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Accumulation, depuration and hepatic mixed-function oxidase enzyme induction in juvenile rainbow trout and lake whitefish exposed to dietary 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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Abstract

Juvenile rainbow trout (*Oncorhynchus mykiss*) and lake whitefish (*Coregonus clupeaformis*) were exposed to three concentrations (40, 190, 400 pg g^{-1}) of dietary 2,3,7,8-[^{3}H]tetrachlorodibenzo-*p*-dioxin (TCDD) to compare bioaccumulation and hepatic monooxygenase enzyme (MO) induction. Fish were exposed for 30 days followed by a 180 day depuration phase. Differences in the accumulation and depuration of TCDD were found between rainbow trout and lake whitefish, despite similar body size and lipid content. Assimilation efficiencies of TCDD were greater in lake whitefish (66–76%) than rainbow trout (43–58%), but TCDD half lives were shorter in lake whitefish (32–39 days) than in rainbow trout (73–83 days). Biomagnification factors (BMF) ranged from 1.6 to 1.8 in rainbow trout and from 0.8 to 0.9 in lake whitefish, confirming the known potential for biomagnification of TCDD in aquatic food webs. Reverse phase HPLC showed that a majority of the radioactivity in the rainbow trout bile was TCDD, with minor amounts present as a hydroxylated TCDD and as a glucuronide conjugate. MO enzyme induction, measured by ethoxyresorufin-*O*-deethylase (EROD), was observed in the rainbow trout after 10 days of exposure to 400 pg g^{-1} TCDD, and in the lake whitefish after 5 days of exposure to 380 pg g^{-1} TCDD. The whole fish threshold concentration for EROD induction by TCDD ranged between 15 and 45 pg g^{-1} (wet weight) for both species. EROD activity returned to control levels 120 and 80 days after the cessation of the treatments in the rainbow trout and lake whitefish, respectively. Growth rates were significantly reduced in trout and whitefish at whole fish concentrations (wet weight) of 150 ± 4.6 and 85 ± 8.3 pg g^{-1}, respectively. Histological effects of the TCDD were found in the spleen and liver of the rainbow trout which had whole fish concentrations (wet weight) of 150 ± 4.6 pg g^{-1} and 72 ± 8.0 pg g^{-1} TCDD, respectively.

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1. Introduction

One of the predominate polychlorinated dioxin and furan (PCDD/F) congeners in lake trout of the Great Lakes (De Vault et al., 1989), in fish and sediment of the Baltic Sea (Rappe et al., 1987), and in fish from most major watersheds of the United States (Kuehl et al., 1989; Kuehl et al., 1994), is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCDD is also considered to be the most toxic PCDD/F congener (Mehrle et al., 1988; Safe, 1990), and is used as a bench mark for the toxicity of other planar organochlorine pollutants to fish and mammals (Janz and Metcalfe, 1991; Clemons et al., 1994; van der Weiden et al., 1994).

Despite the well known presence of TCDD in the aquatic environment, and the high toxicity of this compound, there is a lack of pharmacokinetic data for fish. Published bioaccumulation parameters for TCDD vary greatly. For example, Branson et al. (1985) calculated a half life of 58 days in rainbow trout, whereas Kleeman et al. (1986a) reported TCDD half life in rainbow trout of 105 days, and Opperhuizen et al. (1986) found a half life of 15 days in guppies.

Assimilation efficiencies and depuration rates are necessary for modelling contaminants in food chains and predicting environment exposure (Thomann, 1989). TCDD, like other hydrophobic organochlorine pollutants, is thought to accumulate in fish mainly via the food chain (Thomann and Connolly, 1984; Batterman et al., 1989). The first objective of this work was to measure, and compare, the dietary accumulation parameters (assimilation efficiency, depuration rates, half life and biomagnification factors) of TCDD in juvenile rainbow trout and lake whitefish.

A second objective was to examine the relationship between the induction of cyctochrome P450 hepatic monooxygenase (MO) activity and TCDD concentrations, by measuring ethoxyresorufin-*O*-deethylase (EROD) levels, in both juvenile rainbow trout and lake whitefish. In previous work the authors found sustained EROD activity in rainbow trout following dietary exposure of 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Muir et al., 1992b) and 2,3,4,7,8-pentachlorodibenzofuran (PnCDF) (Muir et al., 1990) at whole body concentrations of $\geq$200 pg g^{-1}. TCDD, along with TCDF and PnCDF, are considered to be among the most potent inducers of MFO enzymes in fish (Parrott et al., 1995), and had been previously implicated as inducers of MFO enzymes in fish exposed to bleached kraft mill effluent (Rogers et al., 1989). However, neither the species variation nor sustained enzyme induction by TCDD in fish has been previously examined with controlled laboratory experiments.

The last objective was to examine the histopathologic effects of TCDD in the liver and spleen of the juvenile rainbow trout. The histopathological effects of TCDD in fish are not well studied (Spitsbergen et al., 1988), and there have been few studies which have integrated enzyme induction with morphological changes.

2. Methods

2.1. Chemicals

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

2.2. Fish and food preparation

Juvenile rainbow trout and juvenile lake whitefish (both species 5–9 g initial weight), supplied by the Freshwater Institute hatchery, were acclimated for 2 weeks in UV dechlorinated, carbon-filtered city tap water (10°C). All fish were fed commercial fish food (Martin's Feed Mills Ltd., Elmira, Ont.) consisting of 41% protein, 15% lipid and 3% fibre. This food was found to contain 33 ng g^{-1} total PCB congeners and sub-ng g^{-1} levels of mono-ortho-substituted PCBs (Muir et al., 1990).

^{3}H-TCDD was added to food by suspending the food particles in a hexane solution (50 mL) containing graded concentrations of TCDD, and slowly evaporating to dryness under reduced pressure in a rotary evaporator. The food was allowed to air-dry (24 h) and was stored at 10°C in sealed glass jars. Food fed to control fish was treated in the same manner but without radiolabelled compound. Final concentrations of the TCDD congener in the spiked food were determined by extraction and analysis as described below for fish tissue.

2.3. Uptake and depuration studies

The design of dietary exposure studies with TCDD was identical to that used previously for TCDF (Muir et al., 1992b), except for exposure concentrations. Briefly, trout ($n=38$) and whitefish ($n=38$) were held in fibreglass aquaria in a continuous flow of water (1 L min^{-1}) and fed TCDD-treated food at 0.015 g g fish^{-1} day^{-1} for 30 days (weight of food was adjusted to the number of fish after each sampling time). Three treatments for each species were used (40, 190 and 410 pg g^{-1} for the rainbow trout; and 41, 190 and 380 pg g^{-1} for the lake whitefish), and concentrations in the food were checked in a similar manner as in the fish. Three fish per treatment were sampled after 5, 10, 20 and 30 days. After 30 days the remaining fish were fed clean food at approximately 0.015 g g fish^{-1} day^{-1} for up to 180 days to study depuration of radioactivity associated with 2,3,7,8-TCDD (weight of food was again adjusted after each sampling time). As well, six fish were kept on the two highest TCDD treatments for 41 additional days, and three fish were sampled on Days 51 and 71. Livers were removed for EROD analysis and the rest of the fish was frozen at −20°C until analysis for ^{3}H-TCDD.

2.4. Sample analysis

Carcass, GI tract and bile samples from the rainbow trout, and carcass (including GI tract) from the lake whitefish, were weighed and lyophilized. Extractable radioactivity was determined by homogenising the dried samples (except bile) with toluene, centrifuging, and assaying portions of the supernatant extract by liquid scintillation counting (LSC). Food samples (0.5 g) were extracted by the same method. Unextractable ^{3}H in carcass, GI tract, and liver in the rainbow trout, and carcass (including GI tract) and liver in the lake whitefish, were determined by combusting a portion of the extracted tissue, after isolating and air drying the residue (Whatman No. 1 paper), on a Packard Model D306 oxidizer (Packard Instrument Co., Downers Grove IL). $^{3}H_2O$ was diluted with Monophase scintillation fluor (Packard Instrument Co.) prior to LSC. Toluene-extractable lipids in fish and food were determined by air-drying portions of the toluene extract to constant weight.

2.5. HPLC analysis of rainbow trout bile

Bile samples from rainbow trout collected on Day 120 of depuration were suspended in water and extracted with an equal volume of dichloromethane (DCM). Portions of the aqueous and DCM phases were assayed by LSC for total radioactivity and by reverse-phase HPLC for TCDD. The aqueous phase was evaporated to about 0.5 mL, diluted with 0.05M phosphate buffer (pH 7.8) and incubated with glucuronidase (Sigma Chemical Co., St. Louis, MO) for 4 h at 37°C. Following the incubation the mixture was re-extracted with DCM and assayed by LSC and by reverse-phase HPLC-fraction collection with detection of ^{3}H by LSC.

2.6. MO assays

Analysis of liver samples for MO enzyme activity was carried out with post mitochondrial supernatants as described previously (Muir et al., 1990). The small size of the livers throughout most of the experiment precluded preparation of microsomal fractions. Livers were subsampled for TCDD analysis if they weighed more than 0.3 g. In brief: samples (0.05–0.3 g) were weighed and immediately homogenised in 0.5–2.0 mL HEPES-KCl (0.02 mol HEPES, 0.15 mol KCl, pH 7.5), depending on sample size, and homogenates were centrifuged for 20 min ($15\,600 \times g$) and the supernatant stored at −80°C until analyzed. All preparative steps were done in a coldroom at 2°C.

The supernatants were analysed for EROD activity using the method of Pohl and Fouts (1980) with several modifications (Muir et al., 1990). The reaction was started by the addition of 10 μL of ethoxyresorufin in DMSO (0.04 mg mL^{-1}). The samples were incubated for precisely 2 min in a water bath at 25°C and then the reaction was stopped by addition of 2.5 mL of methanol. The samples were centrifuged at $24\,000 \times g$ to pellet the precipitated protein, and the amount of resorufin in the supernatant was determined spectrofluorometrically using an excitation wavelength

of 530 nm and an emission wavelength of 585 nm. Protein was determined using the Lowry method as modified by Markwell et al. (1981).

2.7. Histological examination of liver and spleen

Liver and spleen samples were collected for histological examination from rainbow trout after 30 and 40 days of depuration from the control treatment and the highest exposure treatment (410 pg g^{-1}). Additional samples of trout liver and spleen were collected from the highest exposure treatment (410 pg g^{-1}) after 60 days of uptake, and from the medium concentration exposure (190 pg g^{-1}) after 40 days of depuration. No lake whitefish samples were examined.

Liver and spleen samples were excised from the fish and fixed in 2.5% buffered glutaraldehyde and then embedded in eponate for sectioning. Sections (1 μm thickness) were stained with 1% toluidine blue and 1% borax and examined using a light microscope.

2.8. 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). TCDD concentrations were corrected for growth dilution and were lipid-normalized for calculation of bioaccumulation parameters. Depuration rate constants (k_D) were obtained by fitting data from the depuration phase of the experiment to a first-order decay curve (ln concentration = $a+b$ time (days) where a is a constant and b is the depuration rate). The best value of assimilation (α) was calculated by fitting the concentration data to the integrated form of the first order kinetic rate equation for constant dietary exposure (Bruggeman et al., 1981) using iterative non-linear regression (SYSTAT, 1992):

$$C_{fish} = \left(\frac{\alpha F C_{food}}{k_D}\right) \times [1 - \exp(-k_D t)] \quad (1)$$

where C_{fish} is the concentration in the fish, F is the feeding rate (lipid corrected), C_{food} is the concentration in the food (on a lipid basis), and t is the time of uptake (days). The equilibrium biomagnification factor (BMF) was estimated from the equation $BMF = \alpha F/k_D$ (Bruggeman et al., 1981).

Differences between growth rate constants, and differences in depuration rates, 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 at the $P < 0.05$ level of significance (Steel and Torrie, 1980).

3. Results

3.1. Bioaccumulation

TCDD was rapidly accumulated from food by rainbow trout and lake whitefish (Fig. 1). Steady state had not been reached in the trout by Day 70 of uptake in the two highest TCDD treatments (410 and 190 pg g^{-1}) or in whitefish in the two lowest TCDD treatment (41 and 190 pg g^{-1}), or by Day 30 of uptake in any of the treatments. Depuration rates did not vary between treatments for either the trout or whitefish, but did differ significantly between species ($P < 0.05$, ANCOVA) (Table 1). The calculation of depuration rate for the whitefish exposed to 380 pg g^{-1} TCDD used only data for the first 80 days of depuration because the mean TCDD concentrations did not decline significantly beyond 110 days of depuration (Fig. 1). TCDD depuration rates in the lake whitefish were twice those found for the rainbow trout, and therefore TCDD had a greater half life in trout (70 ± 5.7 to 92 ± 15 days) than in whitefish (32 ± 3.2 to 39 ± 3.5 days) (Table 1).

Assimilation efficiencies were greatest in the lake whitefish, ranging from 66 ± 3.0 to 76 ± 3.9% (Table 1). Despite the higher assimilation efficiency, the BMF calculated for the lake whitefish (0.8–0.9) were lower than those BMFs calculated for the rainbow trout (1.6–1.8). Rainbow trout assimilation efficiencies varied from 43 ± 3.9 to 58 ± 2.4% (Table 1).

Table 1
Bioaccumulation parameters[a] for 2,3,7,8-TCDD in juvenile rainbow trout and lake whitefish during dietary exposures

Concentration in food[b] (pg g^{-1})	Depuration rate constant[c] ($day^{-1} \times 10^{-3}$)	$t_{1/2}$[d] (days)	BMF[e]	Assimilation efficiency[f] (%)
Rainbow trout				
40	9.0 ± 1.2 (0.75)[B]	77.3 ± 10.3	1.65	58.4 ± 2.4
192	9.9 ± 0.8 (0.90)[B]	70.4 ± 5.7	1.76	45.4 ± 3.1
413	7.5 ± 1.2 (0.67)[B]	92.2 ± 14.8	1.61	43.2 ± 3.9
Lake whitefish				
41	20.6 ± 2.6 (0.80)[A]	33.7 ± 4.2	0.80	69.7 ± 3.8
189	17.6 ± 1.5 (0.87)[A]	39.3 ± 3.5	0.89	66.0 ± 3.0
380[g]	21.8 ± 2.2 (0.89)[A]	31.8 ± 3.2	0.90	75.7 ± 3.9

[a]Parameters were determined from concentrations of the carcass and GI tract combined; [b]concentration in food is wet weight; [c]the depuration rate constant (k_D) is calculated from the equation ln concentration = $a+b\ t$, where b is the rate constant ± 1 standard error and t is the depuration time (days). Coefficient of determination is in brackets (R^2); [d]half life is calculated from the equation $t_{1/2} = 0.693/k_D$; [e]biomagnification factor (BMF) is calculated from the equation $BMF = \alpha F/k_D$ where F is the feeding rate on a lipid basis; [f]the assimilation efficiency (α) is determined by fitting the data to the integrated form of the kinetic rate equation for constant dietary exposure using iterative nonlinear regression: $C_{fish} = (\alpha F C_{food}/k_D) \times [1-\exp(-k_D t)]$ 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); [g]the depuration rate for the lake whitefish exposed to 0.38 ng g^{-1} TCDD was calculated from the first 80 days of depuration only. Different letters (A, B) indicate significant differences ($P < 0.05$, ANCOVA) in depuration rates for, and between, both fish species.

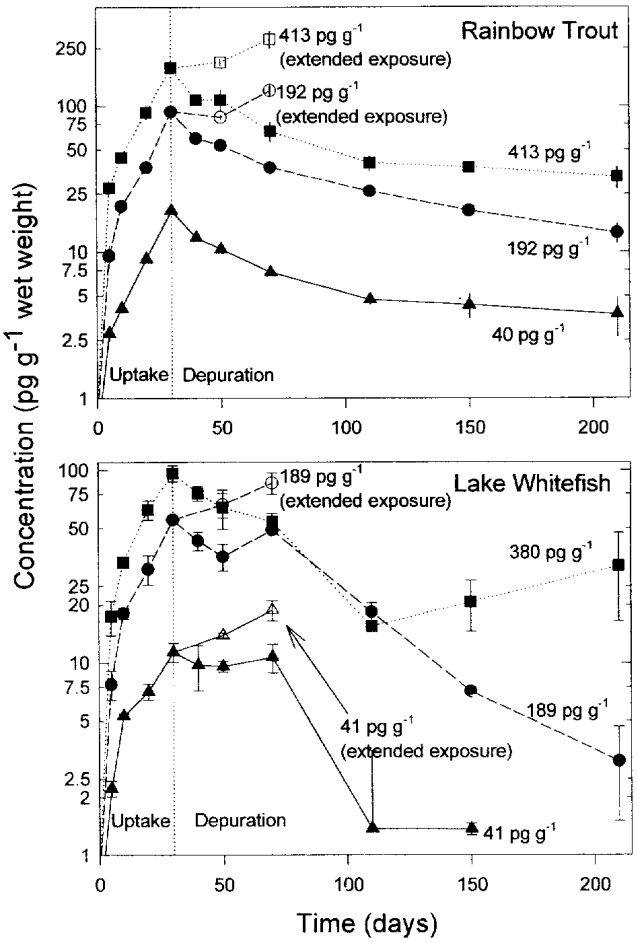

Fig. 1. Accumulation and depuration of 2,3,7,8-TCDD in juvenile rainbow trout and lake whitefish (whole fish). Solid symbols represent 30 day exposures, empty symbols are concentrations in fish exposed up to 70 days. Each point is the mean toluene-extractable concentration (whole fish minus the liver), corrected for growth dilution, from three fish ±1 standard error.

3.2. Tissue distribution and metabolism

Tissue distribution of toluene-extractable and -unextractable TCDD in trout at Days 10, 30, 70 and 150 are summarized in Table 2 (whitefish tissues were not analyzed separately). Greater than 60% of the total radioactivity in the carcass was toluene-extractable in all treatments on most collection days. Toluene-extractable radioactivity in the GI tract initially made up almost 30% of the activity, but eventually decreased to approximately 15% by the end of the experiment. Relative amounts of ^{3}H in the bile and liver were very small. However, the small size of these samples resulted in fairly high concentrations.

During Days 10 and 30 of the uptake phase in the trout, and Day 70 of the depuration phase, over 80% of the radioactivity in the trout was toluene-extractable

Table 2
Percentage of 2,3,7,8-[^{3}H]TCDD in carcass, GI tract, bile, and liver of juvenile rainbow trout during a 30 day exposure and 180 day depuration period

Concentration (pg g^{-1})	Day	Radioactivity in each tissue (%)						
		Carcass		GI tract		Bile		Liver[a]
		Extractable	Unextractable	Extractable	Unextractable	Extractable	Unextractable	Total
40	10	55.8 ± 1.2	11.5 ± 1.5	29.9 ± 2.0	2.9 ± 0.4	NA	NA	NA
	30	67.6 ± 1.0	12.4 ± 1.0	18.1 ± 0.3	1.8 ± 0.3	NA	NA	NA
	70	64.7 ± 0.7	13.1 ± 1.7	18.2 ± 2.4	3.5 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.0
	150	59.4 ± 4.9	24.9 ± 4.5	12.0 ± 0.4	3.6 ± 0.4	NA	NA	0.2 ± 0.1
192	10	53.5 ± 1.1	11.0 ± 1.6	32.3 ± 2.3	3.2 ± 0.5	NA	NA	NA
	30	77.7 ± 1.5	7.7 ± 1.1	13.6 ± 2.4	1.0 ± 0.2	> 0.1	> 0.1	NA
	70	66.1 ± 2.5	11.8 ± 1.4	18.6 ± 0.7	3.0 ± 0.7	0.2 ± 0.1	> 0.1	0.3 ± 0.1
	150	60.8 ± 2.6	22.8 ± 2.9	12.6 ± 1.5	3.5 ± 1.4	> 0.1	> 0.1	0.3 ± 0.1
413	10	58.0 ± 2.5	12.9 ± 0.7	26.4 ± 2.6	2.7 ± 0.7	NA	NA	NA
	30	61.1 ± 1.9	8.6 ± 0.7	27.0 ± 1.6	3.2 ± 0.6	0.1 ± 0.1	> 0.1	NA
	70	64.6 ± 1.5	12.4 ± 0.5	19.8 ± 1.0	3.1 ± 0.6	> 0.1	> 0.1	0.3 ± 0.0
	150	61.0 ± 4.4	19.8 ± 2.7	16.7 ± 2.1	2.1 ± 0.3	> 0.1	> 0.1	0.4 ± 0.1

[a]No toluene-extractable data is available for liver. NA, no sample available for analysis

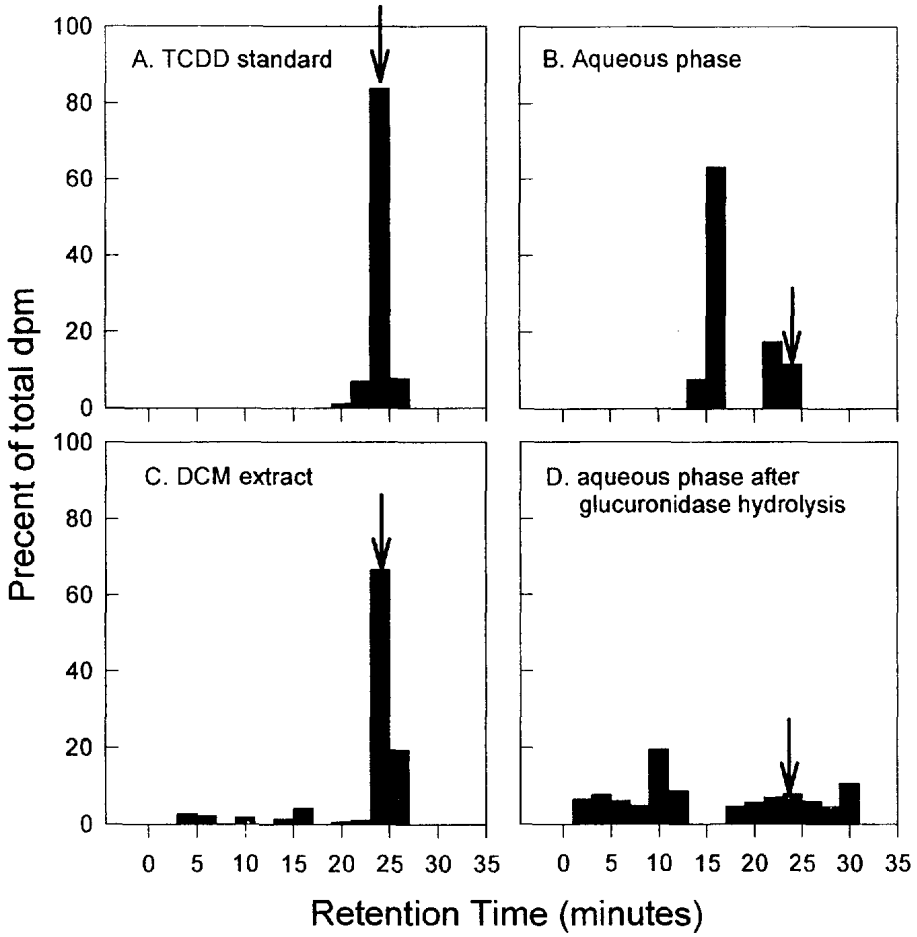

Fig. 2. Reverse-phase HPLC chromatogram of (a) 2,3,7,8-TCDD standard, (b) aqueous phase of bile (following dichloromethane extraction) from rainbow trout exposed to 40 pg g^{-1} TCDD for 30 days followed by 120 days of clean food, (c) dichloromethane extract of the bile sample, and (d) aqueous phase following treatment with glucuronidase.

for all treatments. Greater than 60% of the total radioactivity in the trout bile was in the form of the parent TCDD molecule (Fig. 2(a), Fig. 2(b) and Fig. 2(c)). However, by Day 150 (120 days of depuration) extractable radioactivity in the rainbow trout fell below 80% for all treatments, implying some type of metabolic transformation of the TCDD into a more polar compound or covalent binding of the TCDD to large biological macromolecules. HPLC analysis of bile revealed that a majority of the radioactivity in the aqueous phase was not the TCDD compound, but a more polar metabolite (Fig. 2(b)). Glucuronidase hydrolysis of the aqueous phase yielded a more polar product (Fig. 2(d)).

3.3. MO induction

EROD activity at the two highest exposure concentrations (190 and 400 pg g^{-1}) in both species was found to be greater than control fish. Levels of induction were an order of magnitude less in the whitefish than the trout (Table 3). The magnitude of MO induction, and the length of time required to return to control levels, increased with increasing exposure concentrations in both species (Table 3). EROD levels in trout exposed to 410 pg g^{-1} dietary TCDD became significantly greater than the control fish by Day 10 of the uptake phase, which corresponded to a wet weight whole fish (minus the liver) concentration of 41 ± 0.4 pg g^{-1}. Trout exposed to 190 pg g^{-1} of TCDD had EROD levels greater than the control on Day 20 of uptake when wet weight whole fish (minus the liver) concentrations were 32 ± 1.5 pg g^{-1}. Whitefish EROD levels in the 380 pg g^{-1} treatment were significantly greater than the control whitefish levels after 5 days of uptake, at a whole fish concentration (wet weight) of 17 ± 3.5 pg g^{-1}. EROD levels in the whitefish

Table 3
EROD activity in livers of juvenile rainbow trout and juvenile lake whitefish trout exposed to three concentrations of dietary 2,3,7,8-TCDD

Day	Phase	EROD activity in each exposure concentration (wet weight) in food (nmol mg protein^{-1} min^{-1})(mean ± 1 standard error)			
		Control	40 pg g^{-1}	192 pg g^{-1}	413 pg g^{-1}
Rainbow trout					
5	Uptake	0.022 ± 0.007	0.017 ± 0.001	0.026 ± 0.010	0.031 ± 0.006
10	Uptake	0.015 ± 0.003	0.021 ± 0.006	0.015 ± 0.005	0.027 ± 0.006
20	Uptake	0.021 ± 0.003	0.017 ± 0.003	0.029 ± 0.003	0.028 ± 0.002
30	Uptake	0.021 ± 0.002	0.029 ± 0.007	0.035 ± 0.014	0.078 ± 0.018
10	Depuration	0.012 ± 0.001	0.015 ± 0.003	0.017 ± 0.003	0.038 ± 0.016
20	Depuration	0.014 ± 0.006	0.013 ± 0.002	0.030 ± 0.001	0.033 ± 0.002
41	Depuration	0.020 ± 0.004	0.021 ± 0.002	0.020 ± 0.002	0.033 ± 0.006
80	Depuration	0.022 ± 0.002	0.022 ± 0.001	0.018 ± 0.002	0.041 ± 0.014
120	Depuration	0.030 ± 0.003	0.025 ± 0.003	0.031 ± 0.004	0.028 ± 0.011
180	Depuration	0.024 ± 0.003	0.030 ± 0.007	0.026 ± 0.008	0.029 ± 0.005
		Control	41 pg g^{-1}	189 pg g^{-1}	380 pg g^{-1}
Lake whitefish					
5	Uptake	0.004 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.008 ± 0.001
10	Uptake	0.005 ± 0.001	0.007 ± 0.002	0.006 ± 0.001	0.008 ± 0.002
20	Uptake	0.004 ± 0.000	0.007 ± 0.002	0.008 ± 0.002	0.007 ± 0.001
30	Uptake	0.004 ± 0.001	0.005 ± 0.002	0.010 ± 0.001	0.006 ± 0.003
10	Depuration	> 0.001	0.005 ± 0.001	0.007 ± 0.001	0.008 ± 0.002
20	Depuration	0.003 ± 0.000	0.003 ± 0.001	0.003 ± 0.001	0.006 ± 0.002
40	Depuration	0.003 ± 0.001	0.002 ± 0.000	0.001 ± 0.000	0.006 ± 0.000
80	Depuration	0.005 ± 0.002	0.010 ± 0.001	0.007 ± 0.000	0.006 ± 0.001

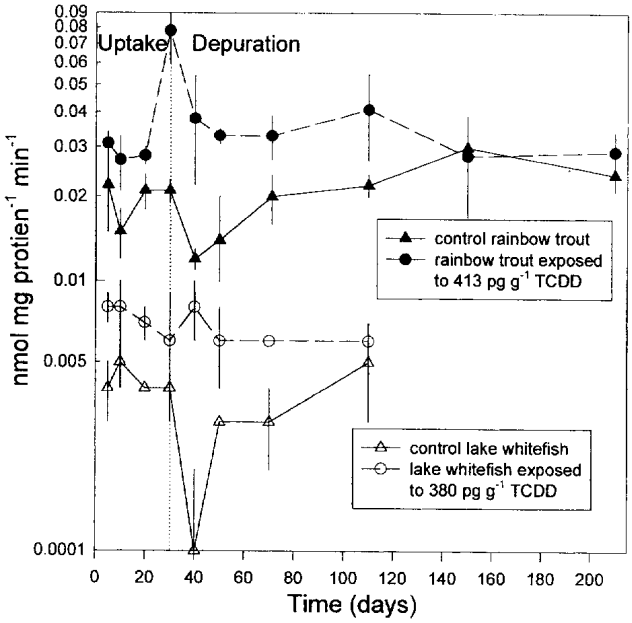

Fig. 3. EROD activity in livers (postmitochondrial supernatants) of juvenile rainbow trout and lake whitefish during a 30 day exposure to a nominal 400 pg g^{-1} dietary concentration followed by 180 (rainbow trout) and 110 (lake whitefish) day depuration period. The 40 and 190 pg g^{-1} treatments are not shown for clarity. Each point is the mean activity of three fish livers ± 1 standard error.

exposed to 190 pg g^{-1} TCDD exceeded the controls on Day 20 at whole fish concentrations (wet weight) of 27 ± 4.6 pg g^{-1}. No sustained EROD induction occurred in either species at the lowest exposure concentration (≈40 pg g^{-1}) despite whole fish concentrations (wet weight) of 15 ± 0.4 and 10 ± 1.1 pg g^{-1} in the trout and whitefish, respectively. At Day 30 of the uptake phase, EROD levels in the highest exposure concentrations (410 and 380 pg g^{-1}) were 4 and 0.5 fold higher than control in the trout and whitefish, respectively. EROD induction by the highest dietary TCDD concentration (410 pg g^{-1}) in rainbow trout required 120 days of depuration to return to control levels (whole fish concentration (wet weight) of 13 ± 1.3 pg g^{-1}), and rainbow trout exposed to 190 pg g^{-1} TCDD required 40 days to return to control levels (Fig. 3 and Table 3). EROD levels in the highest TCDD exposure (380 pg g^{-1}) in whitefish returned to control levels by Day 80 of the depuration phase when carcass (GI tract included) wet weight concentrations averaged 10 ± 0.5 pg g^{-1} (Fig. 3).

3.4. Growth and histopathological effects

Trout and whitefish from the highest exposure treatments (≈400 pg g^{-1}) had significantly lower growth when compared with their respective control fish (Table 4), and had achieved whole body TCDD concentrations of 150 ± 4.6 and 85 ± 8.3 pg g^{-1}, respectively. Reduced growth was not observed in either species at the lower

Table 4
Growth rates, lipid content, liver somatic index and cumulative mortality of juvenile rainbow trout and juvenile lake whitefish exposed to dietary 2,3,7,8-TCDD

Concentration in food[a] ($pg \cdot g^{-1}$)	Duration		Growth rate constants		Lipid[d]	LSI[e]	Mortality
	Uptake (days)	Depuration (days)	Whole fish[b] ($day^{-1} \times 10^{-3}$)	Liver[c] ($day^{-1} \times 10^{-3}$)	(%)		
Rainbow trout							
Control			10.2 ± 0.6 $(0.92)^{A}$	4.4 ± 0.9 $(0.45)^{A}$	7.4 ± 0.4	1.6 ± 0.3	0
40	30	180	9.4 ± 0.5 $(0.94)^{AB}$	4.0 ± 0.7 $(0.47)^{A}$	6.2 ± 0.2	1.3 ± 0.0	3
192	30	180	9.2 ± 0.6 $(0.86)^{AB}$	4.0 ± 1.0 $(0.38)^{A}$	7.0 ± 0.4	1.2 ± 0.1	5
413	30	180	8.4 ± 0.7 $(0.87)^{BC}$	3.7 ± 0.8 $(0.43)^{A}$	6.7 ± 0.3	1.5 ± 0.3	5
Lake whitefish							
Control			7.3 ± 0.8 $(0.76)^{CD}$	1.9 ± 0.3 $(0.03)^{A}$	10.0 ± 2.1	0.9 ± 0.2	11
41	30	180	5.6 ± 1.0 $(0.57)^{DEF}$	1.2 ± 0.2 $(0.02)^{A}$	8.4 ± 0.4	1.0 ± 0.1	6
189	30	180	6.8 ± 0.7 $(0.77)^{CDE}$	2.1 ± 0.2 $(0.07)^{A}$	8.2 ± 0.4	0.9 ± 0.1	0
380	30	180	4.5 ± 0.9 $(0.47)^{F}$	4.0 ± 0.2 $(0.23)^{A}$	8.1 ± 0.3	1.0 ± 0.1	6

[a]Concentration in food is wet weight; [b]whole fish growth rate calculated from the equation ln weight = $a+b$ day, where b is the growth rate ± 1 standard error. Coefficient of determination in brackets (R^2); [c]liver growth rate calculated from the equation ln weight = $a+b$ day, where b is the growth rate ± 1 standard error. Coefficient of determination in brackets (R^2); [d]lipid percentage is the average lipid percentage of all fish in each treatment; [e]LSI is calculated from Day 40 of the depuration phase for all treatments except lake whitefish control, which were calculated for Day 80 depuration. Different letters (A,B,C,D,E,F) represent significant differences ($P < 0.05$, ANCOVA) in body growth rates and liver growth rates for, and between, both fish species.

concentration exposures despite achieving whole body TCDD concentrations of 72 ± 8.0 and 46 ± 1.1 pg g^{-1} in the trout and whitefish, respectively. Trout growth rates were significantly greater than whitefish with the exception of the highest TCDD exposed trout. Whitefish had greater whole body lipid percentages and lower liver somatic indexes (LSI) than trout (Table 4). Mortality rates in trout increased slightly with TCDD exposure concentration, no relationship between mortality rate and TCDD concentration was seen with the whitefish (Table 4).

Histopathological effects in the trout spleen tissue were observed in the highest exposure treatment (410 pg g^{-1}) on Day 30 of depuration and Day 60 of uptake at whole fish TCDD concentrations (wet weight) of 150 ± 4.6 and 190 ± 42 pg g^{-1}, respectively. Splenic veins were congested, distended and filled with blood, and leucocytes (large macrophage-like cells that are a defense against infection) were being extruded into splenic veins. This type of response is usually associated with various toxemias and acute infections. Splenic arteries of all TCDD exposed fish were similar to control fish, and appeared to be healthy. Splenic lymphoid depletion was observed, and an overall splenic atrophy was obvious in the form of degenerating cells. Spleen tissue appeared normal in the high exposure treatment (410 pg g^{-1}) and meduim exposure (190 pg g^{-1}) after 40 days of depuration, despite whole fish TCDD concentrations (wet weight) of 72 ± 8.0 pg g^{-1}.

Histological effects were seen in trout liver tissue at whole body TCDD concentrations averaging 72 ± 8.0 pg g^{-1} (Day 40 uptake). Slight fibrosis of the hepatic sinuses, and evidence of parenchymal cell degeneration (swollen cells with visible hepatocyte perimeters), were observed in the Day 40 depuration rainbow trout livers (medium (190 pg g^{-1}) and high (410 pg g^{-1}) exposure concentrations). Hepatic sinuses were dilated and some fibrosis was evident in the liver of rainbow trout 30 days after exposure to high (410 pg g^{-1}) concentration TCDD treatment. Cellular atrophy was apparent in these livers from lighter staining parenchymal cells, and cells appeared irregular in shape. The hepatic sinuses from rainbow trout exposed to 60 days of dietary TCDD (410 pg g^{-1}) were dilated, and parenchymal cells were swollen or enlarged. Hepatocytes were distinctly outlined by separation and some cell degeneration was evident.

4. Discussion

4.1. Bioaccumulation

Significant differences in the bioaccumulation parameters (depuration rate, assimilation efficiencies and BMF) of TCDD were found between juvenile rainbow trout and lake whitefish. Experimental protocols were kept very similar for both species to reduce confounding effects such as fish size, lipid content and exposure routes in the two species. However, differences in characteristics between the two species were observed. The whitefish, as a benthic feeder, did not feed as a aggressively as the trout, preferring to let the food to sink to the bottom of the tank before feeding. This could have resulted in a small amount of food lost due to the flow-through nature of the aquaria, and thereby reducing the exposure to TCDD. Assimilation

efficiency (Eq. 1) could therefore have been underestimated in the whitefish. Nevertheless, assimilation efficiencies of the whitefish (66 ± 3.0 to 76 ± 3.4%) were > 10% higher than assimilation efficiencies found for the trout (43 ± 3.9 to 58 ± 2.4%). Similar assimilation efficiencies for TCDD of about 50% in juvenile rainbow trout were calculated by Muir et al. (1992a) using data from Kleeman et al. (1986a).

The TCDD half lives determined for the trout are similar to those determined for other 2,3,7,8 chlorine substituted PCDD/F congeners in juvenile rainbow trout (Branson et al., 1985; Muir and Yarechewski, 1988; Muir et al., 1992b). TCDD half lives of both the whitefish, and trout, also fall within a range established for a number of other smaller fish including guppies (Opperhuizen et al., 1986) and juvenile yellow perch (Kleeman et al., 1986b). However, the half lives are much shorter than the TCDD half life of 1 year determined for 1 kg carp by Kuehl et al., 1986. Sijm and van der Linde (1995) found that half life increased with fish size, and the larger size could explain the greater TCDD half life in the carp. Geyer et al. (1995), using published data for a range of species, showed that TCDD half life in fish is positively related to the body size, and lipid content, of the fish. Delorme (1995) measured a half life of 2,3,4,7,8-PnCDF in adult lake trout (620 g) that was 50 times greater than the half life reported for juvenile rainbow trout (≈ 50 g) by Muir et al. (1990). Variation among reported TCDD half lives may therefore be related to differences in body size and lipid content, and suggests caution when applying bioaccumulation parameters derived from different size fish.

Although the whitefish and trout were of equal size and similar lipid content, TCDD half lives in whitefish were approximately half those observed in the trout. This suggests that factors other than body size, and lipid content, can influence the elimination and half life of TCDD. The greater depuration rates in the whitefish suggest either a greater capacity for biotransformation of the TCDD or other physiological differences between trout and whitefish. The lower EROD activity in the whitefish makes it unlikely that greater phase I metabolizing enzymes are responsible for higher depuration rates in the whitefish. Higher MO enzyme activity has been correlated with increased oxidative biotransformation activity (Lech and Bend, 1980; Muir et al., 1992b). Elevated EROD activity was not correlated, for both the trout and whitefish, with increased depuration rate of the TCDD. Muir et al. (1992b) observed greater depuration rates of 2,3,7,8-TCDF in juvenile rainbow trout with greater EROD activity using a wider range (100×) of exposure concentrations than used in the present work. Phase II metabolizing enzymes, glucuronosyl transferase and gluthathione S-transferases, might also be responsible for differences in depuration rate between these two species. Phase II enzyme have been shown to be induced be halogenated aromatics (Safe, 1990); unfortunately data on these enzymes in the whitefish are not available.

TCDD would biomagnify in aquatic food chains based on the BMF values estimated for the trout (BMF = 1.6–1.8). However, the BMF values for the whitefish were < 1 (BMF = 0.8–0.9). Because the whitefish did not consume all available food, as did the trout, these values may be underestimated. TCDD has been found to biomagnify in aquatic food webs in the Baltic Sea (Rolff et al., 1993), and between forage fish and top predator fish in the Great Lakes (Whittle et al.,

1992). TCDD has also been shown to biomagnify in the fish to herring gull food web in Lake Ontario (Braune and Norstrom, 1989).

4.2. Tissue distribution and metabolism of TCDD in rainbow trout

TCDD accumulated in lipid-rich tissues (i.e. GI tract and carcass) of the trout. Although the carcass had the highest burden of TCDD, concentrations of TCDD were higher in the GI tract, as has been observed for other PCDD/Fs in rainbow trout (Kleeman et al., 1986a; Sijm et al., 1990). Hektoen et al. (1992) observed that TCDD accumulated in the fat deposits of rainbow trout, including the abdominal fat and subcutaneous fat. Abdominal fat was included with the GI tract, and the subcutaneous fat was part of the carcass in the present work. Concentrations of TCDD in dorsal muscle tissue of the trout may be much lower than the concentrations established for the carcass as a whole, as the subcutaneous fat of the carcass may be accounting for a large majority of the carcass TCDD burden.

Biotransformation of the TCDD compound was minimal, which is commonly observed with PCDD/F congeners that are substituted at the 2,3,7,8-positions (Kleeman et al., 1986a; Sijm et al., 1990). The only definitive evidence of metabolic transformation of the TCDD compound came from the HPLC analysis of the trout bile, which showed small percentages of TCDD metabolites including a glucuronide conjugate. Kleeman et al. (1986a) also found a glucuronide conjugate in the bile of juvenile rainbow trout exposed to TCDD. The bile accounts for less than 1% of the total ^{3}H in the rainbow trout in this study and less than 2% in Kleeman et al. (1986a) study. However, by the end of the experiment greater than 20% of the ^{3}H was non-toluene-extractable, implying a non-polar metabolite of TCDD. Branson et al. (1985) estimated that 30% of the radioactivity in rainbow trout exposed to TCDD was converted to a polar metabolite. Although toluene non-extractable ^{3}H implies a more polar compound, there is also the possibility that the ^{3}H may still be either non-extracted parent TCDD or part of an intact TCDD compound that has covalently bonded to a biological macromolecule.

4.3. Hepatic MO enzyme induction

The threshold for induction of EROD in trout and whitefish exposed to TCDD occurred at similar whole fish concentrations (wet weight) of between 30–45 pg g^{-1} and 15–30 pg g^{-1}, respectively. Sustained EROD induction did not occur in the lowest exposure treatments in either species, despite whole fish concentrations of 15 and 10 pg g^{-1} in the trout and whitefish, respectively. EROD induction in both species only returned to control levels at the highest exposure when concentrations had fallen below 15 pg g^{-1}. Liver tissue concentrations have been found to correlate closely with EROD induction, unfortunately there was insufficient liver tissue to carry out both EROD and ^{3}H analysis. However, assuming that the liver accounts for 2% of the TCDD burden (Kleeman et al., 1986a), the current study observed a EROD induction threshold concentration of TCDD in liver of approximately 55 pg g^{-1}. This concentration is higher than the EROD induction threshold concen-

tration of TCDD in rainbow trout liver of 16 pg g^{-1} reported by Parrott et al. (1995). However, the current study's estimate of the liver concentration is dependent on the liver burden percentage of TCDD, unlike the measured TCDD concentrations in liver used by Parrott et al. (1995).

Although threshold concentrations for EROD induction were similar in trout and whitefish, differences in the magnitude of MO enzyme activity were observed. EROD activity in the control trout was an order of magnitude greater than the control whitefish, and the level of EROD induction by TCDD in the trout was much greater than in whitefish, despite nearly identical exposure, and whole fish, concentrations. Differential responsiveness in MO enzyme induction in different strains of mice (Safe, 1990), and different species of fish (Stegeman et al., 1981; van der Weiden et al., 1992a), have been reported. Differences in EROD activity between fish species, both at non-induced levels and xenobiotic induced levels, need to be addressed when evaluating EROD activity in the environment. Certain species of fish may be more sensitive to xenobiotic chemicals, and, therefore, would make better biomonitors of MO enzyme inducing chemicals. Other factors can influence EROD activity including contaminant dosage (van der Weiden et al., 1990), sex and age of the fish, annual cycles, condition of the fish and environmental factors (Blanck et al., 1989).

4.4. Growth and histopathological effects

The whole fish concentrations of TCDD (150 and 85 pg g^{-1} (wet weight) in trout and whitefish, respectively) associated with reduced growth are lower than concentrations reported previously (Mehrle et al., 1988; van der Weiden et al., 1990). Mehrle et al. (1988) found reduced growth in all rainbow trout exposed to TCDD. However, the lowest TCDD concentration in the rainbow trout was 98 000 pg g^{-1}. van der Weiden et al. (1990) found reduced growth in juvenile rainbow trout (25–55 g) which were i.p. injected once with 5000 pg g^{-1} TCDD, but did not observe growth effects with lower concentration injections. The continuous 30 day TCDD exposure of the fish in this study, at much lower body burdens than used by van der Weiden et al. (1990) can probably explain the lower growth effect concentration found in this study, indicating that the route of chemical exposure should be considered when comparing toxic effects. The results of this study are in general agreement with the lowest observable adverse effect level of TCDD on lake trout eggs of 55 pg g^{-1} and the LD_{50} concentrations for lake trout swim-ups of 65 pg g^{-1} (Walker et al., 1991). No reports on the effects of TCDD on the growth of lake whitefish were found in published literature.

The TCDD concentrations which reduced growth in the trout and whitefish were lower than tissue levels of 2,3,7,8 substituted PCDFs associated with reduced growth (Muir et al., 1990; Muir et al., 1992b). Reduced growth was observed in juvenile rainbow trout which had 2,3,7,8-TCDF concentrations of 10 000 pg g^{-1} (wet weight), but no reduced growth was found at concentrations of 1400 pg g^{-1} (wet weight) (Muir et al., 1992b), or at 2000 pg g^{-1} (wet weight) of 2,3,4,7,8-PnCDF (Muir et al., 1990). The greater toxicity of TCDD as compared with other

PCDD/Fs is consistent with toxic equivalent factors (TEFs) derived for fishes (Walker and Peterson, 1991; Clemons et al., 1994).

The liver and spleen are both sensitive indicators of the effects of TCDD based on the histopathological examination of these tissues. Unfortunately, the amount of data available on the histopathological effects of TCDD on fish for comparison is limited, and data comparing effects to TCDD tissue burdens are not available. van der Weiden et al. (1992a) and Spitsbergen et al. (1988) observed congestion in juvenile rainbow trout spleen tissue exposed to a single i.p. injection of TCDD (30 and 10 pg g^{-1}, respectively), and van der Weiden et al. (1992b) found lymphoid atrophy at higher exposure concentrations (600 to 3100 pg g^{-1} i.p.). Similar histopathological effects were seen in the spleens of trout exposed for 30 days to 410 pg g^{-1} TCDD in this study, although no effects were observed in the 190 pg g^{-1} TCDD treatment. TCDD concentrations in the spleen were not measured, but histological effects were observed in the spleen of trout which had achieved whole body TCDD concentrations (wet weight) of 150 ± 4.6 pg g^{-1} (wet weight) 30 days prior to the histopathological examination. Effects were not observed in spleen of trout which achieved whole fish concentrations of 72 ± 8.0 pg g^{-1} (wet weight) 40 days prior to examination.

Histopathological effects in the liver were observed at TCDD exposure concentrations that produced no effects in the spleen, suggesting that the liver is a more sensitive organ to TCDD exposure. Helder (1981) observed histological effects in the liver of juvenile rainbow trout (0.85 g) exposed to water borne TCDD at 10 and 100 ng L^{-1}. van der Weiden et al. (1992b) found vacuolisation in the cytoplasm of the hepatocytes of juvenile rainbow trout 3 weeks after a single i.p. injection of 300 pg g^{-1} TCDD, and a decrease in glycogen content of the hepatocytes at doses as low as 60 pg g^{-1}. Assuming that the TCDD burden in liver is approximately 2% of the carcass TCDD burden (Kleeman et al., 1986a), histopathological effects were observed in liver which had experienced a maximum concentration of approximately 55 pg g^{-1} (wet weight), 40 days prior to the histopathological examination.

5. Conclusion

The results demonstrate that species differences in elimination and assimilation exist which cannot be accounted for by differences in lipid content or body size. Greater assimilation efficiency, but shorter half lives, of TCDD were found in the whitefish, but equilibrium BMFs of TCDD were higher in rainbow trout.

Threshold concentrations for MO enzyme induction by TCDD were slightly lower in whitefish (15 to 30 pg g^{-1}) than trout (30 to 45 pg g^{-1}). EROD activity, in both TCDD exposed and control fish, was an order of magnitude less in the whitefish than in the trout. EROD activity in the trout and whitefish returned to control levels 120 and 80 days after the cessation of TCDD exposure, respectively.

TCDD reduced the growth rates of whitefish and trout at whole fish concentrations (wet weight) of 150 ± 4.6 and 85 ± 8.3 pg g^{-1}, respectively. Histopathological effects were also observed in the spleen and liver of the trout at whole fish concentrations (wet weight) of 150 ± 4.6 and 72 ± 8.0 pg g^{-1} TCDD, respectively.

TCDD concentrations found to reduce growth in juvenile trout and whitefish (85–150 pg g^{-1}) or induce MO activity (15–45 pg g^{-1}) in this study are higher than median or mean levels of 2,3,7,8-TCDD in fish (1.4 and 6.9 pg g^{-1}) from US waterways (USEPA, 1992). However, adult lake trout from Lake Ontario have mean whole body TCDD levels which exceed, by about 2-fold, levels we found associated with EROD induction in juvenile fish (De Vault et al., 1995).

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