained by biotransformation. However, amounts of non-extractable ^{14}C in sediment were also high and very similar to the ratios observed in the oligochaetes during the uptake phase. Fisk (chapter 3) found a much smaller percentage of non-extractable ^{14}C in rainbow trout exposed to the same ^{14}C-PCAs. Therefore, it is more likely that PCAs are being transformed in the sediment prior to being accumulated by the oligochaetes. Alternatively, the oligochaetes might have transformed greater amounts than fish, which seems unlikely. However, the proportion of toluene-extractable ^{14}C also decreased throughout the depuration phase for all the PCAs when oligochaetes were in PCA-free sediment, indicating oligochaetes do metabolize PCAs. Further studies on the degradation and metabolism of CPs in sediments, invertebrates, and fish are needed to fully evaluate the fate of CPs in sediment.

There is limited information on the degradation of PCAs in sediment. The susceptibility of PCAs to microbial degradation has been found to decrease with greater chlorine content and carbon-chain-length (Madeley and Birtley 1980; Omori et al. 1987). Madeley and Birtley (1980) reported that microorganisms previously acclimated to specific CPs showed a greater ability to degrade the compounds. Based on the ratios of extractable to non-extractable ^{14}C in this work, lower chlorinated alkanes are more susceptible to degradation in the sediment. However, there are insufficient data to make any conclusions

about differences in susceptibility to microbial degradation due to carbon chain length or about the type of degradation products produced.

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

6.6 Summary and Conclusions

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

7. THE TOXICITY OF C_{10}-, C_{11}-, C_{12}- AND C_{14}-POLYCHLORINATED ALKANES TO JAPANESE MEDAKA (*Oryzias latipes*) EGGS

7.1 Abstract

Japanese medaka (*Oryzias latipes*) eggs were exposed to aqueous concentrations of 6 polychlorinated *n*-alkane (PCAs) standards ($C_{10}H_{15.5}C_{6.5}$, $C_{10}H_{15.3}Cl_{6.7}$, $C_{11}H_{20}Cl_4$, $C_{12}H_{19.5}Cl_{6.5}$, $C_{14}H_{24.9}Cl_{5.1}$ and $C_{14}H_{23.3}Cl_{6.7}$) of known carbon chain length and chlorine content to assess their toxicity. Eggs were also exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to act as a positive control. PCAs, also known as chlorinated paraffins, are complex industrial products for which there is a lack of toxicological data on individual congeners. High aqueous concentrations of $C_{10}H_{15.5}C_{6.5}$ and $C_{10}H_{15.3}Cl_{6.7}$ (9600 and 7700 $ng \cdot ml^{-1}$, respectively) caused 100% mortality in eggs, but no other significant mortalities or lesions were observed at lower concentrations, or in any eggs exposed to the other PCAs. Larvae from eggs exposed to high concentrations of the C_{10}-, C_{11}- and C_{12}-PCAs were extremely lethargic or did not move. The concentrations in these exposures, and the tissue concentrations of the larvae, were at levels which should elicit narcosis. Concentrations of the C_{14}-PCAs in larvae did not reach narcotic levels, and larvae in these exposures appeared normal with no signs of narcosis. Hatching success was low for many of the treatments and control groups, but does not appear to be due to PCA exposure. TCDD was found to be extremely embryotoxic, consistent with past work using Japanese medaka

eggs. The LC_{50} ($ng \cdot mL^{-1}$), the concentration required to kill 50% of the eggs in 20 days, for the PCAs were as follows: 2700 < $C_{10}H_{15.5}C_{6.5}$ < 9600; 5100 < $C_{10}H_{15.3}Cl_{6.7}$ < 7700; $C_{11}H_{20}Cl_4$ > 8900; $C_{12}H_{19.5}Cl_{6.5}$ > 270; $C_{14}H_{24.9}Cl_{5.1}$ > 3400; and $C_{14}H_{23.3}Cl_{6.7}$ > 1600. Toxic equivalency factors of the PCAs, based on acute toxicity and TCDD results, were all < 0.0001 and most were < 0.000001. These results suggest that the acute mechanism of toxicity of lower chlorinated, short (C_{10-13}) and medium (C_{14-18}) carbon chain PCAs is narcosis and that chlorine substitution on the terminal carbons of PCAs does not significantly increase nor decrease the toxicity of C_{10}- and C_{14}-PCAs. However, additional work is needed to assess a greater range of PCAs, as well as their sub-lethal effects and chronic toxicity.

7.2 Introduction

Polychlorinated *n*-alkanes (PCAs) with carbon chains between 10 and 30 are used for a variety of industrial applications including lubricating additives, flame retardants, adhesives, sealants and a number of other miscellaneous applications (Windrath and Stevenson 1985; Government of Canada 1993; Willis et al. 1994). Commercial PCA formulations, also known as chlorinated paraffins (CPs), are classified as short (C_{10-13}), medium (C_{14-17}) and long (C_{18-30}) with varying amounts of chlorination (~35-70% by weight). Annual world production of PCAs is estimated at greater than 300 kilotonnes, and they remain one of the last high molecular weight organochlorines in production and in use in North America and western Europe (Swedish National Chemicals Inspectorate 1991). Because they are produced with free radical chlorination, a single PCA formulation

consists of thousands of different compounds with a range of physical-chemical properties (Tomy et al. 1998).

The toxicity of PCAs to aquatic organisms appears to be low, and in most cases reported effects concentrations are above the water solubility of the PCA formulation (Thompson and Madeley 1983a; Thompson and Madeley 1983c; Madeley and Thompson 1983c; Madeley and Maddock 1983c; Madeley and Maddock 1983d; Madeley and Maddock 1983e; Linden et al. 1979). Most experiments have used mortality as an endpoint; however, death was rarely achieved and reported effects concentrations are usually greater than the highest concentration tested or are based on observational data (Thompson and Madeley 1983a; Thompson and Madeley 1983c; Madeley and Thompson 1983c; Madeley and Maddock 1983c; Madeley and Maddock 1983d; Madeley and Maddock 1983e; Linden et al. 1979). Toxicity is believed to increase with decreasing carbon chain length (Government of Canada 1993; Willis 1994), but with water exposure assays, toxicity may be a function of greater water solubility of the shorter chain PCAs and hence greater exposure. No observable effect concentrations (NOEC) of 5.0 and 7.3 $\mu g \cdot L^{-1}$ for a short chain PCA (C_{10-13}, 58% Cl) using *Daphnia magna* and a marine shrimp (*Mysidopsis bahia*), respectively, are the lowest effect concentrations reported (Thompson and Madeley 1983c; Thompson and Madeley 1983d).

The amount of toxicological data on PCAs is limited, and a majority of these data have been produced using commercial PCA formulations. This presents problems because PCA products consist of thousand of compounds, and therefore differences in the toxicity of individual components cannot be identified. Owing to the selective biodegradation, biotransformation and bioaccumulation of individual compounds of these products in the

environment, the relative abundance of individual PCA compounds to which an organism is exposed may vary from the original PCA product. The lack of appropriate analytical techniques has resulted in uncertain estimates of exposure concentrations and body burdens. Lastly, additives, such as stabilisers, and impurities may cause false positives in toxicity tests. Therefore, it is difficult to use the current PCA toxicological data to evaluate concentrations of PCAs in the environment.

Some individual PCA congeners may have greater toxicity than others, similar to what has been observed with non-ortho substituted polychlorinated biphenyl (PCB) congeners and 2,3,7,8-substituted chlorinated dioxin/furans (Safe 1992), but identifying those congeners will be a very difficult task. To date, there are no published data on the toxicity of PCAs with a single carbon chain length or chlorine content. Therefore, the objective of this work was to generate toxicological data on PCAs of a single carbon chain length and known chlorine content, and in some cases chlorine position, using the Japanese medaka (*Oryzias latipes*) embryo toxicity assay. This assay has been used to assess the toxicity of a number of hydrophobic organochlorines, such as chlorinated dioxins and PCBs (Wisk and Cooper 1990a; Wisk and Cooper 1990b; Harris et al. 1994a; Harris et al. 1994b; Metcalfe et al. 1997), allowing a relative comparison of PCA toxicity. Six PCAs have been synthesised which have a single carbon chain length and known chlorine content. For three of these PCAs the chlorine positions are known. These PCAs cover four carbon chain lengths (C_{10}, C_{11}, C_{12} and C_{14}) and a number of chlorine contents, allowing a comparison of toxicity based on carbon chain length and chlorine content.

7.3 Methods and Materials

7.3.1 Chemicals

Six PCAs were synthesized for this experiment: three by chlorination of an alkene starting material (1,5,9-decatriene; 1,10-undecadiene; and 1,13-tetradecadiene) (Figure 7.1), and three by free radical chlorination of a ^{14}C-labeled alkane starting material (C_{10} and C_{14}). There were fewer individual PCA compounds produced from the alkene starting material than the alkane starting material (Figure 7.2). Also, PCAs produced from the alkenes had chlorines substituted at the double bonds, providing information on chlorine positions. A list of the starting materials, the PCA formed and their relative abundance in the standards is found in Table 7.1.

PCA congeners were synthesized by bubbling SO_2Cl_2, at room temperature, into neat solutions of the respective *n*-alkenes, contained in a flask wrapped in aluminium foil to exclude light. In the absence of light, these conditions were expected to lead, predominantly, to chlorine addition at the double bond(s). Reaction mixtures were shaken with NaOH (0.05M), the aqueous phase was removed, and the organic phase was dried over $MgSO_4$. The drying agent was removed by filtration. In addition to the expected products formed by chlorine additions to each bond, a number of by-products were formed that resulted from free radical substitution of hydrogen atom(s) by chlorine atom(s) (Figure 7.1 and 7.2). Un-reacted starting material and SO_2Cl_2 were removed from the PCAs using Florisil column chromatography (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

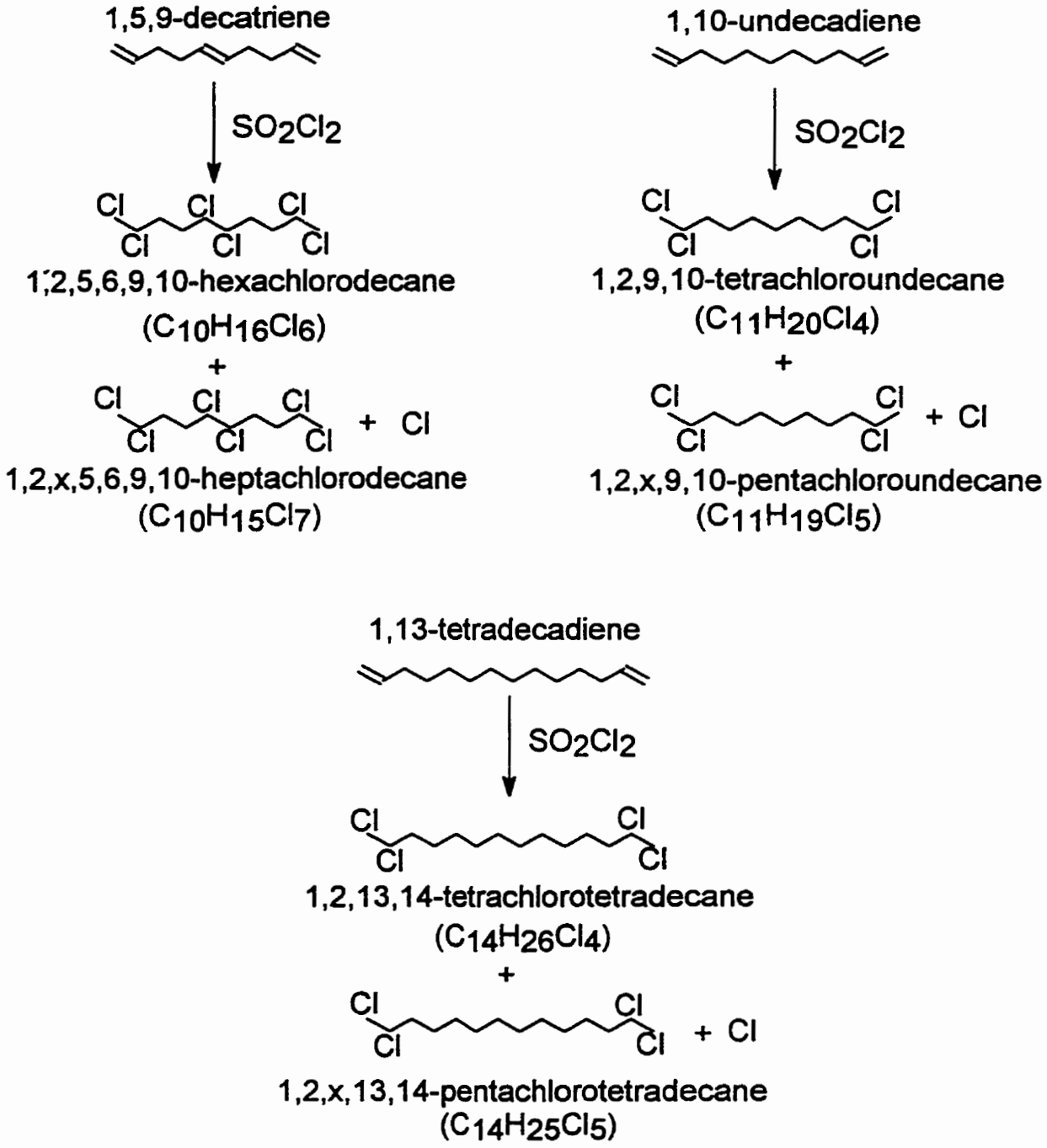

Figure 7.1 Polychlorinated alkanes and their alkene starting material used in Japanese medaka egg exposures.

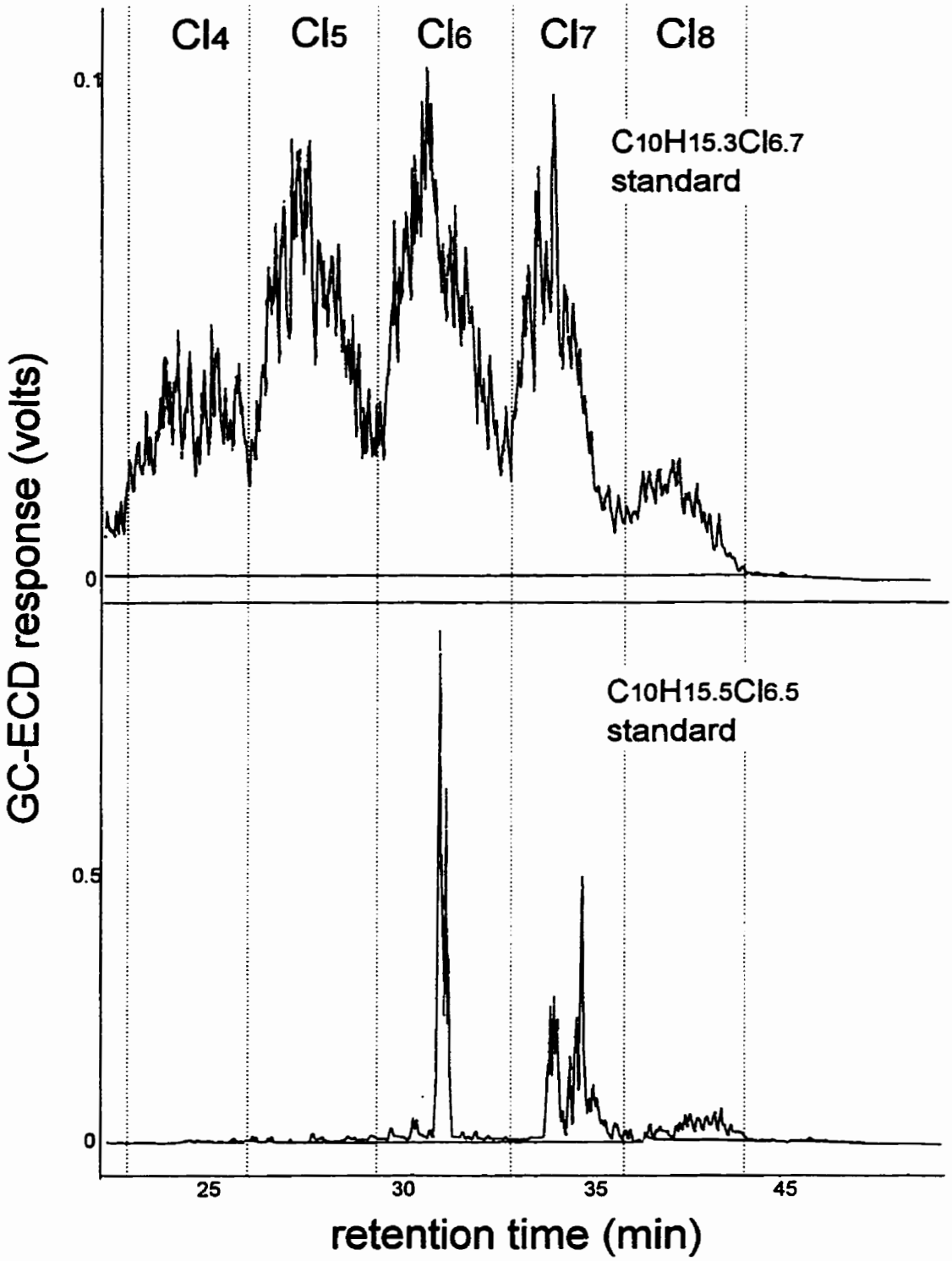

Figure 7.2 GC-ECD chromatograms of 2 PCAs used in Japanese medaka egg exposures. The $C_{10}H_{15.5}Cl_{6.5}$ standard was synthesized by the addition of SO_2Cl_2 to a 1,5,9-decatriene starting material. The $C_{10}H_{15.3}Cl_{6.7}$ standard was synthesized from the addition of SO_2Cl_2 to a C_{10}-*n*-alkane under UV light.

Table 7.1 PCAs used in Japanese medaka embryo toxicity tests.

PCA standard	starting material	PCA formed[A]	% in standard
$C_{10}H_{15.5}Cl_{6.5}$	1,5,9-decatriene	1,2,5,6,9,10-hexachlorodecance ($C_{10}H_{16}Cl_6$)	55
		x,1,2,5,6,9,10-hexachlorodecance ($C_{10}H_{15}Cl_7$)	41
		x,y,1,2,5,6,9,10-hexachlorodecance ($C_{10}H_{14}Cl_8$)	4
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	1-^{14}C-decane	$C_{10}H_{18}Cl_4$	0.1
		$C_{10}H_{17}Cl_5$	4.1
		$C_{10}H_{16}Cl_6$	37
		$C_{10}H_{15}Cl_7$	45
		$C_{10}H_{14}Cl_8$	12
		$C_{10}H_{13}Cl_9$	1.5
$C_{11}H_{18.4}Cl_{5.6}$	1,10-undecadiene	x,1,2,10,11-pentachloroundecance ($C_{11}H_{19}Cl_5$)	40
		x,y,1,2,10,11-hexachloroundecance ($C_{11}H_{18}Cl_6$)	49
		x,y,z,1,2,10,11-hexachloroundecance ($C_{11}H_{17}Cl_7$)	10
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	1-^{14}C-dodecane	$C_{12}H_{19}Cl_5$	14
		$C_{12}H_{18}Cl_6$	31
		$C_{12}H_{17}Cl_7$	50
		$C_{12}H_{16}Cl_8$	5
$C_{14}H_{24.9}Cl_{5.1}$	1,13-tetradecadiene	1,2,13,14-tetrachlortetradecance ($C_{14}H_{26}Cl_4$)	10.5
		x,1,2,13,14-pentachlorotetradecance ($C_{14}H_{25}Cl_5$)	74.3
		x,y,1,2,13,14-hexachlorotetradecance ($C_{14}H_{24}Cl_6$)	14.2
		x,y,,z1,2,13,14-heptachlorotetradecance ($C_{14}H_{23}Cl_7$)	1.0
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	1-^{14}C-tetradecane	$C_{14}H_{26}Cl_4$	0.2
		$C_{14}H_{25}Cl_5$	4.4
		$C_{14}H_{24}Cl_6$	34
		$C_{14}H_{23}Cl_7$	45
		$C_{14}H_{22}Cl_8$	14
		$C_{14}H_{21}Cl_9$	1.9

[A] The positions of chlorine atoms designated with an x or y are unknown.

contained unreacted alkene starting material but no PCAs and was discarded. F2 and F3 contained the PCAs and were combined. After Florisil clean-up, greater than 99% of the standards were found to be PCAs based on chromatograms generated by gas chromatography (GC) with an electron capture detector (ECD) which matched GC-negative ion MS chromatograms conformed as PCAs. GC conditions are described in Chapter 5.

The free radically chlorinated PCAs were synthesized using two methods. The C_{12}-PCA was provided by Å. Bergman (Stockholm University), and was synthesized using a method which has been described previously (Bergman et al. 1981). The C_{10} and C_{14}-PCAs were synthesized by adding a quantity of SO_2Cl_2 into the respective *n*-alkanes that would provide the desired chlorination (i.e. ~ 6-7 Cl per alkane). The alkanes and SO_2Cl_2 were combined in a small glass flask (250 μL) and exposed to UV light for one hour. Un-reacted alkane and SO_2Cl_2 were removed from the standard using the Florisil methods described above. After Florisil clean-up, greater than 99% of the standards were found to be PCAs based on chromatograms generated by GC-ECD which matched GC-NIMS chromatograms confirmed as PCAs. Radiopurity was checked by HPLC and was found to be greater than 99%. Samples were injected on a Varian 5000 liquid chromatograph (Varian Canada Inc., Mississauga, ON, Canada) equipped with a Prep Nova pak HR C-18 column (Waters Division of Millipore, Milford, MA, USA), an autosampler and an automated fraction collector. The mobile phase used for the PCAs consisted of 90% acetonitrile and 10% water; 3 minute fractions were collected over a 60 minute period. Fractions were counted using LSC.

^{3}H-labelled 2,3,7,8-TCDD with specific activity of 223 DPM·pg^{-1} was obtained from Chemsyn Science Laboratories, Lenexa, KS, USA. This TCDD standard was found to be 99.5% radiochemically pure after purification by HPLC.

7.3.2 Adult Japanese medaka

Adult Japanese medaka (*Oryzias latipes*) were purchased from the Carolina Biological Company (Burlington, NC, USA). Japanese medaka were housed in glass aquaria (n = 3, 40 L aquariums) with approximately 20 adults per aquaria at a ratio of 3 females to 2 males. The aquaria had flow-through water (UV and carbon dechlorinated City of Winnipeg tap water), maintained at 20-25°C. During non-breeding periods, the photoperiod was 12:12 h ratio of light:dark and the fish were fed Tetra Standard Mix freshwater tropical food (Tetra Werke, Melke, Federal Republic of Germany) once daily. During breeding periods, the photoperiod was changed to a 16:8 h ratio of light:dark, and the fish were fed Tetra Standard Mix freshwater tropical food and newly hatched brine shrimp once daily.

7.3.3 Egg collection

Eggs were collected from individual females approximately 1 h after the lights came on. Eggs were placed in medaka rearing solution (0.10 g NaCl, 0.03 g KCl, 0.04 g CaCl, 0.16 g $MgSO_4$, 0.0001 g methylene blue and 100 mL distilled water). Unfertilised eggs were discarded, and all remaining fertilized eggs from different parents were mixed to avoid individual fish effects prior to placing in exposure vessels.

7.3.4 Exposure vessels and concentrations

Eggs were exposed to the PCAs or TCDD individually in 1.8 mL GC vials with Teflon lined caps (Supelco, Bellefonte, PA, USA). Before adding the egg, PCAs and TCDD, dissolved in DCM:hexane, were added to the vials and the solvents were allowed to evaporate for 24 hours. Control treatments had 100 μL of DCM:hexane added to each vial, an amount of solvent which was greater than that present in any treatment vial, and allowed to evaporate for 24 h. One mL of rearing solution was added to each vial, and the vials were sonicated for 30 min and allowed to stand overnight in an incubator at 25°C (Blue M Electric Company, Blue Island, IL, USA). One egg was added to each vial.

Four to seven exposure concentrations were established for each PCA and TCDD (Table 7.2). The PCA concentrations were based on their water solubility (WS), as well as 1/100, 1/10, 10X and 100X the WS. The 100X WS exposure could not be performed for the $C_{12}H_{19.5}Cl_{6.5}$ because there was not sufficient standard. The WS of the $C_{11}H_{19}Cl_5$ and ^{14}C-$C_{12}H_{20}Cl_6$ were from Drouillard et al. (1998), and the WS of the remaining PCAs were estimated based on WS of PCAs reported in Drouillard et al. (1998). Due to the hydrophobic nature of the PCAs, the majority of the PCAs should partition onto the GC vial glass. The partitioning ratio of the three ^{14}C-PCAs, at three concentrations (n = 3), between rearing solution and glass were determined and used for establishing the amount of PCA needed to achieve the desired rearing solution concentrations. TCDD concentrations were based on past Japanese medaka embryo toxicity results (Wisk and Cooper 1990a; Metcalfe et al. 1997).

Table 7.2: Concentrations (mean ± 1 SE), and larvae bioconcentration factors (BCF), of PCAs and TCDD in water, egg and larvae from Japanese medaka experiments. The symbol (-) represents samples where concentrations were not determined.

PCA	amount of PCA added (ng)	nominal water conc. ($ng \cdot ml^{-1}$)[A]	measured water conc. ($ng \cdot ml^{-1}$)[B]	estimated water conc. ($ng \cdot ml^{-1}$)[C]	egg conc. ($ug \cdot g^{-1}$)[D]	larvae conc. ($ug \cdot g^{-1}$)[D]	larvae BCF[E]
$C_{10}H_{15.5}Cl_{6.5}$	35	6.3	-	5.9	-	-	-
	350	63	-	62	-	-	-
	3500	630	-	460	-	-	-
	35000	6300	-	2700	-	-	-
	350000	63000	-	9600	-	-	-
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	28	5.0	4.7 ± 0.92	-	8.1	12 ± 2.5	2600
	280	50	50 ± 7.4	-	-	100 ± 4.8	2000
	2800	500	370 ± 20	-	-	1000 ± 100	2700
	28000	5000	2200 ± 140	-	-	3000 ± 130	1400
	140000	25000	5100 ± 690	-	2500	3500 ± 300	690
	280000	50000	7700 ± 620	-	4100 ± 870	-	
$C_{11}H_{20}Cl_4$	32	5.8	-	5.4	-	-	
	320	58	-	57	-	-	
	3200	580	-	420	-	-	
	32000	5800	-	2500	-	-	
	320000	58000	-	8900	-	-	
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	3.3	0.50	0.70 ± 0.030	-	-	0.74 ± 0.09	1100
	33	5.0	9.6 ± 1.7	-	5.4	7.1 ± 1.4	740
	330	50	55 ± 7.9	-	-	62 ± 5.5	1100
	3300	500	270 ± 43	-	360 ± 13	460	1700
$C_{14}H_{24.9}Cl_{5.1}$	13	1.0	-	2.9	-	-	
	130	10	-	25	-	-	
	1300	100	-	250	-	-	

	13000	1000	-	880	-	-	
	130000	10000	-	3400	-	-	
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	6.2	0.50	1.4 ± 0.16	-	0.94 ± 0.21	0.24	170
	62	5.0	12 ± 2.0	-	8.4 ± 0.8	8.2 ± 1.5	680
	620	50	120 ± 32	-	63 ± 3.9	45 ± 13	380
	6200	500	420 ± 78	-	110 ± 1.0	84 ± 19	200
	62000	5000	1600 ± 260	-	72	51 ± 8.3	32
^{3}H-TCDD	1		0.0013 ± 0.0001	-	0.00049 ± 0.00018	-	
	5		0.0017 ± 0.0001	-	0.00085 ± 0.000044	-	
	20		0.0017 ± 0.0001	-	0.0016 ± 0.00016	-	
	30		0.0037 ± 0.0004	-	0.0023 ± 0.00088	-	
	50		0.011 ± 0.0025	-	0.0056 ± 0.00067	0.0081 ± 0.0025	740
	100		0.036 ± 0.012	-	0.015 ± 0.011	0.011 ± 0.0047	310
	100		0.031 ± 0.007	-	0.0067 ± 0.025	0.0072 ± 0.0014	230

[A] The nominal water concentration of the PCAs was estimated based on the partitioning behavior of the ^{14}C-PCAs between rearing solution and the glass of the exposure vessel (see Section 7.3.4). For non-labeled PCAs the nominal water concentration has been estimated based on the partition behavior of the ^{14}C-PCA. The nominal water concentrations of $C_{10}H_{15.5}Cl_{6.5}$ and $C_{11}H_{20}Cl_4$ were based on the partitioning behavior of $^{14}C_{10}H_{15.3}Cl_{6.7}$ and the nominal water concentrations of $C_{14}H_{25.9}Cl_{5.1}$ were based on the partitioning behavior of $C_{14}H_{23.3}Cl_{6.7}$. The partitioning of the TCDD was not determined.

[B] The measured water concentrations of the ^{14}C-PCAs were determined by liquid scintillation counting and for the non-labeled PCAs by GC-ECD (see section 7.3.5).

[C] The estimated water concentration of the non-labeled PCAs was based on the ratio between the measured and nominal water concentrations of the ^{14}C-PCAs. The $C_{10}H_{15.5}Cl_{6.5}$ and $C_{11}H_{20}Cl_4$ water concentrations were estimated based on $^{14}C_{10}H_{15.3}Cl_{6.7}$ and the $C_{14}H_{25.9}Cl_{5.1}$ water concentrations were based on $C_{14}H_{23.3}Cl_{6.7}$.

[D] The larvae and egg concentrations could only be determined for the ^{14}C-PCA exposures.

[E] The larvae BCF = larvae concentration ($ng \cdot g^{-1}$, wet weight) / measured water concentration ($ng \cdot ml^{-1}$).

Ten vials were established for each concentration. One control group (10 vials) was established for every 5 treatments. Eggs were added to the vials on the day of fertilization, and were maintained at 25°C throughout the experiment. During the first 10 days of exposure, vials were checked daily. For days 11-20 vials were checked every other day. Embryos were observed through the GC vial glass using a dissecting microscope. Stages of development of the embryo were evaluated based on *The Japanese Medaka Its Care and Development* pamphlet provided by Carolina Biological Supply Company.

7.3.5 Determination of water, egg and larvae concentrations

Water, or rearing solution, concentrations of the ^{14}C-PCAs and the ^{3}H-TCDD were determined by transferring the rearing solution of a vial (n = 5), minus the egg or fish, to a scintillation vial (7 mL) using a disposable glass pipette. Following the transfer, the glass pipette was rinsed once with toluene and the toluene was added to the scintillation vial. Four mL of Fluor (Atomlight, Dupont Chemical Company, Boston, MA, USA) was added to each vial for ^{14}C or ^{3}H counting. Due to detection limit problems, only the water concentrations of the ^{14}C-PCAs were determined. Water concentrations of the non-labeled PCAs were estimated based on the partition behavior of the ^{14}C-PCAs (Table 7.2).

Owing to detection limit problems, PCA concentrations in eggs and larvae were only determined in the ^{14}C-PCA exposures. Fish and eggs were removed from the exposure vessel, placed in clean distilled water, transferred to a scintillation vial and allowed to dry over night. The dried egg or fish was then pulverized with a stainless steel spatula, 1 mL of toluene was added, and the vials were sonicated for 30 min. Following sonication, 5 mL of fluor (Atomlight) was added for ^{14}C and ^{3}H counting.

Vials were counted on a Beckman LS 7500 liquid scintillation counter (LSC) (Beckman Instruments Inc., Irvine, CA, USA). ^{14}C and ^{3}H counts were corrected for quench using a quench curve prepared from ^{14}C- or ^{3}H-toluene (Dupont Chemical Company), and were automatically corrected for background by the LSC.

7.4 Results

7.4.1 Water, egg and fish concentrations

Measured water concentrations of the ^{14}C-PCAs were close to the desired concentrations for the lower exposure concentrations (< 1,000 ng·mL^{-1}) but were lower than expected for the higher exposure concentrations (> 1,000 ng·mL^{-1}) (Table 7.2). The higher exposure concentrations (> 1,000 ng·mL^{-1}) were well beyond the water solubility of these compounds, and therefore a large percentage of PCA added to these vials was probably sorbed to the glass vial and were not in solution. GC-ECD chromatograms of the non-radiolabeled PCAs standards and extracts from water were similar (Figures 7.3, 7.4 and 7.5).

Larvae and eggs from the same treatment and concentration level had similar ^{14}C-PCA and ^{3}H-TCDD concentrations, suggesting that these chemicals readily diffuse through the chorion (i.e., egg shell) (Table 7.2). With the exception of the very highest exposure, concentrations in the larvae increased in proportion to the water concentrations (Table 7.2).

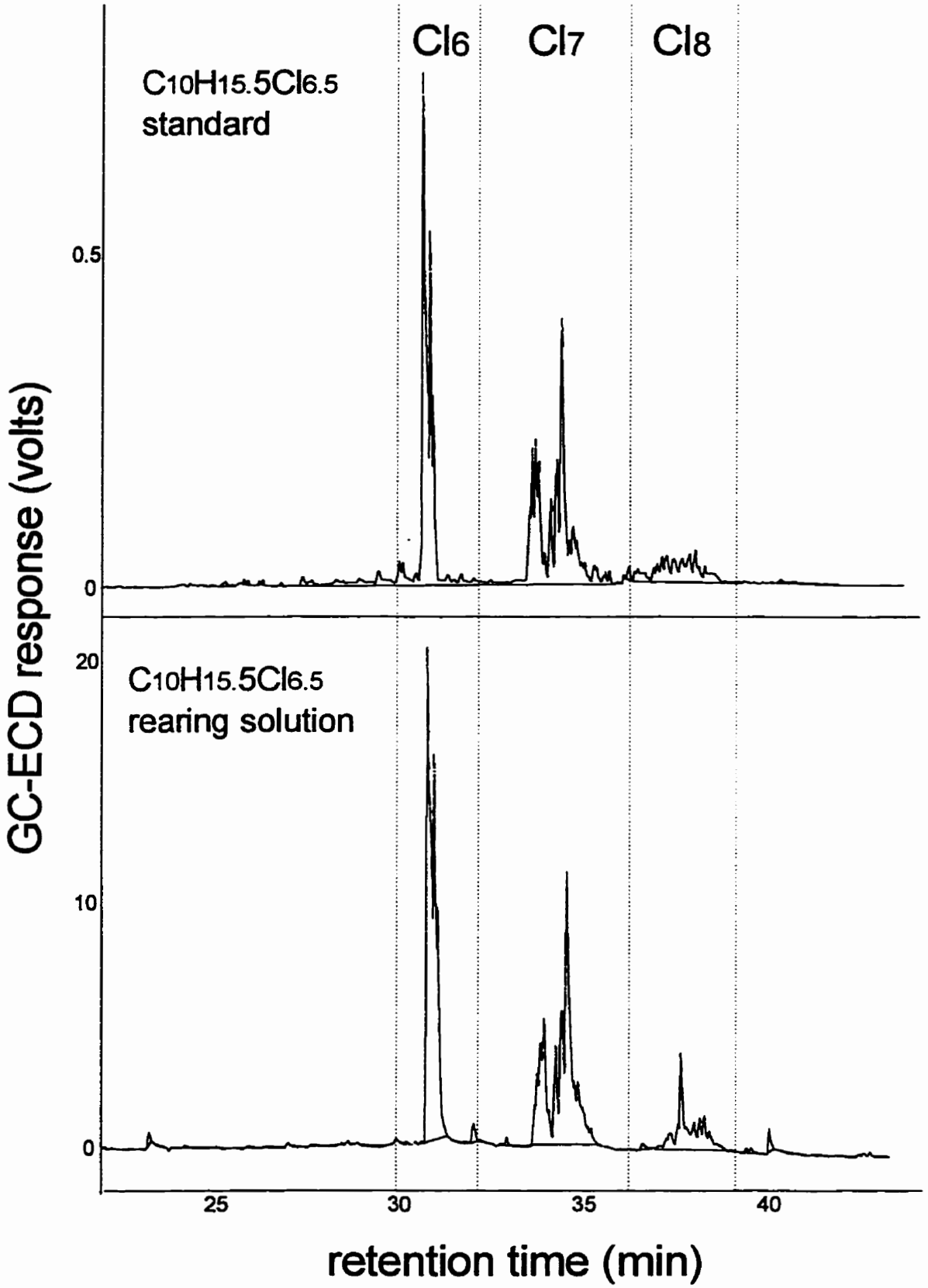

Figure 7.3 GC-ECD chromatograms of the $C_{10}H_{15.5}Cl_{6.5}$ standard and extracts from water exposures collected on day 20.

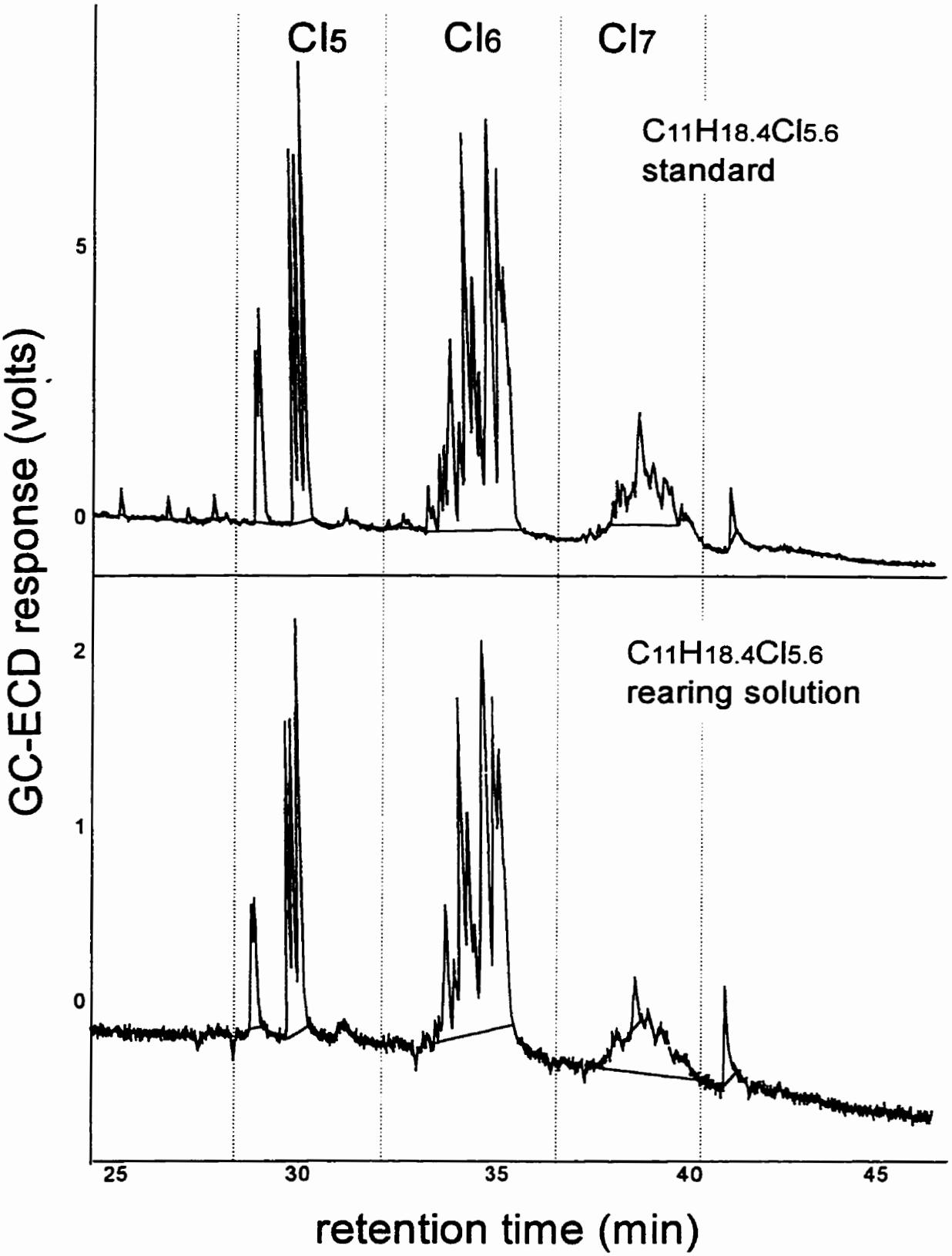

Figure 7.4 GC-ECD chromatograms of the $C_{11}H_{20}Cl_4$ standard and extracts from water exposures collected on day 20.

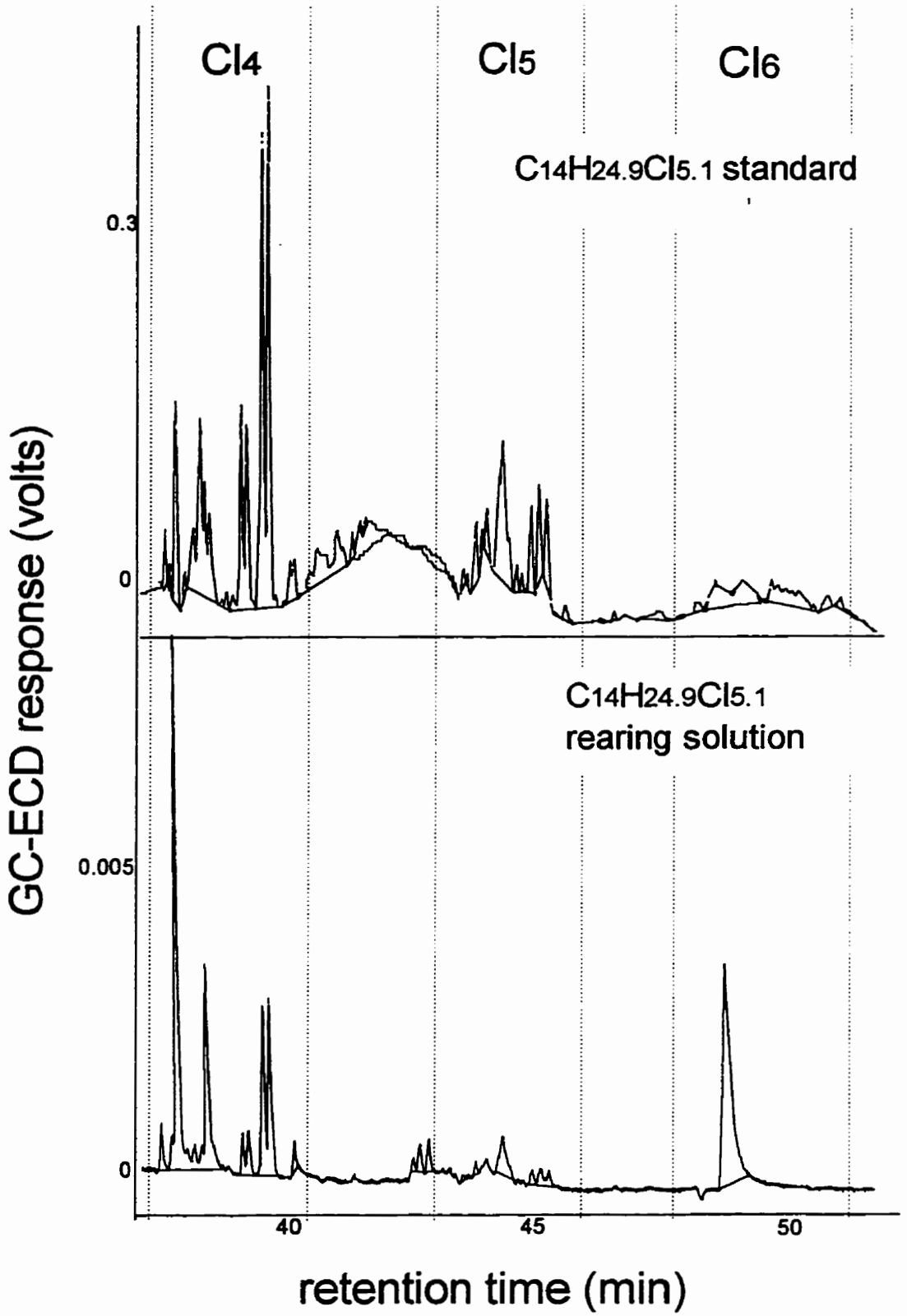

Figure 7.5 GC-ECD chromatograms of the $C_{14}H_{24.9}Cl_{5.1}$ standard and extracts from water exposures collected on day 20.

7.4.2 Effects of PCAs on Japanese medaka eggs

Exposure of the Medaka eggs to $C_{10}H_{15.5}Cl_{6.5}$ and $C_{10}H_{15.3}Cl_{6.7}$ at the highest concentrations (9600 and 7700 $ng \cdot mL^{-1}$, respectively) resulted in 100% mortality in the eggs (Table 7.3). All eggs died on the second day of exposure to the $C_{10}H_{15.3}Cl_{6.7}$, with little or no recognizable development (Figure 7.6). Identical results were produced when this exposure was repeated two days later. Eggs from the $C_{10}H_{15.5}Cl_{6.5}$ exposure died between days 10 and 12. These embryos appeared to develop normally with no indication of lesions or toxic stress, unlike eggs exposed to TCDD (see below), and then died suddenly (Figure 7.7). No significant amount of deaths or recognizable lesions occurred in eggs from any of the other PCA exposures (Table 7.3).

Larvae which were exposed to higher concentrations (> 200 $ng \cdot mL^{-1}$) of the two C_{10}-, the C_{11}- and C_{12}-PCAs were lethargic, with little or no movement, but all had a heart beat which signified that the larvae were alive (Table 7.3). In many of these cases, the larvae had extremely large yolk sacs (Figure 7.8). These effects were not observed in any control treatment larvae nor larvae exposed to any concentration of the C_{14}-PCAs.

Hatching success varied between PCA treatments and control, and in almost all cases un-hatched eggs were still alive on the last observation day (day 20). A majority of the eggs had hatched when the vials were checked 20 days after the last observation day. All larvae which were not sacrificed for concentration determinations survived until they were killed (> 3 d), although no recovery from lethargic behavior was observed for any larvae during this time.

Table 7.3: Effects of PCAs and TCDD on hatching, appearance of lesions and survival of Japanese medaka embryos (n = 10) exposed on the day of fertilization.

PCA	start date	Water conc. ($ng \cdot ml^{-1}$)	Hatched by day 20 (%)	Alive but not hatched by day 20 (%)	Lesions (%)	Lesion type[A]	Larvae behavior and appearance[B]	Death first 2 days (%)	Death after day 2 (%)
Control A	Aug. 21	-	80	20	0	-	N	0	0
Control B	Aug. 22	-	50	50	0	-	N	0	0
Control C	Oct. 12	-	20	80	0	-	N	0	0
Control D	Oct. 13	-	10	90	0	-	N	0	0
Control E	Oct. 14	-	10	90	0	-	N	0	0
Control F	Oct. 15	-	10	90	0	-	N	0	0
Control G	Jan. 9	-	40	60	0	-	N	0	0
Control H	Jan. 10	-	0	100	0	-	N	0	0
Control I	Jan. 11	-	0	100	0	-	N	0	0
$C_{10}H_{15.5}Cl_{6.5}$	Aug. 21	5.9	70	10	0	-	N	20	0%
	Aug. 21	62	50	40	0	-	N	0	10
	Aug. 21	460	90	0	0	-	A,B	0	10
	Jan. 9	2700	90	0	0	-	A,B,C	0	10
	Jan. 9	9700	0	0	0	E	-	0	100
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	Aug. 21	4.7 ± 0.92	70	30	0	-	N	0	0
	Aug. 22	50 ± 7.4	60	40	0	-	N	0	0
	Aug. 22	370 ± 20	70	30	0	-	A,B	0	0
	Jan. 9	2200 ± 140	70	30	0	-	A,B	0	0
	Jan. 12	5100 ± 690	40	50	0	-	A,B,D	0	10
	Jan. 9	7700 ± 620	0	0	0	E	-	100	-
$C_{11}H_{20}Cl_4$	Aug. 22	5.4	50	50	0	-	N	0	0
	Aug. 22	57	90	10	0	-	N	0	0
	Aug. 23	420	80	0	0	-	A	20	0

	Jan. 10	2500	50	50	0	-	B	0	0
	Jan. 10	8900	80	20	0	-	N,A,B	0	0
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	Aug. 23	0.70 ± 0.030	30	70	0	-	N	0	0
	Aug. 23	9.6 ± 1.7	70	20	0	-	N	10	0
	Aug. 23	55 ± 7.9	40	60	0	-	A,B	0	0
	Jan. 10	270 ± 43	20	80	0	-	N	0	0
$C_{14}H_{24.9}Cl_{5.1}$	Oct. 12	2.9	20	80	0	-	N	0	0
	Oct. 12	25	10	90	0	-	N	0	0
	Oct. 12	250	40	60	0	-	N	0	0
	Oct. 13	880	20	80	0	-	N	0	0
	Oct. 13	3400	60	40	0	-	N	0	0
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	Oct. 13	1.4 ± 0.16	10	90	0	-	N	0	0
	Oct. 14	12 ± 2.0	20	80	0	-	N	0	0
	Oct. 14	120 ± 32	50	50	0	-	N	0	0
	Oct. 14	420 ± 78	90	10	0	-	N	0	0
	Oct. 14	1600 ± 260	30	70	0	-	N	0	0
$^{3}TCDD$	Jan. 11	0.0013 ± 0.0001	0	100	0	-	-	0	0
	Jan. 11	0.0017 ± 0.0001	0	100	30	A	-	0	0
	Jan. 11	0.0017 ± 0.0001	0	100	90	A,B	-	0	0
	Jan. 11	0.0037 ± 0.0004	20	40	100	C,D,E	E	0	60
	Jan. 11	0.011 ± 0.0025	20	30	100	C,D,E	A,D,E	0	60
	Oct. 15	0.036 ± 0.012	40	20	50	A,B	B	0	10
	Oct. 15	0.031 ± 0.007	10	30	100	C,D,E,F,G	B	0	70

[A] Lesion types are classified as:

A - oil globule migration away from head

B - thinning of blood vessels between the head and oil globule

C - extreme oil globule migration away from head

D - extreme thinning of blood vessels between the head and oil globule

E - death

F - blood collection in larvae body

G - blood collection in yolk sac

[B] Behavior and appearance of larvae are classified as:

A - large yolk sac

B - lethargic or no movement but heart beat present

C - dead

D - rapid irregular movements

E - partially hatched but dead

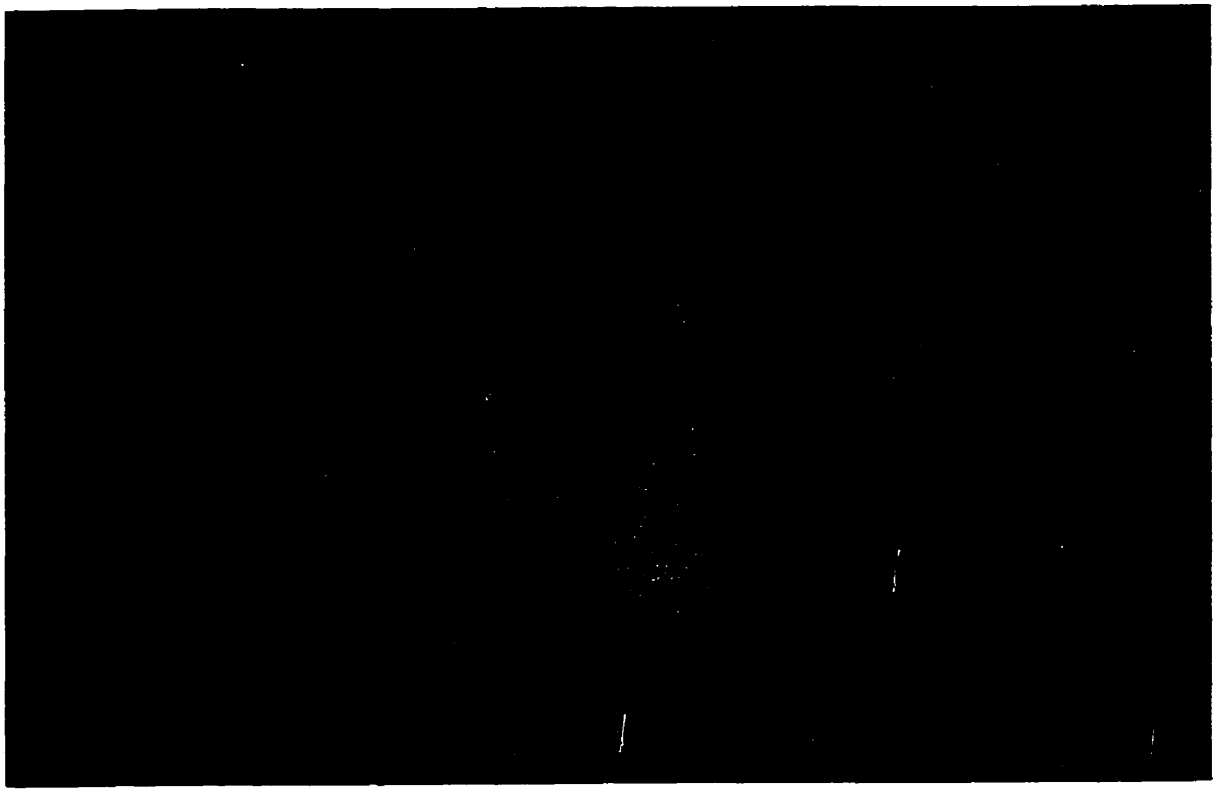

Figure 7.6 Micrographs (mag. ~44X) of a Japanese medaka egg, 2 days after fertilization, exposed to $C_{10}H_{15.3}Cl_{6.7}$ at a water concentration of 7700 $ng \cdot ml^{-1}$. The cloudy section between the two oil globules is the dead embryo.

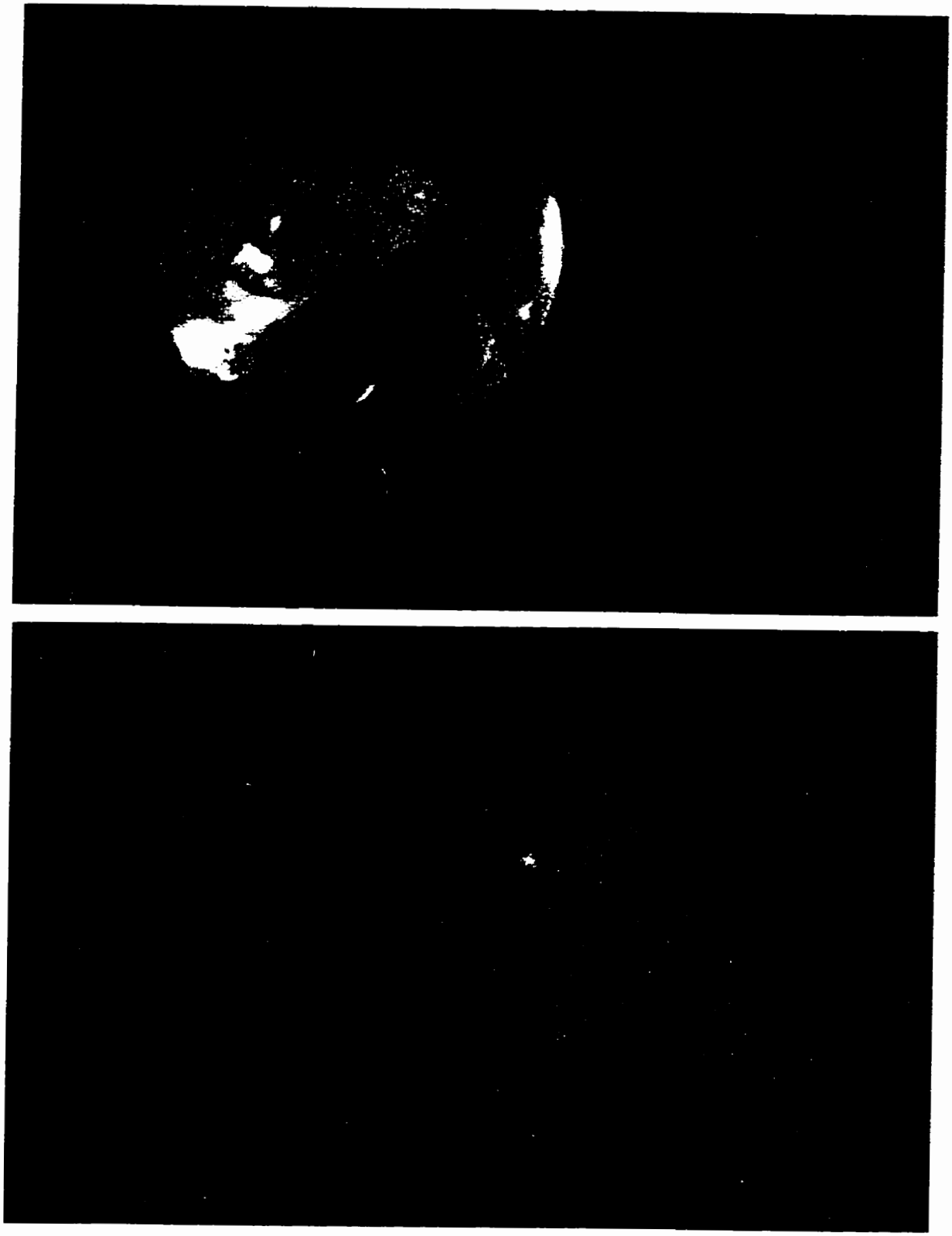

Figure 7.7 Micrographs (mag. ~ 44X) of Japanese medaka eggs, 11 days after fertilization. A. Control egg. B. Egg exposed to $C_{10}H_{15.5}Cl_{6.5}$ at a water concentration of 9600 $ng \cdot ml^{-1}$. This embryo is dead.

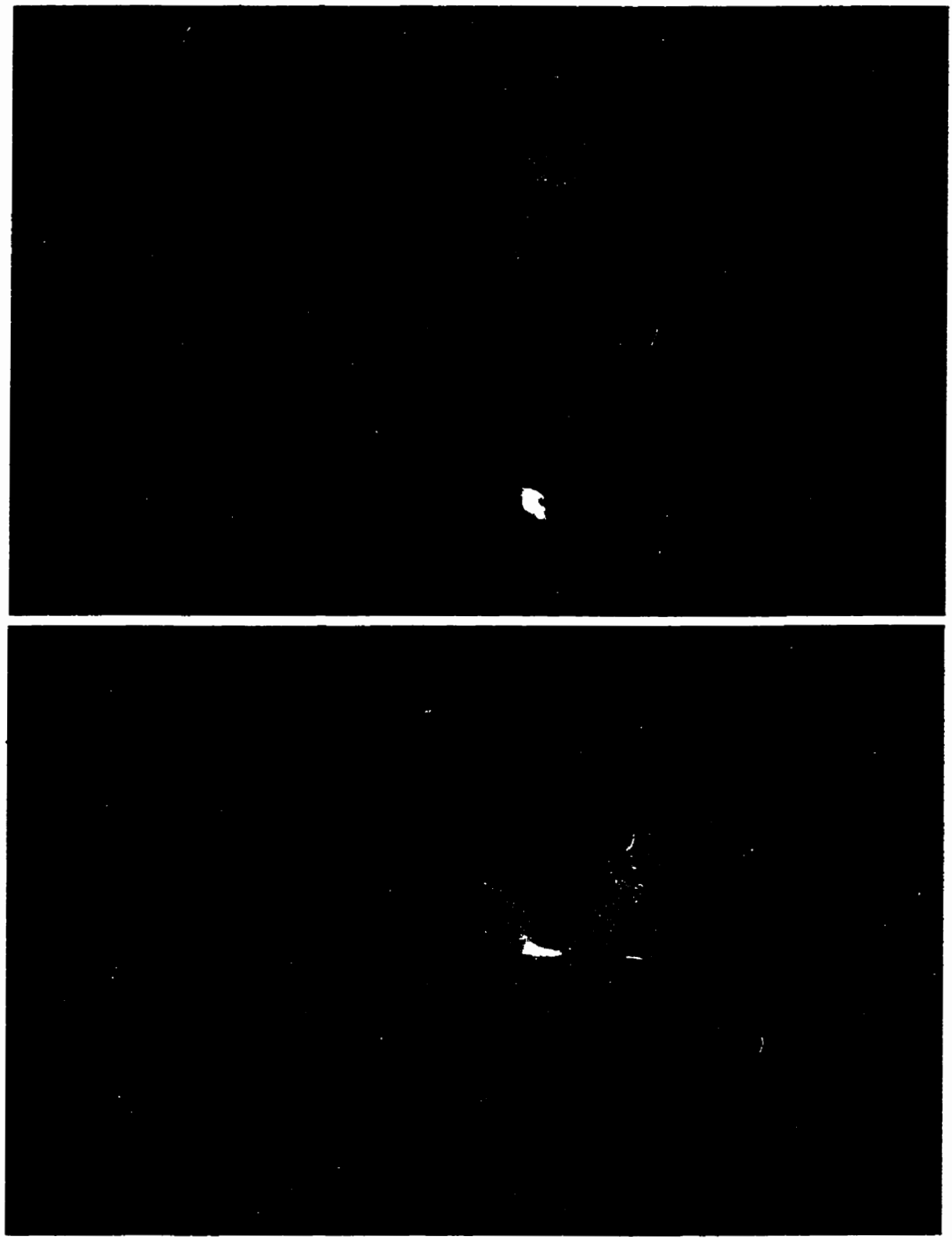

Figure 7.8 Micrographs of Japanese medaka larvae, 1 day post-hatch. A. Control larvae (mag. ~ 20X). B. Larvae (mag. ~ 44X) exposed to $C_{10}H_{15.5}Cl_{6.5}$ at a water concentration of 2700 ng·ml^{-1}. Note the large distended yolk sac.

7.4.3 Effects of TCDD on Japanese medaka eggs

TCDD produced toxic effects in the Japanese medaka at water and tissue concentrations that were orders of magnitude lower than the lowest PCA concentration used. The most common effect was the migration of the oil globule away from the head (Figure 7.9), which was observed in the embryos at all but the lowest TCDD concentration (Table 7.3). Other effects observed include thinning of blood vessels between the head and oil globule (Figure 7.9), slowing of blood flow, collections of blood in the body or yolk sac, low amounts of blood (i.e., transparent blood), and death (Figure 7.10) (Table 7.3). Based on the migration of the oil globule away from the head of the larvae, the lowest observable effects concentration ($ng \cdot ml^{-1}$) is 0.0017, which corresponds to a tissue concentration of 0.49 $ng \cdot g^{-1}$. Based on these results, the LC_{50} value for TCDD would be between 0.0017 and 0.0037 $ng \cdot l^{-1}$.

7.5 Discussion

These results represent the first work on PCA toxicity using PCAs of specific carbon chain lengths and known chlorine content. The C_{10}-, C_{11}- and C_{12}-PCAs produced toxic effects in eggs and larvae but only at extremely high exposure and tissue concentrations. No effects were observed in eggs exposed to C_{14}-PCAs, despite using similar water concentrations to the C_{10}-, C_{11}- and C_{12}-PCAs exposures. These results are consistent with the suggestion that the toxicity of PCAs increases with decreasing carbon chain length (Willis 1994, Government of Canada 1993). It was clear from the low bioconcentration factors that the C_{14}-PCA are not as readily accumulated by the eggs as the shorter chain PCAs. This could be an artifact due to an overestimation of the true

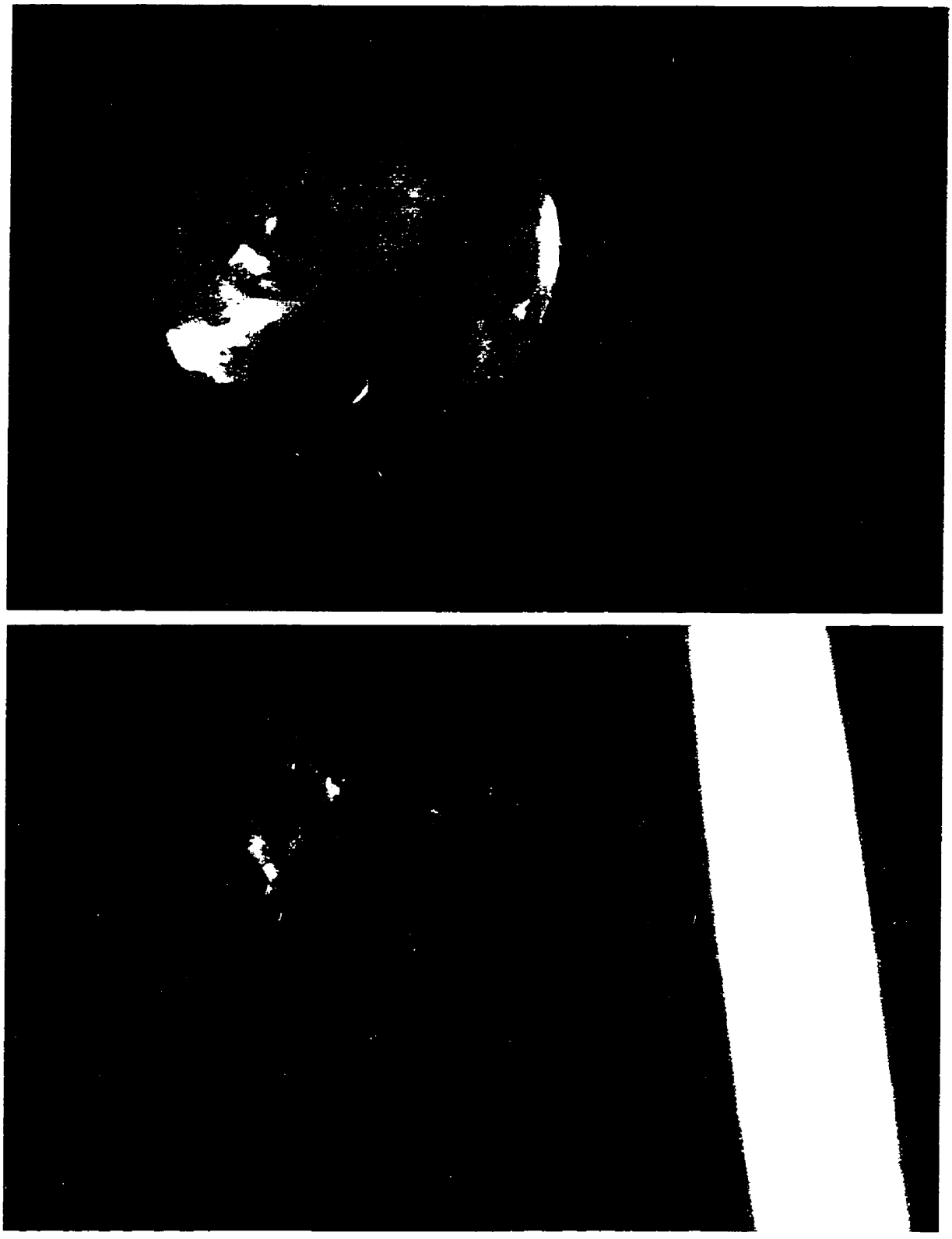

Figure 7.9 Micrographs (mag. ~ 44X) of Japanese medaka eggs, 11 days after fertilization. A. Control egg. B. Egg exposed to TCDD at a water concentration of 50 $pg \cdot ml^{-1}$. Note the movement of the oil globule at the opposite pole of the head and the thin and irregular blood vessels surrounding the yolk sac in the TCDD treated egg.

Figure 7.10 Micrographs (mag. ~44X) of a Japanese medaka egg, 20 days after fertilization, exposed to TCDD at a water concentration of 20 $pg \cdot ml^{-1}$. Note that the head is protruding from the egg although the larva is dead.

bioavailable water concentrations of the C_{14}-PCAs because they are more hydrophobic. Consideration should be given to the dynamics of PCAs when comparing the toxicities of PCAs with different carbon chain lengths and chlorine content determined using water exposure experiments.

These results are similar to results from past work, where PCA toxicity tests exposing fish in water have failed to produce mortalities (Madeley and Maddock 1983c; 1983d; 1983e; 1983f; Linden et al. 1979). Results from this work are also consistent with the only other study to examine PCA toxicity to fish eggs or larvae (Hill and Maddock 1983a and 1983b). Sheepshead minnow (*Cyprinodon variegatus*) embryos (eggs) and larvae were exposed to a short chain commercial PCA formulation (C_{10-13}, 58% Cl) for 32 days at a range of water concentrations (2.4 to 620 $\mu g \cdot L^{-1}$) to determine its effect on hatchability of eggs, and survival and growth of the larvae. At the concentrations tested, the PCAs had no statistically significant effect on hatchability of the eggs or survival of the larvae. Larvae from the highest exposure concentration (620 $\mu g \cdot L^{-1}$) tested, had reduced growth compared to the acetone control group. Based on this reduced growth, the "observed effect concentration (OEC)" for this PCA was reported as 620.5 $\mu g \cdot L^{-1}$, and the no observable effect concentration (NOEC) was set at 280 $\mu g \cdot L^{-1}$. These results are in excellent agreement with LOEC and NOEC values determined for short chain (C_{10-13}) PCA used in this study (Table 7.4).

Hatching success (by day 20) was low for many of the treatments, and was well below the hatching success reported in other studies on Japanese medaka eggs (Wisk and Cooper 1990a; Wisk and Cooper 1990b; Harris et al. 1994a; Harris et al. 1994b; Metcalfe et al. 1997). The average time of hatching (> 15 d) was also longer than what is normally

Table 7.4 Lowest observable effect concentrations (LOEC) and no observable effect concentration (NOEC) for PCAs and TCDD generated with Japanese medaka embryos.

Chemical	LOEC[A] $ng \cdot mL^{-1}$	NOEC $ng \cdot mL^{-1}$
$C_{10}H_{15.5}Cl_{6.5}$	460	62
$C_{10}H_{15.3}Cl_{6.7}$	370	50
$C_{11}H_{20}Cl_4$	420	57
$C_{12}H_{19.5}Cl_{6.5}$	55	9.6
$C_{14}H_{24.9}Cl_{5.1}$	> 3400	3400
$C_{14}H_{23.3}Cl_{6.7}$	> 1600	1600
TCDD	0.0017	0.0013

[A] LOEC and NOEC were based on any effect in egg or larvae.

expected (11-13 d). However, it is unlikely that this is due to exposure to the PCAs as there was no clear relationship with exposure concentration and control treatments were also low. As well, most of the eggs had hatched when the vials were checked 20 days after the final observation day (40 days after fertilization).

The mechanism of acute toxicity of PCAs appears to be narcosis. Most of the concentrations used in these experiments were well above estimated LC_{50} values for PCAs (Table 7.5), determined from equations with K_{ow} developed by Smith and Craig (1983) using rainbow trout (*Oncorhynchus mykiss*) exposed to a range of narcotic compounds. Similar relationships developed by Veith et al. (1983), using the fathead minnow (*Pimephales promelas*), yield LC_{50} concentrations for PCAs that are much higher than those of Smith and Craig (1983) and higher than all but the highest PCA exposure concentrations used in these experiments (Table 7.5). However, with the exception of the highest exposure concentration of $C_{14}H_{24.9}Cl_{5.1}$, PCA water concentrations which exceed the LC_{50} numbers generated from Veith et al. (1983) produced larvae that were lethargic or had no movement at all, suggesting a narcotic state. Further, we calculated the body concentrations that would cause narcosis assuming that all chemicals cause narcosis at body concentrations of 2 to 8 μmol/g (McCarty 1986; Sijm et al. 1993). In all cases where $C_{10}H_{15.3}Cl_{6.7}$ concentrations exceeded these calculated tissue concentrations, larvae appeared to be in a narcotic state. No larva exposed to the $C_{14}H_{23.3}Cl_{6.7}$ achieved these narcotic tissue concentrations, and none of the larvae exhibited any signs of narcosis. Further evidence that the acute toxic mechanism of PCAs is narcosis is the lack of effects in the embryos prior to death and the lack of effects at high but not narcotic exposure and

Table 7.5 Estimated LC_{50} values based on K_{ow} assuming the chemical is a narcotic and tissue concentrations required to cause a narcotic effect for PCAs and TCDD.

chemical	LC_{50} ($ng \cdot ml^{-1}$)[A]	LC_{50} ($ng \cdot ml^{-1}$)[B]	low estimate of narcotic tissue concentration ($\mu g \cdot g^{-1}$)[C]	high estimate of narcotic tissue concentration ($\mu g \cdot g^{-1}$)[C]
$C_{10}H_{15.5}Cl_{6.5}$	9.8	2500	73	290
$C_{10}H_{15.3}Cl_{6.7}$	9.3	2500	74	300
$C_{11}H_{20}Cl_4$	17	2000	58	230
$C_{12}H_{19.5}Cl_{6.5}$	4.7	2700	78	310
$C_{14}H_{25.9}Cl_{5.1}$	3.6	2500	74	300
$C_{14}H_{23.3}Cl_{6.7}$	2.5	2900	85	340
TCDD	4.2	2200	64	260

[A] The LC_{50} values were estimated based on the equation $\log LC_{50} = -0.88 \log K_{ow} - 1.9$ (Smith and Craig 1983) and assuming the chemical is a narcotic .

[B] The LC_{50} values were estimated based on the equation $\log LC_{50} = -0.94 \log K_{ow} + 0.94 \log (0.000068 K_{ow} + 1) - 1.25$ (Veith et al. 1983) and assuming the chemical is a narcotic .

[C] The low and high estimate of narcotic tissue concentrations are based on narcosis occurring at concentrations of 2 to 8 $\mu mol \cdot g^{-1}$ (McCarty 1986; van Wezel et al. 1996).

tissue concentrations. Narcosis is believed to be an all-or-nothing effect and it is believed that a "critical concentration" of narcotic toxin within the target tissue must be reached before toxicity occurs (Abernethy et al. 1988).

In contrast to the PCAs, TCDD caused effects and death in the eggs at concentrations which were orders of magnitude lower than the PCA exposures, as well as calculated narcotic water and tissue concentrations (Tables 7.3 and 7.5). These results are not surprising, because TCDD is an extremely toxic chemical with specific modes of action (Safe 1990; Safe 1992). The LC_{50} determined for TCDD in this study is in the range reported in other studies for TCDD using Japanese medaka eggs (Wisk and Cooper 1990a; Wisk and Cooper 1990b; Metcalfe et al. 1997). As well, Wisk and Cooper (1990a) reported an ED_{50} of 0.24 $ng \cdot g^{-1}$ for TCDD equilivalents in larvae, which is similar to the concentration in eggs at the LOEC in this study. Wisk and Cooper (1990a) also observed many of the same lesions as were observed in this study, including slowing and cessation of blood flow throughout the animal, and collections of blood.

LC_{50} values for the PCAs are at least 3 to 6 orders of magnitude higher than those observed for chlorinated dioxin and furan congeners, polychlorinated biphenyl congeners (PCB) or polychlorinated diphenyl ether congeners (PCDE) using Japanese medaka eggs (Table 7.6). These high LC_{50} values resulted in extremely low toxic equivalency factors (TEF) for the PCAs, which may overestimate the true TEFs of some of the PCAs because no toxic effects were observed. TEFs are commonly used to assess the toxic potential of a compound and to assess the overall toxicity of an organism's organochlorine burden (Safe 1990). It should be noted that these TEFs were based on acute toxicity (LC_{50}) and not on

Table 7.6 LC_{50} values (survival day 20) and toxic equivalency factors (TEFs) for PCAs, TCDD and a number of other hydrophobic organochlorine compounds generated with Japanese medaka embryos.

Chemical	LC_{50} $ng \cdot mL^{-1}$	LC_{50} $pmol \cdot mL^{-1}$	TEF[A]	ref.[B]
$C_{10}H_{15.5}Cl_{6.5}$	> 2700	> 7400	< 0.0000024	1
	< 9600	< 26000	> 0.00000068	
$C_{10}H_{15.3}Cl_{6.7}$	> 5100	> 14000	< 0.0000013	1
	< 7700	< 21000	> 0.00000086	
$C_{11}H_{20}Cl_4$	> 8900	> 30000	< 0.00000058	1
$C_{12}H_{19.5}Cl_{6.5}$	> 270	> 690	< 0.000026	1
$C_{14}H_{24.9}Cl_{5.1}$	> 3400	> 9100	< 0.0000019	1
$C_{14}H_{23.3}Cl_{6.7}$	> 1600	> 3700	< 0.0000047	1
2,3,7,8-TCDD	> 0.0017	> 0.005	< 3.4	1
	< 0.0037	< 0.011	> 1.5	
2,3,7,8-TCDD	0.009	0.028	0.63	2
2,3,7,8-TCDD	0.013	0.040	0.44	3
2,3,7,8-TCDF	0.016	0.052	0.34	3
1,2,3,7,8-PCDD	0.027	0.076	0.23	3
1,2,3,4,7,8-HCDD	2.9	7.4	0.0024	3
2,3,7,8-TCDD	0.020	0.062	0.29	4
CB 126	0.215	0.66	0.027	4
CB 81	16	55	0.00032	4
CB 77	> 250	> 860	0.000021	4
2,3,7,8-TCDD	0.0057	0.018	1	5
PCDE #105	10.8	31.8	0.00054	5
PCDE #77	170.6	557	0.000030	5
PCDE #118	605.2	1,780	0.0000095	5
PCDE #71	> 2,500	> 8,169	< 0.0000021	5

[A] TEFs were calculated as the ratio of the LC_{50} of each chemical ($pmol \cdot ml^{-1}$) to the LC_{50} of TCDD ($pmol \cdot ml^{-1}$) from reference 5.

[B] References: 1 - this work; 2 - Wisk and Cooper 1990b; 3 - Wisk and Cooper 1990a; 4 - Harris et al. 1994; 5 - Metcalfe et al. 1997.

more commonly used criteria such as carcinogenicity studies or Ah receptor-mediated responses (Safe 1990). TEFs were generated assuming that TCDD has a TEF of 1, which is a common method for calculating TEFs.

The major objective of this work was to compare the toxicity of PCAs of different carbon chain length, chlorine content and chlorine position. The acute toxic mechanism of the PCAs used in this experiment appears to be narcosis. Narcosis will occur at tissue concentrations of 2 to 8 $\mu mol \cdot g^{-1}$ (McCarty 1986; van Wezel et al. 1996), and therefore as molecular weight increases, the tissue concentration required to cause narcosis increases. The LC_{50}s of narcotic chemicals decrease with increasing K_{ow} (Smith and Craig 1983; Veith et al. 1983). Therefore, the concentrations of PCAs required to cause narcosis will be greater for PCAs with longer carbon chain lengths and greater chlorine contents because these cause an increase in molecular weight and K_{ow} (Sijm and Sinnige 1995).

It appears that chlorine substitution on the terminal carbons of PCAs does not significantly increase or decrease the toxicity of C_{10}- and C_{14}-PCAs. It would also appear that none of the PCAs used in these experiments are significantly more or less toxic than commercial PCA formulations. The majority of published reports, based almost exclusively on commercial PCA formulations, have found that PCAs have very low acute toxicities to fish (Thompson and Madeley 1983a; Thompson and Madeley 1983c; Madeley and Thompson 1983c; Madeley and Maddock 1983 c; Madeley and Maddock 1983 d; Madeley and Maddock 1983 e; Linden et al. 1979; Tomy et al. 1998).

7.5 Conclusions and Summary

We have reported the first acute toxicity data for PCAs of a specific carbon chain length (C_{10}, C_{11}, C_{12} and C_{14}) and chlorine content using the Japanese medaka embryo toxicity assay. Extremely high concentrations of $C_{10}H_{15.5}C_{6.5}$ and $C_{10}H_{15.3}Cl_{6.7}$ (9600 and 7700 $ng \cdot ml^{-1}$, respectively) caused 100% mortality in eggs, but no other significant mortalities or lesions were observed at lower concentrations, or in any eggs exposed to the other PCAs. Larvae which had tissue concentrations above the threshold for narcosis (2 to 8 μmol/g) were lethargic with little or no movement. Those larvae which did not achieve this concentration appeared normal and active. These results suggest that the acute toxic mechanism of PCAs is narcosis and that chlorine substitution on the terminal carbons of PCAs does not significantly increase or decrease the toxicity of C_{10}- and C_{14}-PCAs. TCDD caused lesions and death in the medaka eggs at water and tissue concentrations which were orders of magnitude less than the PCA concentrations.

These experiments only begin the process of evaluating the toxicity of individual PCAs congeners. Synthesizing all the possible PCA congeners is a monumental task and would produce such a large number of compounds as to make it impossible to test them all. However, these results do begin the task of identifying which, if any, PCA congeners are more toxic and what PCA characteristics cause toxicity. More highly chlorinated PCAs and long chain PCAs need to be tested to provide a more complete examination of PCA toxicity to aquatic organisms.

8. EXAMINATION OF THE BEHAVIOR AND THE LIVER, POSTERIOR KIDNEY AND THYROID HISTOLOGY OF JUVENILE RAINBOW TROUT (*Oncorhynchus mykiss*) EXPOSED TO HIGH DIETARY CONCENTRATIONS OF C_{10}-, C_{11}-, C_{12}- AND C_{14}-POLYCHLORINATED *n*-ALKANES

8.1 Abstract

Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to high dietary concentrations of 6 polychlorinated *n*-alkane (PCAs) standards ($C_{10}H_{15.5}C_{6.5}$, $C_{10}H_{15.3}Cl_{6.7}$, $C_{11}H_{20}Cl_4$, $C_{12}H_{19.5}Cl_{6.5}$, $C_{14}H_{24.9}Cl_{5.1}$ and $C_{14}H_{23.3}Cl_{6.7}$) for 21 and 85 days to assess their effects on behavior and liver, posterior kidney and thyroid histology. This is the first histological work using PCAs of known carbon chain length and chlorine content and the first effort to examine the histolopathology of fish exposed to PCAs. PCAs, also known as chlorinated paraffins, are complex industrial products for which there is a lack of toxicological data on individual congeners. With the exception of trout exposed to $C_{14}H_{24.9}Cl_{5.1}$, many of the trout exposed to the PCAs showed a lack of, or a slowed startle response, loss of equilibrium and dark coloration. These responses are indicative of a narcotic mode-of-action. Histopathological lesions were observed in the liver of trout exposed to $C_{10}H_{15.3}Cl_{6.7}$ at dietary concentrations of 13 $\mu g \cdot g^{-1}$and tissue concentrations of 0.92 ± 0.24 $\mu g \cdot g^{-1}$. These multi-focal lesions included coagulative necrosis of hepatocytes with associated pigmented macrophage proliferation. These changes are an established

biomarker of contaminant exposure in fish. A single trout from this group also had significant histopathological effects in the kidney, which included multiple foci of necrotic, desquamating tubules with extensive accumulation of debris. No lesions were found in the liver or kidney of trout exposed to the other PCAs, or in any thyroid tissue. The relative hepatocyte size of all PCA exposed trout were smaller than control trout, although only a few were statistically significant. It would appear that PCA toxicity is inversely related to carbon chain length, which has been observed in similar studies using mammals. These results suggest that histopathological effects would only occur at extremely high exposure and tissue concentrations of PCAs, but at levels which are well beyond those observed in wild fish or invertebrates.

8.2 Introduction

Polychlorinated *n*-alkanes (PCAs) with carbon chains between 10 and 30 are used for a variety of industrial applications including lubricating additives, flame retardants, adhesives, sealants and a number of other miscellaneous applications (Windrath and Stevenson 1985; Government of Canada 1993; Willis et al. 1994). Commercial PCA formulations, also know as chlorinated paraffins (CPs), are classified as short (C_{10-13}), medium (C_{14-17}) and long (C_{18-30}) with varying amounts of chlorination (~35-70% by weight). Annual world production of PCA is estimated at greater than 300 kilotonnes, and they remain one of the last high molecular weight organochlorines in production and use in North America and western Europe (Swedish National Chemicals Inspectorate 1991). PCAs are prevalent in the environment, although until recently it has been difficult to measure PCAs in environmental matrices (biota, sediment, water, etc) (Tomy 1997). For

example, short chain (C_{10-13}) PCAs were among the most prominent OCs in aquatic and terrestrial biota from Sweden (Jansson et al. 1993) and sediment from mid-latitude and arctic lakes (Tomy et al. 1997b). Because they are produced with free radical chlorination, a single PCA formulation consists of thousands of different compounds, or congeners, with a range of physical-chemical properties (Tomy et al. 1998). Currently, there is a lack of PCA standards with single carbon chain length and chlorine number and/or position.

The toxicity of PCAs to aquatic organisms appears to be low, however a majority of these data has been generated with water exposures using commercial PCA products and death as a toxicological endpoint (Thompson and Madeley 1983c; Madeley and Maddock 1983b; Madeley and Maddock 1983c; Linden et al. 1979; Tomy et al. 1998). Water exposures are problematic because PCAs are very hydrophobic, and in many cases, effects concentrations reported (e.g. LC_{50} or no observable effects concentrations (NOEC)) are above the nominal exposure concentrations used in the experiment (Thompson and Madeley 1983a; Thompson and Madeley 1983b; Thompson and Madeley 1983c; Tomy et al. 1998). Researchers have also reported cloudy water in some studies, due to extremely high concentrations, and the deaths reported may be due a mechanical effect (coating of the organism) and not a toxic effect (Thompson and Madeley 1983c; Madeley and Thompson 1983c). The use of commercial PCA formulations is also problematic because the individual compounds in a commercial PCAs will differ from what is found in the environment due to different rates of degradation, biotransformation and bioaccumulation. Therefore, evaluating the toxicological significance of environmental PCA concentrations is difficult. Death as a toxicological endpoint also presents problems because sub-lethal effects are missed, although a number of studies have reported growth

effects and reduced feeding (Madeley and Thompson 1983c; Hill and Maddock 1983b), and it is difficult to achieve death via water for very hydrophobic compounds. Also, many of these studies have been short term ($\leq$ 28 d) and chronic effects in aquatic organisms have not been studied. This is of concern because a number of sub-lethal effects have been observed in mammals and mammalian cell lines, including peroxisomal proliferation in the liver (Wyatt et al. 1993; Elcombe 1994) and inhibition of gap junction intercellular communication (Kato and Kenne 1996).

There is very little information on the toxic mechanisms of PCAs, although a review of PCA toxiological data suggests that the acute toxic mechanism of PCA is narcosis (Tomy et al. 1998). Therefore, there is a gap in knowledge regarding the toxic mechanism of PCAs, a lack of toxicity data on PCAs of a single carbon chain length and chlorine content, and a lack of data on sub-lethal effects in aquatic organisms. To address these data gaps, juvenile rainbow trout were exposed to high dietary concentrations of 6 PCAs, with single carbon chain lengths (C_{10}, C_{11}, C_{12} and C_{14}) and known chlorine content to assess their short term (21 days) effects on behavior and their short and long term (85 days) effects on the histology of the liver, posterior kidney and thyroid. The trout were exposed to high concentration in an attempt to achieve lethal concentrations. Dietary exposures were chosen to avoid problems with high water concentrations. Behavioral monitoring was chosen because it has shown considerable promise as a screening tool to identify the mode of action of various industrial chemicals (McKim et al. 1987). The advantage of behavioral monitoring is that it does not interfere with the other toxicological testing. Histopathological alterations are the net result of adverse biochemical and physiological changes in an organism, and represent a higher level toxic response (Hinton

and Lauren 1990a; Hinton et al. 1992). The liver, kidney and thyroid were chosen for histological work because there is evidence, based on mammalian studies, that these are target organs of PCA toxicity (Serrone et al. 1987; Bucher et al. 1987; Elcombe et al. 1994;). As well, there is a fairly large data base of toxicological effects of contaminants on the liver and thyroid of fish. Although there is limited data on the toxic effects of contaminants on fish kidney, its function suggests that it may be an important target organ (Hinton et al. 1992).

8.3 Methods and Materials

8.3.1 Chemicals

Six PCAs were synthesized for this experiment; three by chlorination of an alkene starting material (1,5,9-decatriene; 1,10-undecadiene; and 1,13-tetradecadiene) (Figure 8.1) (Sigma-Aldrich, Oakville, ON), and three by free radical chlorination of a ^{14}C-labeled alkane starting material (C_{10} and C_{14}) (Table 8.1) (Sigma, St. Louis, MO). There were fewer individual PCA compounds produced from the alkene starting material than the alkane starting material (Table 8.1 and Figure 8.2). Also, PCAs produced from the alkenes had chlorines substituted at the double bonds, providing information on chlorine positions. A list of the starting materials, the PCA formed and their relative abundance in the standards is found in Table 8.1.

PCA congeners were synthesized by bubbling SO_2Cl_2, at room temperature, into neat solutions of the respective *n*-alkanes, contained in a flask wrapped in aluminium foil to exclude light. In the absence of light, these conditions were expected to lead, predominantly, to chlorine addition at the double bond(s). Reaction mixtures were shaken

with NaOH (0.05M), the aqueous phase was then removed and then the organic phase was dried with $MgSO_4$. The drying agent was removed by filteration. In addition to the expected products formed by chlorine additions to each bond, a number of by-products were formed that resulted from free radical substitution of hydrogen atom(s) by chlorine atom(s) (Figures 8.1 and 8.2). Un-reacted starting material and SO_2Cl_2 were removed from the PCAs using Florisil column chromatography (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 un-reacted alkene starting material but no PCAs and was discarded. F2 and F3 contained the PCAs and were combined. After Florisil clean-up, greater than 99% of the products were found to be PCAs based on chromatograms generated by gas chromatography (GC) with an electron capture detector (ECD) and GC-negative ion MS. GC conditions are described in Chapter 5.

The free radically chlorinated PCAs were synthesized using 2 methods. The C_{12}-PCA was provided by Å. Bergman (Stockholm University), and was synthesized using a method which has been described previously (Bergman et al. 1981). The C_{10} and C_{14}-PCAs were synthesized by adding a quantity of SO_2Cl_2 into the respective *n*-alkanes that would provide the desired chlorination (i.e. ~ 6-7 Cl per alkane). The alkanes and SO_2Cl_2 were combined in a small glass flask (250 μL) and exposed to UV light for one hour. Un-reacted alkane and SO_2Cl_2 was removed from the standard using the Florisil methods describe above. After Florisil clean-up, greater than 99% of the standards were found to be PCAs based on GC-ECD and GC-NIMS. Radio purity was checked by HPLC and was

Table 8.1 PCAs used in juvenile rainbow trout toxicity tests.

PCA standard	starting material	PCA formed[A]	% in standard
$C_{10}H_{15.5}Cl_{6.5}$	1,5,9-decatriene	1,2,5,6,9,10-hexachlorodecance ($C_{10}H_{16}Cl_6$)	55
		x,1,2,5,6,9,10-hexachlorodecance ($C_{10}H_{15}Cl_7$)	41
		x,y,1,2,5,6,9,10-hexachlorodecance ($C_{10}H_{14}Cl_8$)	4
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	1-^{14}C-decane	$C_{10}H_{18}Cl_4$	0.1
		$C_{10}H_{17}Cl_5$	4.1
		$C_{10}H_{16}Cl_6$	37
		$C_{10}H_{15}Cl_7$	45
		$C_{10}H_{14}Cl_8$	12
		$C_{10}H_{13}Cl_9$	1.5
$C_{11}H_{18.4}Cl_{5.6}$	1,10-undecadiene	x,1,2,10,11-pentachloroundecance ($C_{11}H_{19}Cl_5$)	40
		x,y,1,2,10,11-hexachloroundecance ($C_{11}H_{18}Cl_6$)	49
		x,y,z,1,2,10,11-hexachloroundecance ($C_{11}H_{17}Cl_7$)	10
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	1-^{14}C-dodecane	$C_{12}H_{19}Cl_5$	14
		$C_{12}H_{18}Cl_6$	31
		$C_{12}H_{17}Cl_7$	50
		$C_{12}H_{16}Cl_8$	5
$C_{14}H_{24.9}Cl_{5.1}$	1,13-tetradecadiene	1,2,13,14-tetrachlortetradecance ($C_{14}H_{26}Cl_4$)	11
		x,1,2,13,14-pentachlorotetradecance ($C_{14}H_{25}Cl_5$)	74
		x,y,1,2,13,14-hexachlorotetradecance ($C_{14}H_{24}Cl_6$)	14
		x,y,,z1,2,13,14-heptachlorotetradecance ($C_{14}H_{23}Cl_7$)	1.0
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	1-^{14}C-tetradecane	$C_{14}H_{26}Cl_4$	0.2
		$C_{14}H_{25}Cl_5$	4.4
		$C_{14}H_{24}Cl_6$	34
		$C_{14}H_{23}Cl_7$	45
		$C_{14}H_{22}Cl_8$	14
		$C_{14}H_{21}Cl_9$	1.9

[A] The positions of chlorine atoms designated with an x or y are unknown.

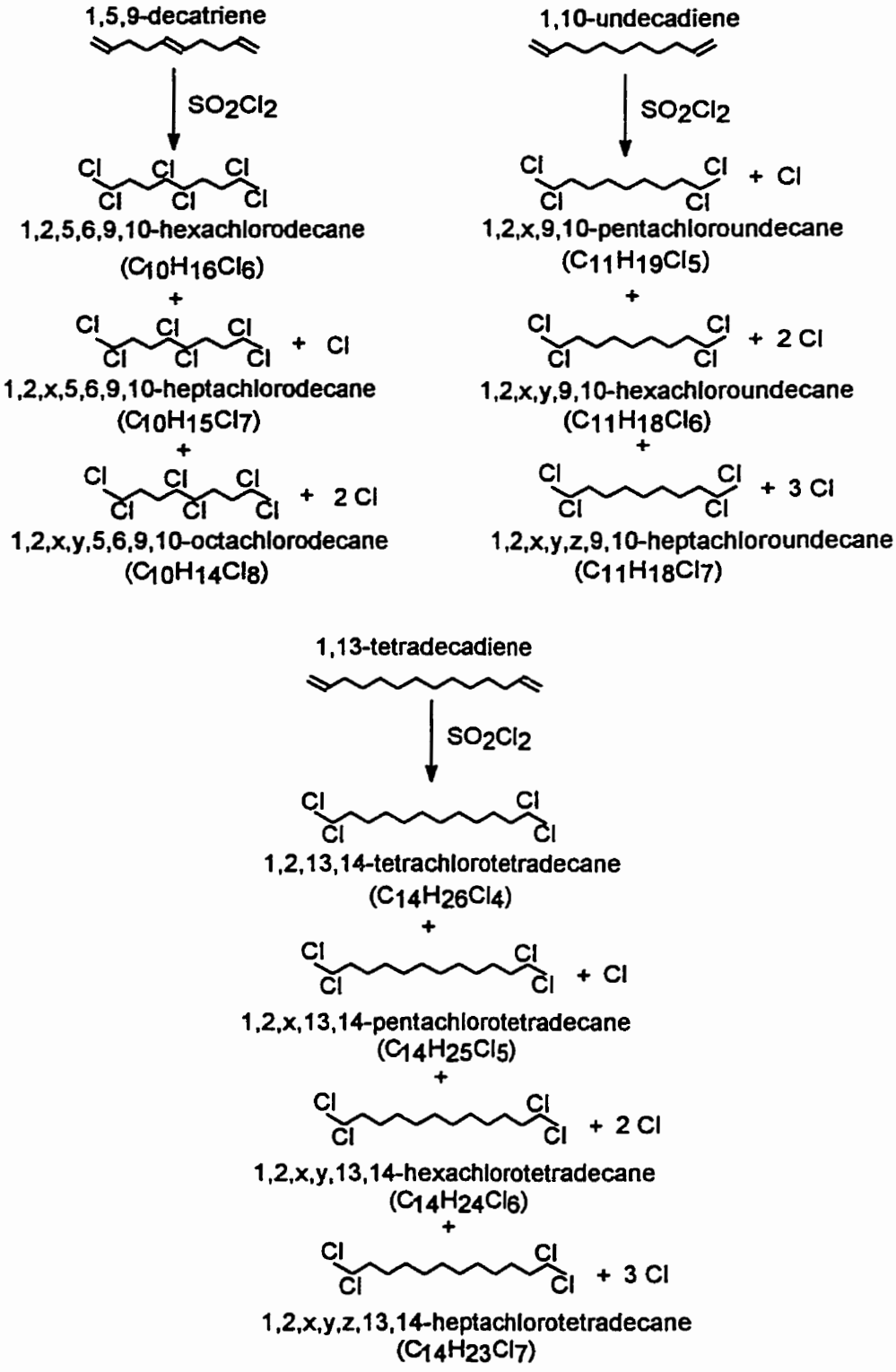

Figure 8.1 Polychlorinated alkanes and their alkene starting material used in rainbow trout toxicity tests.

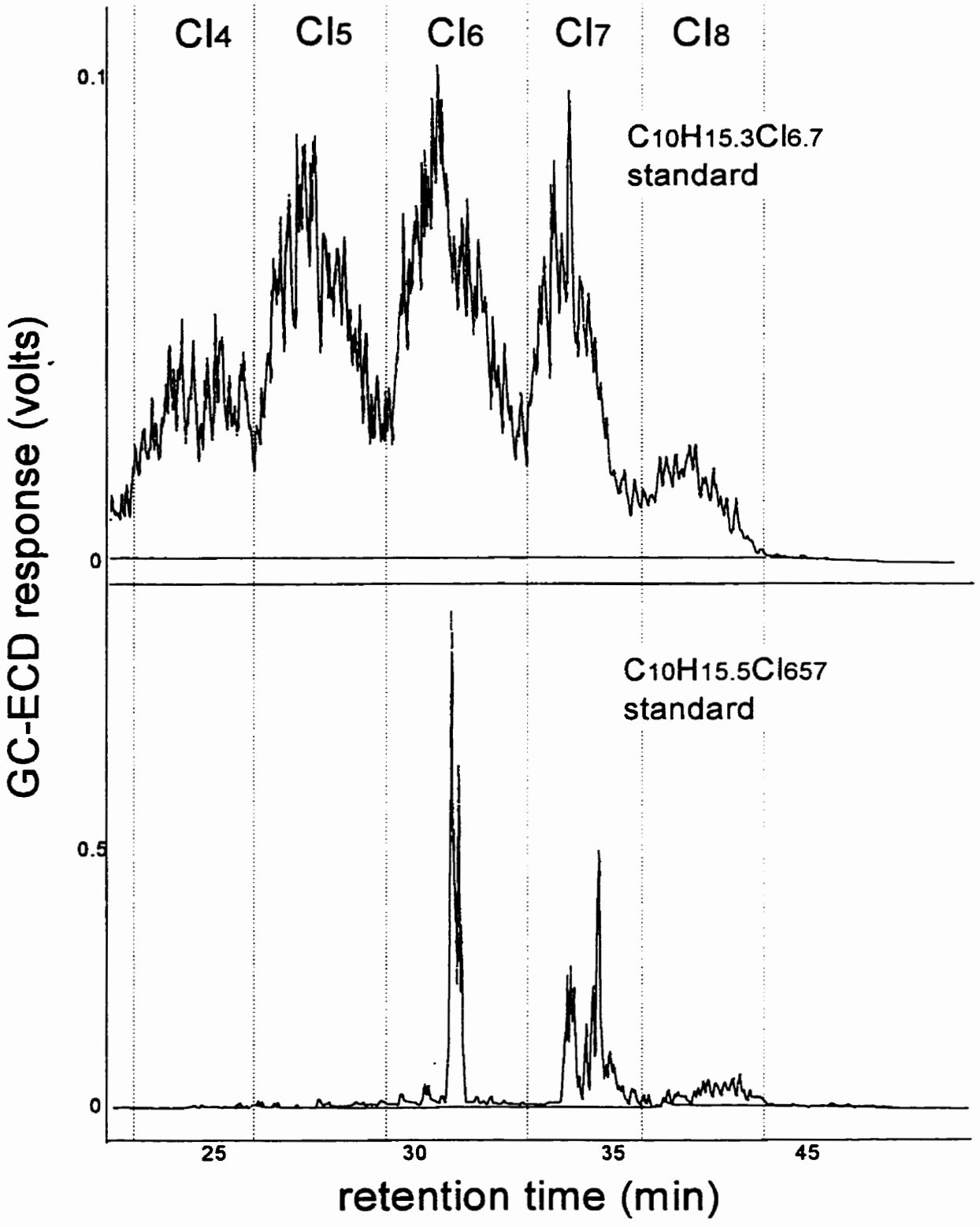

Figure 8.2 GC-ECD chromatograms of the $C_{10}H_{15.3}Cl_{6.7}$ standard (top), a free-radically chlorinated decane product, and of the $C_{10}H_{15.5}Cl_{6.5}$ standard (bottom), a chlorinated alkene product.

found to be greater than 99%. The specific activities ($dpm \cdot ng^{-1}$) of the $C_{10}H_{15.3}Cl_{6.7}$, $C_{12}H_{19.5}Cl_{6.5}$, and $C_{14}H_{23.3}Cl_{6.7}$ were 42.3, 94.2 and 88.3, respectively.

8.3.2 Food preparation and experimental protocol

Food was spiked by suspending a known quantity of each PCA standard in 100 ml of hexane and 50 g of commercial fish food (Martin's Feed Mills Ltd., Elmira, ON, Canada) and slowly evaporating to dryness. Food was air-dried for 24 hours and stored at 10°C. The fish food consisted of 41% protein, 14% lipid and 3% fiber. Concentrations in the food were determined by the same analytical techniques used to determine levels in the rainbow trout tissue (*see below*), and are found in Table 8.2. 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 g) were exposed to three concentrations of each PCA (Table 8.2), along with 3 control groups. The daily rate of feeding was equal to 1.5% of the mean weight of the rainbow trout. Ten rainbow trout were used for each treatment and were housed in separate 10, 20 or 40 L glass aquarium with flow-through UV and carbon dechlorinated City of Winnipeg tap water (11°C). After 21 days of exposure, all the trout from the two highest exposure concentrations, and 2 control groups, were euthanized and 5 trout were designated for histological examination and 5 for determination of PCA concentrations. Three fish were sacrificed from the low exposure groups and the last control group for possible histological examination. The remaining fish (from the low exposure group) were exposed for an additional 61 days (85 days in total). On day 85, 4 fish were sacrificed for histological examination and three for determination of PCA concentrations.

Table 8.2 Concentrations (mean ± 1 standard error, wet weight) of PCAs in food and rainbow trout. Concentrations were not determined in liver, GI tract and carcass of rainbow trout exposed to non-labeled PCAs.

PCA	exposure length (days)	food conc. ($\mu g \cdot g^{-1}$)	whole fish conc. ($\mu g \cdot g^{-1}$)	liver conc. ($\mu g \cdot g^{-1}$)	GI tract conc. ($\mu g \cdot g^{-1}$)	carcass conc. ($\mu g \cdot g^{-1}$)
$C_{10}H_{15.5}Cl_{6.5}$	85	0.87	0.10 ± 0.029	-	-	-
	21	12	0.84 ± 0.14	-	-	-
	21	62	0.92 ± 0.45	-	-	-
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	85	0.84 ± 0.65	0.099 ± 0.036	0.43 ± 0.11	0.44 ± 0.29	0.079 ± 0.035
	21	13 ± 0.21	0.92 ± 0.24	0.50 ± 0.24	1.6 ± 0.22	0.47 ± 0.089
	21	74 ± 23	3.0 ± 1.0	0.76 ± 0.20	9.6 ± 2.0	2.1 ± 0.92
$C_{11}H_{20}Cl_4$	85	3.7	0.10 ± 0.015	-	-	-
	21	53	5.5 ± 1.1	-	-	-
	21	290	4.0 ± 0.50	-	-	-
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	85	1.9 ± 0.042	0.14 ± 0.041	0.17 ± 0.069	0.42 ± 0.12	0.098 ± 0.029
	21	14 ± 0.11	0.79 ± 0.15	0.53 ± 0.23	2.5 ± 0.61	0.61 ± 0.099
	21	58 ± 1.5	1.1 ±0.30	0.50 ± 0.052	4.1 ± 1.5	0.71 ± 0.16
$C_{14}H_{24.9}Cl_{5.1}$	85	0.082	0.018 ± 0.0027	-	-	-
	21	0.78	0.11 ± 0.0018	-	-	-
	21	2.9	0.028 ± 0.0079	-	-	-
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	85	5.7 ± 0.061	0.57 ± 0.18	.41 ± 0.060	1.8 ± 0.74	0.45 ± 0.12
	21	29 ± 0.51	1.3 ± 0.33	0.67 ± 0.10	3.6 ± 1.2	1.1 ± 0.21
	21	78 ± 1.0	0.22 ± 0.057	0.33 ± .098	1.2 ± 0.19	0.15 ± 0.048

8.3.3 Extraction and analysis of PCAs

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

Extraction and analysis of non-radiolabeled PCAs were identical to the methods used by Fisk et al. (1997). In brief: carcass samples were freeze dried and homogenized in toluene, and OCN was added as a recovery standard. 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 were diluted to 10 ml prior to GC analysis.

PCA standards were used as external standards for quantification of food and fish samples using a GC equipped with an electron capture detector (ECD). The response of all

the PCAs in the standard were combined and compared with fish and food extracts. Samples were analyzed on a Varian 3600-GC equipped with a 60 m x 0.25 mm DB-5 column and an ^{63}Ni-ECD. The carrier gas was H_2. Responses of the PCAs were monitored by analyzing standards after every 4 samples. The limits of detection for each PCA standard were approximately 1 $ng \cdot g^{-1}$ by GC-ECD.

8.3.4 Behavioral Monitoring

Behavioral monitoring was carried out daily during the 21 day exposures. The general behavior, coloration and body conditions were checked, followed by tapping the aquarium to observe the startle response behavior. Fish were then fed and their feeding habits were observed until the food was consumed or 5 min had passed. If food had not been consumed within 5 min, the aquarium was checked periodically over the next 2 hours to see if the food had been consumed. Behavioral data were compared with those described by McKim et al. (1987).

8.3.5 Tissue Processing and Staining

After being euthanized, the peritonel wall of rainbow trout were split open to expose the internal organs, and their jaws were cut at the corners, to expose thyroid tissue, and were fixed in Bouin's fixative for 48 hours and stored in 70% ethanol. Liver, posterior kidney and thyroid tissue was excised from the trout, and then rinsed in 4 changes of 70% ethanol and stored until processing.

Tissues were processed in an automated tissue processor (IL MUP Tissue Processor) using an ethanol/butanol series and embedded in Tissue Prep II paraffin.

Tissues were then embedded in paraffin and sectioned at 6 μm for liver, 7 μm for posterior kidney and 7 μm for thyroid.

Liver, posterior kidney and thyroid were stained for light microscopy and basic histopathological analyses with hematoxylin and eosin. All chemicals were obtained from Fisher Scientific (Fisher Scientific, Edmonton, AB), with the exception of phosphotungstic acid which was obtained from Sigma Chemicals (Sigma Chemical, St. Louis, MO). The protocol employed followed the method described in Edwards (1950). Briefly, the procedure involved routine clearing and rehydration, staining in Harris' hematoxylin, differentiation in a 0.33% solution of phosphotungstic acid followed by treatment in a 0.33% solution of sodium citrate, counterstaining in aqueous Eosin Y, dehydration, and clearing. Slides were mounted using Cyto-seal and 1 ½ mm glass coverslips.

Photomicrographs were taken on a Zeiss bright field microscope with a built in camera, using Ilford PANF 135 film (ISO 50/18° DX).

8.3.6 Histological Examinations and Histomorphological Measurements

Only tissue from trout exposed to the medium (21 days of exposure) and low (85 days of exposure) concentrations were examined for histopathological effects and histomorphological measurements. Liver, posterior kidney and thyroid tissue from three trout from each of these PCA-treatment groups, along with two control groups, were examined.

To perform histomorphological measurements, microscope images were projected onto a Summagraphics Bit Pad (Summagraphics, Fairfield, CT, USA) and measured with Sigma-Scan Version 3.90 (Jandel Scientific, Corte Madera, CA, USA).

8.3.6.1 Liver. For each fish, the hepatocyte nuclear diameter was the mean of a minimum of 50, randomly selected, spherical nuclei (Figure 8.3). Relative hepatocyte size were determined by counting the number of cells (by counting nuclei) in two areas (38,000 um^2) of liver parenchyma which appeared "normal". The relative hepatocyte size was equal to the total area (80,000 um^2) divided by the number of hepatocytes in both fields. Areas were chosen that had few structures other than hepatocytes. The nucleus : cytoplasm area ratio was determined by dividing the hepatocyte nuclear area ($\Pi \cdot r^2$) by the cytoplasm area minus the nuclear area. It should be noted that these methods may not give an accurate indication of hepatocyte size heterogeneity (C. Metcalfe, personal communication). This would affect the relative hypatocyte size and nucleus-cytoplasm ratios.

8.3.6.2 Posterior kidney. Tubule epithelium heights were determined by randomly selecting 15 cross sections from the second segment of the proximal tubule (P2) (Figure 8.4). The mean of two epithelial cell heights, which were opposite one another, were measured for each cross section by measuring from the brush border to the outer membrane of the cell. Cells were measured from the basement membrane to the tip of the luminal brush borders.

8.3.6.3 Thyroid. The cells heights of thyroid epithelium were measured in a total of 15 follicles per fish (Figure 8.5). Measurements were made at 4 points within each follicle at 90 degrees from one another.

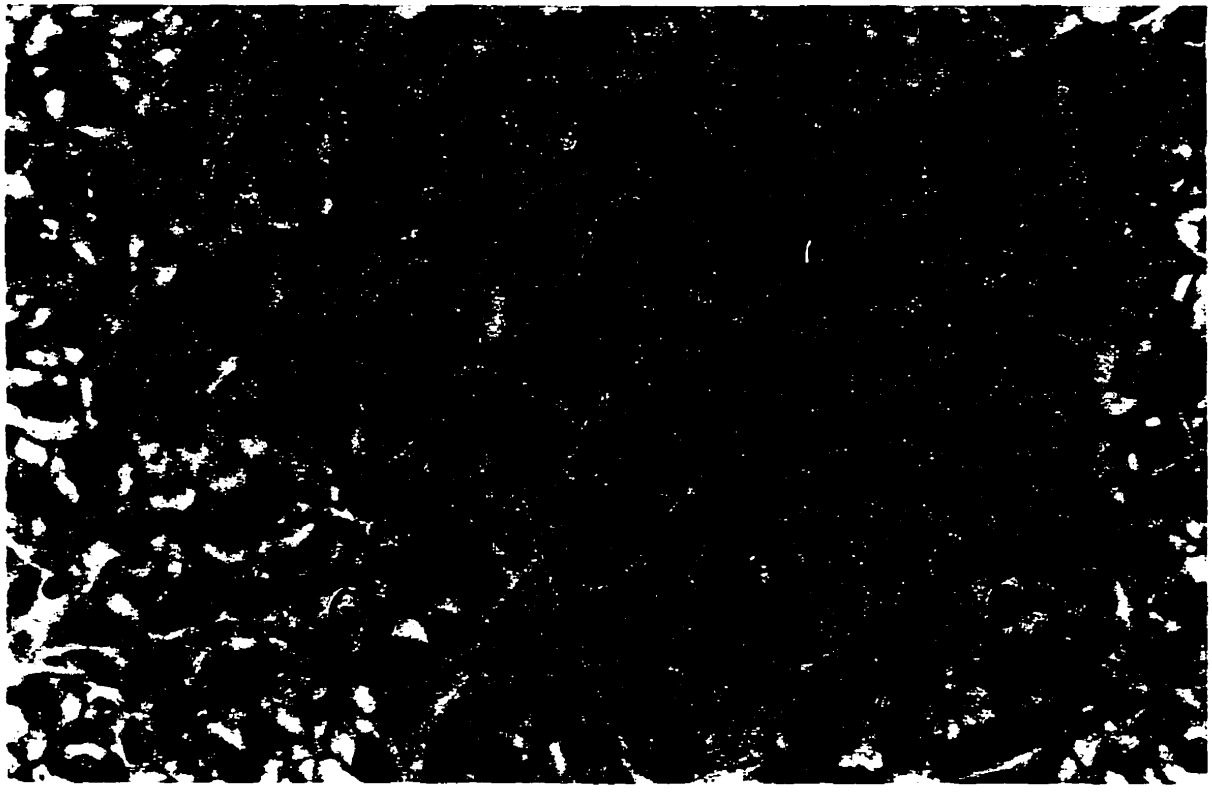

Figure 8.3 Micrograph (mag. 400X) of liver from a control rainbow trout. N: hepatocyte nucleus, G: glycogen or lipid, S: sinusiod, cord or lamina between arrows.

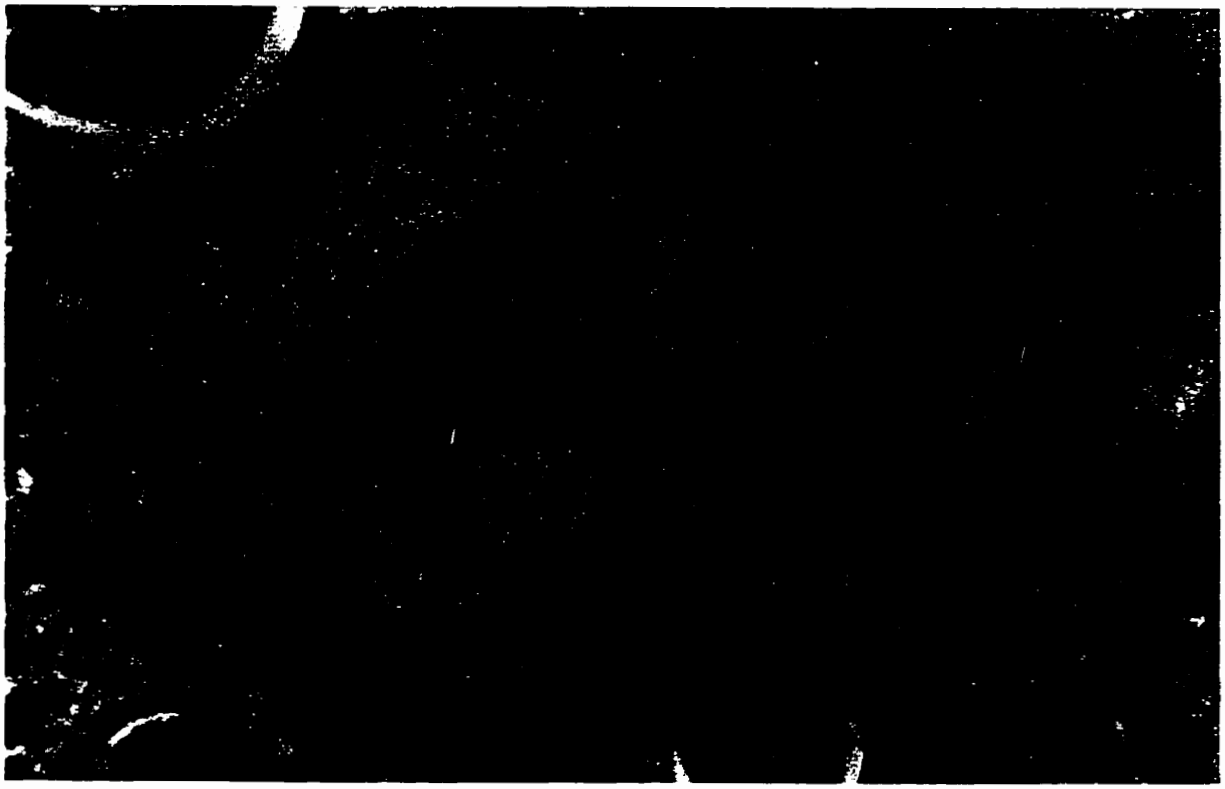

Figure 8.4 Micrograph (mag. X 400) of posterior kidney from a control rainbow trout. P1: first segment of proximal tubule; P2: second segment of proximal tubule; B: brush border, H: hematopoietic tissue.

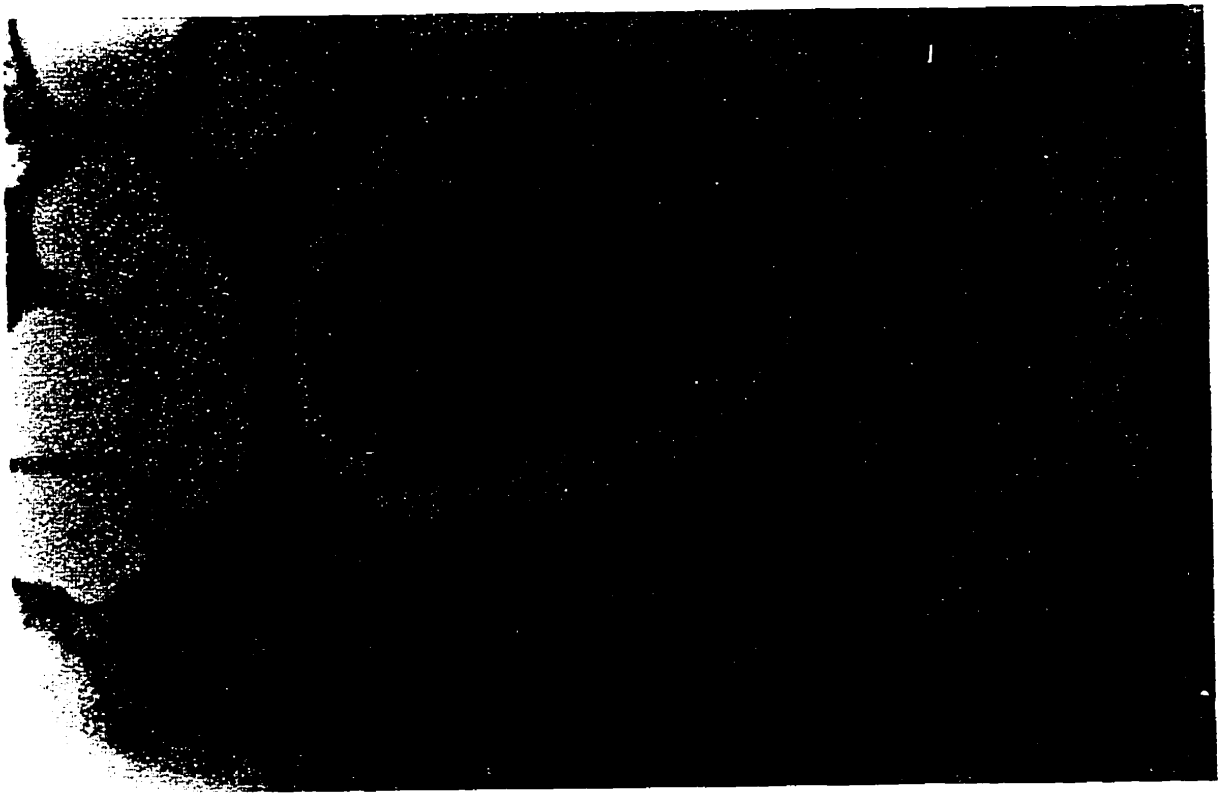

Figure 8.5 Micrograph (mag X 400) of thyroid from control rainbow trout. TF: thyroid follicle; E: thyroid epithelium; C: colloid, V: vacuole.

8.3.7 Statistical analysis

Histological morphometrics means of PCA treatments were compared with the control using analysis of variance and a Dunnett pairwise comparison test. All statistics were performed with SYSTAT for Windows, Version 5 (SYSTAT, Evanston, IL, USA).

8.4 Results

8.4.1 Fish weight and LSI

Mean fish weights (n = 3) and liver somatic indices did not vary between PCA treatment trout and control trout after 21 and 85 days (Table 8.3).

8.4.2 PCA concentrations in food and fish

With the exception of the $C_{14}H_{24.9}Cl_{6.7}$ treatments, PCA concentrations in food were consistent between the various PCA treatments (Table 8.2). Fish from a number of the high concentration exposures fed inconsistently (Table 8.4), which resulted in tissue concentrations that were lower, or similar, to tissue concentrations in medium concentration exposures. The relative composition of individual PCAs remained consistent between standards, food extracts and trout extracts (Figures 8.6, 8.7 and 8.8).

8.4.3 Fish Behavior and general appearance

Observations on the behavior of the rainbow trout during the 21 exposures are summarized in Table 8.4. Trout behavior in the three control aquarium was similar and was used as normal behavior for evaluation of PCA treatment trout. Trout from the lowest exposures of all PCAs exhibited similar behavior to the control fish. With the exception of

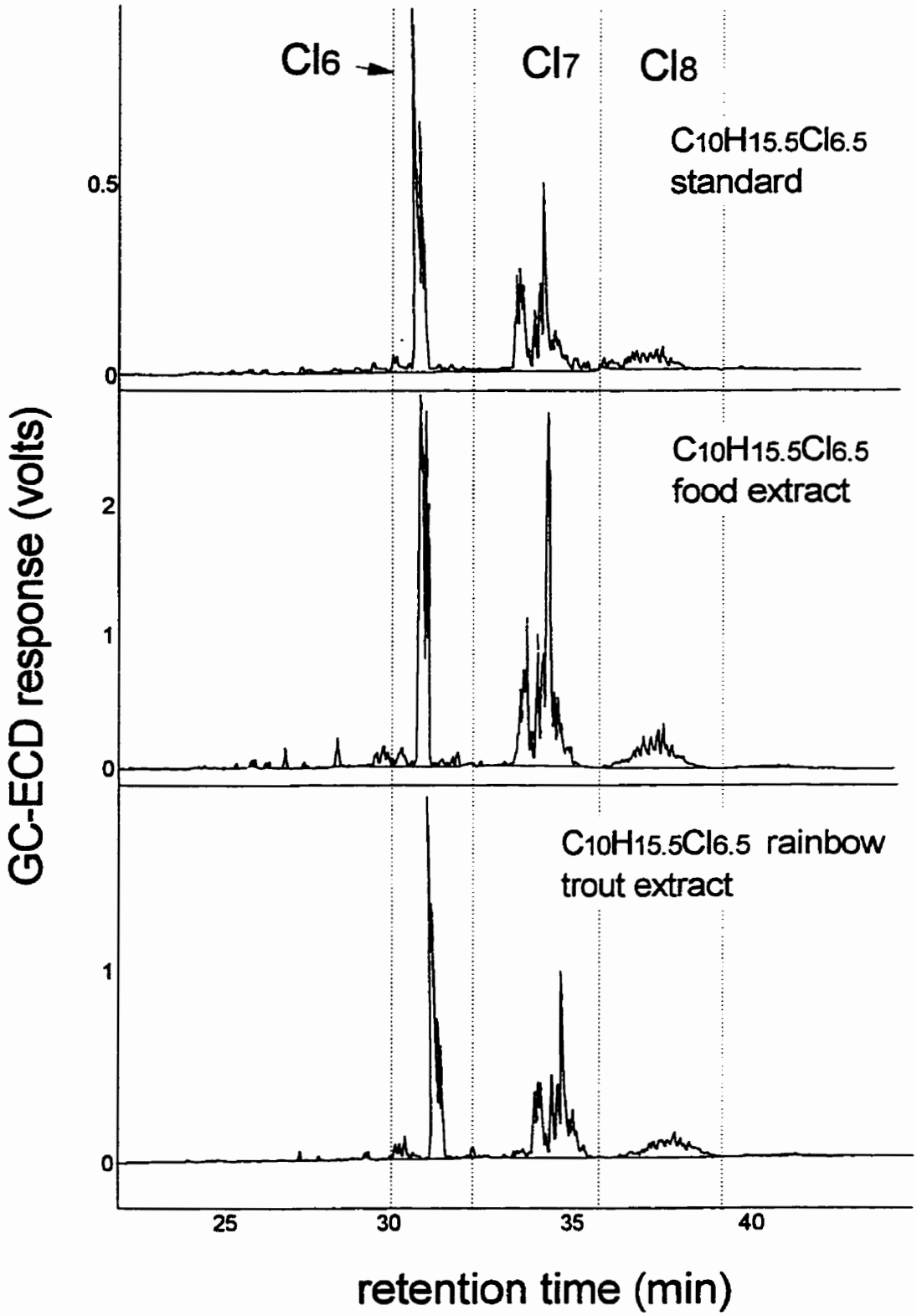

Figure 8.6 GC-ECD chromatograms of the $C_{10}H_{15.5}Cl_{6.5}$ standard (top), extracts of food spiked with $C_{10}H_{15.5}Cl_{6.5}$ (middle), and extracts of rainbow trout exposed to the $C_{10}H_{15.5}Cl_{6.5}$ spiked food for 85 days (bottom).

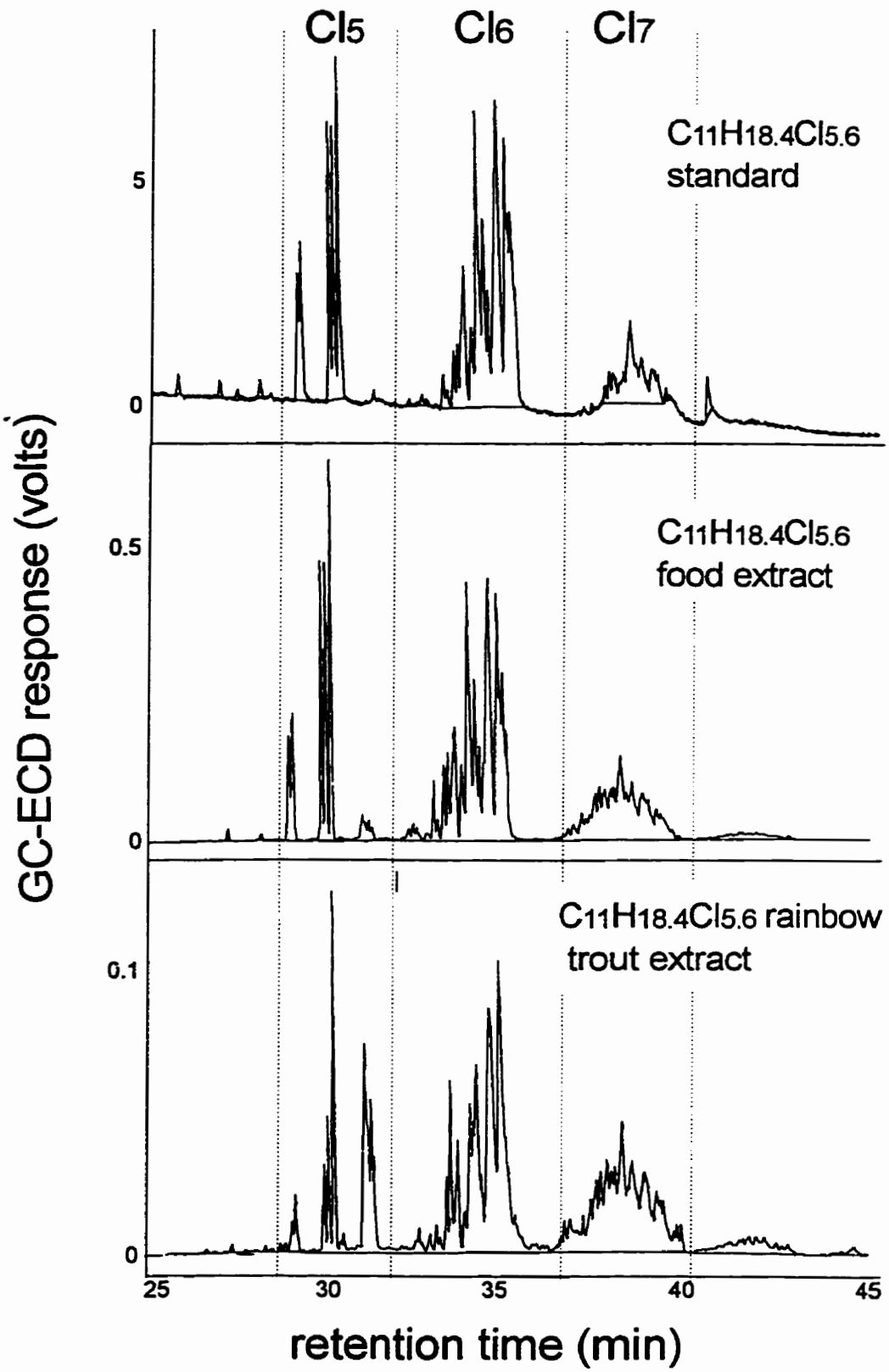

Figure 8.7 GC-ECD chromatograms of the $C_{11}H_{18.4}Cl_{5.6}$ standard (top), extracts of food spiked with $C_{11}H_{18.4}Cl_{5.6}$ (middle), and extracts of rainbow trout exposed to the $C_{11}H_{18.4}Cl_{5.6}$ spiked food for 85 days (bottom).

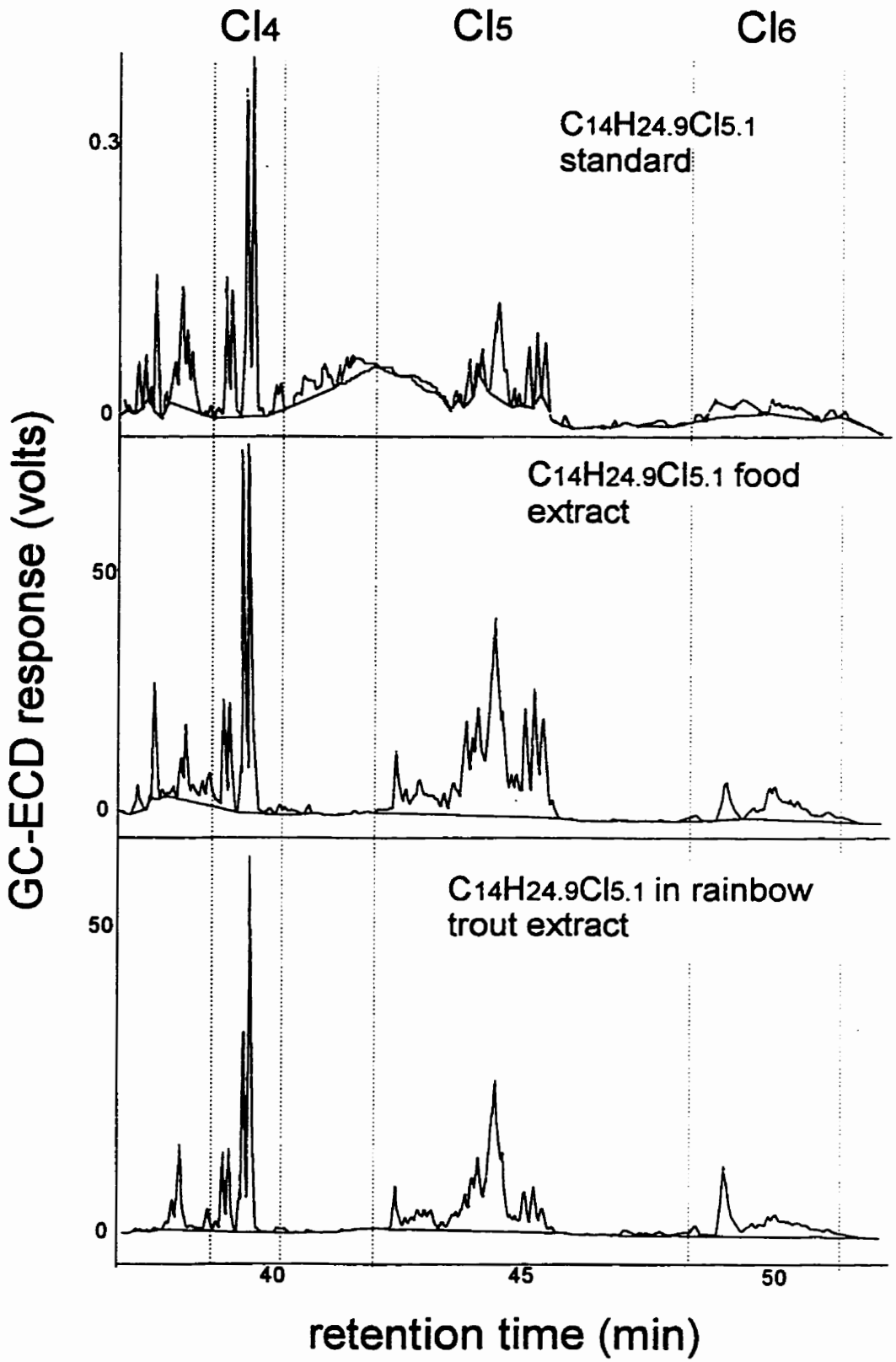

Figure 8.8 GC-ECD chromatograms of the $C_{14}H_{24.9}Cl_{5.1}$ standard (top), extracts of food spiked with $C_{14}H_{24.9}Cl_{5.1}$ (middle), and extracts of rainbow trout exposed to the $C_{14}H_{24.9}Cl_{5.1}$ spiked food for 85 days (bottom).

Table 8.3 Fish weights, liver somatic indices (LSI) and lipid contents of rainbow trout exposed to PCAs for 21 and 85 days.

PCA	exp. conc. ($ng \cdot g^{-1}$)	exp. Length (d)	fish weight (g)	LSI (%)
control	-	21	2.9 ± 0.40	1.3 ± 0.04
	-	85		
$C_{10}H_{15.5}Cl_{6.5}$	0.87	85	5.4 ± 1.6	1.1 ± 0.12
	12	21	2.5 ± 0.24	1.1 ± 0.04
	62	21	2.4 ± 0.26	1.0 ± 0.02
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	0.84	85	6.5 ± 1.6	1.0 ± 0.06
	13	21	2.4 ± 0.44	1.2 ± 0.08
	74	21	1.9 ± 0.17	1.0 ± 0.05
$C_{11}H_{18.4}Cl_{5.6}$	1.8	85	8.4 ± 1.2	0.94 ± 0.06
	2.6	21	2.8 ± 0.18	1.4 ± 0.09
	14	21	2.4 ± 0.37	1.0 ± 0.02
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	1.9	85	5.0 ± 1.4	1.0 ± 0.08
	14	21	2.8 ± 0.81	1.2 ± 0.09
	58	21	3.0 ± 0.49	1.0 ± 0.07
$C_{14}H_{24.9}Cl_{5.1}$	0.082	85	6.1 ± 2.0	1.0 ± 0.09
	0.78	21	2.8 ± 0.29	1.1 ± 0.05
	2.9	21	2.3 ± 0.14	1.1 ± 0.06
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	5.7	85	4.6 ± 1.5	1.2 ± 0.11
	29	21	2.4 ± 0.24	1.2 ± 0.11
	78	21	2.0 ± 0.63	1.0 ± 0.07

Table 8.4 General behavior of rainbow trout exposed to PCAs of varying concentrations over 21 days.

Treatment	Conc. ($\mu g \cdot g^{-1}$)	General behavior	Tap response	Coloration	Feeding behavior	Feeding time
control 1	-	-calm, active swimming	-quick, normal reaction	normal	aggressively fed	< 1 min
control 2	-	-calm, active swimming	-quick, normal reaction	normal	aggressively fed	< 1 min
control 3	-	-calm, active swimming	-quick, normal reaction	normal	aggressively fed	< 1 min
$C_{10}H_{15.5}Cl_{6.5}$	0.87	-calm, active swimming	-quick, normal reaction	normal	aggressively fed	< 1 min
	12	-calm, active swimming -beginning on day 21 a number of trout appeared to have dis-equilibrium	-quick normal reaction -after day 16 some trout did not response to tapping	normal	-fed aggressively on most days	< 5 min
	62	-generally calm, active swimming but some trout had low activity -some aggressive behavior	-quick, normal reaction on most days -some slow response behavior started on day 5	-4 trout developed dark lower jaws and 3 trout developed dark backs on day 18	-trout did not feed aggressively beginning on day 2 and by day 5 few fish were feeding	-beginning on day 4 > 5 min and not all food was consumed
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	0.84	-calm, active swimming	-quick, normal reaction	normal	-aggressively fed	< 1 min
	13	-calm, active swimming	-quick, normal reaction on most	normal	-trout began spitting out their food on day 4	-after day 4 > 5 min

			days -some slow response behavior started on day 5		-not all trout feed at every feeding	-food was not always consumed
	74	-calm, active swimming -a number of trout had rapid ventilation rates	-weak response to tapping began on day 2	-3 trout developed dark coloration on day 8	-beginning on day 3 trout fed slowly, spit out food, and some did not feed	> 5 min beginning on day 8
$C_{11}H_{20}Cl_4$	1.8	-calm, active swimming	-quick, normal reaction	normal	-aggressively fed	< 1 min
	2.6	-on day 11 one trout could not swim off bottom of aquarium -beginning on day 19 all trout had dis-equilibrium	-beginning on day 11 some trout did not response to tapping	-1 trout developed dark coloration on day 5	-most trout fed slowly beginning on day 6 -trout had trouble feeding on day 21 because they were off balance	> 5 min
	14	-calm, active swimming -day 21 one trout began to poke head out of water -day 21 two trout were bumping into walls	-beginning on day 14 some trout did not response to tapping	-1 trout developed dark coloration on day 5	-trout did not fed aggressively and some trout spit food out beginning on day 2	>5 min beginning on day 8 -not all food was consumed beginning on day 16
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	1.9	-calm, active swimming	-quick, normal reaction	-2 trout developed dark spots on back on day 11	aggressively fed	< 1 min

	14	-calm, active swimming -beginning on day 19 some trout had dis-equilibrium	-beginning on day 14 some trout did not response to tapping	-one trout developed black spots on day 4 -3 had orange tint on lateral line	-beginning on day 3 some trout did not feed	> 5 min beginning on day 11 -all food was consumed
	58	-calm, active swimming	-beginning on day 5 some trout did not response to tapping	-4 trout developed dark spots on day 4	-beginning on day 4 trout fed slowly -by day 11 some trout were not feeding at all	> 5 min began on day 10 -not all food was consumed beginning on day 19
$C_{14}H_{24.9}Cl_{5.1}$	11.1	-calm, active swimming	-quick, normal reaction	normal	aggressively fed	< 1 min
	28.6	-calm, active swimming	-quick normal reaction	normal	aggressively fed	< 1 min
	78.0	-calm, active swimming	-quick normal reaction	-1 trout developed dark stomach on day 15	-trout spit out food beginning on day 3 -most were not feeding by day 12	> 30 min beginning on day 3 -not all food was consumed
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	low	-calm, active swimming	-quick, normal reaction	normal	aggressively fed	< 1 min
	med	-calm, active swimming -day 19 three trout had "curved bodies"	-beginning on day 5 some trout did not response to tapping	-3 trout developed dark coloration on day 19	-beginning on day 2 trout fed slowly while most appeared un-interested	> 5 min began on day 4 -all food was consumed

	high	-beginning on day 7, all but 1 trout remain at bottom of aquarium with little activity	-beginning on day 4 all trout showed a weak response to tapping	-3 trout developed dark coloration on stomach on day 15 -all but 1 trout were dark by day 15	-all but one trout stopped feeding on day 3	- > 5 min and not all food was consumed beginning on day 3

the $C_{14}H_{26}Cl_4$, abnormal behavior was observed in rainbow trout from the medium concentration exposures. The most common modification in behavior was a slowed response, or no response at all, to tapping on the aquarium glass, and less aggressive feeding or a stoppage of feeding on certain days. Other changes included a loss of equilibrium and dark coloration. Onset of these changes varied between the PCA treatments. Trout from the highest exposure treatments exhibited many of the same behavior modifications as the medium exposure treatments but the onset of these effects began earlier. Also, trout from the highest exposure treatments did not feed aggressively and in many cases fish stopped feeding completely.

8.4.4 Rainbow trout liver

The liver of teleosts is not arranged in distinct lobes, as it is in mammals. The histology of the lobules are not as well defined as they are in higher vertebrates, and are identified by the central veins and hepatic triads (Yasutake and Wales 1983). Hepatocytes are arranged as tubules of cells (cords) with their apices directed toward the central bile canaliculus and/or bile preductule. The tissue morphology is complicated with branching tunneled mazes of sinusoids, which take the place of capillaries, suspended in an intricate network of connecting cells. Blood enters the sinusoids and moves through hepatocytes which remove nutrients and xenobiotics. Bile is then secreted into the central bile canaliculus and ultimately flows into the gallbladder (Heath 1995).

The major functions of the liver include: interconversion of foodstuffs; storage of glycogen; removal and metabolism of foreign chemicals in the blood; formation of bile;

synthesis of many plasma proteins; synthesis of cholesterol for use in steroid hormones and cell membranes; exocrine pancreatic secretion; and metabolism of hormones (Heath 1995).

Hepatocytes are the most common liver cell of fish (~ 80% of all liver cells) (Hinton and and Lauren 1990) and were the predominant cell observed in the rainbow trout of this study (Figure 8.3). Hepatocytes were arranged in cords, which were two cells thick, and cords were arranged around sinusoids (Figure 8.3).

8.4.5 Rainbow trout posterior kidney

The kidneys of rainbow trout are found along the dorsal position along the length of the body cavity. Although they appear to be a single unit, rainbow trout kidney are composed of two completely fused sections, each with its own mesonephric duct. These ducts leave the kidney and unite to form the common urinary duct and bladder. The anterior section of the kidney, the head, is composed entirely of hematopoietic tissue while the posterior section of the kidney is embedded with tubules or nephrons. Rainbow trout have a typical freshwater glomerular nephron that consists of the following regions: a renal corpuscle; short neck segment; two morphologically distinct segments of the proximal tubule; a variably present intermediate segment; distal segment; and a collecting duct system terminating in the mesonephric duct (Hendricks 1983).

The major functions of the rainbow trout kidney, and most other freshwater teleosts, is conservation of salts and the elimination of excess water. The kidney is also involved in urine pH, glucose reabsorption, and excretion of nitrogenous compounds (Hickman and Trump 1969). Note that most nitrogenous waste is eliminated via the gills.

Histologically, the posterior kidney is composed of hematopoietic tissue embedded with nephrons. The first (P1) and second (P2) segments of the proximal tubules were commonly observed in the rainbow trout posterior kidney (Figure 8.4). Both proximal tubule segments have a brush border and are differentiated by intensity of staining (P2 stain darker) and nucleus position (P2 nucleus are centrally located in the cell). Surrounding the proximal tubules are hematopoietic tissue (Figure 8.4).

8.4.6 Rainbow trout thyroid

The thyroid is part of the endocrine system, the basic components of which are similar in fish and higher vertebrates (Gorbman 1969). The thyroid tissue of the rainbow trout consists of glandular follicles scattered throughout the ventral aorta and branchial arteries that supply the gills. The center of each follicle is filled with colloid, which is a reserve of the protein-bound form of the thyroid hormone. The surrounding epithelial cells are either flattened, cuboidal, or columnar, depending on their activity. Tall, columnar epithelium cells with basophilic colloid containing vacuole-like spaces are indicative of an active thyroid gland (Yasutake and Wales 1983).

The thyroid gland secrete thyroxine (T_4) and triiodothyronine (T_3), hormones which are essential for many functions including: maturation; proper functioning of the nervous system; and the accumulation and storage of iodine (Gorbman 1969; Yasutake and Wales 1983; Peakall 1995). The thyroid is also a source of calcitonin, a polypeptide hormone involved in calcium homeostasis (Peakall 1995).

The thyroid follicles of rainbow trout in this study were filled with colloid, and vacuoles were present in the colloid, and epithelium cells appeared to be columnar (Figure 8.5). This would suggest that the thyroid glands of the rainbow trout were active.

8.4.7 Histological Morphometrics for 21 day exposures

Histological evaluations were only performed on the rainbow trout exposed to the medium concentrations because these trout fed more consistently than treatments with higher exposures, and for a number of the PCAs had achieved higher tissue concentrations (Table 8.2). Histological morphometrics are summarized in Table 8.5. Hepatocyte nuclear diameter, posterior kidney proximal tubule epithelium cell height and thyroid epithelium cell height did not vary between the PCA treatment trout and control trout (ANOVA, Dunnett's pairwise comparison, $p > 0.05$). The relative hepatocyte size of the $C_{10}H_{15.5}Cl_{6.5}$, $C_{12}H_{19.5}Cl_{6.5}$ and $C_{14}H_{23.3}Cl_{6.7}$ trout were significantly smaller than the control (ANOVA, Dunnett's pairwise comparison, $p < 0.05$), but these characteristics in trout exposed to the other PCAs did not vary from the control (ANOVA, Dunnett's pairwise comparison, $p > 0.05$). The nucleus : cytoplasm ratio of heptaocytes was significantly greater in $C_{10}H_{15.5}Cl_{6.5}$ trout than the control trout (ANOVA, Dunnett's pairwise comparison, $p > 0.05$), but did not vary between the other PCA treatment trout and control trout (ANOVA, Dunnett's pairwise comparison, $p > 0.05$).

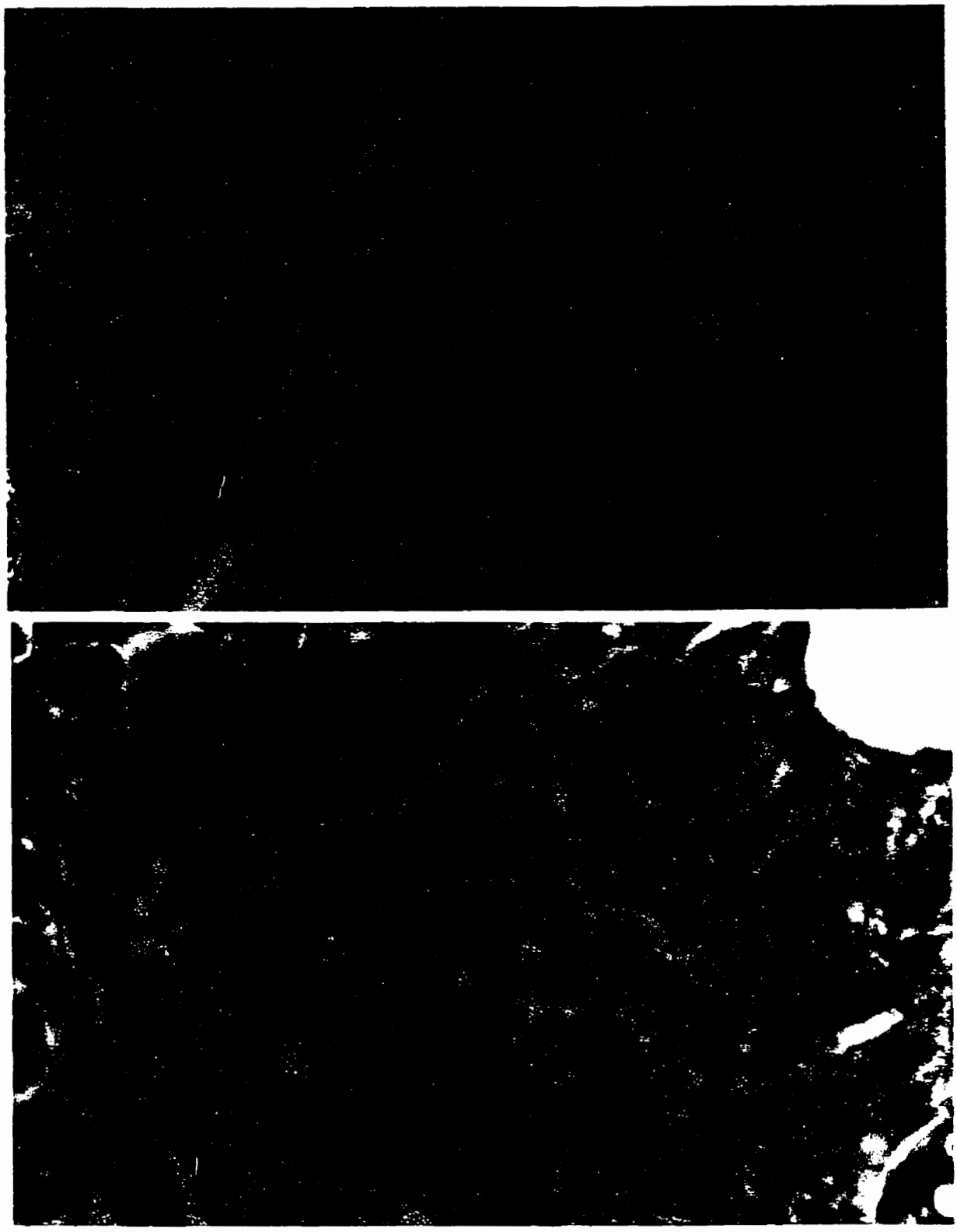

Figure 8.9 Micrographs (top mag. 100X, bottom mag X400) of liver from a rainbow trout exposed to $C_{10}H_{15.3}Cl_{6.7}$ for 21 days at dietary concentrations of 13 $\mu g \cdot g^{-1}$. H: areas of normal hepatocytes; L: focal coagulative necrosis of hepatocytes; PM: pigmented macrophage; P: pyknotic nucleus.

Figure 8.10 Micrograph (mag X250) of posterior kidney from a rainbow trout exposed to $C_{10}H_{15.3}Cl_{6.7}$ for 21 days at dietary concentrations of 13 $\mu g \cdot g^{-1}$. P1: first segment of proximal tubule (normal); P2: second segment of proximal tubule (normal); N: necrotic tubules (either P1 or P2); PN: pyknotic nucleus; D: debris.

Table 8.5 Histological morphometrics (mean ± 1 standard error, n = 3) of liver, kidney and thyroid tissue from rainbow trout exposed to PCAs for 21 days. Exposure concentrations for each PCA are the medium concentration found in Table 8.1. Treatment means which significantly differ from the control mean are indicated by an (* or **)(ANOVA, Dunnett pairwise comparison, * $p < 0.05$, ** $p < 0.1$).

	liver				
PCA	hepatocyte nuclear diameter (μm)	relative hepatocyte size (μm^2)	nucleus : cytoplasm area (μm^2)	kidney proximal tubule (P2) epithelium cell height (μm)	thyroid epithelium cell height (μm)
control	5.51 ± 0.174	160 ± 1.27	0.175 ± 0.012	18.4 ± 1.36	3.57 ± 0.307
$C_{10}H_{15.5}Cl_{6.5}$	5.61 ± 0.0859	118 ± 6.47*	0.270 ± 0.0305*	18.2 ± 0.581	4.40 ± 0.421
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	5.53 ± 0.293	135 ± 12.1	0.216 ± 0.0079	14.8 ± 0.928	2.92 ± 0.422
$C_{11}H_{18.4}Cl_{5.6}$	5.63 ± 0.134	149 ± 10.9	0.240 ± 0.0202	16.7 ± 1.71	3.34 ± 0.209
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	5.30 ± 0.0361	123 ± 2.34*	0.219 ± 0.0036	16.5 ± 1.16	3.95 ± 0.0407
$C_{14}H_{24.9}Cl_{5.1}$	5.58 ± 0.102	134 ± 12.6	0.228 ± 0.0215	14.4 ± 0.351	3.93 ± 0.145
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	5.20 ± 0.162	116 ± 4.28*	0.226 ± 0.0184	15.1 ± 0.233	3.26 ± 0.155

8.4.8 Histological Morphometrics for 85 day exposures

Histoiogical morphometrics for the long term exposures are summarized in Table 8.6. No significant differences were found between the control trout and PCA treatment trout for any of the morphometric measurements (ANOVA, Dunnett's pairwise comparison, $p > 0.05$). Although only marginally significant, trout exposed to the $C_{10}H_{15.3}Cl_{6.7}$ had smaller hepatocyte nuclear diameters and relative hepatocyte sizes than control trout (ANOVA, Dunnett's pairwise comparison, $p < 0.1$). Trout exposed to $C_{14}H_{23.3}Cl_{6.7}$ also had marginally significantly smaller relative hepatocyte size than control trout (ANOVA, Dunnett's pairwise comparison, $p < 0.1$).

8.4.9 General Histological Evaluation of liver, thyroid and posterior kidney

With the exception of trout exposed to $C_{10}H_{15.3}Cl_{6.7}$ at the medium concentration, the liver and posterior kidney of all trout exposed to medium and low concentrations of the PCAs, did not appear to have any overt lesions. No overt lesions were observed in any thyroid glands. Note that trout exposed to the highest PCA concentrations were not examined and more comprehensive evaluations are needed.

Trout exposed to the $C_{10}H_{15.3}Cl_{6.7}$ had focal coagulative necrosis of hepatocytes with associated pigmented macrophage proliferation and pyknotic nuclei (i.e. shrunken and dark) (Figure 8.9). These lesions occurred in multiple foci. One trout from the medium concentration $C_{10}H_{15.3}Cl_{6.7}$ exposure also had significant histopathologies in the posterior kidney (Figure 8.10). The most profound alterations were multiple foci of necrotic, desquamating tubules with extensive accumulation of debris in the tubule lumens. Other alterations included excessive pigmentation, debris in the lumens of tubules and

mesonephric ducts, sites of peritubular inflammation (nephritis) and less severely damaged tubules (pyknosis).

8.5 Discussion

This study represents one of the first attempts at examining the toxic mode of action and sub-lethal effects of PCAs in fish. In general, PCAs appear to have low acute toxicity to rainbow trout with minimal histopathological responses in the liver, posterior kidney and thyroid.

The acute toxic mode of action of PCAs in aquatic organisms would appear to be narcosis. The reduced or non-existent response to the glass tapping, loss of equilibrium and evidence of dark coloration in the trout exposed to the PCAs are all behavioral and morphological responses indicative of a narcotic mode-of-action (McKim et al. 1987). However, tissue concentrations of the PCAs are not nearly high enough to cause narcosis (Table 8.7). Because we exposed the trout to the PCAs via food, unhealthy trout could avoid the exposure by a cessation of feeding. It would appear likely that, unless force fed by methods such as a gavage or at extremely high dietary concentrations, narcosis could not be achieved by exposure via food.

Narcotic-like behavior has also been observed in bleak (*Alburnus alburnus*), a marine fish, when exposed to high concentrations of commercial PCA products in food (Svanberg et al. 1978, Bengtsson et al. 1979, Bengtsson et al. 1982), and in rainbow trout exposed to high water concentrations of a commercial PCA (C_{10-13})(Swigert and Bowman 1986a, 1986b). Further evidence for a narcotic mode of action are the high concentrations

Table 8.6 Histological morphometrics (mean ± 1 standard error, n = 3) of liver, kidney and thyroid tissue from rainbow trout exposed to PCAs for 82 days. Exposure concentrations for each PCA are the low concentration found in Table 8.1. Treatment means which significantly differ from the control mean are indicated by an (* or **)(ANOVA, Dunnett pairwise comparison, * $p < 0.05$, ** $p < 0.1$).

	liver				
PCA	hepatocyte nuclear diameter (μm)	relative hepatocyte size (μm^2)	nucleus : cytoplasm area (μm^2)	kidney proximal tubule epithelium cell height (μm)	thyroid epithelium cell height (μm)
control	5.81 ± 0.0470	183 ± 10.1	0.171 ± 0.0106	17.0 ± 1.22	3.73 ± 0.629
$C_{10}H_{15.5}Cl_{6.5}$	5.40 ± 0.165	155 ± 7.39	0.174 ± 0.00350	16.9 ± 0.991	3.64 ± 0.241
^{14}C-$C_{10}H_{15.3}Cl_{6.7}$	5.30 ± 0.216**	146 ± 8.05**	0.179 ± 0.00610	18.1 ± 0.323	3.84 ± 0.140
$C_{11}H_{18.4}Cl_{5.6}$	5.41 ± 0.0751	160 ± 10.7	0.169 ± 0.0127	19.0 ± 1.36	3.560 ± 0.238
^{14}C-$C_{12}H_{19.5}Cl_{6.5}$	5.48 ± 0.142	154 ± 8.41	0.184 ± 0.0214	17.7 ± 0.441	3.886 ± 0.320
$C_{14}H_{24.9}Cl_{5.1}$	5.63 ± 0.149	151 ± 13.8	0.200 ± 0.0112	16.6 ± 0.586	4.04 ± 0.259
^{14}C-$C_{14}H_{23.3}Cl_{6.7}$	5.60 ± 0.0451	147 ± 0.693**	0.201 ± 0.00300	16.0 ± 0.186	4.03 ± 0.190

used in numerous toxicity tests which have not elicited a toxic response in invertebrates (Madeley and Thompson 1983a, 1983b, 1983c, 1083d), fish (Madeley and Maddock 1983f, 1983c, 1983d, 1983e, Linden et al. 1979, chapter 7) birds (SDS Biotech 1984, as reported in Willis et al. 1993, Madeley and Birtley 1980; Linden et al. 1983) and mammals (Howard et al. 1975, Birtley et al. 1980, Serrone et al. 1987).

There have been a small number of studies which have examined the histopathological effects of PCAs in mammals, but in most cases the exposure durations were longer (13 weeks to 2 yrs) and the exposure concentrations were higher (5-5,000 $ug \cdot g^{-1}$) than in this work, making comparisons tenuous.

Obvious histopathological lesions were only found in trout exposed to $C_{10}H_{15.3}Cl_{6.7}$ at concentrations of 13 $\mu g \cdot g^{-1}$, although some morphometric changes in the liver were observed in trout exposed to the other PCAs. It should be stressed that a more comprehensive evaluation of all tissue is needed and is currently in progress. Because the exposure and tissue concentrations were fairly similar between the PCA treatments, the effects observed in the $C_{10}H_{15.3}Cl_{6.7}$ exposed trout suggest that this compound is more toxic than the other PCAs. This is consistent with past work on PCAs, where toxicity is considered to be inversely related to carbon chain length and potentially chlorine content (Bucher et al. 1987; Serrone et al. 1987).

Although few histopathological effects were observed in the rainbow trout exposed to the PCAs, a majority of these were found in the liver. Notable coagulative necrosis of the hepatocytes was observed in rainbow trout exposed to $C_{10}H_{15.3}Cl_{6.7}$. Hepatocyte necrosis has also been observed in rats exposed to high concentrations of commercial PCA

Table 8.7 Highest mean tissue concentrations of PCAs measured in the rainbow trout and tissue concentrations required to cause narcosis assuming 2 to 8 $\mu mol \cdot g^{-1}$ causes narcosis (McCarty 1986).

chemical	highest mean concentration measured ($\mu g \cdot g^{-1}$)	low estimate of narcotic tissue concentration ($\mu g \cdot g^{-1}$)	high estimate of narcotic tissue concentration ($\mu g \cdot g^{-1}$)
$C_{10}H_{15.5}Cl_{6.5}$	0.92 ± 0.45	73	290
$C_{10}H_{15.3}Cl_{6.7}$	3.0 ± 1.0	74	300
$C_{11}H_{20}Cl_4$	5.5 ± 1.1	58	230
$C_{12}H_{19.5}Cl_{6.5}$	1.1 ± 0.30	78	310
$C_{14}H_{25.9}Cl_{5.1}$	0.028 ± 0.0079	74	300
$C_{14}H_{23.3}Cl_{6.7}$	1.3 ± 0.33	85	340

formulations. Bucher et al. (1987) observed slight to minimal necrosis in the livers of rats exposed to a C_{10-13}-PCA (60% Cl) for 2 years at concentrations of 125-625 $ug \cdot g^{-1} \cdot d^{-1}$. Single-cell necrosis was observed in the hepatic lobes of male and female rats exposed for 13 weeks to 5,000 $ug \cdot g^{-1} \cdot d^{-1}$ of a C_{14-17}-PCA (52% Cl)(Poon et al. 1995). Hepatocellular hypertrophy, evidence of previous necrosis, was observed in rats exposed to a C_{10-13}-PCA (58% Cl) at concentrations of 100-625 $ug \cdot g^{-1} \cdot d^{-1}$ for 90 days (Serrone et al. 1987).

Morphometric changes in the liver of PCA-exposed trout were also observed. Smaller relative hepatocyte size was observed in all PCA treated trout compared with the control trout, although only a few of these differences were statistical significant. Only 3 fish were examined per treatment, and a larger sample size may have provided a more powerful statistical comparison. Although hepatocyte morphometric measurements have never been measured previously in PCA exposed organisms, published data suggests that PCA can cause an increase in hepatocyte size, which is not consistent with the reduced hepatocytes sizes observed in the PCA-exposed rainbow trout. Bucher et al. (1987) observed significantly fewer hepatocytes per given microscope field, suggesting larger hepatocytes, in rats exposed to a C_{10-13}-PCA (60% Cl) for 2 years at concentrations of 125-625 $ug \cdot g^{-1} \cdot d^{-1}$. Increases in liver weights, which is the most common effect observed in PCA-exposed rats, could be a function of larger hepatocytes caused by peroxisome and smooth endoplasmic reticulum proliferation (Nilsen et al. 1981; Wyatt et al.1993). The smaller hepatocytes observed in the rainbow trout in this study do not appear to be due to decreases in food consumption, as most trout in the medium and low exposures fed consistently throughout the experiments. Also, fish weights and LSI did not vary between control and PCA-exposed trout.

No obvious morphometric changes in the posterior kidney were found in trout exposed to any of the PCA treatments, and with the exception of one trout exposed to $C_{10}H_{15.3}Cl_{6.7}$ all posterior kidney appeared normal and healthy. As stated above, this needs to be confirmed by a more extensive evaluation. Significant histopathological changes have been observed in rats exposed to commercial PCA formulations although at higher exposure concentrations and longer exposure periods than were used in this experiment. Rats exposed to 100-625 $ug \cdot g^{-1} \cdot d^{-1}$ of a C_{10-13}-PCA (58% Cl) for 90 days had high incidences of trace-to-mild chronic nephritis and increased pigmentation of the renal tubules (Serrone et al. 1987). Focal dilation of the tubules of the inner medulla of the posterior kidney with focal pyknosis of epithelial nuclei were observed in female rats exposed to a C_{14-17}-PCA (52% Cl) for 13 weeks at concentrations between 50 and 5,000 $ug \cdot g^{-1} \cdot d^{-1}$, although the effects were minimal (Poon et al. 1995). Bucher observed nephropathy and increased pigmentation of the posterior kidney in rats exposed daily to C_{10-13}-PCA (60% Cl) and C_{20-25} (43% Cl) for 2 years at concentrations ranging from 312 to 3750 $\mu g \cdot g^{-1}$. These results are consistent with lesions observed in one trout exposed $C_{10}H_{15.3}Cl_{6.7}$ at food concentrations of 13 $\mu g \cdot g^{-1}$ or approximately 0.6 $\mu g \cdot g^{-1} \cdot d^{-1}$.

No obvious lesions or morphometric changes were seen in the thyroid of PCA exposed trout, although this needs to be confirmed by a more extensive evaluation. Histopathological effects have been observed in the thyroid of rats exposed to higher concentrations of commercial PCA formulations. Poon et al. (1995) observed reduced follicle size and collapsed angularity, increased epithelium cell height, and cytoplasmic vacuolation and nuclear vesiculation in epithelium cells in rats exposed for 13 weeks to 50 to 5,000 $ug \cdot g^{-1} \cdot d^{-1}$ of a C_{14-17}-PCA (52% Cl). These changes were generally minimal to

mild in nature. Hypertrophy and hyperplasia of the thyroid has been reported in rats exposed to a C_{10-13}-PCA (60% Cl) for 90 days at concentrations of 100-625 $ug \cdot g^{-1} \cdot d^{-1}$ (Serrone et al. 1987). Histopathological effects may have been observed in the trout if PCA exposures were longer and/or at greater concentrations.

Three of the PCAs used in this experiment, $C_{10}H_{15.5}C_{6.5}$, $C_{11}H_{20}Cl_4$ and $C_{14}H_{24.9}Cl_{5.1}$, had chlorine substituted at two terminal carbons on both ends of the carbon chain. The other three PCAs, $C_{10}H_{15.3}Cl_{6.7}$, $C_{12}H_{19.5}Cl_{6.5}$ and $C_{14}H_{23.3}Cl_{6.7}$ probably did not, assuming free radical chlorination does not form 1,2 substitutions. This allows an examination of effect of chlorine position on the toxicity of PCAs. It does not appear that the terminal chlorinated PCAs were any less or more toxic than the PCAs which are not chlorinated on the terminal carbons, although further research is needed to validate this statement.

These results suggest that histopathological effects would only occur at extremely high exposure and tissue concentrations, and at levels which are well beyond those observed in wild fish or invertebrates (Jansson et al. 1993, Fisk et al. 1996b, Tomy et al. 1997). However, this statement should be taken with caution. Only a small subset all of all the PCAs were tested, and more PCAs should be examined. Longer exposures are needed to assess the chronic effects of PCAs, using a range of species. As only 5 morphometric parameters and three tissues were examined, it is possible that PCAs may influence other aspects of histology. Lastly, PCAs may disrupt other physiological and/or biochemical processes in aquatic organisms which do not cause a histological change.

8.6 Summary and Conclusions

This work represents one of the first attempts at examining the toxic mode-of-action and sub-lethal effects of PCAs on fish. It is also the first research on the histological effects of PCAs in fish, the first histological data of any kind using PCAs with a single carbon chain length and known chlorine, and the first histological morphometric measurements in organisms exposed to PCAs. The behavior of rainbow trout exposed to high dietary concentrations of all but the $C_{14}H_{24.9}Cl_{5.1}$ PCA, were consistent with a narcotic mode-of-action. Overt histopathological lesions were observed in the liver and kidney of rainbow trout exposed to high dietary concentrations of $C_{10}H_{15.3}Cl_{6.7}$ for 21 days, but no obvious lesions were found in the rainbow trout exposed to the other PCAs or to any of the PCAs at lower concentrations for 85 days. This histological work needs to be verified. Histological morphometric changes, hepatocyte size, were also observed in the livers of rainbow trout exposed to all PCAs for 21 days. No changes were observed in the rainbow trout exposed to the same PCAs at lower concentrations for 85 days. It would appear that the sub-lethal toxicity of PCAs is inversely related to the carbon chain length. The results of this study suggest that the acute toxicity of PCAs to fish is very low, although PCAs may cause histological changes at extremely high dietary concentrations. More work is needed to verify these results and to examine the effects of longer term exposure to PCAs.

9. GENERAL DISCUSSION

The objectives of this thesis were to examine the dietary accumulation, sediment bioavailability, and the toxicity of individual PCAs of single carbon chain lengths and known chlorine content. These types of data are necessary to examine the fate and dynamics of chemicals in the environment and for risk and exposure assessments. However, these data are lacking for PCAs. The dietary accumulation of PCAs was examined using rainbow trout exposed to PCA-spiked food using established experimental protocols. The dietary accumulation of a range of recalcitrant hydrophobic organochlorines was also examined using these same experimental protocols. This provided data for compounds which have been shown to bioaccumulate and biomagnify in the environment, which could be compared to the PCA data. It also provided information on the biotransformation of PCAs. The sediment bioavailability was studied by exposing oligochaetes to sediment spiked with PCAs. The toxicity of PCAs was examined by exposing Japanese medaka eggs and juvenile rainbow trout to high water and dietary concentrations, respectively.

The dynamics, fate and effects of polychlorinated *n*-alkanes (PCAs) in the aquatic environment are expected to vary with carbon chain length and chlorine content. Commercial formulations of PCAs, e.g. Cerelor 49 (C_{10-13}, 49% Cl), are composed of thousands of compounds which vary in carbon chain length and chlorine content. Individual PCA compounds will have different rates of biodegradation, bioavailability, and

bioaccumulation, resulting in different relative combinations of individual PCAs at various trophic levels. Changes in PCA abundance have been observed in invertebrates and fish from the Detroit River and western basin of Lake Erie relative to commercial products (Tomy et al. 1997). These shifts in congener patterns are analogous to what has been observed with polychlorinated biphenyls (PCB) in aquatic food webs (Oliver and Niimi 1988; Koslowski et al. 1994). Therefore, hazard and/or risk assessment of PCAs in the environment based on data generated using commercial formulations of PCAs has proven difficult.

The number of potential PCA compounds in the environment may number in the 10,000s. However, it is impossible to examine the biodegradation, bioavailability, bioaccumulation and toxicity of each of these PCA compounds. If the risk of PCAs to wildlife and man are to be assessed, it is necessary to examine a range of PCA compounds which vary in carbon chain length and chlorine content and develop relationships based on these physical-chemical properties. For example, characteristics of PCAs which may result in greater bioaccumulation or toxicity need to be identified and used for risk and hazard assessment, and government regulation.

It is clear from the PCA dietary accumulation and sediment bioavailability studies from this work that the bioaccumulation of PCAs is directly influenced by carbon chain length and chlorine content. This combination of physical properties directly influences physical-chemical properties and biotransformation rates in aquatic organisms, and ultimately influence PCA bioaccumulation and fate in aquatic systems.

The octanol-water partition coefficient (K_{ow}) of PCAs is positively correlated with carbon chain length and chlorine content (Sijm and Sinnige 1995). Greater K_{ow} results in

longer chemical half lives in aquatic organisms because these hydrophobic chemicals partition predominantly into lipids, and have slow elimination rates out of lipids. Slow elimination rates result in higher biomagnification or BMF values. However, as was shown with a range of hydrophobic organochlorines (Chapter 4), the experimental protocol used for these dietary accumulation studies may underestimate the half lives of very hydrophobic compounds (log K_{ow} > 7.0). Therefore, the relationship observed for PCA half lives and carbon chain length may be less accurate for the very hydrophobic PCAs (i.e. PCAs with long carbon chain and high chlorine content).

All of the PCAs have K_{ow}s which are in a range that should result in biomagnification in aquatic food chains (log K_{ow} > 5.0), but not all PCAs had BMFs >1. However, it appears that all PCAs are also susceptible to biotransformation in rainbow trout and oligochaetes, which reduces the half life, and subsequently the BMF and BSAF, of these chemicals. The susceptibility of PCAs to biotransformation may decrease with increasing chlorine content and carbon chain length, and the low and medium chlorinated short chain PCAs appear to be metabolized sufficiently so that biomagnification is unlikely.

More highly chlorinated (> ~ 60% Cl), medium chain PCAs do not appear likely to biomagnify despite very high K_{ow}s (log K_{ow} > 7.0) and relatively low biotransformation rates. Extremely hydrophobic compounds tend to have low bioavailability from sediment and food (Opperhuizen et al. 1985; Loonen et al. 1997). This could be due to a combination of the strong binding of these hydrophobic compounds to food lipids and sediment organic matter and/or the large molecular size of hydrophobic compounds which may lead to steric hindrance in passing through biological membranes (Opperhuizen et al.

1985). Although we did not examine any long chain PCAs (> C_{16}), these extremely large and hydrophobic compounds are likely to have low bioavailability and bioaccumulation.

Short carbon chain (C_{10-13}), highly chlorinated (> 60% Cl) PCAs and medium carbon chain (C_{14-18}), low and medium chlorinated (< 60% Cl) PCAs have the greatest potential for bioaccumulation in aquatic ecosystems. These compounds had biota sediment accumulation factors and biomagnification factors which were > 1, implying potential for magnification of concentrations from sediment to benthic invertebrates and between various trophic steps. These PCAs have higher K_{ow} and lower biotransformation rates in aquatic organisms, producing longer half lives and higher BSAFs and BMFs.

A majority of the aquatic toxicity data for PCAs indicate that these compounds have low acute toxicity. The data generated with Japanese medaka embryo toxicity assays and experiments with juvenile rainbow trout support the findings that acute toxicity of PCAs is low. These results also indicate that the mode-of-action of PCA toxicity is narcosis. Narcosis will occur at tissue concentrations of 2 to 8 $\mu mol \cdot g^{-1}$ (McCarty 1986; van Wezel et al. 1996), so that as molecular weight increases the tissue concentration required to cause narcosis increases. As well, the LC_{50}s of narcotic chemicals decrease with increasing K_{ow} (Smith and Criag 1983; Veith et al. 1983). Therefore, the concentrations of PCAs which cause acute toxicity, or narcosis, will increase with increasing carbon chain length and chlorine content. This is consistent with past work on PCA toxicity using aqueous exposures. However, because water solubility of PCAs decreases with increasing carbon chain length and chlorine content, water exposures result in much greater tissue concentrations for shorter carbon chain and less chlorinated PCAs.

This work has provided evidence that PCAs can cause sub-lethal effects in fish at very high exposure concentrations. Lesions, which included extensive coagulative narcosis, were found in the liver of rainbow trout exposed to the $C_{10}H_{15.3}Cl_{6.7}$ at high dietary concentrations. Although there were changes in liver cell morphometrics of rainbow trout exposed to a slightly less chlorinated C_{10}-PCA and a C_{12}- and C_{14}-PCA, no lesions were observed in any livers of rainbow trout exposed to a C_{11}-PCA at similar dietary concentrations. As well, lesions were observed in the kidney of one rainbow trout exposed to the $C_{10}H_{15.3}Cl_{6.7}$, but none were found in any other PCA exposed rainbow trout. These results suggest that the acute sub-lethal effects of PCAs increase with decreasing carbon chain length and increasing chlorine content. In studies with mammals, Bucher et al. (1987) and Serrone et al. (1987) both concluded that PCA toxicity is inversely related to carbon chain length and chlorine content.

PCAs were bioaccumulated from food and sediment by rainbow trout and oligochaetes in laboratory experiments. Recalcitrant OCs, such as PCBs, were also found to bioaccumulate in our laboratory exposures. Therefore, PCAs are likely to transfer from sediment to benthic organisms and through aquatic food chains, although there are no data from the environment to verify these results. Experimental data also suggested that some PCAs may even biomagnify. Food chain transfer of PCAs varied with K_{ow} and carbon chain length, and the relative abundance of PCA congeners, and the exposure to PCA congeners should vary between various trophic levels.

Although environmental data are limited, PCAs have been found at relatively high concentrations in lake sediment and in aquatic biota (~ 100's $ng \cdot g^{-1}$ wet wt.) (Tomy et al. 1997a). However, results from this study suggest that the toxicological relevance of these

$ng \cdot g^{-1}$ levels is probably low. It is unlikely that PCAs will cause any acute toxic effects on aquatic organisms at $ng \cdot g^{-1}$ levels because their mode-of-action is likely narcosis. Although this work indicated that PCAs may cause sub-lethal effects, such as histopatholgical lesions, effects only occurred at extremely high exposure concentrations (> 10 $\mu g \cdot g^{-1}$) and were not found in rainbow trout exposed for longer periods at lower exposure concentrations. The concentrations required to cause the histopathological lesions are well above concentrations to which any aquatic organism is likely exposed. The exception could be a spill of PCAs directly into an aquatic system, but even in this case effects would be hard to measure and large scale effects are unlikely.

10. SUMMARY and CONCLUSIONS

This thesis has examined the bioaccumulation, sediment bioavailability and biodegradation, and the toxicity of polychlorinated *n*-alkanes (PCAs). The results of this work represent some of the first data for PCAs with a single carbon chain length and known chlorine content. PCAs are complex industrial formulations which consist of thousands of compounds with varying carbon chain length and chlorine content. To date, it has not been possible to separate individual components from industrial PCA formulations, and until recently there have not been any synthesized PCA standards available. Therefore there has been a lack of any type of data for PCAs of single carbon chain lengths and known chlorine content.

This research has generated assimilation efficiencies, half lives, and biomagnification factors (BMFs) for 23 PCAs which have a range of carbon chain lengths (C_{10}-, C_{11}-, C_{12}-, C_{14}- and C_{16}) and chlorine contents (35-70% by weight) using juvenile rainbow trout. The half lives (range from 7 to 75 days) and BMFs (range from 0.1 to 2.9) of PCAs are positively correlated with carbon chain length and chlorine content. These relationships appear to be driven by the hydrophobicity and biotransformation of the PCAs.

K_{ow} of PCAs is also positively correlated with carbon chain length and chlorine content (Sijm and Sinnige 1995). Greater K_{ow} results in longer chemical half lives in aquatic organisms because these chemicals partition predominantly into, and have slow

elimination rates out of, lipids. Slow elimination rates result in higher biomagnification or BMF values.

All of the PCAs appeared to have been biotransformed by the rainbow trout; however, susceptibility to biotransformation decreased with increasing chlorine content. A significant portion of the total radioactivity in rainbow trout exposed to the ^{14}C-PCAs was not extractable by toluene, a non-polar solvent, implying that these compounds had been biotransformed to more polar metabolites. The percentage of non-extractable ^{14}C decreased with increasing chlorine content for C_{12}- and C_{16}-PCAs. No relationship between extractable and non-extractable ^{14}C was obvious for the carbon chain lengths. Based on half life-K_{ow} relationships developed for a series of non-metabolized organochlorines using juvenile rainbow trout (see below), a majority of the half lives of PCAs were lower than expected for compounds of this range of K_{ow}. Only PCAs with high chlorine content had half lives which were similar to non-metabolized organochlorines of similar K_{ow}.

Highly chlorinated (>60% Cl), short chain (C_{10-13}) and medium chlorinated (~50-60% Cl), medium chain (C_{14-18}) PCAs appear to have the longest half lives in aquatic organisms and the greatest potential for biomagnification in aquatic food webs. Lower chlorinated short chain PCAs are readily biotransformed and do not have sufficient half lives to biomagnify. More highly chlorinated medium chain PCAs have low bioavailability and hence low assimilation efficiency because of extremely high K_{ow}s, and are not likely to biomagnify. Additional research is needed on the bioaccumulation of long chain (C_{18-30}) PCAs. Research on PCAs in aquatic food chains is needed to confirm laboratory results. Biotransformation rates of PCAs are also needed for mammals which may readily

metabolize PCAs and reduce their total burden. As well, information is needed on the enzyme systems that are used to biotransform PCAs and the metabolites formed.

C_{12}- and C_{16}-PCAs failed to elevate CYP 1A1 enzyme activity as measured by ethoxyresorufin-*o*-deethylase (EROD) activities in rainbow trout. CYP1A1 is a mixed function oxygenase (MFO) enzyme which is involved with biotransformation of xenobiotic chemicals, and is used as a biomarker of organochlorine exposure. More work is needed on the enzyme induction potential of PCAs in fish. There is evidence that PCAs can induce phase I and II enzyme systems in mammals (Nilsen and Toftgard 1981; Nilsen et al. 1981; Meijer et al. 1981; Poon et al. 1995).

This thesis also examined the dietary accumulation of a series of hydrophobic, recalcitrant organochlorine compounds to develop relationships between K_{ow} and assimilation efficiency, half life and BMF. Assimilation efficiency, half life and BMF all had significant curvilinear relationships with K_{ow}, with the highest values for chemicals with a log K_{ow} of approximately 7.0. Lower assimilation efficiencies have been reported for compounds with a log Kow above 7.0 (Gobas et al. 1988), which have been attributed to reduced bioavailability of the chemicals or steric hinderance in crossing biological membranes (Opperhuizen et al. 1985). The lower assimilation efficiencies, and shorter half lives of chemicals with log K_{ow} greater than 7.0 resulted in a similar decline in BMF values. A comparable relationship has been observed for hydrophobic organochlorines in Lake Ontario salmonids (Oliver and Niimi 1988). Data from this work and other dietary accumulation studies need to be compiled and used to develop quantitative structure activity relationships (QSARs) for dietary accumulation of hydrophobic chemicals. Currently, there have been few attempts to develop such QSARs despite the need for

robust bioaccumulation relationships that predict the behavior of hydrophobic chemicals in the aquatic environment.

The most surprising result of the K_{ow}-bioaccumulation parameter relationship work was the curvilinear relationship between organochlorine half life in rainbow trout and K_{ow}. As K_{ow} increases the ratio of chemical in lipids to water should increase and partitioning out of lipid should decrease. Therefore, compounds with extremely high K_{ow} should have long half lives. Possible explanations for this phenomenon include insufficient data, inaccurate K_{ow}s, and dis-equilibrium between fish compartments. The latter explanation implies that these results are an artifact of the experimental design. Clearly, bioaccumulation parameters generated in laboratory experiments need to be used with caution when applied to wild populations. More research is needed on the influence of experimental protocol on laboratory-derived bioaccumulation parameters. Models that predict the fate and dynamics of chemicals in the environment are often dependent on such parameters.

Oligochaetes were found to readily accumulate PCAs from spiked sediment in laboratory experiments. Biota sediment accumulation factors (BSAFs) for two C_{12}- (6 and 10 Cl) and a C_{16}-PCA (3 Cl) were > 1, implying magnification of these PCAs between sediment and benthic invertebrates. The BSAF of a higher chlorinated C_{16}-PCA (13 Cl) was < 1, suggesting that this chemical is less bioavailable to benthic invertebrates when associated with sediment. This is likely due to the high hydrophobicity and strong sorption of this chemical which inhibits the movement from sediment into the oligochaete via interstitial water or gut absorption. PCA half lives in oligochaete ($t_{1/2}$ = 12 - 33 d) were shorter than what were observed in the rainbow trout. This may be due to the smaller size

of the oligochaetes, which provides a greater surface-volume ratio and shorter routes for chemicals to travel during depuration. More bioavailability data is needed for a range of PCAs which vary in carbon chain length and chlorine content. Such data could be used to develop relationships between bioavailability and carbon chain length and chlorine content. On a broad scale, more information and understanding are needed on the dynamics of hydrophobic organochlorines in sediment and on how physical properties of the sediment can influence bioavailability.

Oligochaetes appear to be able to biotransform PCAs. A significant portion of the total radioactivity in the oligochaetes was not extractable by toluene, implying that these compounds have been biotransformed to a more polar metabolite. The percentage of non-extractable ^{14}C decreased with increasing chlorine content for C_{12}- and C_{16}-PCAs. No relationship was obvious for the other carbon chain lengths. As with the rainbow trout, research is required on the biotransformation of organochlorine compounds by invertebrates. Information is also needed on the enzymes systems used to biotransform the PCAs and the metabolites formed.

PCAs appear to be readily biodegraded in aerobic sediments. A significant portion of the total radioactivity in sediments spiked with the ^{14}C-PCAs was not extractable by non-polar solvents (toluene and dichloromethane) implying that these compounds have been biodegraded to a more polar compound. Thirty two days after spiking the sediment, only 23-40% of the lower chlorinated C_{12}- and C_{16}-PCAs (6 and 3 Cl, respectively) and 58-64% of the higher chlorinated C_{12}- and C_{16}-PCAs (10 and 13 Cl, respectively) were extracted by toluene. PCA half lives in sediment ranged from 12-13 days for the lower chlorinated C_{12}- and C_{16}-PCAs (6 and 3 Cl, respectively) to 30-58 days for the higher

chlorinated C_{12}- and C_{16}-PCAs (10 and 13 Cl, respectively). Therefore, it appears that PCAs have short half lives in aerobic sediment and that half lives increase with increasing chlorine content. Persistence in sediment is an important regulatory question for short chain PCAs and more research is needed. A greater range of PCAs need to be studied so that relationships between biodegradation and carbon chain length and chlorine content can be more thoroughly examined. PCAs have been found on subsurface slices of lake sediment cores (Tomy 1997a), indicating that research on the biodegradation of PCAs in anaerobic sediments is also needed. The oligochaetes appeared to accumulate the biodegraded PCAs; thus, information on the products of PCA degradation in sediments is also needed.

PCAs have low acute toxicity and their mechanism of acute toxicity appears to be narcosis. Effects were only observed in Japanese medaka eggs and larvae that had been exposed to narcotic concentrations and had achieved tissue concentrations which would cause narcosis. No effects were observed in medaka eggs or larvae which were exposed to concentrations below the narcotic threshold. As well, rainbow trout exposed to high concentrations of the same PCAs exhibited behavior that is indicative of a narcotic toxicity mechanism although actual tissue concentrations were well below levels associated with narcosis.

There was evidence that PCAs can cause sub-lethal effects in rainbow trout at high dietary concentrations. Histopathological lesions were observed in liver and kidney of rainbow trout exposed to a C_{10}-PCA (average of 6.7 Cl) for 21 days. Morphometric changes were observed in the livers of rainbow trout exposed high dietary concentrations of a range of PCAs (C_{10}-, C_{12}- and C_{14}-PCAs) for 21 days. However, no histological

effects were found in rainbow trout exposed to the same PCAs for 85 days at lower concentrations. Additional work is needed to assess the potential for PCAs to cause histological effects in fish with longer exposures (years).

11. CONTRIBUTION TO KNOWLEDGE

1) This work has produced the first bioaccumulation parameter data for PCAs of single carbon chain lengths (C_{10}, C_{11}, C_{12}, C_{14}, and C_{16}) and chlorine content. This data has provided the first clear evidence that the bioaccumulation of PCAs can vary greatly based on the carbon chain length and chlorine content, and that the relative abundance of PCA congeners will vary greatly between different trophic levels or species. These data has also shown that PCAs should move through aquatic food chains, and that certain PCAs have the potential to biomagnify.

2) From the PCA bioaccumulation data, relationships have been created between half life, assimilation efficiency, and biomagnification factor, and carbon chain length, chlorine content and K_{ow}. These relationships can be used to calculate bioaccumulation parameters of PCAs which have not been studied. Bioaccumulation parameters are needed for models that predict fate and dynamics of chemicals in the environment.

3) This work has produced the first laboratory derived bioaccumulation data on TCPMe, TCPMeOH and three important toxaphene congeners. Bioaccumulation parameters are needed for models that predict fate and dynamics of chemicals in the environment.

4) This work has produced one of the first bioaccumulation parameter-K_{ow} relationships developed from dietary accumulation data. These relationships can be used to predict bioaccumulation parameter data for new compounds or compounds which lack bioaccumulation data. It can also identify compounds which may be metabolized by rainbow trout if half life and K_{ow} data are available. The half life-K_{ow} relationship has also identified that laboratory derived half lives of very hydrophobic chemicals in fish may be underestimated if exposure length is short.

5) This work has produced the first data of any kind on the bioavailability of sediment-sorbed PCAs. These results have clearly demonstrated that PCAs in sediment are bioavailable to, and accumulated by sediment ingesting benthic organisms. It has also shown that the accumulation of PCAs from sediment will vary with carbon chain length and that relative abundance of PCA congeners will vary greatly between sediment and benthic invertebrates.

6) Reported PCA half lives in sediment represent some of the first data of this type, and the first for PCAs with a single carbon chain length and known chlorine content. These short sediment half lives suggest that PCAs are not as persistent in sediment as originally believed, and have influenced the interpretation of PCA levels in sediment cores. PCA persistence in the environment is considered a major data gap for government regulation of short chain PCAs in Canada.

7) This work has produced the first data on PCA toxicity using PCAs of a single carbon chain length and chlorine content. This work has provided more evidence that the toxic mode-of-action of PCAs is narcosis.

8) Histological examination of the liver, kidney and thyroid of rainbow trout exposed to high dietary concentrations of PCAs is among the first data on sub-lethal effects of PCAs in fish and the first histological examination of fish exposed to PCAs. The results suggest that PCAs may create sub-lethal stress in fish populations at high exposure concentrations.

12. REFERENCES

Abernethy, S.G., Mackay, D. and McCarty, L.S. 1988. "Volume fraction" correlation for narcosis in aquatic organisms: The key role of partitioning. Environ. Toxicol. Chem. 7: 469-481.

Åhlman, M., Bergman, Å., Darnerud, P.O., Egestad, B. and Sjovall, J. 1986. Chlorinated paraffins: formulation of sulphur-containing metabolites of polychlororhexadecane in rats. Xenobiotica **16**: 225-232.

Atkinson, R. 1986. Kinetics and mechanisms of gas phase reactions of the hdyroxylradical with organic compounds under atmospheric conditions. Chem. Rev. **86**: 69-201.

Belfroid, A.C., Sijm, D.T.H.M. and van Gestel, C.A.M. 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. Environ. Rev. **4**: 276-299.

Bengtsson, B. and Ofstad, E.B. 1982. Long-term studies of uptake and elimination of some chlorinated paraffins in the bleak, *Alburnus alburnus*. Ambio **11**: 38-40.

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

Bergman, Å., Leonardsson, L and Wachmeister, C.A. 1981. Synthesis of polychlorinated [^{14}C] alkanes (PCA) of high specific activity. Chemosphere. **10**: 857-863.

Bidleman, T.F., Walla, M.D., Muir, D.C.G. and Stern, G.A. 1993. Selective accumulation of polychlorocamphenes in aquatic biota from the Canadian arctic. Environ. Toxicol. Chem. **12**: 701-709.

Bintein, S., Devillers, J. and Karcher, W. 1993. Nonlinear dependence of fish bioconcentration on *n*-octanol/water partition coefficient. SAR QSAR Environ. Res. **1**:29-39.

Biessmann, A., Brandt, L and 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.

Birtley, R.D.N., Conning, D.M., Daniel, J.W., Ferguson, D.M., Longstaff, E. and Swan, A.A.B. 1980. The toxicological effects of chlorinated paraffins in mammals. Toxicol. Appl. Pharmacol. **54**: 514-525.

Bremle, G., Okla, L., and Larsson, P. 1995. Uptake of PCBs in fish in a contaminated river system: Bioconcentration factors measured in the field. Environ. Sci. Technol. **29**:2010-2015.

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

Bruggeman, W.A., Opperhuizen, A., Wijbenga, A. and Hutzinger, O. 1984. Bioaccumulation of super-lipophilic chemicals in fish. Toxicol. Environ. Chem. **7**: 173-189.

Bucher, J.R., Alison, R.H., Montgomery, C.A., Huff, J., Haseman, J.K., Farnell, D., Thompson, R. and Prejean, J.D. 1987. Comparative toxicity and carcinogenicity of two chlorinated paraffins in F344/N rats and $B6C3F_1$ mice. Fund Appl Toxicol **9**: 454-468.

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

Carter, M.R. 1993. Soil sampling and method analysis. Canadian Society of Soil Publication.

Chapman, P.M. 1989. Current approaches to developing sediment quality criteria. Environ. Toxciol. Chem. **8**: 589-599.

Chessells, M, Hawker, D.W., and Connell, D.W. 1992. Influence of solubility in lipid on bioconcentration of hydrophobic compounds. Ecotoxicol. Environ. Saf. **23**:260-273.

Chiou, C.T. 1985. Partition coefficients of organic compounds in lipid-water systems and correlations with fish bioconcentration factors. Environ. Sci. Technol. **19**:57-62.

Clark, K.E. and Mackay, D. 1991. Dietary uptake and biomagnification of four chlorinated hydrocarbons by guppies. Environ. Toxicol. Chem. **10**:1205-1217.

Connell, D.W. and Hawker, D.W. 1988. Use of polynomial expressions to describe the bioconcentration of hydrophobic chemicals by fish. Ecotoxicol. Environ. Saf. **16**:242-257.

Connolly, J.P. and Pedersen, C.J. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. Environ. Sci. Technol. **22**:99-103.

Coristine, S., Haffner, G.D., Ciborowski, J.J.H., Lazar, R., Nanni, M.E. and Metcalfe, C.D. 1996. Elimination rates of selected di-*ortho*, mono-*ortho*, and non-*ortho* substituted polychlorinated biphenyls in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. **15**:1382-1387.

Darnerud, P.O. 1984. Chlorinated paraffins: Effect of some microsomal enzyme inducers and inhibitors of the degradation of 1-^{14}C-chlorododecanes to $^{14}CO_2$ in mice. Acta. Pharacol. Toxicol. **55**:110-115

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

Darnerud, P.O., Bengtsson, B.E., Bergman, A. and Brandt, I. 1983. Chlorinated paraffins: Disposition of a polychloro-[1-^{14}C]-hexadecane in carp (Cyprinus carpio) and bleak (*Alburnus alburnus*). Toxicol. Letters. **19**: 345-351.

Darnerud, P.O., Bergman, A., Lind, B.O. and Brandt, I. 1989. Selective accumulation of chlorinated paraffins (C_{12} and C_{16}) in the olfactory organ of rainbow trout. Chemosphere **18**: 1821-1827.

de Boer, J., Wester, P.G., Evers, E.H.G. and Brinkman, U.A.T. 1996. Determination of *tris*(4-chlorophenyl)methanol and *tris*(4-chlorophenyl)methane in fish, marine mammals and sediment. Environ. Pollut. **93**:39-47.

de Wolf, W., de Bruijn, J.H.M., Seinen, W. and Hermens, J.L.M. 1992. Influence of biotransformation on the relationship between bioconcentration factors and octanol-water partition coefficients. Environ. Sci. Technol. **26**:1197-1201.

Devillers, J., Bintein, S. and Domine, D. 1996. Comparison of BCF models based on log P. Chemosphere **33**:1047-1065.

Drouillard, K.G., Hiebert, T., Tran, P., Tomy, G.T., Muir, D.C.G. and Friesen, K.J. 1998. Water solubilities of chlorinated *n*-alkanes. Environ. Toxicol. Chem. (In press).

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. MSc thesis. University of Manitoba, MB, Canada.

Drouillard, K.G., Hiebert, T., Muir, D.C.G. and Friesen, K.J. 1995. Water solubility and Henry's Law constants of short chain chlorinated paraffins. Proceedings, 38th Conference on Great Lakes Research, East Lansing, MI, USA. May 28 - June 1, 1995, pp. 118-119.

E and G Bionomics. 1983. Acute and chronic toxicity of a chloriated paraffin to the midge *Chironomus tentans*. United States Environmental Protection Agency Document # 88-8800549.

Elcombe, C.R., Watson, S.C., Wyatt, I. and Foster, J.R. 1994. Chlorinated paraffins (CP): mechamisms of carcinogensis. Toxicologist **14**:276.

Eriksson, P. and Kilström, J. 1985. Disturbance of motor performance and thermoregulation in mice given two commercial chlorinated paraffins. Bull. Environ. Contam. Toxicol. **34**: 205-209.

Evans, M.S., Noguchi, G.E. and Rice, C.P. 1991. The biomagnification of polychlorinated biphenyls, toxaphene, and DDT compounds in a Lake Michigan offshore food web. Arch. Environ. Contam. Toxicol. **20**: 87-93.

Fisk, A.T., Yarechewski, A.L., Metner, D.A., Lockhart, W.L., Evans, R.E. and Muir, D.C.G. 1997. Accumulation, elimination and hepatic mixed-function oxidase enzyme induction in juvenile rainbow trout and lake whitefish exposed to dietary 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Aquat. Toxicol. **37**:201-220.

Fisk, A.T., Cymbalisty, C.D., Tomy, G.T., Stern, G.A., Muir, D.C.G. and Haffner, G.D. 1996b. 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.

Forbis, A.D., Frazier, S. and Schon, L. 1986. Acute toxicity of Chlorowax 45 LV to *Daphnia magna* Analytical Biochemistry Laboratories, Inc. Static Toxicity Report #34264.

Fox, K., Zauke, G. and Butte, W. 1994. Kinetics of bioconcentration and clearance of 28 polychlorinated biphenyl congeners in zebrafish (*Brachydanio rerio*). Ecotox. Environ. Saf. **28**:99-109.

Franke, C., Studinger, G., Berger, G., Bohling, S., Bruckmann, U., Cohors-Fresesborg, D. and U. Johncke. 1994. The assessment of bioaccumulation. Chemosphere **29**: 1501-1514.

Friedman, D. and Lombardo, P. 1975. Photochemical technique for the elimination of chlorinated aromatic interferences in the gas-liquid chromatographic analysis for chlorinated paraffins. J. Assoc. Offic. Anal. Chem. **58**: 703 706.

Gjøs, N. and Gustavsen, K.O. 1982. Determination of chlorinated paraffins by negative ion chemical mass spectrometry. Anal. Chem. **54**: 1316-1318.

Glassmeyer, S.T., de Vault, D.S., Myers, T.R. and Hites, R.A. 1997. Toxaphene in Great Lakes Fish: A temporal, spatial, and trophic study. Environ. Sci. Technol. **31**:84-88.

Gobas, F.A.P.C., Clark, K.E., Shiu, W.Y. and 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.

Gobas, F.A.P.C., McCorquodale, J.R. and Haffner, G.D. 1993. Intestinal absorption and biomagnification of organochlorines. Environ. Toxicol. Chem. **12**:567-576.

Gobas, F.A.P.C., Shiu, W.Y. and Mackay, D. 1986. Factors determining partitioning of hydrophobic organic chemicals in aquatic organisms. In Kaiser KLE, ed, *QSAR in Environmental Toxicology - II.* , D. Reidel Publishing Company, Dordrecht, Holland, pp 107-124.

Gobas, F.A.P.C., Muir, D.C.G. and Mackay, D. 1988. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. Chemosphere **17**: 943-962.

Gorbman, A. 1969. Thyroid function and its control in fishes. Pages 241-274. *in* W.S. Hoar and D.J. Randall (Eds.). Fish physiology, Volume II, The endocrine system. Academic Press, New York, NY, USA.

Government of Canada. 1993. Priority substances list assessment report: Chlorinated paraffins. Environment Canada, Catalogue No. En40-215/17E, ISBN 0-662-0515 17E, 32 pp.

Government of Canada. 1995. Toxic substances management policy. Ottawa, Ontario (June 1995).

Government of Canada. 1997. Short chain chlorinated paraffins: scientific justification. Environment Canada, Catalogue No. En40-230/5-1997E, ISBN 0-662-25391-4, 16 pp.

Harkley, G.A., Landrum, P.F. and Klaine, S.J. 1994a. Comparison of whole-sediment, elutriate and pore-water exposures for use in assessing sediment-associated organic contaminants in bioassays. Environ. Toxicol. Chem. **13**: 1315-1329.

Harkey, G.A., Lydy, M.J., Kukkonen, J. and Landrum, P.F. 1994b. Feeding selectivity and assimilation of PAH and PCB in *Diporeia* spp. Environ. Toxicol. Chem. **13**: 1445-1455.

Harris, G.E., Metcalfe, T.L., Metcalfe, C.D., and Huestis, S.Y. 1994a. Embryotoxicity of extracts from Lake Ontario rainbow trout (*Oncorhynchus mykiss*) to Japanese medaka (*Oryzias latipes*). Environ. Toxicol. Chem. **13**: 1393-1403.

Harris, G.E., Kiparissis, Y. and Metcalfe, C.D. 1994b. Assessment of the toxic potential of PCB congener 81 (3,4,4',5-tetrachlorobiphenyl) to fish in relation to other non-ortho substituted PCB congeners. Environ. Toxicol. Chem. **13**: 1405-1413.

Haux, C., Larsson, A., Lidman, U., Forlin, L., Hansson, T., and Johansson-Sjobeck, M. 1982. Sublethal physiological effects of chlorinated paraffins on the flounder, *Platichthys flesus* L. Ecotoxicol. Environ. Saf. **6**: 49-59.

Hawker, D.W. and Connell, D.W. 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. Environ. Sci. Technol. **22**:382-387.

Heath, A.G. 1995. Liver. Pages 125-139. *in* A.G. Heath (Ed.). Water pollution and fish physiology. 2nd edition. CRC Lewis Publishers, Boca Raton, FL, USA.

Hickman Jr., C.P. and Trump, B.F. 1969. The kidney. Pages 91-240. *in* W.S. Hoar and D.J. Randall (Eds.). Fish physiology, Volume I, Excretion, ionic regulation, and metabolism. Academic Press, New York, NY, USA.

Hill, R.W. and Maddock, B.G. 1983a. Effect of a chlorinated paraffin on embryos and larvae of the sheepshead minnow (*Cyprinodon variegatus*) - (Study one). Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2326.

Hill, R.W. and Maddock, B.G. 1983b. Effect of a chlorinated paraffin on embryos and larvae of the sheepshead minnow (*Cyprinodon variegatus*) - (Study two). Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2327.

Hinton, D.E. and Lauren, D.J. 1990. Liver structural alterations accompanying chronic toxicity in fishes: Potential biomarkers of exposure. Pages 17-57. *in* J.F. McCarthy and L.R. Shugart (Eds.). Biomarkers of environmental contamination. Lewis Publishers, Boca Raton, FL.

Hinton, D.E., Baumann, P.C., Gardner, G.R., Hawkins, W.E., Hendricks, J.D., Murchelano, R.A. and Okihiro, M.S. 1992. Histopathologic biomarkers. Pages 155-209. *in* R.J. Huggett et al. (Eds.). Biomarkers: Biochemical, physiological, and histological markers of anthropogenic stress. Proceedings of the Eighth Pellston Workshop, Keystone, CO, July 23-28, 1989. SETAC Special Publications Series, Lewis Publishers, Boca Raton, FL.

Hollies, J.I., Pinnington, D.F., Handley, A.J., Baldwin, M.K. and Bennett, D. 1979. The determination of chlorinated long chain paraffins in water, sediment and biological samples. Anal. Chem. Acta. **111**: 201-213

Howard, P.H., Santodonato, J. and Saxena, J. 1975. Investigations of selected potential environmental contaminants: Chlorinated paraffins. Syracuse, New York, Syracuse University Research Corporation, 107 pp (Report No. 68-01-3101).

Isnard, P. and Lambert, S. 1988. Estimating bioconcentration factors from octanol-water coefficient and aqueous solubility. Chemosphere **17**: 21-34.

Jansson, B., Andersson, R., Asplund, L., Bergman, Å., Litzén, K., Nylund, K., Reutergårdh, L., Sellström, U., Uvemo, U-B., Wahhlerg, C. and Wideqvist U.1991. Multi-residue method for the gas-chromatographic analysis of some polychlorinated and polybrominated pollutants in biological samples. Fresenius J. Anal. Chem. **340**: 439-445.

Jansson, B., Andersson, R., Asplund, L., Litzen, K., Nylund, K., Sellstrom, U., Uvemo, U., Wahlberg, C., Wideqvist, U., Odsjo, T. and Olsson, M. 1993. Chlorinated and brominated persistent organic compounds in biological samples from the environment. Environ. Toxicol. Chem. **12**: 1163-1174.

Jarman, W.M., Simon, M., Norstrom, R.J., Burns, S.A., Bacon, C.A., Simonelt, B.R.T. and Risebrough, R.W. 1992. Global distribution of Tris(4-chlorophenyl)methanol in high trophic level birds and mammals. Environ. Sci. Technol. **26**:1770-1774.

Junk, S.A. and Meisch, H.U. 1993. Determination of chlorinated paraffins by GC MS. Fres. J. Anal. Chem. **347**: 361-363.

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

Kidd, K.A., Schindler, D.W., Muir, D.C.G., Lockhart, W.L. and Hesslein, R.H. 1995. High concentrations of toxaphene in fishes from a subarctic lake. Science **269**: 240-242.

Kiriluk, R.M., Servos, M.R., Whittle, D.M., Cabana, G. and Rasmussen, J.B. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Can. J. Fish. Aquat. Sci. **52**:2660-2674.

Knezovich, J.P., Harrison, F.L. and Wilhelm, R.G. 1987. The bioavailability of sediment-sorbed organic chemicals: A review. Wat. Air Soil Pollut. **32**: 233-245.

Koslowski, S.E., Metcalfe, C.D., Lazar, R. and Haffner, G.D. 1994. The distribution of 42 PCBs, including three coplanar congeners, in the food web of the western basin of Lake Erie. J. Gt. Lakes Res. **20**: 260-270.

Landrum, P.F. 1988. Toxicokinetics of organic xenobiotics in the amphipod, *Pontoporeia hoyi*: role of physiological and environmental variables. Aquat. Toxicol. **12**: 245-271.

Landrum, P.F. 1989. Bioavailability and toxicokinetics of polycyclic aromatic hydrocarbons sorbed to sediment for the amphipod *Pontoporeia hoyi*. Environ. Sci. Technol. **23**: 588-595.

Landrum, P.F., Nihart, S.R., Eadie, B.J. and Gardner, W.S. 1984. Reverse-phase separation method for determining pollutant binding to Aldrich Humic Acid and dissolved organic carbon of natural waters. Environ. Sci. Technol. **18**: 187-192.

Landrum, P.F. and Poore, R. 1988. Toxicokinetics of selected xenobiotics in *Hexagenia limbata*. J. Great Lakes Res. **14**: 427-437.

Larsson, P. 1985. Contaminated sediments of lakes and oceans act as sources of chlorinated hydrocarbons for release to water and atmosphere. Nature **317**: 347-349.

Linden, E., Bengtsson, B-E., Svanberg, O. and Sundstrom, G. 1979. The acute toxicity of 78 chemicals and pesticide formulations against two brackish water organisms, the bleak (*Alburnus alburnus*) and the harpacticoid (*Nitocra spinipes*). Chemosphere **11/12**: 843-851.

Lombardo. P., Dennison, J.L. and Johnson, W.W. 1975. Bioaccumulation of chlorinated paraffin residues in fish fed Chlorowax 500C. Journal of the Association of Official Analytical Chemists. **58**: 707-710.

Loonen, H., Muir, D.C.G., Parsons, J.R. and Govers, H.A.J. 1997. Bioaccumulation of polychlorinated dibenzo-*p*-dioxins in sediment by oligochaetes: Influence of exposure pathway and contact time. Environ Toxicol Chem. **16**: 1518-1525.

Lydy, M.J., Oris, J.T., Baulmann, P.C. and Fisher, S.W. 1992. Effects of sediment organic carbon content on the elimination rates of neutral lipophilic compounds in the midge (*Chironomus riparius*). Environ. Toxicol. Chem. **11**: 347-356.

Lyman, W.S., Reehl, W.F. and Rosenblatt, R.H. 1982. *Handbook of chemical physical property estimation methods*. McGraw-Hill Book Company, New York, NY, USA.

Mackay, D. 1982. Correlation of bioconcentration factors. Environ. Sci. Technol. **16**:274-278.

Mackay, D., Shiu, W.Y. and Ma, K.C. 1992a. *Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals*. Vol. I. Lewis Publishers, Chelsea, MI, USA.

Mackay, D., Shiu, W.Y. and Ma, K.C. 1992b. *Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals*. Vol. II. Lewis Publishers, Chelsea, MI, USA.

Madeley, J.R. and Birtley, R.D.N. 1980. Chlorinated paraffins and the environment. 2. Aquatic and avian toxicology. Environ. Sci. Technol. **14**: 1215-1221.

Madeley, J.R. and Maddock, B.G. 1983a. The bioconcentration of a chlorinated paraffin in the tissues and organs of rainbow trout (*Salmo gairdneri*). Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2310.

Madeley, J.R. and Maddock, B.G. 1983b. Toxicity of a chlorinated paraffin to rainbow trout over 60 days. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2203.

Madeley, J.R. and Maddock, B.G. 1983c. Toxicity of a chlorinated paraffin to rainbow trout over 60 days. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2202.

Madeley, J.R. and Maddock, B.G. 1983d. Toxicity of a chlorinated paraffin to rainbow trout over 60 days. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2201.

Madeley, J.R. and Maddock, B.G. 1983e. Toxicity of a chlorinated paraffin to rainbow trout over 60 days. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2200.

Madeley, J.R. and Maddock, B.G. 1983f. Effect of a chlorinated paraffin on the growth of rainbow trout. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2309.

Madeley, J.R., Thompson, R.S. and Brown, D. 1983. The bioconcentration of a chlorinated paraffin by the common mussel (*Mytilus edulis*). Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2351.

Madeley, J.R. and Thompson, R.S. 1983a. Toxicity of chlorinated paraffin to mussels (*Mytilus edulis*) over 60 days. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2291.

Madeley, J.R. and Thompson, R.S. 1983b. Toxicity of chlorinated paraffin to mussels (*Mytilus edulis*) over 60 days. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2289.

Madeley, J.R. and Thompson, R.S. 1983c. Toxicity of chlorinated paraffin to mussels (*Mytilus edulis*) over 60 days. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2288.

Madeley, J.R. and Thompson, R.S. 1983d. Toxicity of chlorinated paraffin to mussels (*Mytilus edulis*) over 60 days. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2290.

Markwell, M.A.K., Haas, S.M., Tolbert, N.E. and Bieber, L.L. 1981. Protein determination in membrane and lipoprotein samples: Manual and automated procedures. Methods Enzymol. **72**: 296-393.

McCarty, L.S. 1986. The relationship between aquatic toxicity QSARs and bioconcentration for some organic chemicals. Environ Toxicol Chem **5**: 1071-1080.

McFarland, V.A. and Clarke, J.U. 1989. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: Considerations for a congener-specific analysis. Environ. Health Perspect. **81**: 225-239.

McKim, J.M., Bradbury, S.P. and Niemi, G.J. 1987. Fish acute toxicity syndromes and their use in the QSAR approach to hazard assessment Environ Health Perspect **71**:171-186.

Meijer, J., Rundgren, M., Astrom, A., DePierre, J.W., Sundvall, A. and Rannug, U. 1981. Effects of chlorinated paraffins on some drug-metabolising enzymes in rat liver and in the Ames test. Adv. Experiment Med. Biol. **136**: 821-828.

Metcalfe-Smith, J.L., Maguire, R.J., Batchelor, S.P., Bennie, D.T. 1995. Occurrence of chlorinated paraffins in the St. Lawrence river near a manufacturing plant in Cornwall, ON. Aquatic ecosystem protection branch. National Water Research Institute. Environment Canada, Burlington, ON.

Metcalfe, C.D., Metcalfe, T.L., Cormier, J.A., Huestis, S.Y. and Niimi, A.J. 1997. Early life-stage mortalities of Japanese medaka (*Oryzias latipes*) exposed to polychlorinated diphenyl ethers. Environ. Toxicol. Chem. **16**: 1749-1754.

Muir, D.C.G., Ford, C.A., Rosenberg, B., Norstrom, R.J., Simon, M. and Beland, P. 1996. Persistent organochlorines in beluga whale (*Delphinapterus leucas*) from the St Lawrence river estuary-I. Concentrations and patterns of specific PCBs,

chlorinated pesticides and polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Environ. Pollut. **93**:219-234.

Muir, D.C.G., Marshall, W.K., and Webster, G.R.B. 1985. Bioconcentration of PCDDs by fish: Effects of molecular structure and water chemistry. Chemosphere **14**:829-833.

Muir, D.C.G., Yarechewski, A.L., Metner, D.A. and Lockhart, W.L. 1992. Dietary 2,3,7,8-tetrachlorodibenzofuran in rainbow trout: Accumulation, disposition, and hepatic mixed-function oxidase enzyme induction. Toxicol. Appl. Pharmacol. **117**:65-74.

Muir, D.C.G., Yarechewski, A.L., Metner, D.A., Lockhart, W.L., Webster, G.R.B. and Friesen, K.J. 1990. Dietary accumulation and sustained hepatic mixed function oxidase enzyme induction by 2,3,4,7,8-pentachlorodibenzofuran in rainbow trout. Environ. Toxicol. Chem. **9**:1463-1472.

Muir, D.C.G., Yarechewski, A.L., Knoll, A. and Webster, G.R.B. 1986. Bioconcentration and disposition of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin and octachlorodibenzo-*p*-dioxin by rainbow trout and fathead minnows. Environ. Toxicol. Chem. **5**:261-272.

Mukherjee, A.B. 1990. The use of chlorinated paraffins and their possible effects in the environment. national board of waters and the environment, Helsinki, Finland. Series A 66, 53pp.

Müller, M.D. and Schmid, P.P. 1984. GC/MS analysis of chlorinated paraffins with negative ion chemical ionization. J. High Resol. Chromatogr. Commun. 7: 33-37.

Murray, T.M., Frankenberry, D.H., Steele, D.H. and Heath, R.G. 1988. Chlorinated paraffins: A report on the findings from two filed studies, Sugar Creek, Ohio and Tinkers Creek, Ohio., Vol.1, Technical Report, U.S. Environmental Protection Agency, EPA/560/5 87/012. 150pp.

Neely, W.B. 1980. *Chemicals in the Environment*. Marcel Dekker, New York, NY, USA.

Niimi, A.J. 1996. Evaluation of PCBs and PCDD/Fs retention by aquatic organisms. Sci. Total Environ. **192**:123-150.

Niimi, A.J. and Oliver, B.G. 1983. Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole fish and muscle of rainbow trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. **40**:1388-1394.

Niimi, A.J. and Oliver, B.G. 1987. Influence of molecular weight and molecular volume on dietary absorption efficiency of chemicals by fishes. Can. J. Fish. Aquat. Sci. **45**: 222-227.

Niimi, A.J. and Oliver, B.G. 1988. Influence of molecular weight and molecular volume on dietary adsorption and assimilation efficiency of chemicals by fishes. Can. J. Fish. Aquat. Sci. **45**:222-227.

Niimi, A.J. and Palazzo, V. 1985. Temperature effect on the elimination of pentachlorophenol, hexachlorobenzene and mirex by rainbow trout (*Salmo gairdneri*). Water Res. **19**:205-207.

Nilsen, O.G. and Toftgard, R. 1981. Effects of polychlorinated terphenyls and paraffins on rat liver microsomal cytochrome P-450 and in vitro metabolic activities. Arch. Toxicol. **47**: 1-11.

Nilsen, O.G., Toftgard, R. and Glaumann, H. 1981. Effects of chlorinated paraffins on rat liver microsomal activities and morphology. Arch. Toxicol. **49**: 1-13.

Opperhuizen, A., van der Velde, E.W., Gobas, F.A.P.C., Liem, D.A.K. and van der Steen, J. M. D. 1985. Relationship between bioconcentration in fish and steric factors of hydrophobic chemicals. Chemosphere **14**: 1871-1896.

Oliver, B.G. 1984. Uptake of chlorinated organics from anthropogenically contaminated sediments by oligochaete worms. Can. J. Fish. Aquat. Sci. **41**: 878-883.

Oliver, B.G. 1987. Biouptake of chlorinated hydrocarbons from laboratory-spiked and field sediments by oligochaete worms. Environ. Sci. Technol. **21**: 785-790.

Oliver, B.G. and Niimi, A.J. 1985. Bioconcentration factors of some halogenated organics for rainbow trout: Limitations in their use for prediction of environmental residues. Environ. Sci. Technol. **19**:842-848.

Oliver, B.G. and 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.

Omori, T., Kimura, T. and Kodama, T. 1987. Bacterial cometabolic degradation of chlorinated paraffins. **Appl. Microbiol. Biotechnol. 25**:553-557.

Opperhuizen, A. and Sijm, D.T.H.M. 1990. Bioaccumulation and biotransformation of polychlorinated dibenzo-p-dioxins and dibenzofurans in fish. Environ. Toxicol. Chem. **9**: 175-186.

Opperhuizen, A. and Schrap, S.M. 1988. Uptake efficiencies of two polychlorobiphenyls in fish after dietary exposure to five different concentrations. Chemosphere **17**(2): 253-262.

Parkerton, T.F. 1993. Estimating toxicokinetic parameters for modeling the bioaccumulation of non-ionic organic chemicals in aquatic organisms. PhD thesis. Rutgers State University, New Brunswick, NJ, USA.

Peakall, D. 1995. Thyroid function, retinols, baem and regulatory enzymes. Pages 108-154. *in* D. Peakall (Ed.). Animal biomarkers as pollution indicators. Chapman and Hall, New York, NY, USA.

Pohl, R.J. and Fouts, J.R. 1980. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal cellular fractions. Anal. Biochem. **107**: 150-155.

Poon, R., Lecavalier, P., Chan, P., Viau, C., Hakansson, H., Chu, I. and Valli, V.E. 1995. Subchronic toxicity of a medium-chain chlorinated paraffin in the rat. J. Appl. Toxicol. **15**: 455-463.

Porte, C. and Albaiges, J. 1993. Bioaccumulation patterns of hydrocarbons and polychlorinated biphenyls in bivalves, crustaceans, and fishes. Arch. Environ. Contam. Toxicol. **26**:273-281.

Rasmussen, J. B., Rowan, D.J., Lean, D.R.S. and Carey, J.H. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*) and other pelagic fish. Can. J. Fish. Aquat. Sci. **47**: 2030-2038.

Renberg, L., Tarkpea, M. and Sundstrom, G. 1986. The use of the bivalve *Mytilus edulis* as a test organism for bioconcentration studies. Ecotoxicol. Environ. Saf. **11**: 361-372.

Rieger, R. and 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). Critical Rev. Toxicol. **21**:51-88.

Safe, S. 1992. Toxicology, structure-function relationships, and human and environmental health impacts of polychlorinated biphenyls: Progress and problems. Environ. Health Perspect. **100**: 259-268.

Sarna, L.P., Hodge, P.E. and Webster, G.R.B. 1984. Octanol-water partition coefficients of chlorinated dioxins and dibenzofurans by reverse-phase HPLC using several C_{18} columns. Chemosphere **13**: 975-983.

Schmid, P.P. and Muller, M.D. 1985. Trace level detection of chlorinated paraffins in biological and environmental samples, using gas chromatography/mass spectrometry with negative-ion chemical ionization. J. Assoc. Offic. Anal. Chem. **68**: 427-430.

Schüürmann, G. and Klein, W. 1988. Advances in bioconcentration prediction. Chemosphere **17**:1551-1574.

Schwarzenbach, R.P., Gschwend, P.M. and Imboden, D.M. 1993. Environmental Organic Chemistry. John Wiley and Sons, Inc. Toronto, ON, 681 pp.

Serrone, D.M., Birtley, R.D.N., Weigand, W. and Millischer, R. 1987. Toxicology of chlorinated paraffins. Food Chem Toxic **25**: 553-562.

Sijm, D.T H.M., Schipper, M. and Opperhuizen, A. 1993. Toxicokinetics of halogenated benzenes in fish: Lethal body burden as a toxicological end point. Environ. Toxicol. Chem. 12: 1117-1127.

Sijm, D.T.H.M. and 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.

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

Sijm, D.T.H.M. and van der Linde, A. 1995. Size-dependent bioconcentration kinetics of hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric relationships. Environ. Sci. Technol. **29**:2769-2777.

Sijm, D.T.H.M., Seinen, W. and Opperhuizen, A. 1992. Life-cycle biomagnification study in fish. Environ. Sci. Technol. **26**:2162-2174.

Smith, I. and Craig, G. 1983. Prediction of organic contaminant aquatic toxicity utilizing intraperitoneal injection and structure-activity relationships. Can. Tech. Report Fish. Aquat. Sci. **1151**:122-131.

Spacie, A. and Hamelink, J.L. 1982. Alternative models for describing the bioconcentration of organics in fish. Environ. Toxicol. Chem. **1**:309-320.

Stainton, M.P., Capel, M.J. and Armstrong, F.A.J. 1977. The chemical analysis of freshwater. Fish. Mar. Serv. Misc. Publ. 25: 180 p.

Stern, G.A., Muir, D.C.G., Ford, C.A., Grift, N.P., Dewailly, E., Bidleman, T.F. and Walla, M.D. 1992. Isolation and identification of two major recalcitrant toxaphene congeners in aquatic biota. Environ. Sci. Technol. **26**:1838-1840.

Stern, G.A., Loewen, M.D., Miskimmin, B.M., Muir, D.C.G. and Westmore, J.B. 1996. Characterization of two major toxaphene components in treated lake sediment. Environ. Sci. Technol. **30**:2251-2258.

Stern, G.A., Tomy, G.T., Muir, D.C.G., Westmore, J.B., Dewailly, E. and 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.

Suntio, L.R., Shiu, W.Y., Mackay, D., Seiber, J.N. and Glotfelty, D. 1988. Critical review of Henry's Law Constants for pesticides. Rev. Environ. Contam. Toxicol. **103**:1-59.

Svanberg, O., Bengtsson, B-E., Linden, E., Lunde, G. and Ofstad, E.B. 1978. Chlorinated paraffins - A case of accumulation and toxicity to fish. Ambio 7: 64-65.

Svanberg, O. and Linden, E. 1979. Chlorinated paraffins: An environmental hazard?. Ambio **8**: 206-209.

Swackhamer, D.L. and Hites, R.A. 1988. Occurrence and bioaccumulation of organochlorine compounds in fishes from Siskiwit Lake, Isle Royale, Lake Superior. Environ. Sci. Technol. **22**:543-548.

Swedish National Chemicals Inspectorate. 1991. KEMI Report No. 1. Environmental protection agency (1991) Office of toxic substances. Rm1 decision package. chlorinated paraffins. Environmental risk assessment. Washington DC.

Swigert, J.P. and Bowman, J. 1986a. Acute toxicity of Chlorowax 45 LV to rainbow trout (*Salmo gairdneri*). Analytical Biochemistry Laboratories, Inc. Static Toxicity Report #34263.

Swigert, J.P. and Bowman, J. 1986b. Acute toxicity of Chlorowax 45 LV to bluegill sunfish (*Lepomis macrochirus*). Analytical Biochemistry Laboratories, Inc. Static Toxicity Report #34262.

Thomann, R.V. and Connolly, J.P. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environ. Sci. Technol. **18**:65-71.

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

Thomann, R.V., Connolly, J.P. and Parkerton, T.F. 1992. An equilibrium model of organic chemical accumulation in aquatic food webs with sediment interaction. Environ. Toxicol. Chem. **11**:615-629.

Thompson, R.S. and Madeley, J.R. 1983a. Toxicity of a chlorinated paraffin to the green alga *Selenastrum capricornutum*. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2321.

Thompson, R.S. and Madeley, J.R. 1983b. Toxicity of a chlorinated paraffin to the marine alga *Skeletonema costatum*. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2328.

Thompson, R.S. and Madeley, J.R. 1983c. Toxicity of a chlorinated paraffin to *Daphnia magna*. Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2358.

Thompson, R.S. and Madeley, J.R. 1983d. The acute and chronic toxicity of a chlorinated paraffin to the mysid shrimp (*Mysidopsis bahia*). Imperial Chemical Industries PLC, Devon, UK, Brixham Report No. BL/B/2373.

Tomy, G.T., Muir, D.C.G., Westmore, J.B. and Stern, G.A. 1993. Liquid chromatography thermospray mass spectrometry for characterization of commercial chlorinated *n* paraffin mixtures. Proceedings of the 41st Annual Conference on Mass Spectrometry and Allied Topics, San Francisco, CA. May 30-June 4.

Tomy, G.T. 1997. The mass spectrometric characterization of polychlorinated *n*-alkanes and the methodology for their analysis in the environment. Ph.D. Thesis. University of Manitoba.

Tomy, G.T., Stern, G.A., Muir, D.C.G., Fisk, A.T., Cymbalisty, C.D. and Westmore, J.B. 1997a. 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.

Tomy, G.T., Stern, G.A., Muir, D.C.G., Lockhart, L. and Westmore, J.B. 1997b. Occurrence of polychloro-*n*-alkanes in Canadian mid-latitude and Arctic lake sediments. Organohalogen Compounds **33**: 220-226.

Tomy, G.T., Fisk, A.T., Westmore, J.B. and Muir, D.C.G. 1998. Environmental chemistry and toxicology of polychlorinated *n*-alkanes. Rev. Environ. Toxciol Chem. (*in press*).

van Wezel, A.P., de Vries, D.A.M., Sijm, D.T.H.M. and Opperhuizen, A. 1996. Use of the lethal body burden in the evaluation of mixture toxicity. Ecotox. Environ. Safety **35**: 236-241.

Veith, G.D., DeFoe, D.L. and Bergstedt, B.V. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. **36**:1040-1048.

Veith, G.D., Call, D.J. and Brooke, L.T. 1983. Structure-toxicity relationships for the fathead minnow, *Pimephales promelas*: Narcotic industrial chemicals. Can. J. Fish. Aquat. Sci. **40**: 743-748.

West, C.W., Ankley, G.T., Nichols, J.W., Elonen, G.E. and Nessa, D.E. 1997. Toxicity and bioaccumulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in long-term tests with the freshwater benthic invertebrates *Chironomus tentans* and *Lumbriculus variegatus*. Environ. Toxicol. Chem. **16**:1287-1294.

Wilcock, R.J., Smith, T.J., Pridmore, R.D., Thrush, S.F., Cummings, V.J. and Hewitt, J.E. 1993. Bioaccumulation and elimination of chlordane by selected intertidal benthic fauna. Environ. Toxicol. Chem. **12**: 733-742.

Willis, B., Crookes, M.J., Diment, J. and Dobson, S.D. 1994. Environmental hazard assessment: chlorinated paraffins. Garston, Watford, Building Research Establishment, 47 pp (Report TSD/19).

Windrath, O.M. and Stevenson, D.R. 1985. Chlorination and bromochlorination paraffins as flame retardants. Plastics Compounding pp. 38-52.

Wisk, J.D. and Cooper, K.R. 1990a. The stage specific toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in embryos of the Japanese medaka (*Oryzias latipes*). Environ. Toxicol. Chem. **9**: 1159-1169.

Wisk, J.D. and Cooper, K.R. 1990b. Comparison of the toxicity of several polychlorinated dibenzo-*p*-dioxins and 2,3,7,8-tetrachlorodibenzofuran in embryos of the Japanese medaka (*Oryzias latipes*). Chemosphere **20**: 361-377.

World Health Organization. 1996. Environmental Health Criteria 181: Chlorinated paraffins, Geneva, Switzerland, pp 181.

Wood, L.W., O'Keefe, P. and Bush, B. 1997. Similarity analysis of PAH and PCB bioaccumulation patterns in sediment-exposed *Chironomus tentans* larvae. Environ. Toxicol. Chem. **16**: 283-292.

Wyatt, I., Coutts, C.T. and Elcombe, C.R. 1993. The effect of chlorinated paraffins on hepatic enzymes and thyroid hormones. Toxicology **77**: 81-90.

Yasutake, W.T. and Wales, J.H. 1983. Microscopic anatomy of salmonids: An atlas. US Fish and Wildlife Service Resource Publication 150.

Zitko, V. 1974. Uptake of chlorinated paraffins and PCB from suspended solids and food by juvenile Atlantic salmon. Bull. Environ. Contam. Toxicol. **12**: 406-412.

Zitko, V. 1980. In: Handbook of environmental chemistry. Springer Verlag. Vol 3A. 149 156.

Zitko, V. and Arsenault, E. 1974. Chlorinated paraffins: Properties, uses, and pollution potential. Fisheries and Marine Service Technical Report No. 491, pp 38.

IMAGE EVALUATION
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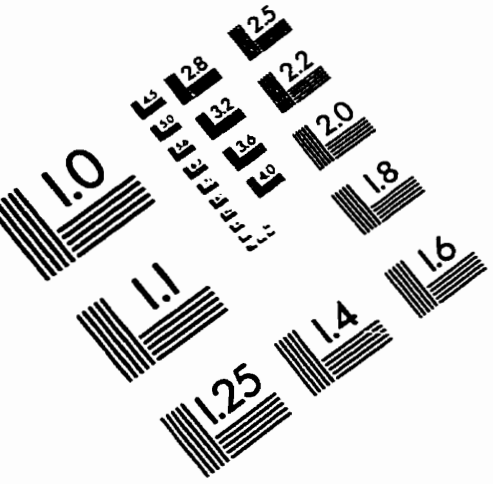

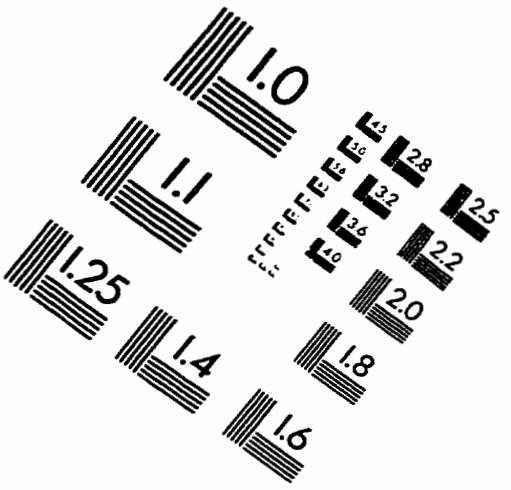

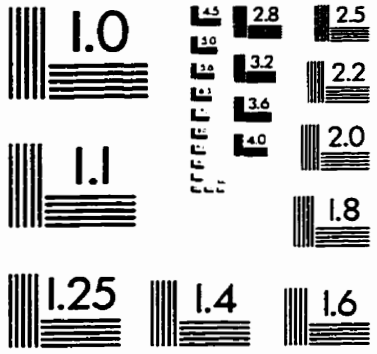

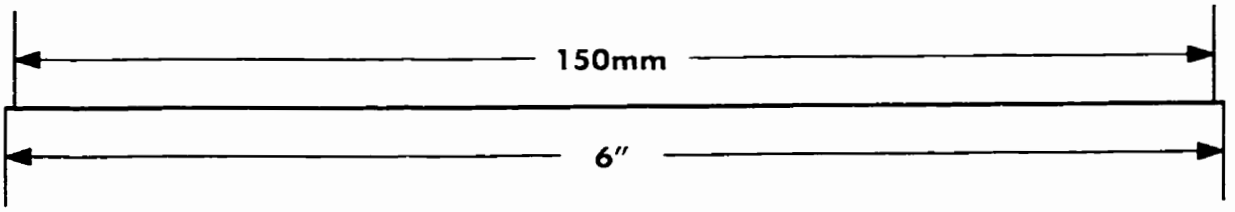

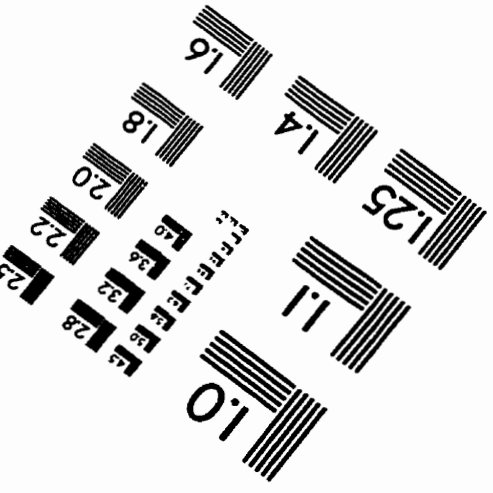

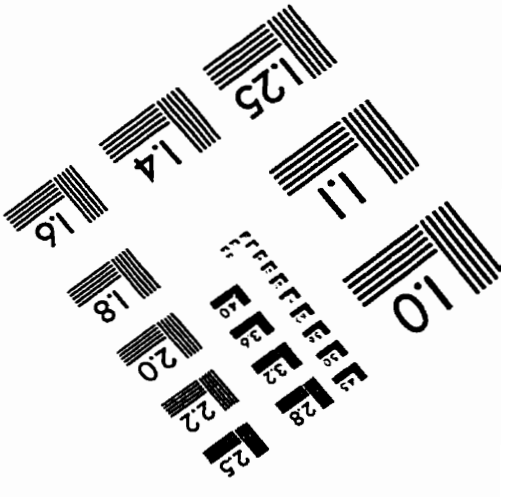

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