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Examination of the behavior and liver 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

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Abstract

Juvenile rainbow trout (*Oncorhynchus mykiss*) were exposed to high dietary concentrations of six polychlorinated *n*-alkane (PCAs) ($C_{10}H_{15.5}C_{6.5}$, $C_{10}H_{15.3}Cl_{6.7}$, $C_{11}H_{18.4}Cl_{5.6}$, $C_{12}H_{19.5}Cl_{6.5}$, $C_{14}H_{24.9}Cl_{5.1}$ and $C_{14}H_{23.3}Cl_{6.7}$) for 21 to assess their effects on behavior and liver and thyroid histology and for 85 days to assess histology for a longer term exposure. This is the first histological work using PCAs of known carbon chain length and chlorine content and the first effort to examine the histopathology 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 $C_{14}H_{24.9}Cl_{5.1}$, which had much lower exposure concentrations, many of the trout exposed to the PCAs (whole fish concentrations 0.22–5.5 μg g^{-1}) showed a diminished or no startle response, loss of equilibrium, and developed a dark coloration. These responses are indicative of a narcotic toxicological mode-of-action. Histopathological lesions were observed in the livers of trout from each exposure group. However, the most severe histopathologies were observed in the livers of fish exposed to $C_{10}H_{15.3}Cl_{6.7}$ and $C_{11}H_{18.4}Cl_{5.6}$ (whole fish concentrations 0.92 and 5.5 μg g^{-1}, respectively), in which extensive fibrous lesions were present that were not observed in any other exposure group. Other alterations observed in all treatment groups included hepatocyte necrosis, sites of inflammation, and glycogen/lipid depletion. The relative sizes of hepatocytes of PCA exposed trout were smaller than control trout, although only a few of the observed differences were statistically significant. No lesions were present in the thyroid, although trout exposed to $C_{10}H_{15.5}Cl_{6.5}$ (whole fish concentration 0.84 μg g^{-1}) had slightly more active thyroids, as indicated by an increased mean thyroid epithelium cell height relative to controls.

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It would appear that PCA toxicity is inversely related to carbon chain length, as has been observed in similar studies using mammals. The concentrations in the fish from this experiment were at levels that have been reported in invertebrates and fish from contaminated sites in the Great Lakes. However, the exposure concentrations were likely much greater in these experiments compared with the environment and require further study.

Keywords: Chlorinated paraffins; Toxicity; Fish; Narcosis

1. Introduction

Polychlorinated *n*-alkanes (PCAs) with carbon chains between 10 and 30 are used for a variety of industrial applications and are components of lubricants, flame retardants, adhesives, sealants and a number of other miscellaneous products (Willis et al., 1994; Tomy et al., 1998a). 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}) chain with varying amounts of chlorination ($\sim 35-70\%$ 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 (OCs) in production and use in North America, western Europe and Japan (Muir et al., 1999). Short chain (C_{10-13}) PCAs were prominent OCs in aquatic and terrestrial biota from Sweden (Jansson et al., 1993), sediments of temperate and arctic lakes (Tomy et al., 1999), fish from the Detroit River (Tomy et al., 1997) and beluga from the St. Lawrence estuary (Tomy et al., 1998b).

Based on present knowledge, the toxicity of PCAs to aquatic organisms appears to be low. However, a majority of available data were obtained from studies using commercial PCA products in aqueous solution with death as a toxicological endpoint (Linden et al., 1979; Madeley and Maddock, 1983a,b; Thompson and Madeley, 1983a; Tomy et al., 1998a). Aqueous exposures are problematic because PCAs are very hydrophobic, and in many cases, concentrations shown to produce effects (e.g. LC_{50}) were above the nominal exposure concentrations used (Thompson and Madeley, 1983a,b,c; Tomy et al., 1998a). Researchers have also observed cloudy water in some studies, using extremely high concentrations of PCAs in water. In these cases, observed deaths may have been due to a mechanical effect (coating of the organism) and not through a chemotoxic effect of the compounds (Madeley and Thompson, 1983a; Thompson and Madeley, 1983c). The use of commercial PCA formulations is also problematic because the individual compounds they contain will differ from those present in the environment due to differences in rates of degradation (Fisk et al., 1998a), biotransformation and bioaccumulation (Fisk et al., 2000). Therefore, the toxicological significance of PCAs in the environment is difficult to evaluate. Furthermore, the use of death as a toxicological endpoint is disadvantageous as it inherently excludes consideration of sub-lethal effects of a contaminant. A number of sub-lethal effects of PCAs, such as alterations of growth and reductions in feeding, have been reported (Hill and Maddock, 1983; Madeley and Thompson, 1983a). Current data are further limited because many studies have been short term (< 28 day) and chronic effects in aquatic organisms have not been adequately evaluated. 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 et al., 1994) and inhibition of gap junction intercellular communication (Kato and Kenne, 1996).

A review of PCA toxiological data indicated that the acute toxic mode-of-action of PCAs is narcosis (Tomy et al., 1998a). Nonetheless, gaps remain in current knowledge regarding the toxic mode-of-action of PCAs, toxicity data on PCAs of a single carbon chain length and chlorine content, and of data on sub-lethal effects in aquatic organisms. To address these data gaps, juvenile rainbow trout (*Oncorhynchus mykiss*) were ex-

posed to high dietary concentrations of six 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 and thyroid. The trout were exposed to high concentrations in an attempt to achieve lethal concentrations. Dietary exposures were chosen to circumvent physico-chemical problems associated with high water concentrations. Behavioral monitoring was chosen because it has shown considerable promise as a screening tool for the identification of the mode of action of various industrial chemicals (McKim et al., 1987). Evaluation of histopathological alterations is advantageous becauses they are integrated biochemical and physiological responses and may provide useful information on sub-lethal toxicity (Hinton and Lauren, 1990; Hinton et al., 1992). The liver and thyroid were chosen for histological evaluation because available data indicate they are target organs of PCA toxicity in mammals (Bucher et al., 1987; Serrone et al., 1987; Elcombe et al., 1994).

2. Materials and methods

2.1. Chemicals

Six PCAs were synthesized for this experiment; three by chlorination of three different alkene starting materials (1,5,9-decatriene; 1,10-undecadiene; and 1,13-tetradecadiene) (Sigma-Aldrich, Oakville, ON, Canada), and three by free radical chlorination of three ^{14}C-labeled alkanes (C_{10}, C_{12} and C_{14}) (Sigma, St Louis, MO, USA) (Table 1). Details of synthesis methods have been reported

Table 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-hexachlorodecane ($C_{10}H_{16}Cl_6$)	55
		x,1,2,5,6,9,10-hexachlorodecane ($C_{10}H_{15}Cl_7$)	41
		x,*y*,1,2,5,6,9,10-hexachlorodecane ($C_{10}H_{14}Cl_8$)	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-pentachloroundecane ($C_{11}H_{19}Cl_5$)	40
		x,*y*,1,2,10,11-hexachloroundecane ($C_{11}H_{18}Cl_6$)	49
		x,*y*,*z*,1,2,10,11-hexachloroundecane ($C_{11}H_{17}Cl_7$)	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-tetrachlortetradecane ($C_{14}H_{26}Cl_4$)	11
		x,1,2,13,14-pentachlorotetradecane ($C_{14}H_{25}Cl_5$)	74
		x,*y*,1,2,13,14-hexachlorotetradecane ($C_{14}H_{24}Cl_6$)	14
		x,*y*,*z*,1,2,13,14-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*, *y* or *z* are unknown.

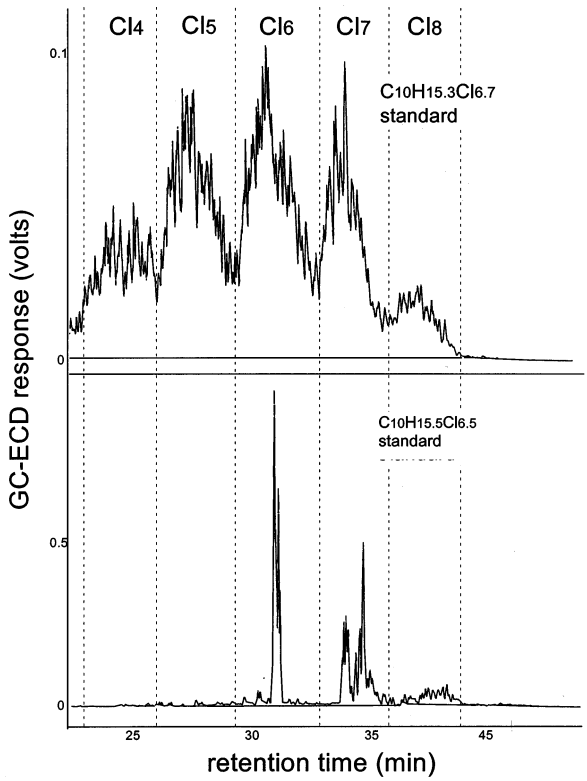

Fig. 1. GC-ECD chromatograms of the $C_{10}H_{15.3}Cl_{6.7}$ standard (top), a free-radically chlorinated decane product, and of the $C_{10}H_{15.5}Cl_{6.5}$ standard (bottom), a chlorinated alkene product.

previously (Fisk 1998b). Standards were found to be $>99\%$ PCAs based on analysis by gas chromatography (GC) with an electron capture detector (ECD) and GC-electron capture negative ion MS (see below for GC conditions). There were fewer individual PCA compounds produced from the alkene starting material than the alkane starting material (Table 1 and Fig. 1). In addition, 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 are reported in Table 1. The specific activities of $C_{10}H_{15.3}Cl_{6.7}$, $C_{12}H_{19.5}Cl_{6.5}$, and $C_{14}H_{23.3}Cl_{6.7}$ were 42.3, 94.2 and 88.3 DPM·ng^{-1}, respectively.

2.2. 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 h and stored at 10°C. The fish food consisted of 41% protein, 14% lipid and 3% fiber. Concentrations of PCAs in the food were determined by the same analytical techniques used to determine levels in rainbow trout tissues (see below), and are presented in Table 2. Control food was treated in an identical manner, but without the addition of a chlorinated alkane compound.

Juvenile rainbow trout (initial weights ~ 2 g) were exposed to each PCA at three concentrations (Table 2). There were also three control groups. The daily rate of feeding was equal to 1.5% of the mean weight of the rainbow trout. Each treatment consisted of 10 fish, contained in separate 10, 20, or 40 l glass aquarium supplied with flow-through UV and carbon dechlorinated City of Winnipeg tap water (11°C). The size of the aquarium varied due to limited availability of each size. After 21 days of exposure, all the trout from the two highest exposure concentrations, and two control groups, were euthanized and five trout were designated for histological examination and five for determination of PCA concentrations, for each treatment group. Three fish were sacrificed from the low exposure groups and the third control group, although they were not analyzed. The remaining fish (from the low exposure group) were exposed for an additional 61 days (85 days in total). On day 85, four fish were sacrificed for histological examination, although only three were processed and evaluated, and three for determination of PCA concentrations.

2.3. Extraction and analysis of PCAs

Extraction and analysis of radiolabeled PCAs were performed according to the methods used by Fisk et al. (1996). In brief: 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 con-

Table 2
Concentrations of PCAs in food and rainbow trout[a]

PCA	Exposure length (days)	n	Food concentration ($\mu g\ g^{-1}$)	Whole fish concentration ($\mu g\ g^{-1}$)	Liver concentration ($\mu g\ g^{-1}$)	GI tract concentration ($\mu g\ g^{-1}$)	Carcass concentration ($\mu g\ g^{-1}$)
$C_{10}H_{15.5}Cl_{6.5}$	85	3	0.87	0.10 ± 0.029	–	–	–
	21	5	12	0.84 ± 0.14	–	–	–
	21	5	62	0.92 ± 0.45	–	–	–
^{14}C–$C_{10}H_{15.3}Cl_{6.7}$	85	3	0.84 ± 0.65	0.099 ± 0.036	0.43 ± 0.11	0.44 ± 0.29	0.079 ± 0.035
	21	5	13 ± 0.21	0.92 ± 0.24	0.50 ± 0.24	1.6 ± 0.22	0.47 ± 0.089
	21	5	74 ± 23	3.0 ± 1.0	0.76 ± 0.20	9.6 ± 2.0	2.1 ± 0.92
$C_{11}H_{18.4}Cl_{5.6}$	85	3	3.7	0.10 ± 0.015	–	–	–
	21	5	53	5.5 ± 1.1	–	–	–
	21	5	290	4.0 ± 0.50	–	–	–
^{14}C–$C_{12}H_{19.5}Cl_{6.5}$	85	3	1.9 ± 0.042	0.14 ± 0.041	0.17 ± 0.069	0.42 ± 0.12	0.098 ± 0.029
	21	5	14 ± 0.11	0.79 ± 0.15	0.53 ± 0.23	2.5 ± 0.61	0.61 ± 0.099
	21	5	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	3	0.082	0.018 ± 0.0027	–	–	–
	21	5	0.78	0.11 ± 0.0018	–	–	–
	21	5	2.9	0.028 ± 0.0079	–	–	–
^{14}C–$C_{14}H_{23.3}Cl_{6.7}$	85	3	5.7 ± 0.061	0.57 ± 0.18	0.41 ± 0.060	1.8 ± 0.74	0.45 ± 0.12
	21	5	29 ± 0.51	1.3 ± 0.33	0.67 ± 0.10	3.6 ± 1.2	1.1 ± 0.21
	21	5	78 ± 1.0	0.22 ± 0.057	$0.33 \pm .098$	1.2 ± 0.19	0.15 ± 0.048

[a] Values represent mean ($\pm$ SE), wet weight of whole fish, liver, GI tract and carcass. Whole fish concentrations for rainbow trout exposed to the ^{14}C-PCAs are a summation of individual tissues. Concentrations were not determined in individual tissues (liver, GI tract and carcasses) of rainbow trout exposed to non-labeled PCAs.

tents), and carcass (whole fish minus liver and GI tract). Each tissue was weighed, frozen, freeze dried and analyzed separately for ^{14}C-radioactivity. To extract and analyze ^{14}C, samples were homogenized in toluene, centrifuged, a fraction of the supernatant was added to fluor (Atomlight, Dupont Chemical Company, Boston, MA, USA), and ^{14}C was counted 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 performed according to the methods used by Fisk et al. (1998c). In brief: carcass samples were freeze dried and homogenized in toluene, and octachloronaphthalene (OCN) was added as a recovery standard. The mean recovery of OCN through all fish analyzed was $79 \pm 3.8\%$, concentrations were not corrected for recovery. 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 OCs, 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 diluted to 10 ml prior to GC analysis.

PCA standards were used as external standards for analysis of food and fish tissues using a GC equipped with an ECD. The responses 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 $\times$ 0.25 mm DB-5 column and an ^{63}Ni-ECD. The carrier gas was H_2. PCAs were quantified using a single point calibration based on analyzed standards after every four samples. The limits of detection for each PCA standard were approximately 1 ng g^{-1}.

2.4. Behavioral monitoring

Behavioral monitoring was conducted daily throughout the 21 day exposures. The general behavior, skin coloration, and external appearance of fish were monitored, and their startle response behaviour was examined by tapping the aquarium. Fish were then fed and their feeding habits were observed until either the food had been consumed or 5 min had passed. If food had not been consumed within 5 min, the aquarium was checked periodically over the next 2 h to see if the food had been consumed (non-consumed food was then removed). Behavioral data were compared with those described by McKim et al. (1987).

2.5. Tissue processing and staining

Rainbow trout were euthanized and processed for histological analysis. The peritoneal wall was cut open to expose the internal organs and the jaws were cut at the corners to expose thyroid tissue. The whole fish was fixed for 48 h in Bouin's fixative, and liver and thyroid tissues containing thyroid follicles were excised, rinsed in four changes of 70% ethanol (ETOH), and stored in 70% ETOH until further processing.

Liver and thyroid tissue samples were processed in an automated tissue processor (IL MVP Tissue Processor) using an ethanol/butanol series, embedded in Tissue Prep II paraffin, and sectioned at 6 and 7 μm, respectively. Tissue sections were mounted on glass slides and stained with Harris' hematoxylin and eosin, following the method described by Edwards (1967). All chemicals were obtained from Fisher Scientific (Fisher Scientific, Edmonton, AB, Canada), with the exception of phosphotungstic acid which was obtained from Sigma Chemicals (Sigma Chemical, St. Louis, MO). Photomicrographs were taken on a Zeiss photomicroscope III, using Kodak TMAX 100 (ISO 100/21° DX) film.

2.6. Histological examinations and relative histomorphological measurements

Trout tissue from the medium (21 days of exposure) exposure, and the respective control fish, were examined both qualitatively and quantitatively for histological alterations. Liver and thyroid from three fish exposed to low PCA concentrations (85 days of exposure), and the respective control fish, were subject to histomorphological measurements, only.

Qualitative assessments of liver and thyroid were made by first defining alterations in an initial evaluation of the slides and subsequently confirmed by a second, thorough examination. Slides of liver from each fish were examined for the types of alterations identified in the primary assessment, which included fibrous lesions, sites of inflammation, hepatocyte necrosis, and depletion of glycogen/lipids. The severity of the alteration was ranked according to the categories: absent (−), minor (+), moderate (++), or severe (+++). The assignation of severity was made via comparison to the appearance of the control slides. As no histopathological alterations were observed in the thyroid of fish exposed to PCAs, no ranking was required.

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

2.6.1. Liver

The relative mean diameter of hepatocyte nuclei were determined for each fish by measuring the diameter of the first 50 spherical nuclei encountered while moving downward from the top of a tissue section. It should be noted that the reported mean diameter is slightly small because there were no correction factors applied for any nuclei not sectioned through the center or due to shrinkage during processing. The relative hepatocyte size was estimated by counting the number of hepatocytes (by counting nuclei) in two fields of standard area (18 000 μm^2). The relative hepatocyte size was calculated by dividing the total area (36 000 μm^2) by the number of hepatocytes in both fields and was not corrected for shrinkage. Nuclear areas were calculated from the formula ($\pi * r^2$). The nucleus: cytoplasm area ratio was determined by dividing the hepatocyte nuclear area by the relative hepatocyte area minus the nuclear area (i.e. cytoplasm area).

2.6.2. Thyroid

Thyroid epithelial cell heights were measured in a total of 15 follicles per fish. Measurements were made of four cells within each follicle, lying 90° from one another. Mean cell height was calculated by the direct method of Kališnik et al. (1977). No correction factor for shrinkage was used.

2.7. Statistical analysis

Treatment group means of the histological morphometrics were compared with the control fish using a one-way analysis of variance and a Dunnett pairwise comparison test. All statistics were performed with SYSTAT for Windows, Version 5 (SYSTAT, Evanston, IL).

3. Results

3.1. Fish weight and LSI

Mean weights and liver somatic indices of fish exposed to PCAs after 21 and 85 days were not significantly different from controls (Table 3).

3.2. PCA concentrations in food and fish

PCA concentrations in food were lowest in the $C_{14}H_{24.9}Cl_{5.1}$ treatments and highest in the $C_{11}H_{18.4}Cl_{5.6}$ and similar between the other PCA treatments (Table 2). Fish from a number of the high concentration exposures fed inconsistently (Table 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 (Fig. 2).

3.3. Fish behavior and general appearance

Observations on the behavior of the rainbow trout during the 21 exposures are provided in Table 4. A summary is provided below. The behavior of trout in the three control groups was similar and was considered 'normal' for comparisons to the behavior of trout exposed to PCAs. Trout from the lowest exposures of all PCAs exhibited similar behavior to the control fish. With the exception of trout exposed to $C_{14}H_{24.9}Cl_{5.1}$, which used lower exposure concentrations, abnormal behavior was observed in rainbow trout from all medium exposure concentrations. The most common modifications in behavior were delayed or absent startle response, reduced aggressive behavior when feeding, and failure to feed on certain days. Other changes included a loss of equilibrium and development of dark skin 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. In addition, trout from the highest exposure treatments did not exhibit 'normal' aggressive behavior when feeding and in many cases fish stopped feeding altogether.

3.4. Histopathology in liver

Hepatocytes are the most common cell in fish liver ($\sim 80\%$ of all liver cells) (Hinton and Lauren, 1990) and are the predominant cell in rainbow trout liver (Fig. 3). The hepatocytes are arranged as tubules of cells with their apices directed toward the central bile canaliculus and/or bile preductule (Hinton and Lauren, 1990). Bile, synthesized in the liver parenchyma, is secreted into the central bile canaliculus and ultimately flows into the gallbladder (Heath, 1995). Blood circulates through the sinusoids that separate the tubules of hepatocytes and the hepatocytes remove nutrients and xenobiotics.

A number of histopathologies were observed in the livers of rainbow trout fed diets contaminated with moderate concentrations of chlorinated paraffin congeners for 21 days (Table 5, Figs. 4–6) but the occurrence, extent, and types/stages of alterations differed between the various expo-

Table 3
Final fish weights (mean ± SE) and liver somatic indices (LSI) (mean ± SE) of rainbow trout exposed to PCAs for 21 and 85 days

PCA	*n*	Food concentration (ng g^{-1})	Exposure length (days)	Fish weight (g)	LSI (%)
Control	23	–	21	2.9 ± 0.40	1.3 ± 0.04
	7	–	85		
$C_{10}H_{15.5}Cl_{6.5}$	7	0.87	85	5.4 ± 1.6	1.1 ± 0.12
	10	12	21	2.5 ± 0.24	1.1 ± 0.04
	10	62	21	2.4 ± 0.26	1.0 ± 0.02
^{14}C–$C_{10}H_{15.3}Cl_{6.7}$	7	0.84	85	6.5 ± 1.6	1.0 ± 0.06
	10	13	21	2.4 ± 0.44	1.2 ± 0.08
	10	74	21	1.9 ± 0.17	1.0 ± 0.05
$C_{11}H_{18.4}Cl_{5.6}$	7	1.8	85	8.4 ± 1.2	0.94 ± 0.06
	10	2.6	21	2.8 ± 0.18	1.4 ± 0.09
	10	14	21	2.4 ± 0.37	1.0 ± 0.02
^{14}C–$C_{12}H_{19.5}Cl_{6.5}$	7	1.9	85	5.0 ± 1.4	1.0 ± 0.08
	10	14	21	2.8 ± 0.81	1.2 ± 0.09
	10	58	21	3.0 ± 0.49	1.0 ± 0.07
$C_{14}H_{24.9}Cl_{5.1}$	7	0.082	85	6.1 ± 2.0	1.0 ± 0.09
	10	0.78	21	2.8 ± 0.29	1.1 ± 0.05
	10	2.9	21	2.3 ± 0.14	1.1 ± 0.06
^{14}C–$C_{14}H_{23.3}Cl_{6.7}$	7	5.7	85	4.6 ± 1.5	1.2 ± 0.11
	10	29	21	2.4 ± 0.24	1.2 ± 0.11
	10	78	21	2.0 ± 0.63	1.0 ± 0.07

Table 4
General behavior of rainbow trout exposed to PCAs of varying concentrations over 21 days

Treatment	Concentration ($\mu g\ g^{-1}$)	General behavior	Tap response	Coloration	Feeding behavior	Feeding time
Control 1	–	Calm, active swimming	Quick, normal reaction	Normal	Fed aggressively	<1 min
Control 2	–	Calm, active swimming	Quick, normal reaction	Normal	Fed aggressively	<1 min
Control 3	–	Calm, active swimming	Quick, normal reaction	Normal	Fed aggressively	<1 min
$C_{10}H_{15.5}Cl_{6.5}$	0.87	Calm, active swimming	Quick, normal reaction	Normal	Fed aggressively	<1 min
	12	Calm, active swimming	Quick, normal reaction	Normal	Fed aggressively on most days	<5 min
		Beginning on day 21 a number of trout appeared to have dis-equilibrium	After day 16 some trout did not respond to tapping			
	62	Generally calm, active swimming but some trout had low activity	Quick, normal reaction on most days	Four trout developed dark lower jaws and three trout developed dark backs on day 18	Trout did not feed aggressively beginning on day 2 and by day 5 few fish were feeding	>5 min beginning on day 4
		Some aggressive behavior	Some slowed response behavior beginning day 5			Not all food was consumed
^{14}C–$C_{10}H_{15.3}Cl_{6.7}$	0.84	Calm, active swimming	Quick, normal reaction	Normal	Fed aggressively	<1 min
	13	Calm, active swimming	Quick, normal reaction on most days	Normal	Trout began spitting out their food on day 4	>5 min after day 4
			Some slowed response behavior beginning on day 5		Not all trout fed at every feeding	Food was not always consumed
	74	Calm, active swimming	Weak response to tapping began on day 2	Three 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
		A number of trout had rapid ventilation rates				
$C_{11}H_{18.4}Cl_{5.6}$	1.8	Calm, active swimming	Quick, normal reaction	Normal	Fed aggressively	<1 min
	2.6	On day 11 one trout could not swim off bottom of aquarium	Beginning on day 11 some trout did not response to tapping	One trout developed dark coloration on day 5	Most trout fed slowly beginning on day 6	>5 min
		Beginning on day 19 all trout exhibited dis-equilibrium			Trout had trouble feeding on day 21 due to disequilibrium	

Table 4 (*Continued*)

Treatment	Concentration ($\mu g\ g^{-1}$)	General behavior	Tap response	Coloration	Feeding behavior	Feeding time
	14	Calm, active swimming	Beginning on day 14 some trout did not respond to tapping	One trout developed dark coloration on day 5	Trout did not feed aggressively and some trout spit food out beginning on day 2	>5 min beginning on day 8
		Day 21 one trout began to poke head out of water Day 21 two trout were bumping into walls				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	Two trout developed dark spots on back on day 11	Fed aggressively	<1 min
	14	Calm, active swimming	Beginning on day 14 some trout did not respond to tapping	One trout developed black spots on day 4	Beginning on day 3 some trout did not feed	>5 min beginning on day 11
		Beginning on day 19 some trout exhibited dis-equilibrium		Three had orange tint on lateral line		Not all food was consumed
	58	Calm, active swimming	Beginning on day 5 some trout did not respond to tapping	Four trout developed dark spots on day 4	Beginning on day 4 trout fed slowly	>5 min beginning on day 10
					By day 11 some trout were not feeding	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	Fed aggressively	<1 min
	28.6	Calm, active swimming	Quick normal reaction	Normal	Fed aggressively	<1 min
	78.0	Calm, active swimming	Quick normal reaction	One trout developed dark belly on day 15	Trout spit out food beginning on day 3	>30 min beginning on day 3
					Most were not feeding by day 12	Not all food was consumed
^{14}C–$C_{14}H_{23.3}Cl_{6.7}$	5.7	Calm, active swimming	Quick, normal reaction	Normal	Fed aggressively	<1 min
	29	Calm, active swimming	Beginning on day 5 some trout did not respond to tapping	Three 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
		On day 19 three trout had spinal curvature				All food was consumed
	78	Beginning on day 7, all but one trout remain at bottom of aquarium with little activity	Beginning on day 4 all trout showed a weak response to tapping	Three trout developed dark coloration on belly on day 15	All but one trout stopped feeding on day 3	>5 min
				All but one trout were dark by day 15		Not all food was consumed beginning on day 3

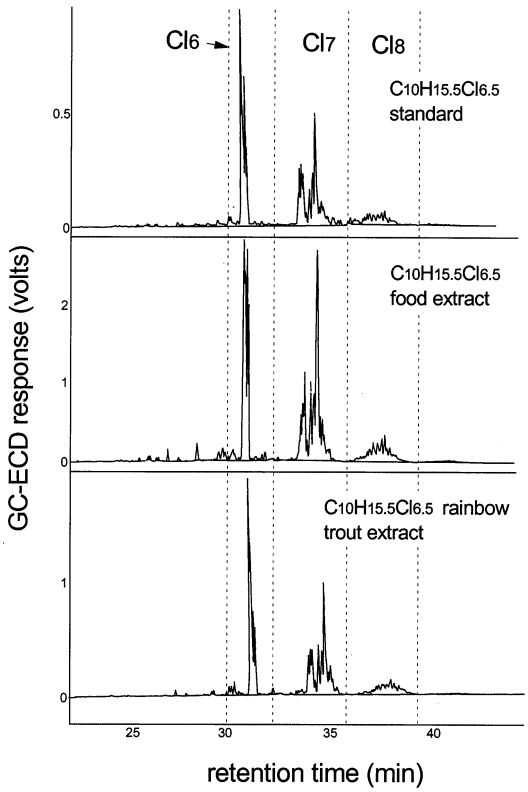

Fig. 2. GC-ECD chromatograms of the $C_{10}H_{15.5}Cl_{6.5}$ standard (top), extracts of food spiked with $C_{10}H_{15.5}Cl_{6.5}$ (middle), and extracts of rainbow trout exposed to the $C_{10}H_{15.5}Cl_{6.5}$ spiked food for 85 days (bottom).

sure groups (Table 5). It should be noted that no gonadal development was observed in any fish and the sex of the fish is not likely to influence these results. The most severe and advanced lesions occurred in fish from the ^{14}C–$C_{10}H_{15.3}Cl_{6.7}$ exposure group, and to a slightly lesser extent, the $C_{11}H_{18.4}Cl_{5.6}$ group. In all treatment groups, fish had hepatocytes with fewer vacuoles, perhaps due to reduced glycogen and lipid stores.

Histopathologies included sites of inflammation (Fig. 4), composed largely of lymphocytes, hepatocyte necrosis (Fig. 5), and fibrosis (Fig. 6). Fibrous lesions, which occurred in all fish from the ^{14}C–$C_{10}H_{15.3}Cl_{6.7}$ group and four of five fish from the $C_{11}H_{18.4}Cl_{5.6}$ group, were composed of fibrous tissue, necrotic hepatocytes, inflammatory cells, and cell debris. Lesions were notably associated with bile ductules and blood vessels but were not limited to these sites; in severe instances the affected areas surrounding adjacent bile ductules were interconnected by bands of fibrous tissue. Necrotic hepatocytes were seen within these lesions, along the periphery of the fibrotic zone, and as 'spotty necrosis' (i.e. scattered throughout).

Necrotic hepatocytes were also observed in fish from the remaining treatment groups, although these lesions were not associated with fibrosis. Typically, necrotic hepatocytes were most frequent or limited to areas surrounding bile ductules, blood vessels, most notably veins, and liver nearest to the gall bladder. However, spotty necrosis was also present in numerous fish. Inflammation was occasionally associated with necrotic lesions but also occurred in areas of normal liver parenchyma.

3.5. Histopathology in thyroid

The structure of the teleost thyroid, an endocrine organ, although similar to that of higher vertebrates, differs in its lack of a discrete organization (Gorbman, 1969). The thyroid tissue of rainbow trout consists of glandular follicles scattered around the ventral aorta and branchial arteries that supply the gills. The follicle consists of an outer layer of thyroid epithelium that surrounds an inner lumen filled with colloid (Fig. 7). Colloid contains a reserve of the protein-bound

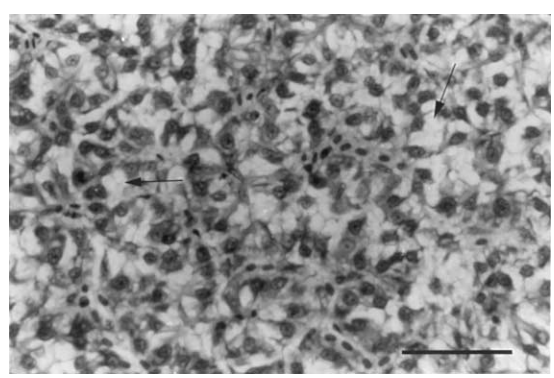

Fig. 3. Photomicrograph of a liver section from a control juvenile rainbow trout. Areas occupied by glycogen/lipid deposits in hepatocyte cytoplasm (vacuoles) are indicated by arrows. Bar = 50 μm. H&E stain.

Table 5
Occurrence and severity of histological alterations in livers of rainbow trout fed PCAs for 21 days[a]

Treatment group	Fish #	Fibrous lesions	Sites of inflammation	Hepatocyte necrosis	Depletion of glycogen/lipids
Controls	6	–	–	–	–
	7	–	–	–	–
	8	–	–	–	–
	9	–	–	–	–
	10	–	–	–	–
$C_{10}H_{15.5}Cl_{6.5}$	6	–	+	+	+++
	7	–	–	++	++
	8	–	–	+	++
	9	–	–	+	++
	10	–	+	+	+++
^{14}C–$C_{10}H_{15.3}Cl_{6.7}$	6	+++	+	+++	+++
	7	++	++	+++	++
	8	+++	+	+++	++
	9	+++	+++	+++	++
	10	+++	+	+++	++
$C_{11}H_{18.4}Cl_{5.6}$	6	–	+	–	–
	7	+++	++	+++	–
	8	++	–	++	++
	9	+	–	+	++
	10	++ to +++	–	+	+
^{14}C–$C_{12}H_{19.5}Cl_{6.5}$	6	–	+	++	++
	7	–	++	+	++
	8	–	+ to ++	–	+++
	9	–	–	–	++
	10	–	–	–	++
$C_{14}H_{24.9}Cl_{5.1}$	6	–	–	+	+++
	7	–	–	+	++
	8	–	+	+	++
	9	–	–	–	+++
	10	–	+	–	++
^{14}C–$C_{14}H_{23.3}Cl_{6.7}$	6	–	–	+	++
	7	–	+	+	++
	8	–	–	–	++
	9	–	+++	++	+++
	10	–	–	–	+++

[a] –: no alterations observed; +: minor; ++: moderate; +++: severe.

form of 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 follicles of all rainbow trout in this study were characterized by a reduction or complete absence of colloid, vacuoles that were present in the colloid, and epithelium cells that were generally cuboidal or columnar (Fig. 7). These observations indicate thyroid glands of

rainbow trout from all exposure groups were active. No lesions or anomalies were observed in the thyroids of fish exposed to PCAs at the medium concentration treatment.

3.6. Relative histological morphometrics in liver and thyroid

Histological evaluations were only performed on the rainbow trout exposed to the low and medium concentrations of PCAs because these trout fed more consistently than fish exposed to higher concentrations, and because a number of the PCAs from these exposures were accumulated in higher concentrations in fish tissues (Table 2). Note that correction factors for shrinkage were not applied to any histological morphometrics and that all values should be considered relative. Relative histological morphometrics for liver and thyroid of trout exposed for 21 and 85 days are summarized in Table 6.

Following 21 days of exposure, hepatocyte nuclear diameters of trout exposed to PCAs were not significantly different from controls ($P > 0.05$). The mean hepatocyte volume index of trout from the $C_{10}H_{15.5}Cl_{6.5}$, ^{14}C–$C_{12}H_{19.5}Cl_{6.5}$, and $C_{14}H_{24.9}Cl_{5.1}$ groups were significantly smaller than controls ($P < 0.05$). The relative hepatocyte size of trout exposed to ^{14}C–$C_{10}H_{15.3}Cl_{6.7}$ was marginally different from controls ($P < 0.1$). The mean of the index nucleus:cytoplasm area ratio of hepatocytes was significantly greater in trout fed $C_{10}H_{15.5}Cl_{6.5}$ than the control trout ($P < 0.05$), but did not vary between the other PCA treatment trout and control trout ($P > 0.05$). Thyroid epithelium cell heights were significantly higher in trout fed $C_{10}H_{15.5}Cl_{6.5}$ for 21 days than controls ($P > 0.05$). No other differences were observed between PCA treated and control fish at this time.

No significant differences were found between the unexposed trout and trout exposed to PCAs for any of the morphometric measurements at a significance level of 0.05. Marginally significant ($P < 0.1$) differences were observed for hepatocyte nuclear diameters and hepatocyte volume index between trout exposed to ^{14}C–$C_{10}H_{15.3}Cl_{6.7}$ and control trout. The smaller mean relative hepato-

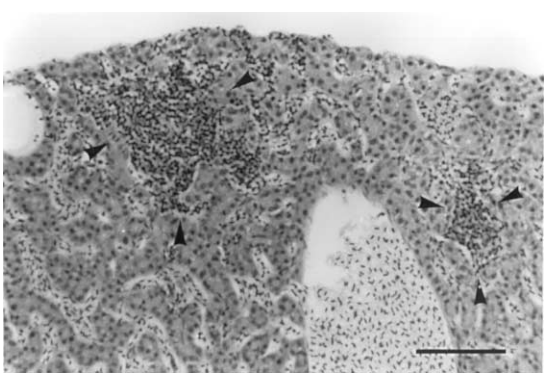

Fig. 4. Liver of a juvenile rainbow trout administered the medium concentration (13 ng g^{-1}) of ^{14}C–$C_{14}H_{23.3}Cl_{6.7}$ in the diet for 21 days, exhibiting glycogen/lipid depletion (absence of clear vacuoles) in hepatocytes, and sites of inflammation (between the arrowheads). Bar = 100 μm. H&E stain.

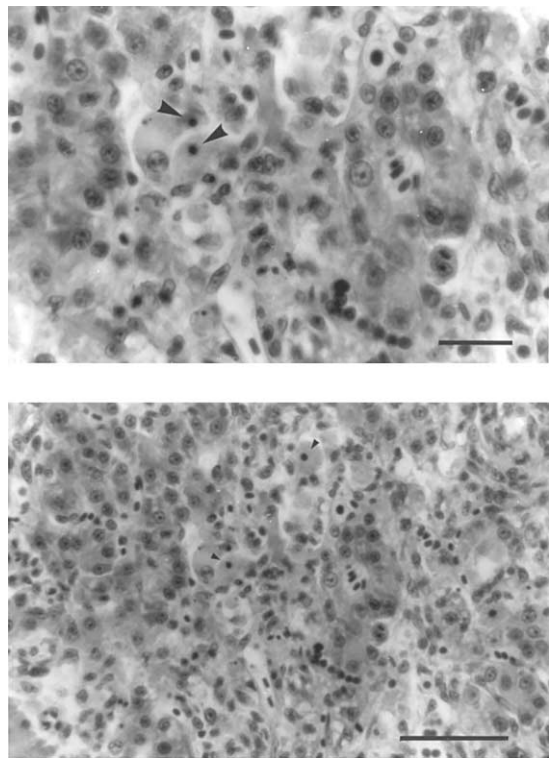

Fig. 5. Hepatocyte necrosis in the liver of a juvenile rainbow trout administered the medium concentration (13 ng g^{-1}) of ^{14}C–$C_{10}H_{15.3}Cl_{6.7}$ in the diet for 21 days. Necrotic hepatocytes contain pyknotic nuclei (arrowheads). Bar = 20 μm (for the high magnification) or 100 μm (for the low magnification). H&E stain.

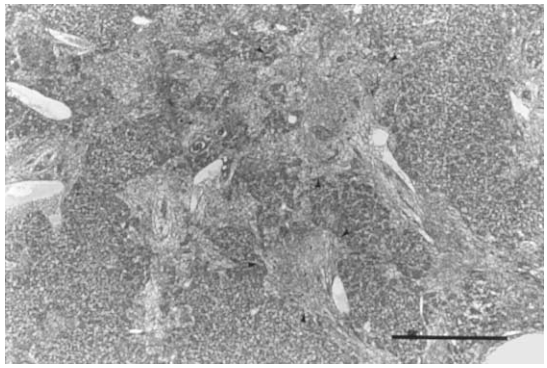

Fig. 6. Low magnification micrograph of the liver of a juvenile rainbow trout administered the medium concentration (2.6 ng g^{-1}) of $C_{11}H_{18.4}Cl_{5.6}$ in the diet for 21 days, exhibiting extensive fibrous lesions throughout the parenchyma (between the arrowheads). Bar = 500 μm. H&E stain.

cyte size of trout exposed to ^{14}C–$C_{14}H_{23.3}Cl_{6.7}$ was marginally significant, relative to control trout ($P < 0.1$). No differences were observed between the heights of thyroid epithelium of trout exposed to PCAs and the controls ($P < 0.1$).

4. Discussion

This study represents one of the first attempts at examining the toxic mode-of-action and sublethal effects of PCAs in fish. In general, the acute toxicity of PCAs to rainbow trout is low. However, under moderate exposures, significant histopathological responses were observed in the liver, but not thyroid, of trout.

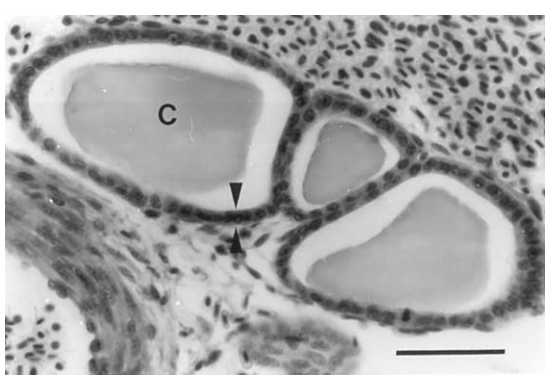

Fig. 7. Thyroid follicles of an unexposed juvenile rainbow trout (d 21). Colloid (C) is surrounded by thyroid epithelium (between the arrowheads). Bar = 50 μm. H&E stain.

The acute mode-of-action of PCA toxicity in aquatic organisms appears to be narcosis. The diminished or lack of a startle response, the loss of equilibrium, and the darkening of skin coloration observed in trout exposed to PCAs, are characteristic responses indicative of a narcotic mode-of-action (McKim et al., 1987). However, according to the literature, tissue concentrations of PCAs observed in trout from this study are not high enough to cause narcosis (McCarty, 1986). Because we exposed trout to PCAs via the diet, unhealthy or stressed trout could avoid or reduce exposure by not feeding. It appears likely that, unless force-fed by methods such as a gavage, or by using extremely high dietary concentrations of PCAs, narcosis could not be achieved for this substance in fish exposed via the diet.

The results from a number of studies support the conclusion that the acute mode-of-action of PCA toxicity is narcosis. Narcotic-like behavior was 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 and Ofstad 1982), and in rainbow trout exposed to high waterborne concentrations of a commercial PCA (C_{10-13}) (Swigert and Bowman, 1986a,b). Using the same PCA standards that were used in this study, Fisk et al. (1999) concluded from Japanese medaka (*Oryzias latipes*) embryo toxicity assays that acute mode-of-action of PCA toxicity was narcosis. Further anecdotal evidence for a narcotic mode of action are the high concentrations used in numerous toxicity tests which have not elicited a toxic response in invertebrates (Madeley and Thompson, 1983a,b,c,d), fish (Linden et al., 1979; Madeley and Maddock, 1983a,b,c,d,e) birds (Linden et al., 1979; Madeley and Birtley, 1980 SDS Biotech, 1984; as reported in Willis et al., 1994) and mammals (Howard et al., 1975; Birtley et al., 1980; Serrone et al., 1987). It is likely that if PCAs had any specific toxic mode-of-actions they would have been observed in experiments which used high exposure concentrations.

Table 6
Relative histological morphometrics (mean ± standard error) of liver and thyroid tissue from rainbow trout exposed to PCAs for 21 ($n = 5$) and 82 ($n = 3$) days[a]

PCA	Liver			
	Hepatocyte nuclear diameter[b] (μm)	Hepatocyte volume index (μm^2)	Nucleus: cytoplasm area ratio	Thyroid epithelium cell height[c] (μm)
Short exposure				
Control	5.7 ± 0.13	151 ± 6.40	0.20 ± 0.02	4.0 ± 0.30
$C_{10}H_{15.5}Cl_{6.5}$	5.6 ± 0.05	129 ± 9.80**	0.25 ± 0.02**	4.3 ± 0.24
^{14}C–$C_{10}H_{15.3}Cl_{6.7}$	5.9 ± 0.28	124 ± 9.67*	0.30 ± 0.05	3.2 ± 0.28
$C_{11}H_{18.4}Cl_{5.6}$	5.7 ± 0.10	145 ± 6.49	0.22 ± 0.02	3.7 ± 0.29
^{14}C–$C_{12}H_{19.5}Cl_{6.5}$	5.4 ± 0.07	125 ± 4.97**	0.22 ± 0.01	3.9 ± 0.04
$C_{14}H_{24.9}Cl_{5.1}$	5.3 ± 0.11	124 ± 5.56**	0.22 ± 0.01	3.5 ± 0.15
^{14}C–$C_{14}H_{23.3}Cl_{6.7}$	5.7 ± 0.08	133 ± 6.73	0.23 ± 0.01	3.7 ± 0.17
Long exposure				
Control	5.8 ± 0.047	183 ± 10.1	0.17 ± 0.011	3.7 ± 0.63
$C_{10}H_{15.5}Cl_{6.5}$	5.4 ± 0.17	155 ± 7.39	0.17 ± 0.004	3.6 ± 0.24
^{14}C–$C_{10}H_{15.3}Cl_{6.7}$	5.3 ± 0.22*	146 ± 8.05*	0.18 ± 0.006	3.8 ± 0.14
$C_{11}H_{18.4}Cl_{5.6}$	5.4 ± 0.08	160 ± 10.7	0.17 ± 0.013	3.6 ± 0.24
^{14}C–$C_{12}H_{19.5}Cl_{6.5}$	5.5 ± 0.14	154 ± 8.41	0.18 ± 0.021	3.9 ± 0.32
$C_{14}H_{24.9}Cl_{5.1}$	5.6 ± 0.15	151 ± 13.8	0.20 ± 0.011	4.0 ± 0.26
^{14}C–$C_{14}H_{23.3}Cl_{6.7}$	5.6 ± 0.05	147 ± 0.693*	0.20 ± 0.003	4.0 ± 0.19

[a] Exposure concentrations for each PCA in the short and long exposures are the medium and low concentrations, respectively, reported in Table 1. Treatment means which significantly differ from the control mean are indicated by an asterisk (ANOVA, Dunnett pairwise comparison, ** $P < 0.05$, * $P < 0.1$).

[b] No correction factors were applied to the nuclear diameter. Therefore, mean diameters are slightly small because not all nuclei were sectioned through the center and shrinkage during processing would have occurred.

[c] No correction for shrinkage was applied to the thyroid epithelium cell height.

Histopathological lesions were present in livers of trout from all treatment groups, except controls, sampled after 21 days of exposure. The most extensive (i.e. area of liver affected), advanced (i.e. stage), and most serious type of lesion were observed in fish exposed to $C_{10}H_{15.3}Cl_{6.7}$ and $C_{11}H_{18.4}Cl_{5.6}$. Because the exposure and tissue concentrations were fairly similar between the PCA treatments, the liver histopathologies observed in these two treatment groups suggest that these compounds are more toxic than the other PCAs. This is consistent with past work on PCAs, where toxicity has been considered to be inversely related to carbon chain length and potentially chlorine content (Bucher et al., 1987; Serrone et al., 1987). That livers of trout from the $C_{10}H_{15.5}Cl_{6.5}$ group, the shortest carbon chain length, were less affected is likely due to the lower bioaccumulation of PCAs in these fish, relative to the aforementioned treatments, as well as to poor feeding.

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 years) and the exposure concentrations were higher (5–5000 ug g^{-1}) than in this work, making comparisons difficult. However, hepatocyte necrosis has been observed in rats exposed to high concentrations of commercial PCA 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 g^{-1} per day. Single-cell necrosis was observed in the hepatic lobes of male and female rats exposed for 13 weeks to 5000 ug g^{-1} per day of a C_{14-17}-PCA (52% Cl) (Poon et al., 1995). Hepatocellular hyperplasia, evidence of previous necrosis, was observed in rats exposed to a C_{10-13}-PCA (58% Cl) at concentrations of 100–625 ug g^{-1} per day for 90 days (Serrone et al., 1987).

These observed morphometric changes conflict with mammalian data. Bucher et al. (1987) observed significantly fewer hepatocytes per given microscope field, in rats exposed to a C_{10-13}-PCA (60% Cl) for 2 years at concentrations of 125–625 ug g^{-1} per day. 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). However, liver weights may also increase due to hyperplasia of hepatocytes, which is typically characterized by an increased number but decreased size of cells. The smaller hepatocytes observed in the rainbow trout in this study do not appear to be due to decreased 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. Conversely, depletion of glycogen and/or lipid reserves was observed in the livers of almost every fish exposed to PCAs that were examined histologically. This depletion may be either a manifestation of reduced feeding and/or stress imparted by PCA exposure, as has been suggested for rainbow trout exposed to other contaminants (e.g. Cd; Lowe-Jinde and Niimi, 1984). Diameters of hepatocyte nuclei measured in the present study, which were not affected by PCA treatment, fell within the range (5.06–6.66 μm) reported for four populations of hatchery-reared rainbow trout (Simon et al., 1967).

No obvious lesions or morphometric changes were observed in the thyroid of PCA exposed trout. Relative histomorphometric measurements indicated that fish exposed to medium concentrations of $C_{10}H_{15.5}Cl_{6.5}$ for 21 days had thyroids that were more active than control fish. Similar 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 50–5000 ug·g^{-1} per day 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 g^{-1} per day (Serrone et al., 1987). Histopathological effects in thyroid may have been observed in trout if PCA exposures were longer and/or at greater concentrations.

Three of the PCAs used in this experiment, $C_{10}H_{15.5}C_{6.5}$, $C_{11}H_{18.4}Cl_{5.6}$ and $C_{14}H_{24.9}Cl_{5.1}$, had chlorine substituted at two terminal carbons on both ends of the carbon chain. The remaining 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 produce 1,2 Cl substitutions. This facilitated an examination of the effects of chlorine position on the toxicity of PCAs. It does not appear that the toxicity of terminal chlorinated PCAs was different than that of PCAs which are not chlorinated on the terminal carbons. However, further research is needed to validate this observation.

The results of this study indicate that histopathological effects would occur at high exposure concentrations or at high body burdens. These concentrations are in the range of those reported in zebra mussels and yellow perch from the Detroit River (Tomy et al., 1997) and carp from Hamilton Harbour, Ontario (Muir et al., 1999), suggesting that short chain PCAs may be causing adverse effects in some wild populations of fish. However, only a small subset of all the PCAs were tested in the present study and information is needed on a wider range of PCAs and whether the effects of the PCAs are additive. As well, the relationship between exposure level and tissue concentrations needs to be explored. The concen-

trations observed in wildlife fish were likely accumulated over a much longer time period at a lower exposure concentration than used in this experiment. These results suggest that additional work on PCA toxicity in fish is warranted. Longer exposures are needed to assess the chronic effects of PCAs, using a range of test species. As only two tissues were examined here, the possibility that PCAs produce histopathologies in other tissues should be examined. Lastly, PCAs may disrupt other physiological and/or biochemical processes in aquatic organisms which do not cause a visible/discernible histological change.

5. 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 content, 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, which used lower exposure concentrations, were consistent with a narcotic mode-of-action. Overt histopathological lesions were observed in the liver of rainbow trout exposed to medium concentrations of all PCA's but were most notable in fish exposed to $C_{10}H_{15.3}Cl_{6.7}$ and $C_{11}H_{18.4}Cl_{5.6}$ for 21 days. Alterations of histological morphometrics were also observed in the livers of rainbow trout exposed to all PCAs for 21 days. Few changes were observed in the livers of rainbow trout exposed to the same PCAs at lower concentrations for 85 days. Our results indicate that the sub-lethal toxicity of PCAs is inversely related to carbon chain length and that the acute toxicity of PCAs to fish is very low. PCAs may cause histological changes in liver of rainbow trout at high dietary and tissue concentrations, but at concentrations which have been observed in wild fish of the Great Lakes. Further research is required to verify these results and to examine the effects of long term exposure to PCAs.

Acknowledgements

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