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Altered thyroid status in lake trout (*Salvelinus namaycush*) exposed to co-planar 3,3′,4,4′,5-pentachlorobiphenyl

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Abstract

Recent studies indicate that co-planar 3,3′,4,4′,5-pentachlorobiphenyl (PCB) congeners or their metabolites may disrupt thyroid function in fishes. Although co-planar PCB have been detected at microgram per kilogram levels in fish from contaminated areas, few studies have examined mechanisms whereby, co-planar PCBs may alter thyroid function in fish. We treated immature lake trout by intraperitoneal (i.p.)-injection or dietary gavage with vehicle containing 0, 0.7, 1.2, 25 or 40 μg 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) per kg BW. Blood and tissue samples were collected at various times up to 61 weeks following exposure. The treatments produced sustained dose-dependent elevations of tissue (PCB 126) concentrations. Thyroid epithelial cell height (TECH), plasma thyroxine (T_4) and 3,3′,5-triiodo-L-thyronine (T_3) concentrations, hepatic 5′-monodeiodinase, hepatic glucuronidation of T_4 and T_3, as well as plasma T_4 kinetics and fish growth were analyzed. Exposure to the highest doses of PCB 126 caused increased TECH, plasma T_4 dynamics and T_4-glucuronidation (T_4-G). PCB 126 did not affect 5′-monodeiodinase and T_3-glucuronidation (T_3-G) and there were no effects on fish growth or condition. Because T_3 status and growth were unaffected, the thyroid system was able to compensate for the alterations caused by the PCB 126 exposure. It is clear that concentrations of co-planar PCBs similar to those found in predatory fish from contaminated areas in the Great Lakes are capable of enhancing metabolism of T_4. These changes may be of significance when T_4 requirements are high for other reasons (e.g. periods of rapid growth, warm temperatures, metamorphosis, and parr-smolt transformation).

Keywords: Endocrine disruption; Non-ortho PCBs; Thyroid hormones

1. Introduction

The co-planar 3,3′,4,4′,5-pentachlorobiphenyl PCBs (i.e., congeners with lateral but no *ortho* chlorine substitution) can adopt a planar shape similar in structure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD).

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These planar TCDD-like compounds bind to a cytosolic protein (ah-receptor) that has been connected with a variety of toxic responses: thymic involution, teratogenicity and metabolic disturbances leading to "wasting" (Goldstein and Safe, 1989). In the predatory fishes of the Great Lakes, the co-planar PCBs represent a predominant source of dioxin-like toxic equivalents (TEQs) (Janz and Metcalfe, 1991; Huestis et al., 1996; Giesy et al., 1997). Of the various co-planar PCBs, 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126) often contributes most significantly to observed TEQs (Huestis et al., 1997; Giesy et al., 1997). The co-planar PCBs and other TCDD-like compounds are also known disruptors of thyroidal function in mammals (Brouwer et al., 1998). There are few studies assessing the consequences of these compounds on the thyroidal status of fish (Besselink et al., 1997; Brown et al., 1998, 2002; Adams et al., 2000; Grinwis et al., 2000) and they are generally limited in their overall scope.

Thyroid hormones together with other hormone systems, promote growth, development and early reproductive events, and are generally associated with protein anabolism in fish (Eales and MacLatchy, 1989). The thyroid of fish, under the control of thyroid stimulating hormone (TSH) from the pituitary, secretes L-thyroxine (T_4) into the circulation. T_4 enters target cells where it undergoes outer-ring 5′-monodeiodination (T4ORD) to 3,3′,5-triiodo-L-thyronine (T_3). Because T_3 is the most effective endogenous ligand for hormone receptor binding, it is considered the active thyroid hormone (Eales and Brown, 1993). One of the routes for inactivation and excretion of T_4 and T_3 is via their conjugation (glucuronidation and sulfation) and elimination in the bile and urine.

There is no single biomarker capable of examining all aspects of fish thyroid function. Eales et al. (1999) recommend that screening for xenobiotic effects on the fish thyroid system requires monitoring at three levels: (i) the centrally-controlled thyroidal secretion of T_4 prohormone to the plasma (T_4 dynamics), (ii) the peripherally-controlled conversion of T_4 to active T_3 (T_3 dynamics), and (iii) the post-receptor effects of T_3. The centrally-controlled thyroidal secretion of T_4 can be monitored from the plasma total and free T_4 levels and from thyroid or thyrotrope histological appearance. The peripherally-controlled conversion of T_4 to T_3 and the T_3 availability to target cell receptors cannot be monitored reliably from plasma T_3 level alone. However, the potential for producing T_3 can be assessed in vitro from the activities of a suite of rate-limiting deiodinations in liver and brain. These deiodinases are highly sensitive to environmental factors including stressors (Johnston et al., 1996). Hence deiodination activities in conjunction with plasma T_4 and T_3 levels may more adequately evaluate peripheral thyroidal (T_3) status (Eales et al., 1999). Assays for the post-receptor effects of T_3 are currently under development and have not been routinely applied to assess the impacts of environmental factors on fish thryoidal status (Eales et al., 1999).

Recently, we reported that dietary exposure of young (0+) rainbow trout to PCB 126 lowered muscle T_3 and T_4 stores (Brown et al., 2002). To further investigate the potential of PCB 126 to impact thyroidal status in fish, we examined the consequences of long-term exposure to PCB 126 on thyroidal status of juvenile lake trout (*Salvelinus namaycush*) given sublethal doses by intraperitoneal injection or orally by gelatin gavage. The dosages were designed to produce tissue burdens of PCB 126 comparable to those reported in predatory fishes of the Great Lakes (Huestis et al., 1997; Giesy et al., 1997) as well as higher concentrations more characteristic of possible historical concentrations. We assessed a variety of indices of thyroidal status (thyroidal histology, plasma thyroid hormone (T_4 and T_3) concentrations, hepatic thyroid enzymes and plasma T_4 kinetics) that are capable of detecting the effects of environmental factors on both central and peripheral control mechanisms of the thyroid axis. Although we did not monitor for specific post-receptor effects of T_3, we did use fish growth and condition to assess the overall functional integrity of the various systems associated with protein anabolism in fish.

2. Materials and methods

2.1. Fish maintenance

Lake trout, *S. namaycush*, (3 + years, weight, 336 ± 7 g, fork length 32.8 ± 0.4 cm (X ± S.E.), and $N = 240$) were held at the Freshwater Institute, Winnipeg, Manitoba. The stock was obtained as eggs

from brood-stock in White Swan Lake, Saskatchewan. Fish were reared in 500 l fibre glass tanks with flowing, aerated, dechlorinated Winnipeg city water containing the following major ion concentrations: 0.51 mmol Ca^{2+}/l, 0.24 mmol Mg^{2+}/l, 0.14 mmol Cl^{-}/l, 0.08 mmol Na^{+}/l, and 0.04 mmol K^{+}/l. Water hardness was equivalent to 0.75 mmol $CaCO_3$/l (75 mg/l) with an average pH 7.7. Fish were fed Martin Feeds trout food (Elmira, Ontario) at a ration of 1.5% wet BW every second day.

2.2. Chemicals

Uniformly ring-labelled [^{14}C]-3,3′,4,4′,5-pentachlorobiphenyl (specific activity $= 8.7 \times 10^{11}$ Bq/mol) was obtained from Sigma Chemical Co. Unlabelled PCB 126 was purchased from Ultra Scientific. Reverse-phase HPLC with radiochemical detection and capillary GC analysis, with electron-capture detection showed a radiochemical purity of >99%.

2.3. Experimental fish

Treated fish were held in fibre glass tanks (130 l). Temperature was maintained between 11.5 and 13.1 °C and the photoperiod was adjusted to 12 h light and 12 h darkness. Fish were fed trout food at a ration of 1% wet BW every second day. Initially, fish were lightly anaesthetized in water containing tricaine methanesulphonate (TMS, 0.38 mmol/l) solution neutralized to tank pH with ammonium hydroxide and added NaCl (150 mmol/l) approximately iso-osmotic with fish plasma. Visual implant tags (Northwest Marine Technology, Shaw Island, WA, USA) were implanted in the clear cartilage posterior to the eye and fish were allowed 4 weeks recovery and acclimation prior to dosing. Treated fish were given either a single intraperitoneal injection (Experiment 1) or a single oral dose (Experiments 2 and 3) containing vehicle or PCB 126. Fish recovered from the procedures in anesthetic-free water within 3 min. Both control and treated fish were housed together in each tank. Secondary uptake of excreted PCB was limited by removing the feces and continuous recirculation and filtration of the tank holding water with activated charcoal. There was no evidence of any radiolabelled materals in hexane/ethyl acetate extracts prepared from 2 l samples of the tank water taken the day after and at 1, 2 or 4 weeks after dosing.

2.4. Experiment 1: i.p.-injection

Injection solutions were prepared by evaporating a hexane solution of PCB 126 to near dryness under N_2 and then mixing with corn oil. After acclimation, fish were lightly anaesthetized in pH-neutralized TMS and NaCl. When the fish lost equilibrium, they were blotted to remove excess moisture, weighed and injected (1 ml/kg) into the peritoneal cavity with corn oil (controls) or corn oil containing doses of PCB 126 that produced exposures of 0.7 or 25 μg [^{14}C] PCB 126 per kg BW. For each sampling period, five fish from each treatment group were randomly allocated to a pair of holding tanks. After 1, 3, 6, 13, 20, and 30 weeks, trout were anaesthetized directly in the paired test tanks by adding pH-neutralized TMS (0.76 mmol/l) solution. All fish were starved for 3 days before sampling. Blood was removed from the caudal vessels in 2–3 min using preheparinized 3 or 5 ml syringes and 18 gauge needles. Plasma was immediately separated by centrifugation and stored at −90 °C in polyethylene vials. To minimize potential diurnal effects, samples were taken between 0830 and 1000 h each day. Immediately after final blood sampling, tissues were quickly dissected, weighed and processed as required for thyroid histology, liver enzyme analysis, and tissue PCB 126 levels.

2.5. Experiment 2: oral gavage

Oral gavage solutions were prepared by dissolving PCB 126 in ethanol and warmed gelatin (Sjim et al., 1990). Fish were lightly anaesthetized in water containing pH-neutralized TMS with NaCl weighed and given gelatin/ethanol solution (2 ml/kg BW) containing PCB 126 to produce exposure concentrations of 0 (control), 1.2 or 40 μg [^{14}C] PCB 126 per kg BW. The warmed gavage solution (approximately 40 °C) was directly introduced to the stomach through the mouth using a syringe and a polyethylene catheter (approximately 10 cm) that had been heat-flared to prevent tissue damage. The gelatin solution rapidly solidified on the stomach walls of the much cooler fish. For each sampling period, five fish from each treatment group were randomly allocated to a pair of holding tanks. After 1, 3, 8, 19, 40, and 61 weeks,

trout were anaesthetized directly in the test tanks by adding pH-neutralized TMS (0.76 mmol/l) solution and sampled similar to Experiment 1.

2.6. Experiment 3: plasma [^{125}I]T_4 kinetics

Trout (five per treatment) were given vehicle and PCB 126 (40 μg/kg BW) by the oral gavage procedure. After 7 weeks, fish were cannulated and allowed to recover from surgical procedure free-swimming in individual 120 l tanks for 7 days prior to their use in kinetic experiments (Brown et al., 1986). Fish plasma was used to suspend [^{125}I]T_4 (33.3 GBq/μmol). The [^{125}I]T_4 in the plasma vehicle (50 μl) was injected into the fish through the catheter without the need for further manipulation or anaesthesia. The contents of the tubing were also immediately flushed into the fish with an additional 100 μl of injection vehicle and 100 μl of physiological saline. After corrections for iodide contamination, it was calculated that each fish received 54.0 ± 0.7 MBq [^{125}I]T_4. Preliminary experiments indicated that residual radioactivity in the cannula attached to the fish was low (<3 Bq) and did not contaminate subsequent blood samples. Blood samples (200 μl) were removed after discarding the cannula dead volume at 5, 15, 45, 120, 420, 900, and 1440 min. The total blood volume removed prior to the last sample was <10% based on a blood volume of 5.0% BW (Sefkow et al., 1996). To determine kinetics, plasma [^{125}I]T_4 was isolated from plasma samples using specific antibodies and column separation techniques as described previously by Eales (1977).

2.7. PCB extraction from tissue

Tissue samples were freeze dried prior to extraction. To extract ^{14}C, muscle (5–10 g) or liver (0.2–0.5 g) samples were homogenized in toluene, centrifuged (10,000 × g). 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. Counts were automatically corrected for background by the counter and were corrected for quench using a quench curve prepared from ^{14}C-toluene (DuPont). Given the specific activity of the [^{14}C]-PCB 126, detection limits were 0.005 μg/kg in muscle tissue and 0.05 μg/kg in liver tissue.

2.8. Histology

Lake trout thyroid tissue consists of glandular follicles scattered about the ventral aorta at the base of the branchial arches (particularly arches 1 and 2). Tissues were fixed for 24 h in Bouin's fluid, washed in 70% ethanol, dehydrated in 1-butanol and embedded in paraffin as described by Brown et al. (1998). The slide preparations (10 μm sections) were stained with Harris' hematoxylin and eosin, and examined for pathological alterations. For morphometry, the microscopic image of each tissue was projected onto a digitizing board interfaced with a PC. Thyroid epithelial cell thickness (TECH) determinations were made for each fish. The first 10 follicles observed with the microscope were measured. Four measurements of cell height (from basement membrane to surface adjacent to colloid) were made at opposite horizontal and vertical extremes of each follicle (40 cells per fish). Mean cell height was calculated by the direct method of Kalisnik et al. (1977).

2.9. Plasma thyroid hormone analyses

Radioimmunoassays for T_3 and T_4 used established procedures (Brown and Eales, 1977).

2.10. Hepatic enzymes for thyroid hormones

High-Km microsomal outer-ring 5′-deiodination of [^{125}I]T_4 (T_4ORD) was determined at a substrate concentration of 0.05 nM using the method outlined in MacLatchy and Eales (1992). Measurements of microsomal glucuronidation of T_4 and T_3 followed procedures described by Finnson and Eales (1997). Microsomal uridine-diphosphoglucuronosyl transferase activity (UDPGT) was determined using the procedures outlined by Palace et al. (1996).

2.11. Statistics

PCB 126 concentration data were corrected for growth dilution. Growth rate was calculated from the equation (specific growth = (ln(wt2) − ln(wt1))/(t2 − t1)) outlined by Bagenal (1978). Bartlett's test for homogeneity of variance was applied to the data. For clarity of presentation, arithmetic means and standard errors are given in results. Two-way ANOVA was

computed and Tukey's studentized range test was used to evaluate differences between group means. Probabilities of <0.05 were considered significant. A three-compartment model was used for the analysis of T_4 kinetics. The nonlinear regression equation $Y(t) = A_1 e^{-a1t} + A_2 e^{-a2t} + A_3 e^{-a3t}$, where Y represents the fraction of $[^{125}I]T_4$ in 1 ml of plasma was used to calculate T_4 plasma clearance rate, T_4 degradation rate and T_4 pool sizes for a three-pool model (Sefkow et al., 1996).

3. Results and discussion

3.1. Tissue concentrations of PCB 126

Both i.p.-injection and oral gavage of PCB 126 produced dose-dependent increases in tissue PCB levels (Fig. 1). The lower PCB 126 doses resulted in tissue concentrations similar to those reported for predatory fish (salmonids and walleye) in the Great Lakes (Janz et al., 1992; Giesy et al., 1997). The tissue levels produced by the high dose are more representative of possible historical levels reported for predatory salmonids from Lake Ontario (Huestis et al., 1997) and Lake Michigan (Smith et al., 1990). If TCDD–TEQs are considered (Williams et al., 1992; Brown et al., 2002), our low and high dose produced muscle TEQs that between experiments averaged 0.095 (low dose group) and 2.60 (high dose group) nanogram per kilogram after 8–13 weeks exposure. Thus the highest experimental doses produced muscle TCDD–TEQs that are about 20 times lower than some estimates for predatory fish in Lake Ontario (Huestis et al., 1997) and Lake Michigan (Giesy et al., 1997).

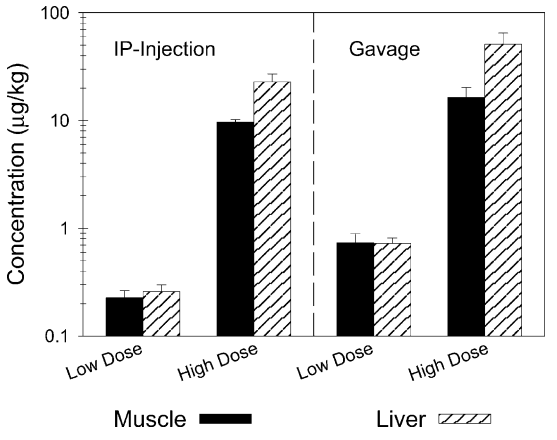

Fig. 1. Tissue PCB 126 concentration in lake trout exposed by i.p.-injection to vehicle, a low dose (0.7 μg/kg) and a high dose (25 μg/kg) of PCB 126 for 13 weeks or by gavage to vehicle, a low dose (1.2 μg/kg) and a high dose (40 μg/kg) of PCB 126 for 8 weeks. Values represent mean (±S.E.) of five fish in each group.

3.2. Thyroid histology

Measurements of epithelial cell height of thyroid follicular cells showed that the higher PCB 126 doses were capable of temporarily inducing mild hypertrophy at 13 weeks exposure in Experiment 1 and at 8 weeks exposure in Experiment 2 (Fig. 2). There was no evidence of development of overt goitre or microfollicular hyperplasia that has been associated with introduced salmonids from the Great Lakes

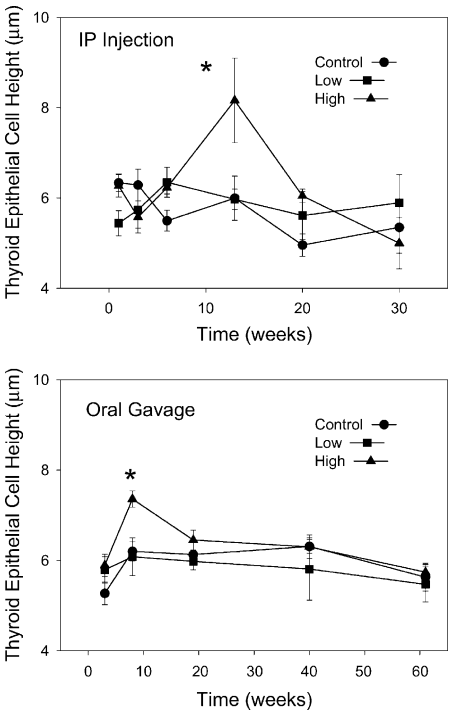

Fig. 2. Thyroid epithelial cell height in lake trout exposed by i.p.-injection to vehicle, a low dose (0.7 μg/kg) and a high dose (25 μg/kg) of PCB 126 or orally to vehicle, a low dose (1.2 μg/kg) and a high dose (40 μg/kg) of PCB 126. Values represent mean (±S.E.) of five fish in each group.

(Leatherland, 1993). There have been few other studies where thyroid histology has been examined in fishes exposed to planar TCDD-like compounds. Brown et al. (2002) found no difference in thyroid histology after 4 weeks of dietary exposure to PCB 126 in juvenile rainbow trout and Brown et al. (1998) found no effect of 2,3,4,7,8-pentachlorodibenzofuran on thyroid histology 40 weeks following initial exposure of adult rainbow trout. Owing to the transitory nature of the response it is highly likely that changes in TECH were missed in these previous studies. In teleosts, the thyroid tissue generally consists of diffuse follicules scattered around the base of the ventral aortic gill arches (Eales, 1979). Therefore, any minor proliferation of thyroid tissue is fairly difficult to determine. In flounder, there were few changes apparent in thyroid histology 1–8 weeks following oral TCDD exposures (Grinwis et al., 2000). Long-term exposure to dietary PCBs given as Aroclor mixtures for 4–12 weeks also showed no evidence of thyroid hyperplasia in coho salmon (Leatherland and Sonstegard, 1978) and rainbow trout (Leatherland and Sonstegard, 1979).

3.3. Plasma thyroid hormones

Plasma concentrations of T_4 were elevated at 6 and 13 weeks and depressed relative to the controls at 30 weeks by the high dose of PCB 126 in i.p.-injected fish (Fig. 3). Plasma concentrations of T_3 followed a similar pattern but did not differ from controls. In contrast, there were no changes in plasma levels when fish were exposed by oral gavage (Fig. 4). The reason for this is not clear but may related to potential differences in pharmacokinetics and tissue distribution associated with the different routes of exposure. In Arctic grayling, single dietary 3,3′,4,4′-tetrachlorobiphenyl (PCB 77) doses (10, 100, and 1000 μg/kg BW) did not alter plasma thyroid hormones or thyroid histology at 30 or 90 days post-treatment (Palace et al., 2001). Plasma T_4 levels were elevated in plaice following short-term i.p.-injections (1-week) of a high dose (500 μg/kg BW) of co-planar PCB 77 but not PCB 126 (Adams et al., 2000). In the same experiment, plasma T_3 levels were unaffected. Besselink et al. (1997) exposed flounder to TCDD (up to 100 μg/kg BW) by oral gavage and reported that after 10 days the highest dose lowered plasma concentrations of

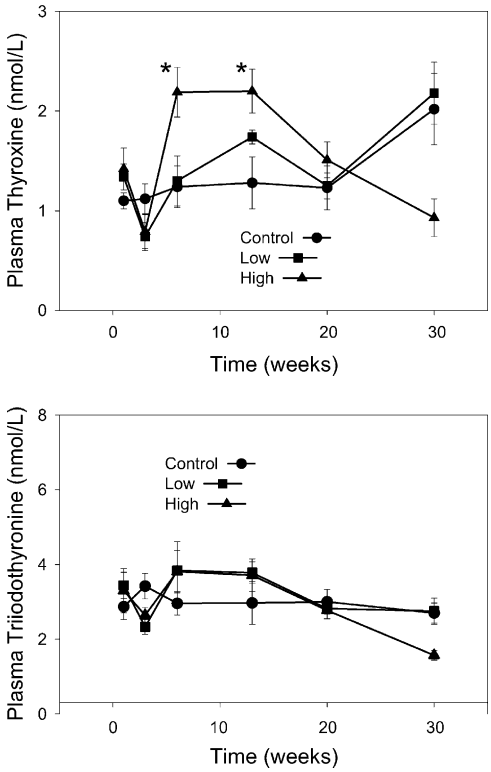

Fig. 3. Plasma T_4 and T_3 in lake trout exposed to vehicle, a low dose (0.7 μg/kg) and a high dose (25 μg/kg) of PCB 126 by i.p.-injection. Values represent mean (±S.E.) of five fish in each group.

total T_4 but did not affect the concentration of free T_4 or total T_3. In an earlier study, Besselink et al. (1996) exposed flounder to Clophen A50 by i.p.-injection and after 10 days observed some changes in plasma T_4 and T_3. Long-term exposure (4–12 weeks) of coho salmon to Aroclor mixtures produced lower plasma T_3 concentrations but did not affect plasma T_4 levels (Leatherland and Sonstegard, 1978). There was no effect of Aroclor mixture 1254 on plasma thyroid hormone levels in one study on rainbow trout (Leatherland and Sonstegard, 1979), while in a second study plasma thyroid hormone levels were depressed (Leatherland and Sonstegard, 1980). Exposure of coho salmon smolts to Aroclor mixture 1254 and fuel oil increased circulating levels of thyroid hormones (Folmar et al., 1982). Therefore, PCB exposures in fishes do not appear to elicit consistently detectable responses in levels of plasma thyroid hormones.

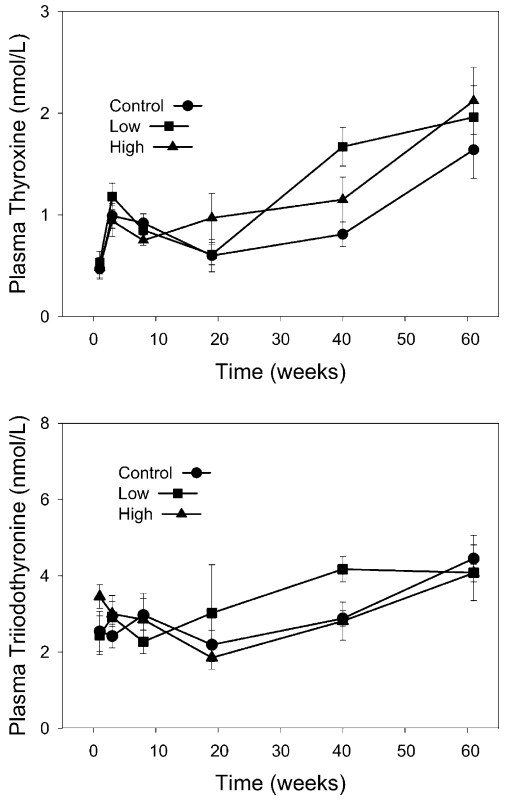

Fig. 4. Plasma T_4 and T_3 in lake trout exposed to vehicle, a low dose (1.2 μg/kg) and a high dose (40 μg/kg) of PCB 126 by gavage. Values represent mean (±S.E.) of five fish in each group.

3.4. Hepatic enzymes

The activity of T_4ORD, the enzyme responsible for conversion of T_4 prohormone to T_3, increased between 1 and 3 weeks (Fig. 5). The initial very low levels may be related to stress effects associated with handling for dosing (Johnston et al., 1996). However, the exposure to PCB 126 by oral gavage did not alter T_4ORD for up to 19 weeks following treatment. Adams et al. (2000) examined thyroid hormone deiodination in plaice following short-term exposure to both PCB 126 and PCB 77 by i.p.-injection. They found that PCB 77 could influence T_4ORD but consistent with our findings in lake trout, PCB 126 did not alter T_4ORD in plaice. This difference in effect between PCB 126 and PCB 77 may be due to the greater potential for the latter to be hydroxylated (Brouwer and van den Berg, 1986). Adams et al. (2000) also investigated the inner-ring

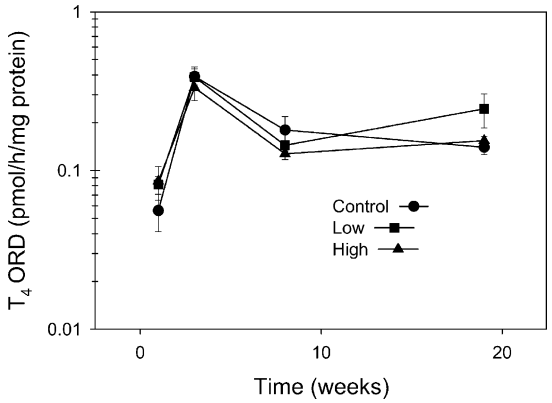

Fig. 5. Hepatic T_4ORD in lake trout exposed to vehicle, a low dose (1.2 μg/kg) and a high dose (40 μg/kg) of PCB 126 by oral gavage. Values represent mean (±S.E.) of five fish in each group.

deiodination (IRD) pathways responsible deactivation of T_4 and T_3. Enhancement of these inactivating pathways was a significant effect of PCB 126 in plaice. In plaice and trout, it is likely that effects of PCBs on deiodinating activities occur via indirect mechanisms because in vitro exposure of microsomes to co-planar PCBs or hydroxy-PCBs had no effect on deiodination or plasma binding (Adams et al., 2000). In mammals, TCDD and co-planar PCBs generally depressed hepatic T_4ORD but enhanced brain T_4ORD (Brouwer et al., 1998).

Thyroid hormone conjugation with glucuronic acid inactivates the thyroid hormones, increases their solubility and facilitates their excretion in bile and urine (Visser, 1990). Glucuronidation of thyroid hormones also represents a major pathway for the hepatic metabolism of thyroid hormones in rainbow trout (Finnson and Eales, 1996). In lake trout exposed to PCB 126 by oral gavage, there was a dose related increase in hepatic T_4-glucuronidation (T_4-G) (Table 1) but not T_3-glucuronidation (T_3-G) after 8 weeks. Enhanced hepatic elimination and excretion of T_4-glucuronide has been identified as the most important mechanistic response for predicting TCDD (Kohn et al., 1996) and co-planar PCB (van Birgelen et al., 1995) impacts on thyroid hormone economy in mammals. The lack of any effect on T_3-glucuronidation is consistent with the hypothesis that similar to mammals (Visser et al., 1993), trout possess a different glucuronosyltransferase enzyme for T_3 metabolism (Finnson and Eales, 1997). Moreover, exposure of

Table 1
Hepatic T_4-glucuronidation and T_3-glucuronidation activity in lake trout exposed to vehicle control, a single low (1.2 μg/kg) or a single high (40 μg/kg) oral dose of PCB 126 for 8 weeks

Parameter	Exposure		
	Control	Low	High
T_4-G (nmol/h/mg protein)	15 (1)	25 (6)	54 (12)[a]
T_3-G (nmol/h/mg protein)	19 (3)	27 (9)	25 (6)

Values represent mean (±S.E.) of five fish in each group.
[a] Significant differences from control values are indicated.

lake trout to PCB 126 by oral gavage produced a prolonged stimulation of UDPGT activity (Fig. 6). In mammals, induction of UDPGT activities by substrates capable of interacting with the Ah-receptor were most effective at stimulating T_4-glucuronidation (Saito et al., 1991).

3.5. Plasma [^{125}I]T_4 kinetics

Exposure of lake trout by dietary gavage showed that PCB 126 accelerated plasma clearance of [^{125}I]T_4 (Fig. 7). Kinetic analysis indicated that the plasma clearance rate and T_4 degradation rate increased following 8 weeks exposure to PCB 126 (Table 2). However, these kinetic changes were not reflected by the plasma concentrations of T_4 which, similar to those in Experiment 2, were unaffected by exposure to PCB 126. The estimated T_4 plasma clearance and

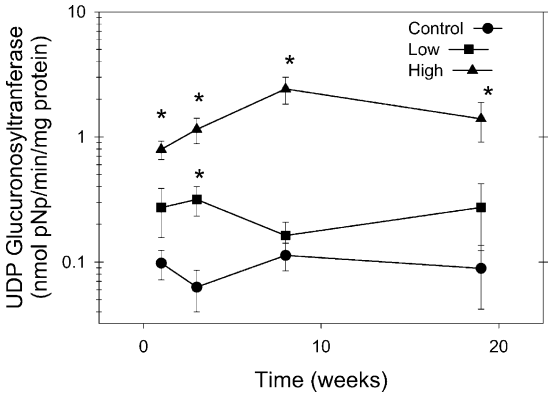

Fig. 6. Hepatic UDPGT activity in lake trout exposed to vehicle, a low dose (1.2 μg/kg) and a high dose (40 μg/kg) of PCB 126 by gavage. Values represent mean (±S.E.) of five fish in each group. Significant differences from control values are indicated (∗).

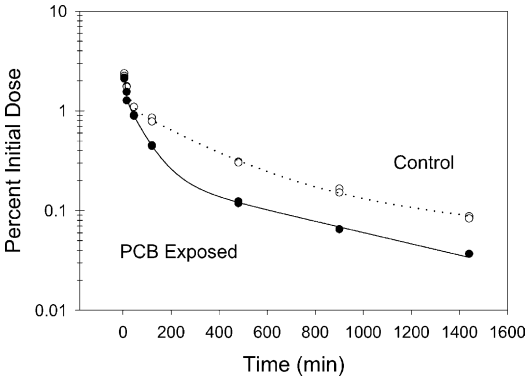

Fig. 7. Disappearance of [^{125}I]T_4 from plasma of representative lake trout following 8 weeks exposure to vehicle control or a single high dose (40 μg/kg) of PCB 126.

degradation rates in lake trout fall in the same range as those previously reported for rainbow trout (Eales et al., 1982; Sefkow et al., 1996). The change in T_4 plasma clearance and degradation caused by PCB 126 was about half that observed during a 6 °C elevation in ambient temperature (Eales et al., 1982). Overall, the findings are consistent with the hypothesis that like mammals exposed to TCDD there is enhanced metabolism of T_4 in peripheral tissues caused by higher glucuronosyl tranferase activity in PCB 126 exposed trout.

3.6. Growth rates and mortality

Growth rates in all groups were initially depressed (Fig. 8). This was likely due to the stress of handling for dosing. After 3 weeks growth rates reached about 0.2% BW per day in all groups and remained

Table 2
Effects of PCB 126 exposure (40 μg/kg) for 8 weeks on plasma levels, plasma clearance and plasma degradation of thyroxine in cannulated lake trout

Plasma parameter	Exposure	
	Control	High
T_4 (nmol/l)	0.67 (0.10)	0.84 (0.05)
Clearance rate (ml/h)	9.20 (1.42)	13.57 (0.92)[a]
Degradation rate (pmol/h)	6.10 (1.14)	11.33 (0.92)[a]

Values represent mean (±S.E.) of five fish and are based on a BW of 1 kg.
[a] Significant differences from control values are indicated.

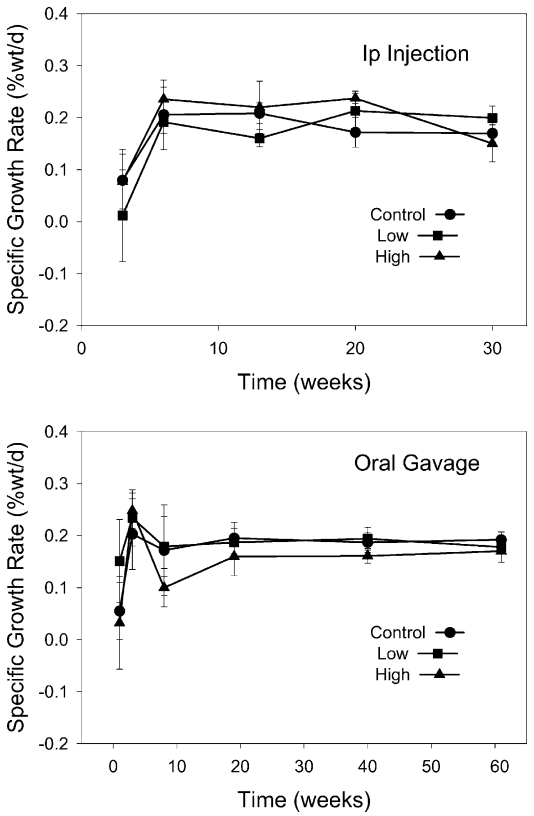

Fig. 8. Specific growth in lake trout exposed to vehicle, a low dose (1.2 μg/kg) and a high dose (40 μg/kg) of PCB 126. Values represent mean (±S.E.) of five fish in each group.

steady throughout the remainder of the experimental period. Fish condition (data not shown) did not show any treatment or time related differences. There was no evidence of impaired growth that has been associated with exposure to high doses of TCDD (van der Weiden et al., 1990). Overall the mortality of experimental fish was extremely low (0.5%).

3.7. Conclusions

Exposure to the highest doses of PCB 126 altered thyroid histology, plasma T_4 dynamics and in vitro T_4-glucuronidation. However, the changes in thyroid follicle morphology were temporary and exposure to PCB 126 did not affect, hepatic 5′-monodeiodinase and T_3-glucuronidation. Also, there were no effects on fish growth or condition. Owing to the extensive autoregulatory feedbacks at both central and peripheral levels (Eales and Brown, 1993), a clinically euthyroid state was preserved, despite the PCB induced changes in T_4 dynamics. The specific induction of T_4-glucuronidation and UDPGT by exposure to PCB 126 suggests that dioxin-like contaminants exert their influence on thyroid status of fish by a mechanism similar to that for TCDD in mammals (Kohn, 2000). The long-term induction of UDPGT by PCB 126, and most likely T_4-glucuronidation, is significant because it chronically elevates T_4 excretion thereby increasing overall demand for thyroidal T_4 production. This may be of significance when T_4 requirements are high for other reasons (e.g. periods of rapid growth, warm temperatures, metamorphosis, parr-smolt transformation). Given the tissue burdens of PCB 126 in our experimental fish, it is certain that historical concentrations of PCB 126 occuring in Great Lakes salmonids were capable of altering thyroidal status. Moreover, based on current estimates of TEQs, it is probable that planar dioxin-like compounds continue to influence thyroidal status of predatory fish in the Great Lakes.

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References

Adams, B.A., Cyr, D.G., Eales, J.G., 2000. Thyroid hormone deiodination in tissues of American plaice, *Hippoglossoides platessoides*: characterization and short-term responses to polychlorinated biphenyls (PCBs) 77 and 126. Comp. Biochem. Physiol. Part C 127, 367–378.

Bagenal, T.B., 1978. Methods for Assessment of Fish Production in Fresh Waters, third ed. Blackwell Scientific Publications, Oxford.

Besselink, H.T., van Beusekom, S., Roex, R., Vethaak, A.D., Koeman, J.H., Brouwer, A., 1996. Low hepatic 7-ethoxyresorufin-*O*-dethylase (EROD) activity and minor alterations in retinoid and thyroid hormone levels in flounder (*Platichthys flesus*) exposed to the polychlorinated biphenyl mixture. Clophen A50. Environ. Pollut. 92, 267–274.

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.

Brown, S., Eales, J.G., 1977. Measurement of L-thyroxine and 3,5,3′-triiodo-L-thyronine levels in fish plasma by radioimmunoassay. Can. J. Zool. 55, 293–299.

Brown, S.B., Eales, J.G., Hara, T.J., 1986. A protocol for estimation of cortisol plasma clearance in acid-exposed rainbow trout (*Salmo gairdneri*). Gen. Comp. Endocrinol. 62, 493–502.

Brown, S.B., Delorme, P.D., Evans, R.E., Lockhart, W.L., Muir, D.C.G., Ward, F.J., 1998. Biochemical and histological responses in rainbow trout (*Oncorhynchus mykiss*) exposed to 2,3,4,7,8-pentachlorodibenzofuran. Environ. Toxicol. Chem. 17, 915–921.

Brown, S.B., Fisk, A.T., Brown, M., Villella, M., Evans, R.E., Metner, D.A., Lockhart, W.L., Muir, D.C.G., Cooley, M., 2002. Dietary accumulation and biochemical responses of juvenile rainbow trout *Oncorhynchus mykiss*) to 3,3′,4,4′,5-pentachlorobiphenyl (PCB 126). Aquat. Toxicol. 59, 139–152.

Brouwer, A., Morse, D.C., Lans, M.C., Schuur, A.G., Murk, A.J., Klasson-Wehler, E., Bergman, A., Visser, T.J., 1998. Interactions of persistent environmental organohalogens with the thyroid hormone system: mechanisms and possible consequences for animal and human health. Toxicol. Ind. Health 14, 59–84.

Brouwer, A., van den Berg, K.J., 1986. Binding of a metabolite of 3,4,3,4-tetrachlorobiphenyl to transthyretin reduces serum vitamin A transport by inhibiting the formation of the protein complex carrying both retinol and thyroxin. Toxicol. Appl. Pharmacol. 85, 301–312.

Eales, J.G., 1977. Use of T_4 and T_3-specific antibodies to study T_4 kinetics in rainbow trout. Gen. Comp. Endocrinol. 52, 89–98.

Eales, J.G., 1979. Thyroid functions in cyclostomes and fishes. In: Barrington, E.J.W. (Ed.), Hormones and Evolution, vol. 1. Academic Press Inc., New York, pp. 341–436.

Eales, J.G., Chang, J.P., Van Der Kraak, G., Omeljaniuk, R.J., Uin, L., 1982. Effects of temperature on plasma thyroxine and iodide kinetics in rainbow trout, *Salmo gairdneri*. Gen. Comp. Endocrinol. 47, 295–307.

Eales, J.G., Brown, S.B., 1993. Measurement and regulation of thyroidal status in teleost fish. Rev. Fish. Biol. 3, 299–347.

Eales, J.G., Brown, S.B., Cyr, D.G., Adams, B.A., Finnson, K.R., 1999. Deiodination as an index of chemical disruption of thyroid hormone homeostasis and thyroidal status in fish. In: Henshel, D.S. Black, M.C., Harrass, M.C. (Eds.), Environmental Toxicology and Risk Assessment: Standardization of Biomarkers for Endocrine Disruption and Environmental Assessment, vol. 8, ASTM STP 1364. American Society for Testing and Materials, West Conshohocken, PA, pp. 136–164.

Eales, J.G., MacLatchy, D.L., 1989. The relationship between T_3 production and energy balance in salmonids and other teleosts. Fish Physiol. Biochem. 7, 289–293.

Finnson, K.W., Eales, J.G., 1996. Identification of thyroid hormone conjugates produced by isolated hepatocytes and excreted in bile of rainbow trout, *Onchorynchus mykiss*. Gen. Comp. Endocrinol. 101, 145–154.

Finnson, K.W., Eales, J.G., 1997. Glucuronidation of thyroxine and 3,5,3′-triiodo-L-thyronine by hepatic microsomes in rainbow trout, *Onchorhynchus mykiss*. Comp. Biochem. Physiol. 117C, 193–199.

Folmar, L.C., Dickhoff, W.W., Zaugg, W.S., Hogins, H.O., 1982. The effect of Aroclor 1254 and No. 2 fuel oil on smoltification and seawater adaptation of coho salmon (*Oncorhynchus kisutch*). Aquat. Toxicol. 2, 291–299.

Giesy, J.P., Jude, D.J., Tillitt, D.E., Gale, R.W., Meadows, J.C., Zajieck, J., Peterman, P., Verbrugge, D.A., Sanderson, J.T., Schwartz, T.R., Tuchman, M.L., 1997. Polychlorinated dibenzo-*p*-dioxins, dibenzofurans, biphenyls and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents in fishes from Saginaw Bay, Michigan. Environ. Toxicol. Chem. 16, 713–724.

Goldstein, J.A., Safe, S., 1989. Mechanism of action and structure-activity relationships for the chlorinated dibenzo-*p*-dioxins and related compounds. In: Kimbough, R.D, Jensen, A.A. (Eds.), Halogenated Biphenyls, Terphenyls, Napthalenes, Dibenzodioxins and Related Products. Elsevier Science Publishers, New York, pp. 239–293.

Grinwis, G.C.M., Besselink, H.T., van den Brandhof, E.J., Bulder, A.S., Englesma, M.Y., Kuiper, R.V., Wester, P.W., Vaal, M.A., Vethaak, A.D., Vos, J.G., 2000. Toxicity of TCDD in Euorpean flounder (*Platichthys flesus*) with emphasis on histopathology and cytochrome P450 1A induction in several organ systems. Aquat. Toxicol. 50, 387–401.

Huestis, S.Y., Servos, M.R., Whittle, D.M., Dixon, D.G., 1996. 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.

Huestis, S.Y., Servos, M.R., Whittle, D.M., van den Heuval, M., Dixon, D.G., 1997. Evaluation of temporal and age-related trends of chemically and biologically generated 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalent in Lake Ontario lake trout, 1977–1993. Environ. Toxicol. Chem. 16, 154–164.

Janz, D.M., Metcalfe, C.D., 1991. Relative induction of aryl hydrocarbon hydroxylase by 2,3,7,8-TCDD and two co-planar PCBs in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 10, 917–923.

Janz, D.M., Metcalfe, T.L., Metcalfe, C.D., Haffner, G.D., 1992. Relative concentrations of cytochrome P450-active compounds in the liver and muscle of rainbow trout from lake Ontario. J. Great Lakes Res. 16, 759–765.

Johnston, C.E., Gordillo, C., Eales, J.G., 1996. Transition from a hatchery to a laboratory environment induces inner-ring monodeiodination pathways for thyroid hormones in liver of rainbow trout, *Oncorhynchus mykiss*. Can. J. Zool. 74, 2178–2183.

Kalisnik, M., Jakopin, P., Sustarsic, J., 1977. On the methodology of the thyroid epithelial cell thickness determination. J. Microsc. 110, 157–162.

Kohn, M.C., 2000. Effects of TCDD on thyroid hormone homeostasis in the rat. Drug Chem. Toxicol. 1, 259–277.

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. 136, 29–48.

Leatherland, J.F., 1993. Field observations on reproductive and developmental dysfunction in introduced and native salmonids from the Great Lakes. Great Lakes Res. 19, 737–751.

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.

Leatherland, J.F., Sonstegard, R.A., 1979. Effects of dietary mirex and PCB (Aroclor 1254) on thyroid activity and lipid reserves in rainbow trout, *Salmo gairdneri*. J. Fish Dis. 2, 43–48.

Leatherland, J.F., Sonstegard, R.A., 1980. Effect of dietary polychlorinated biphenyls (PCBs) or mirex in combination with food deprivation and testosterone administration on serum thyroid hormone concentration and bioaccumulation of organochlorines in rainbow trout, *Salmo gairdneri*. J. Fish Dis. 3, 115–124.

MacLatchy, D.L., Eales, J.G., 1992. Properties of T_4 5′-deiodinating systems in various tissues of the rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 86, 313–322.

Palace, V.P., Dick, T.A., Brown, S.B., Baron, C.L., Klaverkamp, J.F., 1996. Oxidative stress in juvenile lake sturgeon orally exposed to 2,3,7,8-tetrachlordibenzofuran. Aquat. Toxicol. 35, 79–82.

Palace, V.P., Allen-Gill, S.M., Brown, S.B., Evans, R.E., Metner, D.A., Landers, D.H., Curtis, L.R., Klaverkamp, J.F., Baron, C.L., Lockhart, W.L., 2001. 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. Chemosphere 45, 185–193.

Sefkow, A.J., Distefano III, J.J., Himick, B.A., Brown, S.B., Eales, J.G., 1996. Thyroid hormone secretion and interconversion in the five-day fasted rainbow trout, *Oncorhynchus mykiss*. Gen. Comp. Endocrinol. 101, 123–138.

Saito, K., Kaneko, H., Saito, K., Yoshitake, A., Yamada, H., 1991. Hepatic UDP-glucuronyltransferase(s) activity toward thyroid hormones in rats: induction and effects in serum thyroid hormone levels following treatment with various enzyme inducers. Toxicol. Appl. Pharmacol. 111, 99–106.

Sjim, D.T.H.M., Yarechewski, A., Muir, D.C.G., Webster, G.R.B., Seinen, W., Opperhuizen, A., 1990. Biotransformation and tissue distribution of 1,2,3,7-tetrachlorodibenzo-*p*-dioxin, 1,2,3,4,7-pentachlorodibenzo-*p*-dioxin and 2,3,4,7,8-pentachlorodibenzofuran in rainbow trout. Chemosphere 21, 5–866.

Smith, L.M., Schwartz, T.R., Feltz, K., Kubiak, T.J., 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.

van Birgelen, A.P.J.M., Smit, E.A., Kampen, I.M., Groeneveld, C.N., Fase, K., van der Klok, J., van den Berg, M., Koeman, J.H., Brouwer, B., 1995. Subchronic effects of 2,3,7,8-TCDD and PCBs on thyroid hormone metabolism: use in risk assessment. Eur. J. Pharmacol. 293, 77–85.

van der Weiden, M.E.J., van der Klok, J., Pennicks, A.H., Seinen, W., van den Berg, M., 1990. A dose/respnse study with 2,3,7,8-TCDD in the rainbow trout (*Onchorhynchus mykiss*). Chemosphere 20, 1053–1058.

Visser, T.J., 1990. Importance of deiodination and conjugation in hepatic metabolism of thyroid hormone. In: Greer, M.A. (Ed.), The Thyroid Gland. Raven Press, New York, pp. 255–283.

Visser, T.J., Kaptein, E., von Toor, H., van Raaij, J.A.G.M., van den Berg, K.J., Joe, C.T.T., van Englen, J.G.M., Brouwer, A., 1993. Glucuronidation of thyroid hormone in rat liver: effects of *in vivo* treatment microsomal inducers and in vitro assay conditions. Endocrinology 133, 2177–2186.

Williams, L.L., Giesy, J.P., DeGalan, M., Verbrugge, D.A., Tillitt, D.E., Ankley, G.T., 1992. Prediction of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents from total concentrations of polychlorinated biphenyls in fish fillets. Environ. Sci. Technol. 26, 1151–1159.