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Dietary accumulation and biochemical responses of juvenile rainbow trout (*Oncorhynchus mykiss*) to 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126)

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Abstract

Juvenile rainbow trout (*Oncorhynchus mykiss*) (initial weights 2–5 g) were exposed to three dietary concentrations (0, 12.4 and 126 ng g^{-1}, wet weight) of a ^{14}C-labelled 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) for 30 days followed by 160 days of clean food. We assessed bioaccumulation, histology (liver and thyroid) and biochemical responses (liver ethoxyresorufin-*O*-deethylase (EROD), liver vitamins (retinoids and tocopherol) and muscle thyroid hormone levels) along with growth and survival. The half-life of PCB 126 in the rainbow trout ranged from 82 to 180 days while biomagnification factors (BMF) ranged from 2.5 to 4.1 providing further evidence that PCB 126 is among the most bioaccumulative PCB congeners. Toluene extractable ^{14}C declined with time in the trout suggesting the possibility of some biotransformation and/or covalent bonding with biological macromolecules. The threshold for liver EROD induction by PCB 126 was approximately 0.1 ng g^{-1} (wet weight). EROD activities in the low- and high treatments were 9 and 44 times greater than control, respectively, and remained elevated throughout the experiment. EROD activity was correlated with whole body concentrations of PCB 126 although there was evidence of EROD activity suppression in the highly exposed fish. Liver didehydroretinoids and tocopherol concentrations were depressed by the high PCB 126 dose after 30 days exposure. Initially, muscle concentrations of thyroxine (T_4) and triiodo-L-thyronine (T_3) declined as the fish grew during the experiment, and exposure to PCB 126 accelerated the growth related decline. More information is needed to assess the functional significance of the reduced muscular stores of thyroid hormones. Despite the changes in liver EROD, liver vitamins and muscle thyroid hormones, liver and thyroid histology in trout examined after 30 days exposure and growth parameters were unaffected by PCB 126. This indicates that the functional competences of the physiological factors associated with growth were maintained under the experimental conditions. Crown Copyright © 2002 Published by Elsevier Science B.V.

Keywords: Endocrine disruption; Non-*ortho* PCBs; Mixed function oxidase; Thyroid hormones; Vitamin A

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1. Introduction

Non-*ortho* substituted PCB congeners are a sub-group of the PCB congeners that are the most potent inducers of the mixed function oxygenase (MFO) cytochrome P450 CYP 1A enzymes and are considered to be among the most toxic congeners. The toxicity and MFO induction ability of non-*ortho* PCB congeners has been related to their planar configuration and structural similarity to dioxin and furan compounds (2,3,7,8-TCDD/F) (Safe, 1990). Induction of MFO has been associated with a number of toxic responses including thymic involution, teratogenicity, altered vitamin A metabolism and changes in thyroid function (Goldstein and Safe, 1989). Of the three non-*ortho* PCBs (PCBs 77, 126 and 169), PCB 126 is commonly found at the greatest concentration in the environment (Huestis et al., 1996; Smith et al., 1990) and accounts for a large percentage of toxic equivalency factors (TEFs) (Janz and Metcalfe, 1991; Huestis et al., 1997; Atuma et al., 1998). Also, planar PCBs have been found to disrupt thyroid function (Brouwer et al., 1998) and alter hepatic vitamins in both fish (Palace and Brown, 1994) and mammals (Zile, 1992).

Numerous physiological processes depend upon adequate levels of retinoids (vitamin A), tocopherol (vitamin E) and thyroid hormones. The most commonly studied process associated with vitamin A is vision. However, other significant physiological functions are also served by vitamin A including roles in growth and differentiation of epithelial cells, general growth, reproduction, immuno-competence, hepatic pathology and bone metabolism (Halver, 1982; Taveekijakarn et al., 1994). Tocopherol plays an important role as an antioxidant in protecting cellular and subcellular membranes (Burton and Trabler, 1990) and deficiencies cause growth suppression and liver histopathology in salmon (Taveekijakarn et al., 1996). Thyroid hormones together with other hormone systems and vitamins promote growth, development and early reproductive events, and are generally associated with protein anabolism in fish (Eales and Brown, 1993).

Despite the toxic potential of PCB 126 and its relatively high concentrations in the environment, there have been few studies linking bioaccumulation and biochemical effects in fish of this important environmental contaminant. To address this gap in knowledge, we exposed juvenile rainbow trout to three dietary concentrations of PCB 126 (0, 12.4 and 126 ng g^{-1}, wet weight) to assess bioaccumulation parameters; liver CYP1A1 induction; vitamin (A and E) status, thyroidal indices and liver histology. CYP 1A induction was assessed by measuring ethoxyresorufin-*O*-deethylase (EROD) activity. To monitor for disturbances in vitamin metabolism (Palace et al., 1997), we measured hepatic stores of retinoids and tocopherol. Muscle thyroxine (T_4) and 3,3′,5-triiodo-L-thyronine (T_3) levels were measured to determine their potential utility as indices of thyroid status during PCB 126 exposure because it can alter thyroid gland morphology and plasma T_4 dynamics (Brown et al., 1997). The protocols for this experiment were similar to past bioaccumulation work on dioxin, furan and PCB congeners carried out in our labs (Fisk et al., 1998; Muir et al., 1990, 1992) allowing for direct comparisons of bioaccumulation parameters and EROD induction.

2. Methods and materials

2.1. Chemical

[^{14}C]-3,3′,4,4′,5-pentachlorobiphenyl (PCB 126, specific activity of 8.7×10^{11} Bq mol^{-1}) was purchased from Sigma Chemical Co. The PCB 126 standard was found to be $>99.5\%$ radiochemically pure by reverse-phase high performance liquid chromatography (HPLC) and GC-ECD analysis.

2.2. Experimental protocol

Juvenile rainbow trout (initial weight 2–5 g) were simultaneously exposed to PCB 126 at three dietary concentrations (0, 12.4 and 126 ng g^{-1}). Fish were fed the spiked food (0.015 g g^{-1} body weight per day) for 30 days followed by 160 days

of non-spiked food. On days 0, 5, 10, 20 and 30 of the uptake phase and days 5, 10, 20, 40, 80 and 160 of the depuration phase three fish from each treatment (control, low and high) were sacrificed by cervical section. Tissues were collected and separated into liver, GI tract (stomach, intestines, spleen and associated fat) and remaining carcass for ^{14}C analysis. On days 0, 10, 30 of uptake and days 5, 20, 40 and 160 of depuration phase three fish were sampled from each treatment for liver EROD analysis and five fish were collected for determination of liver vitamins and muscle T_3 and T_4. On day 30 of uptake, five fish from each treatment were collected for histological analysis.

2.3. ^{14}C analysis

Fish samples were freeze dried 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 extract to fluor (Atomlight, Dupont, Boston, MA, USA), and counting by liquid scintillation counter (LSC). Counts were corrected for quench using a quench curve prepared from ^{14}C-toluene (Dupont), and were automatically corrected for background by the LSC. Lipids were determined gravimetrically after evaporating 1 ml of the toluene supernatant.

Non-toluene extractable ^{14}C was determined in carcass samples from the high treatments on day 40 of uptake and after 40 days of depuration. 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 then oxidized on a Packard Model 306 Oxidizer (Packard Instruments, Downers Grove, IL, USA) to determine non-toluene extractable ^{14}C.

2.4. Histological examination of liver and thyroid

Following cervical section, rainbow trout were opened to expose the internal organs and the jaws were cut at the corners to expose thyroid tissue. Fish were fixed in Bouin's fixative for 48 h and stored in 70% ethanol. Liver and thyroid tissue was then excised from the trout, and then rinsed in four changes of 70% ethanol and stored until processing. Tissues were dehydrated using an ethanol/butanol series and embedded in paraffin. Tissues were then sectioned at 6 μm for liver and 7 μm for thyroid. Liver and thyroid were stained for light microscopy and basic histopathological analyses with hematoxylin and eosin. The protocol employed followed the methods described in Brown et al. (1998). To perform morphological 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).

To measure liver parenchymal nuclear size, 50 round nuclear diameters for each fish were measured from two or three different sites. Cell size (area) was measured from a count of cell nuclei in a standard area using four tissue sites. Areas were chosen that had few structures other than hepatocytes. The nucleus:cytoplasm area ratio was determined by dividing the hepatocyte nuclear area (πr^2) by the cytoplasm area minus the nuclear area. The cell height of thyroid epithelium were measured in a total of 15 follicles per fish. Measurements were made at four points within each follicle at 90° from one another. Cell height is reported as the mean of corrected values (Kalisnik et al., 1977).

2.5. Biochemical analyses

Analysis of liver samples for EROD enzyme activity was carried out with post-mitochondrial supernatants as described previously (Muir et al., 1990). Retinoids and tocopherol were measured in liver tissue by isocratic HPLC (Palace and Brown, 1994). For tissue thyroid hormone analyses, skeletal muscle was excised, minced and homogenized and extracted with methanolic ammonia containing propylthiouracil (Brown et al., 1991). The supernatant was evaporated to dryness, redissolved in 0.1 N NaOH and assayed for T_3 and T_4 using established procedures (Brown and Eales, 1977).

2.6. Data analysis

Growth rates were determined by fitting all fish and liver weight data to an exponential model (ln weight $= a + b$ time (days), where a is a constant and b is the growth rate). Concentrations were corrected for growth dilution and were lipid-normalized for calculation of bioaccumulation parameters (Muir et al., 1990). Assimilation efficiencies (α) were calculated by fitting the concentration data to the integrated form of the kinetic rate equation for constant dietary exposure using iterative nonlinear regression (Systat Version 5.0, Evanston, IL):

$$C_{\text{fish}} = \left(\frac{\alpha F C_{\text{food}}}{k_{\text{d}}}\right)[1 - \exp(-k_{\text{d}}t)]$$

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 based on the amount of food given to each treatment tank (see Section 2.2 above), 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). The equilibrium biomagnification factor (BMF) was predicted from the equation $\text{BMF} = \alpha F/k_{\text{d}}$.

Differences in depuration rates and differences between growth rate constants, among treatments were examined by testing the homogeneity of slopes in an analysis of covariance (ANCOVA). The Student's t-test was used to compare pairs of growth, and depuration, rate constants. Regression analysis between EROD activity in liver and whole body concentrations used the mean value of three livers for EROD and three whole fish for concentrations for each sampling time because EROD and concentration data were not available for the same fish. Bartlett's test for homogeneity of variance was applied to the biochemical data. Where required, data were transformed according to Taylor's power law to obtain more uniform variance. For clarity of presentation, arithmetic means and their S.E. are given in results. ANOVA using time and treatment as categorical factors followed by Tukey's test to evaluate differences between group means. Probabilities of < 0.05 were considered to be significant.

3. Results

3.1. Bioaccumulation and biotransformation

Rainbow trout rapidly accumulated PCB 126 and contained measurable concentrations by day 5 of uptake (Fig. 1). There was no ^{14}C detected in any of the control fish. Estimated uptake and assimilation efficiencies were similar between treatments (Table 1). The concentrations in the trout had not leveled off by the final day of uptake (day 30) and, therefore, trout had not reached steady state with the food (Fig. 1). Depuration appeared bi-phasic, with an initial more rapid rate followed by a slower elimination (Fig. 1). Depuration rates of PCB 126 calculated from whole fish concentrations differed significantly between low- and high treatments, but depuration rates calculated from carcass only values (no liver or GI tract) data did not differ between treat-

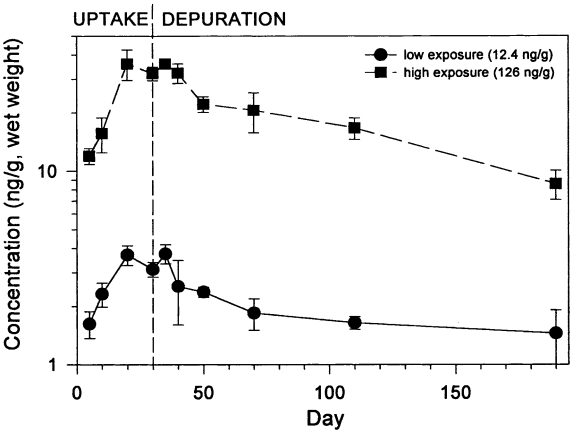

Fig. 1. Concentrations (whole fish, ng g^{-1} wet weight) of ^{14}C-PCB 126 in juvenile rainbow trout exposed to dietary concentrations of 12.4 and 126 ng g^{-1} of PCB 126 for 30 days followed by 160 days of non-spiked food. Each point is the mean concentration of three fish ± S.E.M.

Table 1
Bioaccumulation parameters of PCB 126 from dietary exposures using juvenile rainbow trout

Food concentration (ng g^{-1})	Depuration rate 10^{-2} (per day)[a]	Half life (day)[b]	BMF[c]	Assimilation efficiency (%)[d]
Whole fish				
12.4 ± 0.175	0.39 ± 0.12 (0.39)	180 ± 38	5.6	71 ± 8.6
126 ± 0.739	0.79 ± 0.13 (0.67)	88 ± 14	2.6	68 ± 6.1
Carcass				
12.4 ± 0.175	0.57 ± 0.12 (0.55)	120 ± 26	4.1	62 ± 5.5
126 ± 0.739	0.85 ± 0.15 (0.63)	82 ± 14	2.5	54 ± 3.2

[a] Depuration rate constants (k_d) ($\pm$S.E.M.) 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).

[b] Half life ($\pm$S.E.M.) is calculated from the equation $t_{1/2} = 0.693/k_d$.

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

[d] The assimilation efficiency (α) ($\pm$S.E.M.) 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).

ments. Estimated BMFs exceeded 1, suggesting that PCB 126 will biomagnify in aquatic food chains.

The percentage of toluene non-extractable ^{14}C in carcass increased from about 15% on day 30 of uptake to about 30% by day 80 of depuration (data not shown).

3.2. Liver size and histology

Individual group means did not differ between PCB 126 exposed rainbow trout and control rainbow trout for the morphometric parameters assessed in liver at 30 days exposure (Table 2). However, following exposure, ANOVA and pairwise comparisons indicated that there was an overall trend for greater liver size in fish exposed to the high PCB 126 dose ($P < 0.037$).

3.3. CYP 1A activity

EROD activity was elevated in rainbow trout exposed to the PCB 126 (Fig. 2). EROD activity in the low exposure group had increased by day 10 of uptake when liver concentrations were near detection limits (~ 0.1 ng g^{-1}, wet weight, in two livers and at 0.28 ng g^{-1}, wet weight, in one liver) for our radioisotope methodology. EROD activity in the low-treatment rainbow trout were not elevated relative to the control trout on days 5 and 20 of depuration but were higher on days 40 and 160 of depuration when liver concentrations were again below the detection limits (~ 0.1 ng g^{-1}, wet weight) (Fig. 2). In the high exposure group, EROD had increased by the first sampling day (day 10 of the uptake period) when liver concentrations were 12 ± 6.2 ng g^{-1} (wet weight, $n = 3$), and remained elevated through day 160 of depuration despite liver concentrations falling to 0.95 ± 0.78 ng g^{-1} (wet weight, $n = 3$). At 30 days of exposure, EROD levels were 9- and 44-fold greater in low- and high-exposure rainbow trout than control. Strong relationships between EROD activity in liver and whole body concentrations were found in the PCB 126 exposed rainbow trout (Fig. 3). The slopes of these relationships were steeper in the individual exposure treatments, low and high, when compared with regression of all data (Fig. 3).

3.4. Liver vitamins

At 30 days exposure to PCB 126 didehydroretinol, didehydroretinyl palmitate and toco-

Table 2
Morphometrics of liver tissue in rainbow trout exposed to PCB 126 for 30 days

Treatment	Number of hepatocytes	Relative hepatocyte size (μm^2)	Nuclear diameter (μm)	$N:C$ ratio[a]	Liver somatic index (%)
Control	122 ± 6	146 ± 7	5.63 ± 0.12	0.207 ± 0.005	1.26 ± 0.12
Low (12.4 ng g^{-1})	141 ± 8	130 ± 8	5.62 ± 0.09	0.239 ± 0.016	1.29 ± 0.11
High (126 ng g^{-1})	130 ± 8	142 ± 11	5.58 ± 0.13	0.211 ± 0.012	1.41 ± 0.13

[a] The $N:C$ ratio is the ratio of the nucleus area to the cell area.

pherol concentrations in the livers of rainbow trout exposed to the high PCB 126 concentration were significantly lower than in the liver of control fish (Fig. 4). Levels of these vitamins did not vary between the low treatment group and control. Retinol and retinyl palmitate concentrations did not significantly differ between any treatments.

3.5. Indices of thyroid status

Concentrations of T_3 and T_4 declined rapidly in the muscle of control and PCB 126 exposed rainbow trout during the first 30–35 days of the experiment (Fig. 5). This loss in muscle T_3 and T_4 occurred more rapidly in the PCB 126 rainbow trout than controls. Muscle T_4 concentrations were significantly lower in the PCB 126 exposed rainbow trout than the control throughout the uptake phase and the first 40 days of depuration, and only returned to control levels 160 days after the cessation of PCB 126 exposure. Muscle T_3 concentrations in PCB 126 trout differed from control fish only during the exposure period. Group means (data not shown) did not differ significantly between PCB 126 exposed rainbow trout and control rainbow trout for thyroid epithelial cell height after 30 days exposure to PCB 126.

3.6. Growth and survival

Growth rates did not differ between PCB 126 exposed rainbow trout and control fish (Table 3). No mortalities were observed in any of the treatments.

4. Discussion

4.1. Tissue PCB 126 concentrations

The PCB 126 concentrations in Great Lakes fish are roughly 20- (low-exposure) and 200 (high exposure) fold lower (Giesy et al., 1997) than the exposure concentrations used in this experiment. If TCDD-TEQs are considered (Williams et al., 1992), our low-and high-doses represent TCDD-TEQ values about 0.27 and 2.8 ng g^{-1} (wet weight), respectively. These doses fall near the range of tissue concentrations of TCDD-TEQ reported historically in predatory salmonids from Lake Ontario (Huestis et al., 1997) and Lake Michigan (Smith et al., 1990) but do exceed those reported for prey species. However, the tissue

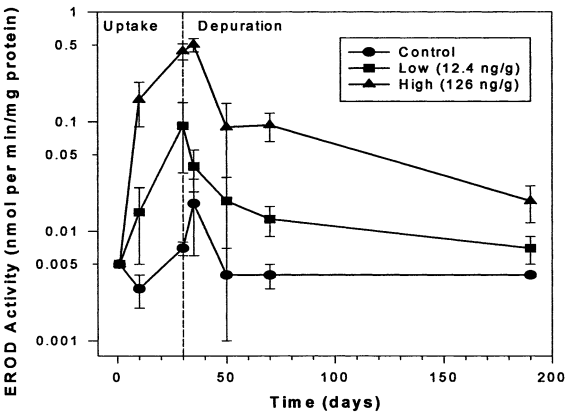

Fig. 2. EROD activity in livers (postmitochondrial supernatants) of juvenile rainbow trout exposed to dietary concentrations of 12.4 and 126 ng g^{-1} of PCB 126 for 30 days followed by 160 days of non-spiked food. Each point is the mean ± S.E.M. activity in three fish livers.

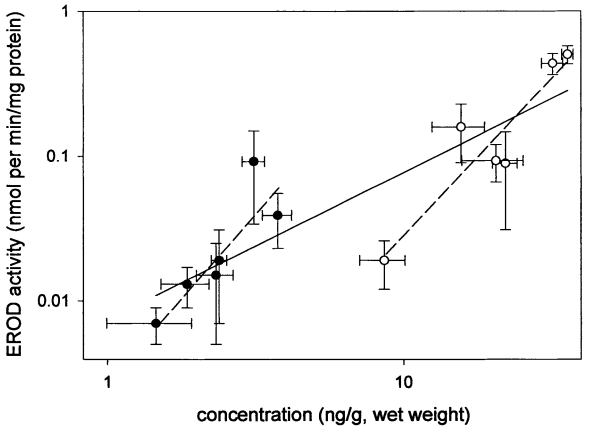

Fig. 3. EROD activity in livers (postmitochondrial supernatants) relationships with whole body concentrations of PCB 126 in juvenile rainbow trout exposed to dietary concentrations of 12.4 and 126 ng g^{-1} of PCB 126 for 30 days followed by 160 days of non-spiked food. Each point is the mean ± S.E.M. of the EROD activity in three fish livers and PCB 126 concentration of three fish. Dashed lines are the liner regression of the low (closed circles, slope = 2.30; $r^2 = 0.76$; $P = 0.02$) and high (open circles, slope = 2.14; $r^2 = 0.85$; $P < 0.01$) exposed fish and the solid line is the regression for all points (slope = 1.01, $r^2 = 0.76$, $P < 0.001$).

TCDD-TEQ concentrations produced following the 30 days exposure to the low-dose group (0.06 ng g^{-1}) were similar to recent reports of TCDD-TEQs in predatory species (Giesy et al., 1997). The tissue concentrations produced in the high-dose group exceeded this by a factor of 10 and are more representative of possible historical values (Huestis et al., 1997).

4.2. Bioaccumulation and biotransformation

Half-lives determined for PCB 126 ($t_{1/2} = 82$–180 days) in juvenile rainbow trout in this experiment indicate that this is one of the most persistent PCB congeners in fish. In a similar experiment, Fisk et al. (1998) found the half-lives of PCB 138 and 153, in juvenile rainbow trout (carcass tissue only) to be 64 and 69 days, respectively. Coristine et al. (1996) also observed a longer half-life for PCB 126 ($t_{1/2} = 210$ days) than PCB 153 ($t_{1/2} = 88$ days) in slightly larger juvenile rainbow trout (final weights 168 g). The greater half-lives of PCB 126 compared with PCBs 138 and 153 may suggest that this congener is 'held' by the fish, potentially by the aromatic (aryl) hydrocarbon receptor (*AhR*) and/or the P450 1A cytochrome(s). CYP 1A activity, as measured by the EROD assay, was increased in the PCB 126 exposed rainbow trout in this experiment (see below). Evidence for such a retention mechanism for fish is lacking, but there is support for such a mechanism in mammals. Studies with rats have found that 2,3,7,8-TCDD was sequestered but PCB 153 (i.e. neither an inducer nor susceptible to metabolism by CYP 1A) was not in hepatic tissue of rats carrying a CYP1A2 gene. However, TCDD was not sequestered in hepatic tissue of rat lacking the CYP 1A2 gene (Diliberto et al. 1999). As well, Leung et al. (1990) found that the distribution ratio of liver:fat (4:1) in rats was primarily due to the binding of TCDD with microsomal TCDD-binding factor in liver. Although this study does not provide direct support for such a mechanism in fish it does present sufficient evidence to recommend further study.

The different PCB 126 half-lives in the two exposure groups suggests that the level of exposure can influence dynamics of *biologically active* organochlorines in fish. The shorter half-life of PCB 126 in the high exposure treatments does provide some evidence that PCB 126 was metabolized by the rainbow trout, potentially due to greater CYP 1A activity (see below) in the high exposure group. In general, fish need two vicinal positions free of chlorine to biotransform PCB congeners (Boon et al., 1989), although there is recent evidence that suggests fish can slowly metabolise these types of PCB congeners (White et al., 1997). The time related increase in the portion of toluene non-extractable ^{14}C compared with extractable ^{14}C in the carcass suggests some biotransformation of PCB 126. This is based on the premise that as a non-polar solvent toluene should mostly extract the very non-polar parent PCB 126 but not PCB 126 that had been biotransformed to more polar metabolites formed by the addition of a hydroxy or carboxyl group. This type of analogy has been used previously with other ^{14}C compounds (Fisk et al., 1997). Although greater toluene non-extractable ^{14}C may represent more polar compounds, it is also possible that the

^{14}C may still be either non-extracted parent PCB 126 or part of an intact PCB 126 compound that has tightly associated with biological macromolecules, such as the *Ahr* and/or P450 cytochrome. Regardless, even if some PCB 126 was biotransformed by the rainbow trout, the long half-life we found suggests that the biotransformation rate is very slow.

The long half-life and high assimilation efficiency of PCB 126 result in a BMF that is >1, suggesting that PCB 126 will biomagnify in aquatic food chains. This is consistent with other studies carried out on PCB 126 in aquatic food chains (Koslowski et al., 1994). BMFs calculated for this experiment fell above relationships developed between BMF and log K_{ow} for juvenile rainbow trout and a series of recalcitrant PCB congeners and organochlorines (Fisk et al., 1998), providing further verification that PCB 126 is one of the most bioaccumulative PCB congeners.

4.3. Liver size and histology

Elevated liver somatic index has been a commonly observed response in fish exposed to *AhR* inducing compounds (Muir et al., 1992). Because liver histology after 30 days exposure to PCB 126 showed no differences in estimates of liver cell size, greater overall liver size is likely to be the result of cell hyperplasia in the juvenile trout. Brown et al. (1998) noted a similar hepatic response in adult rainbow trout given injections of EROD inducing 2,3,4,7,8-pentachlorodibenzofuran.

4.4. P450 1A activity

The induction of EROD activity in the low PCB 126 treatment rainbow trout after 5 days exposure and 160 days after the cessation of expo-

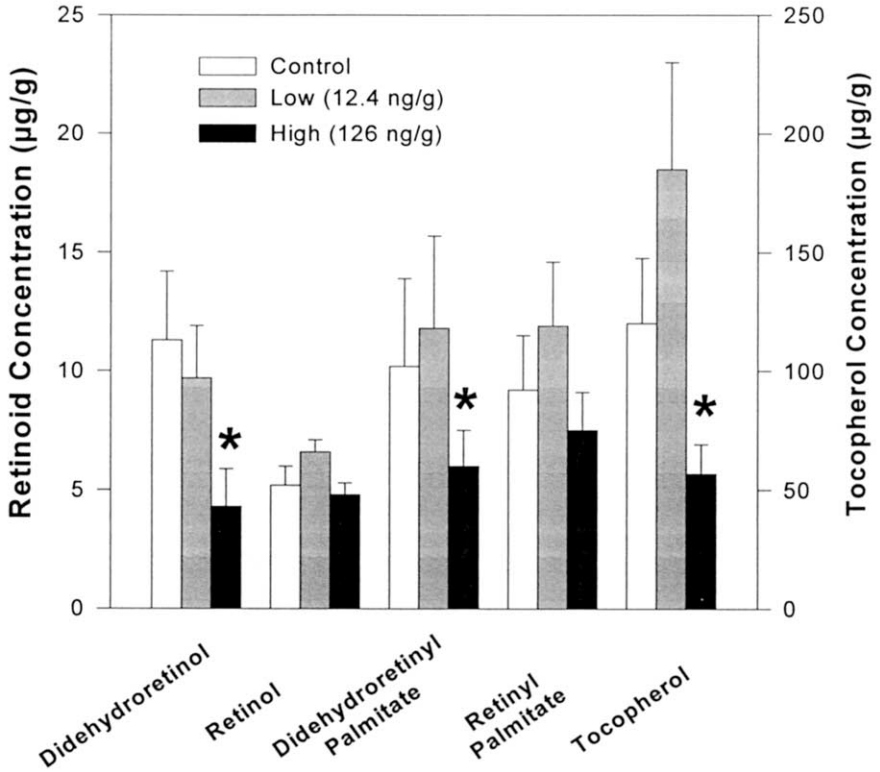

Fig. 4. Mean values for retinoid stores and tocopherol in livers of rainbow trout exposed to PCB 126 for 30 days. Histogram bars represent mean and S.E.M. Significant differences ($P < 0.05$) between reference and exposed sites are indicated by an asterisk.

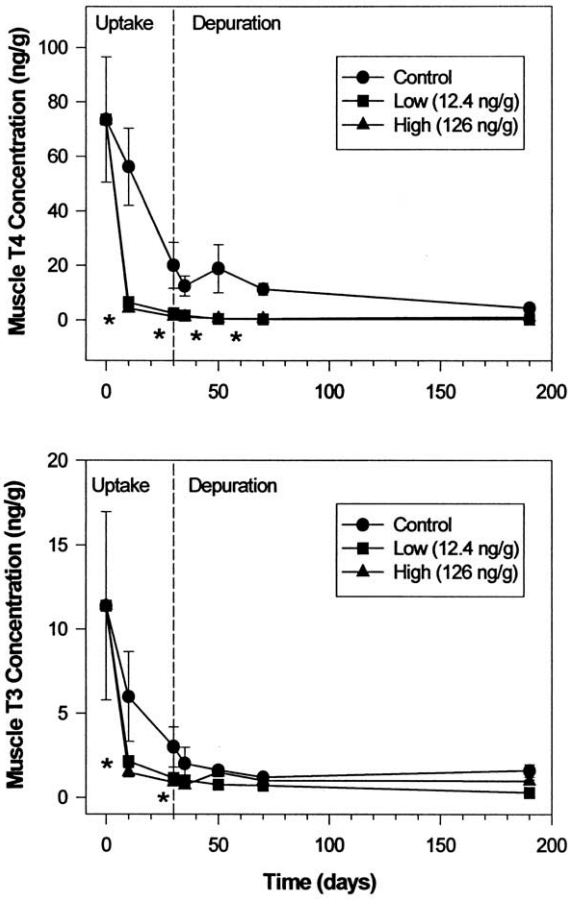

Fig. 5. Concentrations of T_4 and T_3 in muscle of juvenile rainbow trout exposed to dietary concentrations of 12.4 and 126 ng g^{-1} of PCB 126 for 30 days followed by 160 days of non-spiked food. Each point is the mean $\pm$ S.E.M. of six fish. Asterisk indicates significant difference from control mean.

sure, when concentrations were below detection limits in all but one sample (detection limit 0.1 ng g^{-1}), suggested that the liver tissue threshold concentration for EROD induction by PCB 126 in juvenile rainbow trout was ≤ 0.1 ng g^{-1} (wet weight). The threshold concentration could be lower because PCB 126 concentrations were below our isotopic detection limits in two of the three livers and we did not measure EROD activity prior to day 10 of exposure. This threshold concentration is almost twice the threshold concentrations reported for 2,3,7,8-TCDD in juvenile rainbow trout, although liver concentration was only estimated from whole fish concentrations in that experiment (Fisk et al., 1997).

Based on EROD induction, TEFs of PCB 126 is 0.16 using TCDD threshold concentrations reported by Parrott et al. (1995) for rainbow trout following oral gavage (Table 4). This TEF is at least 32 $\times$ greater than the PCB 126 TEFs previously reported in rainbow trout exposed by intraperitoneal injection (Janz and Metcalfe, 1991; Newsted et al., 1995). The reason for this difference is unclear but it could be related to differences between interperitoneal and diet as a route for exposure. Our findings place PCB 126 in the range of 2,3,7,8-Cl substituted dioxin and furan congeners and similar to TEFs reported for mammals and birds in a recent review of TEFs (van den Berg et al., 1998).

Relationships between EROD activity and PCB 126 concentrations suggest that EROD activity in the highly exposed rainbow trout is either suppressed or has reached a maximum induction level. The expected EROD activity in the highly exposed trout based on the regression developed for the lower exposed trout is much higher than what was measured. As well, the slope of the lower exposed rainbow trout is greater than the slope observed for all trout, low and high exposure, combined (Fig. 3). Lower than expected EROD activity due to a maximum level in the trout seems unlikely because higher EROD activity has been measured in similar sized rainbow trout exposed to polychlorinated dibenzofurans

Table 3
Growth parameters (mean $\pm$ S.E.M.) and mortality of juvenile rainbow trout exposed to ^{14}C-PCB 126

Chemical	Growth rate 10^{-3} (per day)[a]	Mortality (%)
Control	11.2 ± 1.63 (0.63)	0
Low (12.4 ng g^{-1})	12.6 ± 2.19 (0.54)	0
High (126 ng g^{-1})	12.5 ± 1.93 (0.60)	0

[a] 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).

Table 4
Threshold liver concentrations of PCB 126 and selected dioxin and furan congeners for induction of EROD activity above control levels in rainbow trout and toxic equivalency factors (TEFs) based on TCDD

Compound	Threshold liver concentration (ng g^{-1})	TEFs[a]	Rainbow trout size (g)	References
PCB 126	~0.10	0.16	20	This work
2,3,7,8-TCDD	0.055	0.29	20	Fisk et al. (1997)
1,2,3,4,7,8-HxCDD	0.039	0.41	200	Parrott et al. (1995)
1,2,3,4,6,7,8-HpCDD	0.35	0.098	200	Parrott et al. (1995)
2,3,7,8-TCDF	0.038	0.54	200	Parrott et al. (1995)
1,2,3,7,8-PnCDF	0.049	0.42	200	Parrott et al. (1995)
2,3,4,7,8-PnCDF	0.0047	4.4	200	Parrott et al. (1995)
1,2,3,4,7,8-HxCDF	0.046	0.44	200	Parrott et al. (1995)
2,3,7,8-TCDD	0.016	1	200	Parrott et al. (1995)

[a] TEFs of chemical X = (threshold liver concentration of chemical X)/(threshold liver concentration of 2,3,7,8-TCDD as reported in Parrott et al., 1995).

(Muir et al., 1990). There is, however, good evidence that at higher concentrations PCB 126 can inhibit the EROD assay and the activity of the P450 1A cytochrome. Schlezinger and Stegeman (2001) showed that CYP 1A mRNA was induced strongly by high PCB 126 exposure but that EROD rates remained at control levels in marine teleost scup (*Stenotomus chrysops*). Similar findings have also been reported in fish cell lines for PCB 77 (Hahn et al., 1993). This has implications for the use of EROD activity, and potentially other similar assays, as quantitative tools in biomonitoring because suppression or inhibition by exposure to high concentrations of inducing chemicals will result in an underestimation of exposure.

4.5. Liver vitamins

The ability of planar PCB's and other dioxin-like compounds lower hepatic retinoids in liver of juvenile rainbow trout has also been observed in lake trout (Palace and Brown, 1994) exposed to PCB 126 and in adult rainbow trout exposed to 2,3,4,7,8-pentachlorodibenzofuran (Brown et al., 1998). Reductions of hepatic retinods have also been observed in rats exposed to PCB 126 (Chen et al., 1992). In fish these disturbances have been hypothesized to result from accelerated metabolism and breakdown of target tissue retinoids and metabolites resulting in a greater demand for vitamin A in fish (Gilbert et al., 1995; Palace et al., 1997; Doyan et al., 1998). In contrast, Besselink et al. (1997) found that flounder (*Platichthys flesus*) exposed to 2,3,7,8-TCDD exhibited EROD induction but few changes in glucuronosyltranferase activity and retinoids. It may be that phase II metabolism is required to elicit changes in retinoid dynamics in fish exposed to co-planar organochlorines (Boyer et al., 2000).

Similar to results reported for long-term exposures of juvenile lake trout to PCB 126 (Palace et al., 1996), lower retinoids and tocopherol in the rainbow trout may also reflect greater oxidative stress. Tocopherol plays a role in preventing oxidative damage (Packer, 1991) and is important in fish owing to their high concentrations of polyunsaturated fatty acids that are susceptible to lipid peroxidation (Singh et al., 1992; Baker et al., 1997). Thus it is probable that declines in tocopherol are related to general utilization for quenching free radicals produced by the high MFO activity found in the PCB-exposed trout (Palace et al., 1996). Similar mechanisms may also account for depletion of retinoid and carotinoid stores (Miki et al., 1995) in the PCB exposed trout. Further work is required to clarify the exact role of retinoids in fish experiencing oxidative stress.

4.6. Thyroid status

Changes in the thyroid hormone content of major body compartments during juvenile growth

and development has received only limited attention (Fok et al., 1990; Specker et al., 1992). In contrast, changes in plasma T_3 and T_4 has been the most common method for evaluating thyroid status (reviewed in Eales and Brown, 1993). The body-content of thyroid hormones and their role in the development in larval fishes has also been the subject of previous investigations (Brown et al., 1987; Raine and Leatherland, 1999).

Because the muscle represent as much as 80% of the body burden of T_3 in salmonids (Fok et al., 1990), the large declines in muscle thyroid hormones of control fish during the first experimental month suggests that there may be significant changes occurring in overall thyroid status and economy during this period. Compartment expansion due to growth and greater fish size is nearly sufficient to account for the decline in thyroid hormones over the first month. Also, the higher levels of thyroid hormones in muscle tissue at the beginning of the experiment may have been partially due to tissue sampling. Although efforts were made to sample consistently throughout the experiment the much smaller size of the fish at the beginning of the experiment may have resulted in the inclusion of a greater percentage of non-muscular tissue. This could have occurred because a much greater proportion of the total body mass was required for analytical purposes in small fish. However, maintenance of the tissue levels over the remainder of the experiment requires significant hormone production and release from the thyroid.

Although effects of PCBs on muscle T_3 and T_4 concentrations have not been reported, minor declines in plasma T_3 and T_4 have been observed previously in fish exposed to PCBs (Leartherland and Sonstegard, 1978; Folmar et al., 1982; Besselink et al., 1996). There are a number of possible mechanisms, which may cause a reduction in T_3 and T_4 levels in rainbow trout exposed to co-planar organochlorines. Induction of CYP1A via the *AhR* is associated with induction of numerous other enzymes and proteins (Stegeman and Hahn, 1994). Among these is glucuronosyltranferase, which is found in teleosts (Clarke et al., 1991). In rats exposed to *AhR* agonists, reductions in plasma T_4 were associated with induction of UDP-glucuronosyltransferase activity (Kohn, 2000), which accelerates T_4 excretion. Because, we observed little change in histology of the thyroid gland after the 30-day exposure period, thyroid disruption in the PCB 126 exposed trout likely occurs in the peripheral components of the system (Eales and Brown, 1993). PCB 126 or metabolites formed from CYP 1A induction could alter hormone interactions with transport proteins (Brouwer et al., 1998), specific enzymes (e.g. 5′-monodeiodinase) and other cellular receptors thereby influencing overall thyroid hormone dynamics. Further research on indicators of peripheral thyroid function(s) in exposed fish is required to identify the site(s) whereby PCB126 disrupts thyroidal status. While lower muscle stores of T_4 and T_3 did not result in any detectable changes in fish growth rate or overall survival, other physiological consequences associated with normal thyroid status (e.g. reproduction, parr–smolt transformation and behavior) need to be tested.

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