



Vitamin and thyroid status in arctic grayling (*Thymallus arcticus*) exposed to doses of 3,3',4,4'-tetrachlorobiphenyl that induce the phase I enzyme system [☆]

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Abstract

Induction of phase I biotransformation enzymes is recognized as a hallmark response in fish exposed to coplanar PCBs. Depletions of vitamins A and E and disrupted thyroid hormone and glandular structure secondary to this induction have not yet been examined in an arctic fish species. Arctic grayling were exposed to a single oral dose of 0 (control), 10, 100 or 1000 ng 3,3',4,4'-tetrachlorobiphenyl (TCB) g⁻¹ bodyweight, a contaminant found in most arctic fish. After 30 and 90 days of exposure, TCB concentrations in tissues, hepatic phase I activity (as ethoxyresorufin-O-deethylase (EROD)), plasma and tissue vitamin A and E concentrations, plasma thyroid hormone levels and thyroid glandular structure were examined. Total plasma osmolality, as an indicator of overall fish health was also monitored. TCB recovery in tissues was low and extremely variable, making comparisons between intended dose groups inappropriate. Therefore, correlation analysis between actual recovered TCB concentrations and biochemical responses was employed. Hepatic EROD activity correlated strongly with liver TCB concentrations. Liver concentrations of vitamin A were altered as a function of TCB concentrations and EROD activity, but plasma vitamin A status was not affected. Vitamin E was depleted by TCB accumulation in blood and EROD induction in liver of males only at 90 days post-exposure. Thyroid hormones status and glandular structure were not affected by the short duration TCB exposures used in this experiment. TCB concentrations were correlated with an elevation in plasma osmolality. Results from this experiment indicate that the vitamin status and osmoregulation of arctic grayling exposed to TCB can be compromised. Further studies of field populations exposed to this type of contaminant are warranted. Crown Copyright © 2001 Published by Elsevier Science Ltd. All rights reserved.

Keywords: PCB; Arctic grayling; Vitamins; Thyroid; Osmolality

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1. Introduction

Polychlorinated biphenyls (PCBs) account for a significant portion of the TCDD toxic equivalents (TEQs) in feral fish collected from contaminated sites in North America (Niimi and Oliver, 1989; Spies et al., 1990). Among PCBs, the non-*ortho* coplanar congeners (IUPAC PCBs 77, 126, and 169) are considered the most toxic (Safe, 1990) and of these three congeners, PCB 77 (TCB) is present at the highest concentration in commercial PCB mixtures (Kannan et al., 1987). In terms of cytochrome P450 enzyme induction in fish, TCB is more potent than mono-*ortho* and di-*ortho* substituted coplanar PCB congeners, respectively (Gooch et al., 1989). In addition to its potent toxicity, TCB has been detected in virtually all samples of fish collected from arctic waters.

Fish exposed to TCB have induced activities of the biotransformation enzymes ethoxyresorufin-O-deethylase (EROD) and aryl hydrocarbon hydroxylase (AHH) activities (Janz and Metcalfe, 1991; Lindstrom-Seppa et al., 1994), delayed sexual maturation, and reduced offspring survival (Monosson et al., 1994). Exposure to coplanar PCBs has also been shown to deplete vitamin stores, to disrupt circulating thyroid hormone levels, and to alter thyroid glandular structure (Palace and Brown, 1994; Brown et al., 1997). We have also recently reported that exposure to doses of PCB congener 126 which increase Phase I biotransformation enzyme activity also elevate oxidative stress and the metabolism of vitamins A and E in fish (Palace et al., 1996a,b; Palace et al., 1998). However, organochlorine exposures in other species of fish do not greatly alter vitamin A or thyroid status even when Phase I enzyme activity is significantly induced (Besselink et al., 1996, 1997).

Since PCB 77 is more readily metabolized by fish than PCB 126, fundamental differences have been proposed regarding the toxicity of these two congeners. This is especially relevant to their effects on vitamin A and E metabolism (Brouwer and van den Berg, 1986). The current study examines vitamin and thyroid hormone status in an arctic fish species exposed to environmentally relevant doses of PCB 77 (Muir et al., 1993). Specifically, we monitored the induction of the Phase I biotransformation enzyme, EROD, and tissue concentrations of vitamins A and E, 30 and 90 days after a single oral dose of 0 (control), 10, 100 or 1000 ng TCB g⁻¹ bdwt. Circulating thyroid hormone levels and thyroid glandular structure were also considered, as was plasma osmolality, a measure of overall contaminant-induced stress.

2. Materials and methods

2.1. Fish maintenance and dosing

Arctic grayling ($n = 70$, weight = 291 ± 61 g, length = 303 ± 23 mm) were reared from fingerlings in

500-l fiberglass tanks receiving at least 2 l of aerated and dechlorinated Winnipeg city water ($10^\circ\text{C} \pm 0.5^\circ\text{C}$) per gram of fish per day. A 12 h light: 12 h dark photoperiod was maintained for holding and experimental periods, and fish were fed Martin Feeds commercial chow at a ration of 1% of body weight every second day.

Two weeks prior to dosing, fish were lightly anesthetized with tricaine methanesulfonate (MS222 = 0.38 g/l in iso-osmotic NaCl and neutralized to pH = 7.0 with ammonium hydroxide), fitted with visual implant tags (Northwest Marine Technology, Seattle, WA) and randomly redistributed into groups of 12 fish each. All fish were held in 168-l fiberglass tanks and the same water flow, photoperiod and feed regimen were maintained. Before dosing, all fish were starved for three days. For dosing, fish were lightly anesthetized, weighed, and given a single dose of one of four concentrations of TCB in warmed ethanol:gelatin. Gavage solutions were prepared by dissolving uniformly ring-labelled [¹⁴C]-3,3',4,4'-tetrachlorobiphenyl (PCB 77) (Sigma Chem., St. Louis, MO) and unlabelled PCB 77 (UltraScientific, N. Kingstown, RI) in ethanol and warmed (30°C) 60 Bloom gelatin (Sigma Chem., St. Louis, MO) (10:90 v/v). Labeled TCB was used as a marker to quantify unlabeled TCB as previously described (Palace et al., 1996a,b). Approximately 1 ml of ethanol:gelatin was delivered directly to the stomach through polyethylene tubing (ID 1.57 mm) attached to a 16 gauge needle (Sijm et al., 1990) to achieve target concentrations of 0 (control), 10, 100 or 1000 ng total TCB per g of fish weight. Fish recovered from the procedure in anesthetic-free water within 3 min.

2.2. Blood and tissue sampling

After 30 or 90 days, all of the fish in randomly selected tanks were anaesthetized by adding pH-neutralized MS222 (0.76 g/l) solution directly to the tanks. Immobilization was complete within 90 s, after which blood was obtained from the caudal vessels with heparin rinsed syringes and 18 gauge needles. To minimize potential diurnal effects, samples were always taken during the same morning hours. Plasma was immediately separated from the red cells by centrifugation and was stored at -90°C until analyzed. Immediately after blood sampling, tissues were quickly dissected, weighed and processed or frozen until analyzed. Specific growth was calculated as $(dw/dt)/w$ as described by Ricker (1979).

2.3. Disposition of TCB

Subsamples (0.01–0.5 g) of bile, blood, gill, gonad, kidney, liver, adipose tissue and muscle were weighed, rinsed with deionized water, and oxidized using a sample oxidizer (Model 306, Packard Instrument, Downer Grove, IL) as previously described (Muir et al., 1992).

Radioactivity was collected using 4–5 ml Carbosorb and 12–13 ml PCS (phase combining scintillant):xylene (Amersham International, Arlington Heights, IL). Burn times ranged from 15 to 45 s depending on the amount of tissue combusted and moisture content of that tissue. Calibration was performed using a 5000 dpm nominal standard (Amersham International). Radioactivity was measured in a liquid scintillation counter (Model LS 7500, Beckman). Blanks assayed with tissue paper were consistently below detection limits and the calibration standards fell within acceptable ranges in all cases. Gonad and muscle samples were analyzed in replicate with the average coefficient of variation being 11% and 13%, respectively.

To test recovery of the radioactive ^{14}C in wet tissues, subsamples of select tissues (gonad, liver, muscle, and carcass) were freeze-dried, pulverized, and extracted with toluene. The toluene extracts were analyzed using the same methods as described above. Values from toluene-extracted samples were typically lower (28–77%) than oxidized (non-extracted) samples in tissues other than muscle, in which toluene extracted samples were higher than oxidized samples (38%). Values from the extracted samples likely represent TCB and any lipophilic metabolites, whereas oxidation values may also include more polar excretion products, including glucuronide conjugates of TCB.

Mass balance calculations were performed on a subset of individuals ($n = 3$ for each dose and treatment time). To determine the total disposition in these fish, whole carcasses were homogenized and oxidized. The percentage of the total dose recovered in each tissue was calculated by multiplying the concentration of TCB determined for each tissue by the total weight of that tissue. In performing these calculations, the following factors were used: (1) muscle comprises 70% of the body weight (Fok et al., 1990), (2) 1-ml blood = 1.1 g, and (3) blood comprises 3% of the body weight (Gingerich and Pityer, 1989). The total weight of visceral adipose tissue was not determined, and could not be included in the calculation of total dose recovered.

2.4. Biochemistry

Microsomes were prepared immediately from fresh livers and were analyzed for Phase I activity, as EROD, using the methods of Hodson et al. (1991). Vitamin E (tocopherol) and Vitamin A (retinol, dihydroretinol, retinyl palmitate) were measured in plasma, liver and kidney using the HPLC method described by Palace and Brown (1994). Plasma triiodothyronine (T3) and thyroxine (T4) were determined using established radioimmunoassays, and thyroid epithelial cell heights were measured using procedures previously outlined (Brown et al., 1997). Plasma osmolality was measured using a

Precision System Micro-Osmette Model 5004 instrument.

2.5. Statistical analysis

Tissue TCB concentrations, EROD, vitamin A and osmolality values were log-transformed prior to statistical analysis, because residuals were proportional to the means for these parameters. Due to the high variability in TCB recovered from tissues, comparison of group means for biochemical and histological endpoints between the intended dose groups were not relevant. To examine the effects of TCB on biochemical and histological endpoints, regression analysis against recovered concentrations of TCB were performed. Additionally, since induced Phase I enzyme activity has been correlated with enhanced metabolism of vitamin A and thyroid hormones, regression analysis between EROD and these parameters was also performed. For these calculations, tissue concentrations in untreated fish were approximated by using a random number generator to assign concentration equivalents which were below the analytical detection limit.

3. Results

3.1. Disposition

Only $21 \pm 14\%$ of the initial dose was recovered in fish for which mass balance sampling and analysis were conducted at both the 30 and 90 day intervals. Fish experienced little or no growth during the experiment (specific growth 0.004 ± 0.0004 g/day) and there were no significant differences between fish weight and length associated with either the TCB exposure or sampling time. Therefore, no corrections for growth dilution were included in the disposition calculations. Total recovered dose was typically highest at 30 days, with some elimination by 90 days after the exposure. Bile and adipose tissue contained the highest levels of TCB while extractable concentrations of TCB in gonad, liver, and muscle were typically 5–10 times lower than the administered oral dose (Table 1). A larger percentage of the recovered dose was recovered in the gonads of females than males, and the percent of dose recovered in the gonads of female fish was strongly correlated with GSI ($r_p = 0.94$) (data not shown). This relationship was not evident in males. Since there were no other significant differences in tissue burdens between males and females, the data for Table 1 are for both sexes combined. For all other tissues, the proportion of TCB dose recovered did not differ as a function of the administered dose. These proportions did not significantly change between treatment times.

Table 1
TCB in selected tissues of arctic grayling (ng/g w.w.)^a

Tissue	30 day exposure			90 day exposure		
	10 ng/g	100 ng/g	1000 ng/g	10 ng/g	100 ng/g	1000 ng/g
Adipose	166.7 ± 39.3	400.0 ± 40.6	3490.0 ± 1172.3	34.2 ± 1.0	319.2 ± 75.4	3661.4 ± 1043.2
Bile	48.3 ± 23.5	153.9 ± 28.7	2413.7 ± 502.1	4.0 ± 0.1	105.4 ± 44.6	2637.1 ± 836.8
Blood	1.1 ± 0.3	2.0 ± 0.3	44.4 ± 9.3	0.4 ± 0.2	3.0 ± 1.5	39.1 ± 10.1
Gill	3.5 ± 1.1	6.8 ± 2.0	60.4 ± 15.6	0.3 ± 0.1	4.9 ± 1.9	31.2 ± 6.4
Gonad	2.9 ± 0.9	6.8 ± 2.0	118.3 ± 17.3	1.2 ± 0.2	7.3 ± 2.0	152.4 ± 1.3
Kidney	na ^b	na	na	0.8 ± 0.2	9.1 ± 2.3	53.1 ± 22.9
Liver	3.8 ± 1.8	15.8 ± 5.4	274.7 ± 46.8	0.4 ± 0.1	14.2 ± 5.3	179.3 ± 51.6
Muscle	1.6 ± 0.2	5.1 ± 1.0	81.8 ± 22.4	0.7 ± 0.1	10.8 ± 2.9	47.0 ± 18.0
Whole Carcass				<i>2.3 ± 0.65</i>	<i>35.0 ± 13.7</i>	<i>168.4 ± 81.1</i>

^a Data are presented as mean ± SEM ($n = 6$ for all treatment levels). Italicized means represent combined data from the analysis of carcasses taken at both the 30 and 90 day exposure times. All values for controls (dose = 0) were below the detection limit of 0.1 ng/g.

^b na: data not available.

3.2. EROD activity

EROD activity was positively correlated with TCB tissue concentrations after 30 and 90 days in males ($r = 0.941$, $P < 0.001$ and $r = 0.901$, $P < 0.001$, respectively) and females ($r = 0.966$, $P < 0.001$ and 0.937 , $P < 0.001$, respectively). When the data from both sexes and treatment times were combined and plotted on a log-log scale, the relationship between EROD activity and liver [¹⁴C]TCB burdens was best described by a linear regression model. Induction threshold was below 2.9 ng/g in this model (Fig. 1).

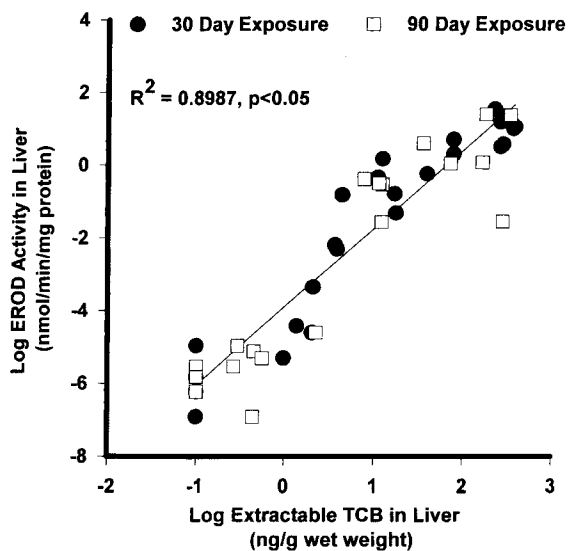


Fig. 1. Linear regression plot of the [log] Phase I enzyme, EROD, activity against [log] extractable 3,3',4,4'-tetrachlorobiphenyl (TCB) in the livers of male and female arctic grayling 30 ($n = 23$) and 90 days ($n = 19$) after a single oral dose of TCB.

3.3. Vitamins

Plasma retinol and didehydroretinol were not significantly altered by TCB exposure, based on regression analysis results. Liver concentrations of retinol and didehydroretinol palmitate storage forms were also not significantly affected by TCB after 30 or 90 days. However, after 90 days liver retinol was correlated with liver concentrations of TCB ($r = 0.659$, $P = 0.027$) (Fig. 2(a)) and activities of EROD ($r = 0.783$, $P = 0.004$) (Fig. 2(b)) in female fish. A similar relationship was not evident in male fish at either exposure time.

Hepatic concentrations of the antioxidant, vitamin E, were inversely correlated with blood concentrations of TCB ($r = -0.615$, $P = 0.044$) and EROD activity ($r = -0.701$, $P = 0.015$) in the liver of males but only at the 90-day sample period.

3.4. Thyroid histology and hormones

Regression analysis revealed no significant relationships between tissue TCB concentrations and circulating levels of T3 or T4. Similarly, there was no relationship between the induction of hepatic EROD and effect on plasma thyroid hormones. Histological analysis of thyroid epithelial cell heights showed no relationship to the recovered doses of TCB in any of the tissues analyzed.

3.5. Osmolality

There was a significant relationship between plasma osmolality and the recovered dose of TCB in the liver of male and female fish at 30 days (Fig. 3). Females and males at 90 days post-exposure showed no significant relationship between these parameters ($r = 0.208$ and 0.07 , $P = 0.21$ and 0.56 , respectively).

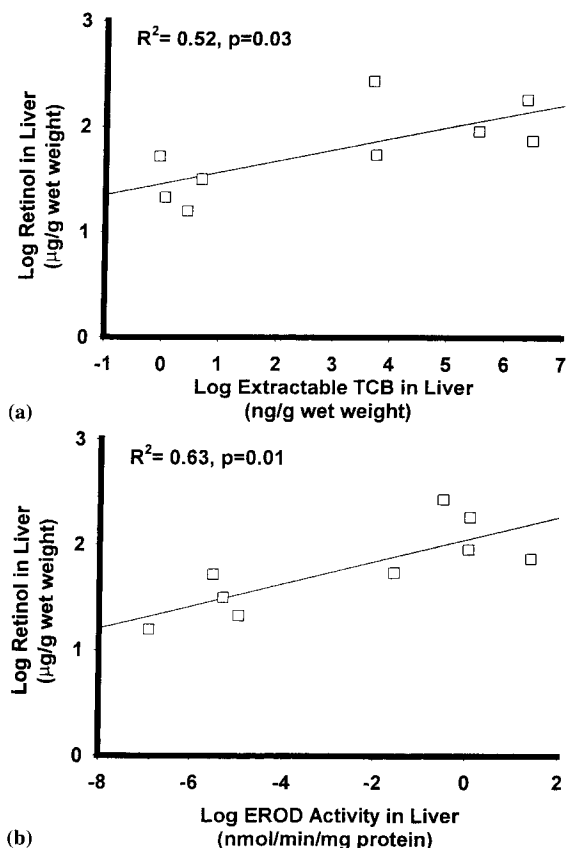


Fig. 2. Linear regression plots of: (a) [log] Retinol in liver against [log] extractable 3,3',4,4'-tetrachlorobiphenyl in the livers of female arctic grayling 90 days ($n = 9$) after a single oral dose of TCB. (b) [log] Retinol in liver against [log] Phase I enzyme, EROD in livers of female arctic grayling 90 days ($n = 9$) after a single oral dose of TCB.

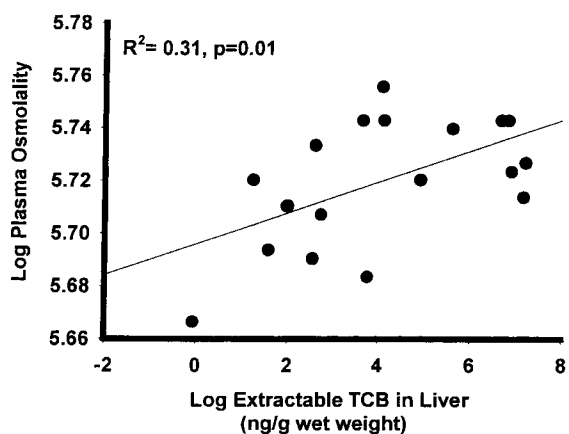


Fig. 3. Linear regression plot of [log] plasma osmolality against [log] extractable 3,3',4,4'-tetrachlorobiphenyl in the livers of female arctic grayling 30 ($n = 23$) after a single oral dose of TCB.

4. Discussion

4.1. TCB exposure and recovery

The doses of TCB used in this experiment are environmentally relevant. Specifically, if the current doses are multiplied by toxic equivalency factors (TEQ) of between 0.002 and 0.0006 for TCB that were derived by Newted et al. (1995) and Janz and Metcalfe (1991) for salmonid fish, the anticipated toxicity of TCB doses used in this experiment can be expressed relative to the most potent Ah-inducer, tetrachlorodibenzodioxin (TCDD). These calculations yield a TEQ of 0.0018–0.02 µg TCDD kg⁻¹. Since TEQs of between 0.2 and 1.7 µg of TCDD kg⁻¹ have been detected in fish from the Great Lakes (Huestis et al., 1997; Smith et al., 1990) the doses from this experiment fall well within the range considered to be environmentally relevant.

Recoveries of TCB from tissues and whole fish in the present study were low (<35%) and highly variable. Niimi and Oliver (1983) also reported variable recovery of TCB, but their mean recovery was 68% of a single oral dose in rainbow trout. An older study in rats recovered only 36% of an oral dose of TCB (Yoshimura and Yamamoto, 1973). Notwithstanding the low recoveries of TCB in the present study, concentrations that were achieved in tissues were also relevant to current environmental levels. For example, muscle concentrations were between 0.7 and 81.8 ng TCB per wet g of muscle tissue. By comparison, salmonids have been reported to contain between 0.16 and 10 ng TCB per g bodyweight from the Great Lakes (Smith et al., 1990; Williams et al., 1992; Janz et al., 1992; Mac et al., 1993; Huestis et al., 1996) and between 7 and 34 ng TCB per g in Baltic waters (Koistinen, 1990). Concentrations of TCB in livers of fish from this study (0.4–180 ng g⁻¹) are also relevant to reported concentrations of TCB in rainbow trout from the Great Lakes (Janz et al., 1992).

4.2. Phase I enzyme activity

The induction of Phase I enzyme activity, measured as EROD, and its positive correlation with TCB concentrations in the liver ($r^2 = 0.90$, $P < 0.05$), was an anticipated outcome. TCB has previously been shown to induce both AHH and EROD in fish (Melancon and Lech, 1976; Janz and Metcalfe, 1991; Monosson and Stegeman, 1991; Tyle et al., 1991; Murk et al., 1994). In fact, EROD induction is a reliable surrogate measure of exposure for planar organochlorine residue analysis in fish, birds and mammals (Hodson et al., 1991). EROD activity was detected in the livers of fish from this study at TCB concentrations below 3 ng/g w.w., which is similar to previous reports from other fish species (Lindstrom-Seppa et al., 1994; Gooch et al., 1989). While TCB has been shown to both induce EROD and

post-transcriptionally inhibit its activity (White et al., 1997), saturation of the EROD response was not achieved even at higher TCB concentrations in this experiment based on regression analysis.

4.3. Vitamins

Tissue concentrations of vitamin A have increasingly been examined in organisms exposed to planar organochlorines (Zile, 1992). Previous work has established that vitamin A declines in exposed fish (Ndayibagira et al., 1995), mammals (Mercier et al., 1990; Hakansson et al., 1992) and birds (Murk et al., 1994; Spear et al., 1990). Our earlier work has shown that lake trout orally exposed to pentachlorobiphenyl had depletions of vitamin A in the major storage organs, liver and kidney (Palace et al., 1996a,b, 1997). It is important to note that in the present study, there were no significant effects of TCB on plasma vitamin A. The plasma serves as a vehicle for delivery of vitamin A to the peripheral tissues, and likely because of this role, concentrations are maintained within a relatively narrow range (Blumhoff et al., 1990). When a disruptive factor like coplanar PCB exposure is introduced, major stores of vitamin A are often mobilized from the liver, and possibly even the kidney, to maintain steady plasma levels (Zile, 1992). Plasma concentrations are, therefore, only affected when storage organs have become severely depleted.

Vitamin A is often depleted in tissues of organisms exposed to organochlorines. Proposed mechanisms for this depletion include loss or transformation of stellate storage cells in the liver (Chen et al., 1992), loss of appetite (Spear et al., 1994) and increased utilization as an antioxidant (Palace et al., 1996a). Alternatively, induced phase I and II enzymes may directly increase metabolism of several vitamin A forms (Gilbert et al., 1995). However, in the current study, the opposite relationship was observed. Specifically, both recovered TCB in liver and hepatic EROD activity were positively correlated with higher concentrations of the free alcohol form of vitamin A in livers of female arctic grayling 90 days after exposure. The fact that this relationship was more prevalent in female fish compared with males is interesting given that Branchaud et al. (1995) also showed that female fish were more sensitive to vitamin A depletion from organochlorine exposure. It is possible that higher concentrations of the free alcohol form of vitamin A in livers of these fish was due to an inhibition of the enzyme system responsible for storing vitamin A by TCB or the endproducts of EROD activity. Alternatively, an activation of the enzyme system that liberates vitamin A from stored esters in the liver could also be responsible (Zile, 1992). Regardless of the mechanism, elevated concentrations of free vitamin A would not likely be maintained over the long term in liver without

subsequent loss from the stored vitamin A ester pools (Palace et al., 1997).

Vitamin E is used as a cellular antioxidant to protect membranes from oxidation (Packer, 1991). Since each vitamin E molecule protects about 1000 lipid molecules, even small reductions in concentration can significantly permit oxidative damage (Liebler, 1993). We have previously correlated elevated oxidative stress and depleted tissue vitamin E in fish exposed to a coplanar PCB (Palace et al., 1996a). Similar results have also been obtained in other aquatic invertebrates (Ribera et al., 1991). Only males exhibited a significant relationship in this experiment. We have not previously detected sexual differences in the susceptibility to oxidative stress from PCB exposure (Palace et al., 1999). Sexual differences in vitamin A and E sensitivity to TCB exposure in arctic species also require further study.

4.4. Thyroid hormones and glandular structure

TCB exposure and the induction of EROD had no effect on thyroid hormone levels or thyroid glandular structure in the present study. These were surprising results given the wealth of previous studies that have shown reduction in plasma thyroid hormones in fish (Leatherland and Sonstegard, 1978; Brown et al., 1997) and mammals (Kohn et al., 1996; Schuur et al., 1997) exposed to Ah-active organochlorines. Reduction of thyroxine in these models is probably mediated through the Ah receptor by induction of phase II conjugation enzymes that increase clearance rates of the hormone (Schuur et al., 1997). Lower thyroxine concentrations in plasma may result in chronically stimulated TSH levels that can subsequently induce glandular hyperplasia (Kohn et al., 1996; Brown et al., 1997). Although phase I activity was greatly induced, exposure times in the present study may not have been sufficient to induce phase II activity to an extent required to deplete thyroxine or to alter glandular structure. Later onset of phase II activity compared with phase I induction has long been established (Andersson et al., 1985). Other previous studies where short-term exposures to Ah-inducers have been utilized, also showed no effects on phase II enzyme activity or thyroid hormones in fish (Besselink et al., 1996, 1997).

4.5. Osmolality

The relationships between TCB tissue residues and elevated plasma osmolality in fish from this study are a unique finding. Quabius et al. (1997) also examined plasma ion content in fish exposed to exposed to PCBs but found no significant alterations. Sodium and chloride ions contribute between 80% and 90% of the total plasma osmolality. While disrupted plasma osmolality has frequently been used as a general indicator of stress

in fish (Folmar, 1993), there have been few reports that consider the specific mechanism by which PCB exposure could affect osmoregulation. Speculation that fish exposed to organochlorines have altered plasma ionic content as a secondary effect of lowered thyroid status are not consistent with the present study, where thyroid hormone levels were unaffected. We have previously shown that Ah-inducing contaminants elevate oxidative stress and membrane damage (Palace et al., 1996a). Increased permeability of the vascular endothelium as a result of oxidative metabolism induced by organochlorines has also been demonstrated in early life stages of fish (Cantrell et al., 1996). This type of damage could account for increased membrane permeability or the reduced capacity of ion pumps to maintain transmembrane potentials. Regardless of the mechanism involved, loss of ion homeostasis could be expected to adversely affect other physiological and biochemical processes in exposed fish (Vethaak, 1992).

5. Summary and conclusions

Dispositions of single, environmentally relevant doses of TCB in arctic grayling were extremely variable. Correlation analysis revealed a strong relationship between actual tissue concentrations of TCB and activity of the phase I enzyme EROD. Concentrations of the free alcohol form of vitamin A concentrations in liver increased in response to TCB accumulation and/or EROD induction in that tissue. While we have previously reported that oxidative stress secondary to phase I induction can result in depleted vitamin E concentrations, this effect was seen only in male fish from the present study. Coplanar PCBs can affect thyroid hormones in plasma and induce glandular hyperplasia, but neither effect was evident after the short exposure durations of this experiment. Total plasma osmolality was elevated by TCB at 30 days, but not after 90 days. If this effect is operating even transiently in field populations of fish, it has the potential to disrupt physiological and biochemical processes, including growth and reproduction. Given the potential for altered vitamin metabolism in TCB exposed grayling, studies regarding the nutritional status of field populations are needed. In this regard, sexual differences in susceptibility to vitamin depletion also require further consideration.

References

- Andersson, T., Pesonen, M., Johansson, C., 1985. Differential induction of cytochrome P-450-dependent monooxygenase, epoxide hydrolase, glutathione transferase and UDP-glucuronosyl transferase activities in the liver of rainbow trout by β -naphthoflavone and clophen A-50. *Biochem. Pharmacol.* 34, 3309–3314.
- Besselink, H.T., van Santen, E., Vorstman, W., Vethaak, A.D., Koeman, J.H., Brouwer, A., 1997. High induction of cytochrome P4501A activity without changes in retinoid and thyroid hormone levels in flounder (*Platichthys flesus*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ. Toxicol. Chem.* 16, 816–823.
- Besselink, H.T., vanBeusekom, S., Roe, E., Vethaak, A.D., Koeman, J.H., Brouwer, A., 1996. Low hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity and minor alterations in retinoid and thyroid hormone levels in flounder (*Platichthys flesus*) exposed to the polychlorinated biphenyl (PCB) mixture, Clophen A50. *Environ. Pollut.* 92, 267–274.
- Blumhoff, R., Green, M.H., Berg, T., Norum, K.R., 1990. Transport and storage of vitamin A. *Science* 250, 399–404.
- Branchaud, A., Gendron, A., Fortin, R., Anderson, P.D., Spear, P.A., 1995. Vitamin A stores teratogenesis and EROD activity in white sucker, *Catostomus commersoni*, from Riviere des Parairies near Montreal and a reference site. *Can. J. Fish. Aquat. Sci.* 52, 1703–1713.
- Brouwer, A., van den Berg, K.J., 1986. Binding of a metabolite of 3,3',4,4'-tetrachlorobiphenyl to trans-thyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxine. *Toxicol. Appl. Pharmacol.* 85, 301–312.
- Brown, S.B., Evans, R.E., Finsson, K.W., Vandenbyllardt, L., 1997. Thyroid function in lake trout (*Salvelinus namaycush*) exposed to co-planar 3,3',4,4',5-pentachlorobiphenyl. In: Proceedings of the 24th Annual Aquatic Toxicity Workshop, 20–22 October. Niagara Falls, Ontario. Canadian Technical Report of Fisheries Aquatic Science 2192, p. 135.
- Cantrell, S.M., Lutz, L.H., Tillitt, D.E., Hannink, M., 1996. Embryotoxicity of 2,3,7,8-tetrachloro-*p*-dioxin (TCDD): the embryonic vasculature is a physiological target for TCDD-induced DNA damage and apoptotic cell death in medaka (*Orizias latipes*). *Toxicol. Appl. Pharmacol.* 141, 23–34.
- Chen, L.C., Berberian, I., Koch, B., Mercier, M., Azais-Braesco, V., Glauert, H.P., Chow, C.K., Robertson, L.W., 1992. Polychlorinated and polybrominated biphenyl congeners and retinoid levels in rat tissues: structure-activity relationships. *Toxicol. Appl. Pharmacol.* 114, 47–55.
- Fok, P., Eales, J.G., Brown, S.B., 1990. Determination of 3,5,3'-triiodo-L-thyronine (T3) levels in tissues of rainbow trout (*Salmo gairdneri*) and the effect of low ambient pH and aluminum. *Fish. Physiol. Biochem.* 8, 281–290.
- Folmar, L.C., 1993. Effects of chemical contaminants on blood chemistry of teleost fish: a bibliography and synopsis of selected effects. *Environ. Toxicol. Chem.* 12, 337–375.
- Gilbert, N.L., Cloutier, M.J., Spear, P.A., 1995. Retinoic acid hydroxylation in rainbow trout (*Oncorhynchus mykiss*) and the effect of a coplanar PCB, 3,3',4,4'-tetrachlorobiphenyl. *Aquat. Toxicol.* 32, 177–187.
- Gingerich, W.H., Pityer, R.A., 1989. Comparison of whole body and tissue blood volumes in rainbow trout (*Salmo gairdneri*) with 125-I bovine serum albumin and 51-Cr-erythrocyte tracers. *Fish. Physiol. Biochem.* 6, 39–47.
- Gooch, J.W., Elkus, A.A., Klopper-Sams, M.E., Stegeman, J.J., 1989. Effects of ortho- and non-ortho-substituted polychlorinated biphenyl congeners on the hepatic monooxygenase system in scup (*Stenotomus chrysops*). *Toxicol. Appl. Pharmacol.* 98, 422–433.

- Hakansson, H., Manzoor, E., Ahlborg, U.G., 1992. Effects of technical PCB preparations and fractions thereof on vitamin A levels in the mink (*Mustela vison*). *Ambio* 21, 588–590.
- Hodson, P.V., Kloepper-Sams, P.J., Munkittrick, K.R., Lockhart, W.L., Metner, D.A., Luxon, P.L., Smith, I.R., Gagnon, M.M., Servos, M., Payne, J.F., 1991. Protocols for measuring mixed function oxygenases of fish liver. Canadian Technical report of Fisheries and Aquatic Sciences #1829, 51 pp.
- Huestis, S.Y., Servos, M.R., Whittle, D.M., van den Heuvel, M., Dixon, D.G., 1997. Evaluation of temporal and age-related trends of chemically and biologically generated 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents in Lake Ontario lake trout, 1977–1993. *Environ. Toxicol. Chem.* 16, 154–164.
- Huestis, S.Y., Servos, M.R., Whittle, D.M., Dixon, D.G., 1998. Temporal and age-related trends in levels of polychlorinated biphenyl congeners and organochlorine contaminants in Lake Ontario lake trout (*Salvelinus namaycush*). *J. Great Lakes Res.* 22, 310–330.
- Janz, D.M., Metcalfe, T.L., Metcalfe, C.D., 1992. Relative concentrations of cytochrome P450-active organochlorine compounds in liver and muscle of rainbow trout from Lake Ontario. *J. Great Lakes Res.* 18, 759–765.
- Janz, D.M., Metcalfe, C.D., 1991. Relative induction of aryl hydrocarbon hydroxylase by 2,3,7,8-TCDD and two coplanar PCBs in rainbow trout (*Oncorhynchus mykiss*). *Environ. Toxicol. Chem.* 10, 917–923.
- Kannan, N., Tanabe, S., Wakimoto, T., Tatsukawa, R., 1987. Coplanar polychlorinated biphenyls in aroclor and kenecolor mixtures. *J. Assoc. Off. Anal. Chem.* 70, 451–454.
- Kohn, M.C., Sewall, C.H., Lucier, G.W., Portier, C.J., 1996. A mechanistic model of effects of dioxin on thyroid hormones in the rat. *Toxicol. Appl. Pharmacol.* 165, 29–48.
- Koistinen, J., 1990. Residues of planar polyaromatic compounds in Baltic fish and seal. *Chemosphere* 20, 7–9.
- Leatherland, J.F., Sonstegard, R.A., 1978. Lowering of serum thyroxine and triiodothyronine levels in yearling coho salmon, *Oncorhynchus kisutch* by dietary Mirex and PCBs. *J. Fish. Res. Bd. Can.* 35, 1285–1289.
- Lieber, D.C., 1993. The role of metabolism in the antioxidant function of vitamin E. *Crit. Rev. Toxicol.* 23, 147–169.
- Lindstrom-Seppa, P., Korytko, P.J., Hahn, M.E., Stegeman, J.J., 1994. Uptake of waterborne 3,3',4,4'-tetrachlorobiphenyl and organ and cell-specific induction of cytochrome P4501A in adult and larval fathead minnow *Pimephales promelas*. *Aquat. Toxicol.* 28, 147–167.
- Mac, M.J., Schwartz, T.R., Edsall, C.C., Frank, A.M., 1993. Polychlorinated biphenyls in great lakes lake trout and their eggs: relations to survival and congener composition 1979–1988. *J. Great Lakes Res.* 19, 752–765.
- Melancon, M.J.J., Lech, J.J., 1976. Isolation and identification of a polar metabolite of tetrachlorobiphenyl from bile of rainbow trout exposed to 14-C tetrachlorobiphenyl. *Bull. Environ. Contam. Toxicol.* 15, 181–189.
- Mercier, M., Pascal, G., Azais-Braesco, V., 1990. Retinyl esterhydrolase and vitamin A status in rats treated with 3,3',4,4'-tetrachlorobiphenyl. *Biochim. Biophys. Acta.* 1047, 70–76.
- Monosson, F., Fleming, W.J., Sullivan, C.V., 1994. Effects of the planar 3,3',4,4'-tetrachlorobiphenyl (TCB) on ovarian development, plasma levels of sex steroid hormones and vitellogenin, and progeny survival in the white perch (*Morone americana*). *Aquat. Toxicol.* 29, 1–19.
- Monosson, F., Stegeman, J.J., 1991. Cytochrome P450E (P4501A) induction and inhibition in winter flounder by 3,3',4,4'-tetrachlorobiphenyl: comparison of response in fish from Georges Bank and Narragansett Bay. *Environ. Toxicol. Chem.* 10, 765–774.
- Muir, D.C.G., Ford, C., Rosenberg, B., Grift, B., 1993. Coplanar PCBs in arctic marine mammals and fish. In: Murray, J.L., Shearer, J.L. (Eds.), *Synopsis of Research Conducted Under the 1992–1993 Northern Contaminants Program*. Environmental Studies No. 70, pp. 99–104.
- Murk, A.J., vandenBerg, H.J., Fellingner, M., Rozemeijer, M.J.C., Swennen, C., Duiven, P., Boon, J.P., Brouwer, A., Koeman, J.H., 1994. Toxic and biochemical effects of 3,3',4,4'-tetrachlorobiphenyl (CB-77) and Clophen A50 on eider ducklings (*Somateria mollissima*) in a semi-field experiment. *Environ. Pollut.* 86, 21–30.
- Muir, D.C.G., Yarechewski, A.L., Metner, D.A., 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.
- Ndayibagira, A., Cluotier, M.J., Anderson, P.D., Spear, P.A., 1995. Effects of 3,3',4,4'-tetrachlorobiphenyl on the dynamics of vitamin A in brook trout (*Salvelinus fontinalis*) and intestinal retinoid concentrations in lake surgeon (*Acipenser fulvescens*). *Can. J. Fish. Aquat. Sci.* 52, 512–520.
- Newted, J.L., Giesy, J.P., Ankley, G.T., Tillitt, D.E., Crawford, R.A., Gooch, J.W., Jones, P.D., Denison, M.S., 1995. Development of toxic equivalency factors for PCB congeners and the assessment of TCDD and PCB mixtures in rainbow trout. *Environ. Toxicol. Chem.* 14, 861–871.
- Niimi, A., Oliver, B.G., 1989. Assessment of relative toxicity of chlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls in Lake Ontario salmonids to mammalian systems using toxic equivalent factors (TEF). *Chemosphere* 18, 1413–1423.
- Niimi, A., 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.
- Packer, L., 1991. Protective role of vitamin E in biological systems. *Am. J. Clin. Nutr.* 53, 1050S–1055.
- Palace, V.P., Baron, C.L., Klaverkamp, J.F., 1998. An assessment of Ah-inducible phase I and phase II enzymatic activities and oxidative stress indices in adult lake trout (*Salvelinus namaycush*) from Lake Ontario and Lake Superior. *Aquat. Toxicol.* 42, 149–168.
- Palace, V.P., Klaverkamp, J.F., Baron, C.L., Brown, S.B., 1997. Metabolism of ³H-retinol by lake trout (*Salvelinus namaycush*) pre-exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB 126). *Aquat. Toxicol.* 39, 321–332.
- Palace, V.P., Brown, S.B., 1994. HPLC determination of tocopherol, retinol, dihydroretinol and retinyl palmitate in tissues of lake char (*Salvelinus namaycush*) exposed to coplanar 3,3',4,4',5-pentachlorobiphenyl. *Environ. Toxicol. Chem.* 13, 473–476.
- Palace, V.P., Klaverkamp, J.F., Lockhart, W.L., Metner, D.A., Muir, D.C.G., Brown, S.B., 1996a. Mixed-function oxidase

- enzyme activity and oxidative stress in lake trout (*Salvelinus namaycush*) exposed to 3,3',4,4',5-pentachlorobiphenyl (PCB-126). *Environ. Toxicol. Chem.* 15, 955–960.
- Palace, V.P., Dick, T.A., Brown, S.B., Baron, C.L., Klaverkamp, J.F., 1996b. Oxidative stress in lake sturgeon (*Acipenser fulvescens*) orally exposed to 2,3,7,8-tetrachlorodibenzofuran. *Aquat. Toxicol.* 35, 79–92.
- Quabius, E.S., Balm, P.H.M., Bonga, S.E.W., 1997. Interrenal stress responsiveness of tilapia (*Oreochromis mossambicus*) is impaired by dietary exposure to PCB 126. *Gen. Comp. Endocrin.* 108, 472–482.
- Ribera, D., Narbonne, J.F., Michel, X., Livingstone, D.R., O'Hara, S., 1991. Responses of antioxidants and lipid peroxidation in mussels to oxidative damage exposure. *Comp. Biochem. Physiol.* 100C, 177–181.
- Ricker, W.E., 1979. Growth rates and models. In: Hoar, W.S., Randall, D.J., Brett, J.R. (Eds.), *Fish Physiology*. American Fisheries Society, Bethesda, MD, pp. 301–324.
- Schuur, A.G., Bockhorst, F.M., Brouwer, A., Visser, T.J., 1997. Extrathyroidal effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on thyroid hormone turnover in male Sprague–Dawley rats. *Endocrinology* 138, 3727–3734.
- Sijm, D.T.H.M., Yarechewski, A., Muir, D.C.G., Webster, G.R.B., Seinen, W., Opperhuizen, A., 1990. Biotransformation and tissue distribution of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin, 1,2,3,4,7-pentachlorodibenzofuran in rainbow trout. *Chemosphere* 21, 845–866.
- Smith, L.M., Schwartz, T.R., Feltz, K., 1990. Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran in Lake Michigan sediment and biota. The question of their relative toxicological significance. *Chemosphere* 21, 1063–1085.
- Spear, P.A., Bourbonnais, D.H., Norstrom, R.J., Moon, T.W., 1990. Yolk retinoids (vitamin A) in eggs of the herring gull and correlations with polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Environ. Toxicol. Chem.* 9, 1053–1061.
- Spies, R.B., Stegeman, J.J., Rice, D.W.J., Woodin, B., Thomas, P., Hose, J.E., Cross, J.N., Prieto, M., 1990. Sublethal responses of *Platichthys stellatus* to organic contamination in San Francisco Bay with emphasis on reproduction. In: McCarthy, J.F., Shugart, L.R. (Eds.), *Biomarkers of Environmental Contamination*. Lewis Publication, Chelsea, MI, pp. 87–121.
- Tyle, H., Egsmore, M., Harri, N., 1991. Mixed-function oxygenase in juvenile rainbow trout exposed to hexachlorobenzene or 3,3',4,4'-tetrachlorobiphenyl. *Comp. Biochem. Physiol.* 100C, 43–48.
- Vethaak, A.D., 1992. Diseases of flounder (*Platichthys flesus*) in the Dutch Wadden Sea and their relation to stress factors. *Neth. J. Sea Res.* 29, 257–272.
- White, R.D., Shea, D., Solow, A.R., Stegeman, J.J., 1997. Induction and post-transcriptional suppression of hepatic cytochrome P4501A1 by 3,3',4,4'-tetrachlorobiphenyl. *Biochem. Pharmacol.* 53, 1029–1040.
- Williams, L.L., Giesy, J.P., DeGalan, N., Verbrugge, D.A., Tillitt, D.E., Ankley, G.T., 1992. Prediction of concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents from total concentrations of polychlorinated biphenyls in fish filets. *Environ. Sci. Technol.* 26, 1151–1159.
- Yoshimura, H., Yamamoto, H., 1973. Metabolic studies of polychlorinated biphenyls. I. Metabolic fate of 3,3',4,4'-tetrachlorobiphenyl in rats. *Chem. Pharm. Bull.* 21, 2237–2238.
- Zile, M., 1992. Vitamin a homeostasis endangered by environmental pollutants. *Proc. Soc. Exp. Biol. Med.* 201, 141–153.