ELSEVIER

Aquatic Toxicology 59 (2002) 35–53

AQUATIC TOXICOLOGY

www.elsevier.com/locate/aquatox

Effects of chronic dietary exposure to environmentally relevant concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on survival, growth, reproduction and biochemical responses of female rainbow trout (*Oncorhynchus mykiss*)

John P. Giesy [a,b,d], Paul D. Jones [b,*], Kurunthachalam Kannan [b], John L. Newsted [c,1], Donald E. Tillitt [c,2], Lisa L. Williams [c,3]

[a] *Department of Zoology, Michigan State University, East Lansing, MI 48824, USA*
[b] *National Food Safety and Toxicology Center, Michigan State University, Food Safety and Toxicology Bldg., East Lansing, MI 48824, USA*
[c] *Department of Fisheries and Wildlife, Michigan State University, East Lansing, MI 48824, USA*
[d] *Institute of Environmental Toxicology, Michigan State University, East Lansing, MI 48824, USA*

Received 14 March 2001; received in revised form 16 July 2001; accepted 3 August 2001

Abstract

Adult female rainbow trout were exposed to dietary 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) at concentrations of 1.8, 18 and 90 ng TCDD/kg (ww) food for up to 300 day. At the end of the exposure fish were spawned and the reproductive outcomes were assessed. TCDD was accumulated into tissues and eggs in a dose-dependent manner with steady state being achieved after 50–100 day of exposure. Biochemical and hematological parameters were monitored at 50, 100, 150, and 200 day after the beginning of exposure. The survival of adult female trout was reduced in a dose-dependent manner by exposure to TCDD in the diet. Fish fed 1.8 ng TCDD/kg, moist weight of diet, showed significantly reduced survival compared with those fed the control diet. TCDD also affected survival of fry from females fed 1.8 ng TCDD/kg. Observed adverse effects in adult fish were as sensitive as early life-stage endpoints. Liver EROD activity was only moderately increased in all exposure groups after 250+ day of exposure. Low rates of edema and deformities were observed in fry from all treatment groups including controls. This study has demonstrated adverse effects of TCDD to both adults and fry at concentrations comparable to current environmental

* Corresponding author. Tel.: +1-517-432-6333; fax: +1-517-432-2310.
E-mail address: jonespa7@msu.edu (P.D. Jones).
[1] ENTRIX, 4295 Okemos Rd, Suite 101, Okemos, MI 48864, USA.
[2] USGS-Biological Resources Division, Columbia Environmental Research Center, 4200 New Haven Rd., Columbia, MO 65201, USA.
[3] US Fish & Wildlife Service, Coolidge Road, East Lansing, MI. 48823, USA.

PII: S0166-445X(01)00235-1

concentrations. This suggests that direct adult toxicity as well as reproductive endpoints need to be incorporated in the current risk assessment paradigm for these compounds.

Keywords: Risk; EROD; Fish; Reproduction; Dioxin

1. Introduction

Polychlorinated dibenzo-*p*-dioxins and related halogenated diaromatic hydrocarbons (HDH) have been shown to adversely effect the reproduction of fish (Walker and Peterson, 1991; Giesy and Snyder, 1998). Of the various classes and congeners of HDH, 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) is the most potent (Walker and Peterson, 1991; Spitsbergen et al., 1991). The adverse effects of HDHs have been observed in laboratory studies as well as in field studies where reproductive effects have been correlated with concentrations of HDH in tissues of fish and the media to which they were exposed (Zabel et al., 1995; Walker et al., 1990; van der Weiden et al., 1990). HDHs have been implicated in several large-scale fish population disease events (Giesy and Snyder 1998). The most notable of these are the 'blue sac' disease and early mortality syndrome (EMS) (Wright and Tillitt 1999) observed in salmonids of the North American Great Lakes and the M74 syndrome identified in salmon from the Baltic Sea (Bengtsson et al., 1999; Fitzsimons et al., 1999). Of these syndromes, only blue-sac disease has been definitively linked to exposure to dioxin like compounds (Wright and Tillitt, 1999). EMS in the North American Great Lakes and M74 syndrome are associated with a deficiency of thiamine, and can be treated by administration of thiamine (Bengtsson et al., 1999). There is no apparent correlation between the occurrence of M74 syndrome and organohalogen concentrations in muscle, blood or eggs of female salmon (Asplund et al., 1999). In contrast to these two syndromes that affect fry approaching or at the swim-up stage, exposure of fish to dioxin-like chemicals results in greater degrees of embryo-mortality and mortality at the 'sac-fry' stage (Mac et al., 1993). To date, salmonids, (Guiney et al., 1996; Walker et al., 1990; Walker and Peterson, 1991) including lake trout (*Salvelinus namaycush*) and rainbow trout (*Onchorhynchus mykiss*), have been demonstrated to be the most sensitive fish species to the adverse effects of HDHs (Walker and Peterson, 1994). In particular, reproductive endpoints have been shown to be the most sensitive events (Walker and Peterson, 1994; Giesy and Snyder, 1998).

Exposures to HDHs under environmental conditions are usually chronic and, at least for larger species of fish, the major pathway of exposure is via the diet (Jones et al., 1993; Batterman et al., 1989). To perform accurate risk assessments of concentrations of HDHs in the environment requires the use of quantitative dose-response relationships linking toxic effects with measurements or estimates of exposure. Since the mechanism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity is chronic, the duration and pathway of exposure is critical to obtaining an accurate dose-response relationship. To date, neither laboratory nor field studies using an appropriate exposure duration and/or pathway have adequately determined quantitative dose-response relationships for HDHs. In general laboratory studies are of relatively short-term duration and expose organisms to relatively great doses of HDHs followed by an effects assessment period. In addition, studies using single large-dose exposures administered in water or by i.p. injection, do not represent relevant exposure pathways. Due to logistical problems of managing long-term exposures with HDHs, laboratory studies have generally focused on immature, juvenile (Mehrle et al., 1988; Muir et al., 1986; Fisk et al., 1997) or smaller species of fish (Gobas and Schrap, 1990; Muir et al., 1986) instead of adult fish. Other studies have evaluated the hatching and development of eggs into which TCDD had been injected (Walker and Peterson, 1994). Even though it has been demonstrated that the effects observed in these studies are similar to those where TCDD was maternally deposited, these egg injection studies do not consider the potential effect of TCDD on the physiology and biochemistry of the

adults, which might affect reproduction (Walker and Peterson, 1994). Furthermore, while some useful information is available from these types of studies, they do not provide insight into the long-term effects of physiologically accumulated TCDD on survival, growth or reproduction. In addition, these studies focused mainly on HDH accumulation and did not investigate effects on some of the most sensitive reproductive responses. To more accurately determine the effects of TCDD on salmonids, a long-term (up to 342 day) dietary exposure of adult female rainbow trout (*O. mykiss*) to environmentally relevant concentrations of TCDD was performed. Accumulation and disposition of TCDD in tissues and physiological and histological effects of exposure have been presented previously (Walter et al., 2000; Jones et al., 2001). Here we present the results of a study of the effects of TCDD on the survival, growth and reproduction of the trout as well as biochemical and physiological responses of the adult fish.

2. Methods

Methods used to culture and expose fish to food-born tritium labeled TCDD (^{3}H-TCDD) have been presented in detail elsewhere (Walter et al., 2000; Jones et al., 2001). ^{3}H-TCDD was synthesized and purified at the Pesticide Research Center, Michigan State University. Adult (age class II; 350 g), female rainbow trout of the spring-spawning *Shasta* strain were collected from the rearing ponds at the Stoney Creek Trout Farm (Grant, MI). Fish were acclimated for 60 day in the exposure tanks before exposure. Fish were exposed in 1700 l flow-through tanks. Tanks were situated in a negative pressure facility with three levels of containment for water and one for air. Control fish were held at the same facility in the same tank configuration and with the same containment, but in an adjacent room to prevent TCDD carry over between tanks. The experiment was initiated with 35 females in each of the four exposure groups (3 dietary concentrations of TCDD and 1 control). Logistic considerations, mainly the size and expense of the negative pressure containment facility, prevented duplication of exposure doses. Fish were checked daily for adult mortality during feeding and tank cleaning procedures. For sampling, fish were anesthetized by submersion in MS-222 (tricaine methane sulfonate; Argent Chemicals, Redmond, WA). Blood was collected by venipuncture, anaesthetized fish were killed by concussion and cervical spinal cord transection, tissues were stored at -20 °C until analysis. Condition factor (CF) was calculated as body weight divided by the cube of length and multiplied by 100 to give a percentage ($CF = (\text{weight})/(\text{length}^3) \times 100$). LSI and OSI were calculated as organ weight divided by body weight multiplied by 100 to give a percentage ((organ weight/body weight) $\times$ 100).

Fish were fed Silver Cup Fish Feed (Murray Elevators, Murray UT) with or without ^{3}H-TCDD for up to 320 day. TCDD-spiked food containing 5.4, 54, or 270 ng TCDD/kg was fed on every third day, which resulted in average dietary concentrations of 0, 1.8, 18 or 90 ng TCDD/kg moist weight (mw) of food (Walter et al., 2000). Food was spiked with both ^{3}H-labeled TCDD and non-labeled TCDD such that while the TCDD dose and TCDD specific activities (DPM/pg TCDD) were varied, the radiometric dose remained constant (Table 1). Control fish were not exposed to ^{3}H. Untreated (control) food contained < 0.2 ng TCDD/kg, moist weight (mw) and < 0.2 mg/PCB/kg, ww (data not shown).

The sampling scheme involved two phases. In the first phase, representative fish (usually 4) were collected from each exposure group after 50, 100, 150 or 200 day of exposure. By 250 day some of the fish were ready to spawn and the sampling entered phase 2. During phase two weekly checks were made to determine which fish were ready to spawn. Fish that were ready were spawned and tissue samples were collected as for phase one samples. Due to the different times in which the fish became ready to spawn the last phase of the exposure extended from 250 day to approximately 320 day.

2.1. Hematology

Blood samples were either collected in EDTA for blood cell enumeration or without anticoagulant for serum chemistry. Clinical pathology was assessed on the same day as blood collection. A 1:200 dilution in modified Dacie's solution was used to enumerate leukocytes (Blaxhall and Daisley, 1973; Campbell, 1988b). Erythrocyte counts and hemoglobin concentrations were determined by use of voltage impedance (Counter Z1, Beckman,) (Campbell, 1988a). Leucocytes were enumerated by microscopic evaluation of Wright's stained blood smears. Serum from two fish of the same treatment group were pooled to provide adequate sample volume for the quantification of sodium, potassium, total carbon dioxide, anion gap, iron, albumen, alkaline phosphatase, amylase, total billirubin, urea nitrogen, calcium, cholesterol, creatine kinase, creatinine, gamma glutamyl transferase, glucose, magnesium, phosphorus, sorbitol dehydrogenase, aspartate aminotransferase, alanine aminotransferase, total protein and osmolality. Blood chemistry measurements were performed with a tandem access analyzer (Abbott Diagnostics, Spectrum, Abbott Park, IL). Tests were not modified from standard methodologies used for mammals. Enzyme analysis was performed at 37 °C. Osmolality was determined by freezing point depression.

2.2. EROD measurement

CYP IA, cytochrome-requiring monooxygenase activity, was determined by measuring the activity of ethoxyresorufin-*O*-deethylase (EROD) in liver microsomes. Microsome preparation, EROD activity and total protein were determined using previously described methods (Newsted et al., 1995; Munkittrick et al., 1992).

2.3. Reproduction

Eggs from each female were collected and fertilized with pooled milt from non-treated male fish. At the time of collection, eggs were randomly placed into aliquants for incubation, determination of egg quality, or measurement of TCDD concentrations. Eggs were incubated in Heath Technica® vertical incubators using previously described techniques (Giesy et al., 1986). Eggs were monitored daily and dead eggs were removed and enumerated. Temperature and dissolved oxygen were monitored throughout the incubation. After hatching, fry were left in the incubators and monitored until the 'swim-up' stage.

2.4. Fecundity, production and egg quality

Number of eggs produced and egg viability was measured for each female. Size, density, caloric content, and lipid content were measured as indicators of egg quality. Egg diameter was measured with a Vernier caliper. The average volume of each egg was determined from the diameter by assuming the eggs were spherical. Density of eggs was determined by dividing the mass of a fixed number of eggs by the water displaced by the same eggs in a tared and volume calibrated vessel. This method provided simultaneous mass and vol-

Table 1
Concentrations of 2,3,7,8-TCDD and radiometric doses in food

	ng TCDD/kg diet			
Dose Group	Control	1.8	18	90
Σ TCDD in food (ng/kg)[a,b]	<0.2	5.4	54	270
Specific activity (DPM/pg TCDD)	na	541	57.0	10.5
Radiometric dose (DPM/g food)	na	974	1026	949

na = Not applicable.
[a] ΣTCDD equals non-radiolabelled and radiolabelled TCDD.
[b] Moist weight (mw).

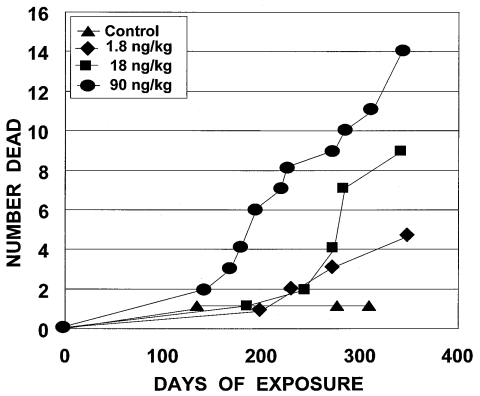

Fig. 1. Cumulative mortality of adult females as a function of time.

ume determinations to allow calculation of egg density. Caloric content was determined by bomb calorimetry. Lipid content of eggs was determined gravimetrically from hexane extracts. Egg survival was determined as the number of eggs that hatched. For the purpose of this study 'production' was defined as the number of eggs per female that survived to 28 day post-hatch. The incidence of deformities of fry was also enumerated.

2.5. Statistical methods

Differences among means of parameter values were examined by use of ANOVA followed by Tukey's HSD multiple range test (PROC GLM; SAS Institute, Carey, NC). Due to the small sample size, and the fact that individuals died at different times, no rigorous statistical tests could be applied to some parameters. Therefore, a one-way contingency table was used with the chi-squared statistic. Relationships between parameters as well as between parameters and treatments were examined by correlation analysis (PROC CORR; SAS Institute, Carey, NC).

3. Results and discussion

3.1. Survival and growth of adults

Some mortality of adult females was observed in all treatment groups, however mortality was least in the control group (Fig. 1). A single fish in the control group died of unknown causes on day 135 resulting in a mortality rate of 2.9%, no other adult fish died in the control exposure group through day 312. Mortality exceeded 5% in the 90 ng/ TCDD per kg, 18 and 1.8 ng TCDD/kg exposure groups on days 143, 243, and 224, respectively. By day 342, adult mortality showed a strong dose–response relationship with mortality rates of 14.3, 25.7 and 40% in the 1.8, 18 and 90 ng TCDD/kg exposure groups, respectively. This indicates that the dietary NOEC for mortality in adult rainbow trout is less than 1.8 ng TCDD/kg, (mw) for long-term exposure in food. However, the NOEC could be as great as 4.2 ng/kg because of the background concentration of TCDD and PCBs in the untreated food (discussed further in section on toxicity reference values). In a long-term exposure of brook trout (*Salvelinus fontinalis*) to TCDD, no significant mortality of adults was observed at dietary treatments that resulted in whole body concentrations burdens of up to 1200 ng TCDD/kg (Tietge et al., 1998). However, the exposure duration in that study was 26 week (182 day). That is approximately the time when mortality was first observed in the present study.

No significant differences in growth of adult females were observed among the exposure groups (Fig. 2). This was partly due to the considerable variability observed in growth among individual fish. This resulted in greater variability in size at the end of the exposure compared with the beginning (see standard deviations in Table 2). While there were no statistically significant differences in condition factor (CF) among exposure groups or duration of exposure, CF tended to be greater at longer exposure times (Table 2). No statistical differences were observed for either liver somatic index (LSI) or ovary somatic index (OSI) among the treatment groups. However, the OSI was greater at longer exposure times for all treatment groups. It was noted throughout the experiment that some individuals showed no ovarian development. For example after 200 day 2/4, 1/4, 3/3, and 2/4 individuals from the control, 1.8, 18, and 90 ng TCDD/kg groups, respectively, showed no or minimal (< 20 g) ovarian development. OSI was unusually low in the 18 ng TCDD/

Table 2
Mean length, whole body, liver and ovary weights, condition factor, liver somatic index, and ovary somatic index as a function of duration and intensity of dietary exposure to ^{3}H-2,3,7,8-TCDD

Dietary exposure (d)	Concentration (ng TCDD/kg)	*N*	Weight (g)[a]	Length (cm)	Liver weight (g)	Ovary weight (g)	Condition factor	Liver somatic index	Ovary somatic index
50	0	3	364 (48)	32.5 (1.9)	4.93 (0.34)	1.88 (0.69)	1.06 (0.12)	1.38 (0.25)	0.539 (0.24)
	1.8	4	428 (52)	33.6 (1.3)	5.38 (1.1)	2.83 (1.9)	1.12 (0.02)	1.25 (0.15)	0.667 (0.41)
	18	4	401 (168)	32.0 (4.1)	4.4 (1.1)	3.18 (1.8)	1.16 (0.08)	1.17 (0.28)	0.766 (0.32)
	90	4	411 (57)	32.5 (1.9)	5.83 (0.72)	2.08 (1.1)	1.20 (0.09)	1.43 (0.19)	0.535 (0.30)
100	0	4	505 (64)	36.1 (0.85)	6.08 (1.1)	5.15 (3.0)	1.07 (0.10)	1.20 (0.06)	1.01 (0.61)
	1.8	4	337 (99)	31.5 (2.9)	4.56 (1.1)	4.25 (3.5)	1.05 (0.12)	1.42 (0.40)	1.33 (0.97)
	18	4	402 (53)	32.9 (0.85)	5.28 (0.64)	7.23 (4.3)	1.13 (0.07)	1.32 (0.07)	1.86 (1.2)
	90	3	573 (169)	35.9 (2.7)	8.47 (3.4)	10.9 (7.0)	1.21 (0.1)	1.47 (0.50)	1.81 (0.73)
150	0	4	555 (109)	36.4 (2.7)	6.99 (1.8)	10.3 (7.4)	1.15 (0.06)	1.27 (0.34)	1.75 (1.01)
	1.8	4	468 (97)	33.3 (1.9)	6.79 (1.4)	17.4 (16)	1.26 (0.15)	1.46 (0.24)	3.55 (2.8)
	18	3	615 (175)	36.4 (3.6)	8.31 (2.7)	25.7 (12)	1.25 (0.10)	1.35 (0.29)	4.28 (1.8)
	90	4	639 (77)	37.3 (2.1)	9.25 (2.7)	21.1 (22)	1.23 (0.09)	1.49 (0.57)	3.60 (3.9)
200	0	2	759 (249)	38.8 (5.5)	11.1 (4.5)	69.5 (60)	1.29 (0.18)	1.37 (0.14)	10.9 (9.6)
	1.8	6	653 (205)	37.9 (3.6)	8.15 (3.0)	35.4 (32.4)	1.26 (0.01)	1.25 (0.17)	5.84 (6.1)
	18	3	809 (238)	40.3 (3.2)	10.1 (2.2)	1.3 (0.42)	1.21 (0.06)	1.27 (0.23)	0.10 (0.09)
	90	4	588 (103)	36.0 (2.3)	8.38 (3.0)	39.0 (32.4)	1.25 (0.03)	1.41 (0.35)	7.44 (6.6)

[a] Each value represents the mean with the standard deviations given in parentheses.

kg food exposure group after 200 d of exposure. All three fish sampled from this group exhibited essentially no ovary development. While this lack of development was observed in some fish from all exposure groups, 100% of individuals sampled from the 18 ng TCDD/kg treatment group at this time point exhibited no ovarian development. The reason for this lack of ovary development in certain individuals is unknown. The relatively small OSI in the 18 ng TCDD/kg, exposure group was most likely due to the small sample size and not a TCDD-related effect. The lack of dose-related effects on growth, CF, LSI, and OSI are similar to the findings observed with the dietary exposure of brook trout to TCDD (Tietge et al., 1998). In that study, there were no statistically significant effects on these endpoints in pre-spawn female brook trout. The fact that the TCDD concentration in the ovary of individuals exposed for 200 day was similar to that measured at the other sampling times indicates that the ovaries were still in chemical equilibrium (Jones et al., 2001). However, there was a statistically significant increase in LSI post-spawn when whole body TCDD concentrations were ≥ 300 ng/kg.

3.1.1. Liver EROD activity

One of the most consistently studied responses of fish when exposed to HDH is the induction of specific cytochrome P450 (CYP450) isozymes (Stegeman et al., 1990). This response has been shown to be regulated through a specific cellular receptor, the arylhydrocarbon receptor (AhR) that binds some dioxins and related planar, chlorinated hydrocarbons with varying affinities. This variation in affinity results in the chemicals having different potencies for inducing CYP450 isozymes (Newsted et al., 1995). The physiological significance of CYP450 induction on toxicity of TCDD-like compounds has not been clearly elucidated (Sweeney et al., 1979; Rifkind et al., 1985). More recently, the ability of TCDD to affect the endocrine system by altering circulating hormone levels (Sivarajah et al., 1978; Goldstein et al., 1989) or by altering expression of hormone receptors (Umbreit and Gallo, 1988) has increased interest in these compounds as 'xenohormones'. Induction of some hepatic CYP450 isozymes can cause changes in plasma hormones. The utility of the CYP450 assay procedure has lead to its being widely used as a biomarker of adverse effects of contaminant exposure, despite the uncertainty about the cause/effect relationship between CYP450 and reproductive endpoints (Stegeman et al., 1992).

Liver EROD activity was greater in fish exposed to TCDD relative to that in unexposed fish (Table 3, Fig. 3). However, when the relationship between EROD activity and TCDD concentration in liver was examined across all treatment groups

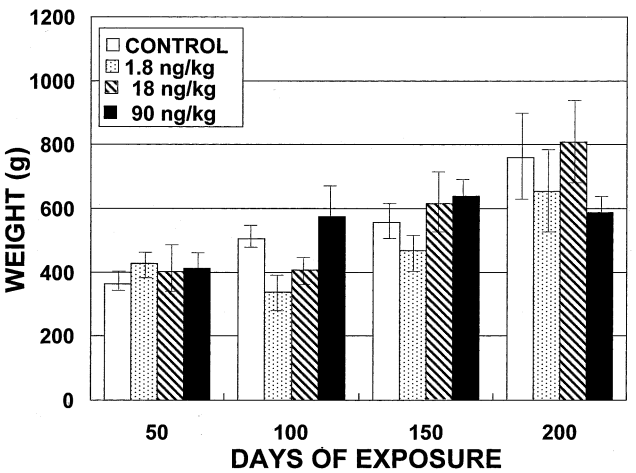

Fig. 2. Average weights of adult female rainbow trout exposed to each treatment as function of time.

Table 3
EROD activity in adult female rainbow trout activities as a function of duration and magnitude of dietary dose of TCDD

Days treatment	Dietary concentration	*N*	Liver concentration (ng TCDD/kg)	Liver EROD activity (pmol/min per mg)	EROD fold-induction
50	0	3	–	29.70 (6.72)	1.0
	1.8	4	0.26 (0.06)	45.23 (28.6)	1.5
	18	4	1.65 (0.57)	67.58 (50.9)	2.3
	90	4	–	57.9 (40.5)	1.9
100	0	4	–	5.193 (5.75)	1.0
	1.8	4	0.31 (0.05)	12.46 (2.31)	2.4
	18	4	2.88 (0.71)	11.21 (7.15)	2.2
	90	3	12.92 (2.75)	24.09 (16.2)	4.6
150	0	4	–	32.27 (24.5)	1.0
	1.8	4	0.21 (0.08)	17.75 (13.1)	0.6
	18	3	1.72 (0.56)	33.67 (36.3)	1.1
	90	4	9.93 (0.53)	60.12 (52.0)	1.9
200	0	2	–	1.41 -	1.0
	1.8	6	0.2 -	34.95 (5.56)	24.8
	18	3	2.85 (0.64)	16.46 (15.5)	11.7
	90	4	16.24 (2.21)	1.912 (2.17)	1.4

Each value represents the mean with the standard deviation in parentheses.

there was no correlation ($r^2 = 0.0006$). Mean overall (for all time points) EROD activities were 20, 28, 35 and 35 pmol/min per mg protein for control, 1.8, 18 and 90 ng TCDD/kg food dose groups, respectively. This represents a maximum level of induction of 1.7 fold over control. EROD activities decreased in all groups between 50 and 100 day of exposure, even though the concentration of TCDD in the liver increased in the treatment groups. After 150 day of exposure, the greatest EROD activity was observed in fish exposed to the greatest concentration of TCDD in the diet, but there was essentially no difference among those exposed to the two least doses and the control group. After 200 day of exposure, EROD activities were less than at any other time period. For instance, the mean EROD activity in livers of females exposed to the maximum dose was 2 pmol/min per mg protein while that in the controls was 1.4 pmol/min per mg protein. This decrease in activity after 200 day is consistent with other studies that have shown that CYP1A1 activities vary seasonally. Furthermore, declines in CYP1A1 activities in female fish have been observed with the approach of spawning and are influenced by the health, reproductive developmental status and condition of the fish and by environmental temperature (Stegeman and Hahn, 1993). In gravid females, suppression of EROD has been linked to the presence of estradiol (Monosson and Stegeman, 1991), and to plasma hormone levels (Anderson and Forlin, 1992). Several studies have reported suppression of EROD activities in reproductive female fish including; sunfish (Jiminez and Stegeman, 1990), pre-spawning dab from the North Sea (Sleiderink et al., 1995), and brook trout (Cormier et al., 2000). In female brook trout with whole body concentrations ≥ 600 ng TCDD/kg, EROD activity was biphasic in that an attenuated inductive response was noted in late maintenance (near-spawn) fish that was followed by a return to previous activity levels at the last post-spawn sampling time (Cormier et al., 2000). This biphasic response was not observed in male brook trout exposed to similar concentrations of TCDD.

The magnitude of EROD induction, reported in this study, differs from other studies that have shown relatively great increases in EROD activity after a single, larger dose of TCDD (van der Weiden et al., 1992; Newsted et al., 1995). However, it should be remembered that many of these single dose experiments were i.p. injection studies which may result in different time-dependent

doses being delivered to the liver. In an oral gavage study with rainbow trout, fish were dosed with 0.06–84 μg TCDD/kg (Parrott et al., 1995a). EROD activity ranged from approximately 1.9 pmol/mg protein per min in control fish to approximately 500 pmol/mg protein per min at the greatest dose. The TCDD oral dose causing greater EROD activity than that of controls was 0.072 μg TCDD/kg. This oral dose corresponds to a liver threshold concentration for induction of EROD activity of approximately 16 ng TCDD/kg. This threshold liver concentration was greater than most liver concentrations observed in our study. Thus, based on the results obtained from Parrott et al. (1995a), accumulation of TCDD in trout livers from our study were at or near the threshold for EROD induction. The results from the current study are also in agreement with previous studies that resulted in a maximal EROD induction of 3.7 fold induction in rainbow trout exposed to 413 ng TCDD /kg in the food for 30 day (Fisk et al., 1997). In this same study, EROD activity was only 1.3-fold greater than controls when fish were exposed to 40 ng TCDD/kg food for 30 day. The threshold whole body concentration for induction of EROD activity was 30–45 ng TCDD/kg, wet weight, with EROD activity returning to control levels at a whole body concentration of approximately 15 ng TCDD/kg. Since liver TCDD concentrations were not measured in their study. Fisk et al. (1997) assumed a liver TCDD concentration equivalent to 2% total body burden to estimate a liver threshold of approximately 55 ng TCDD/kg for induction of EROD activity. This liver threshold value is approximately 3.5-fold greater than the threshold liver concentration needed in our study to induce EROD activity. In contrast, as great as 46-fold induction over control EROD activity was observed in female brook trout with a whole body burden of approximately 1200 ng TCDD/kg (Cormier et al., 2000). However, the liver TCDD concentration, at this whole body concentration, was 1060 ng TCDD/kg which was approximately 65-fold greater than the maximal TCDD liver concentration achieved in our present study. If TCDD liver concentrations are compared with EROD activities for both brook trout and rainbow trout, the EROD induction responses are similar. In brook trout, a mean liver concentration of 17.7 ng TCDD/kg of liver resulted in a 2.3-fold induction of EROD activity relative to control. In the current study, rainbow trout exposed for 150 day, had a mean liver concentration of 16.2 ng TCDD/kg that resulted in a 2.1-fold induction of EROD activity relative to control. The maximal induction of EROD activity in female trout has been reported to be more than 80-fold relative to control after a 30 day exposure to 9000 ng/kg 2,3,4,7,8-PnCDF (Muir et al., 1990). In that experiment, even after 180 day of depuration, EROD activity in the livers of exposed fish was still more than 10-fold greater than in control fish. However, liver concentrations of 2, 3, 4, 7, 8-PnCDF were not reported which makes it difficult to compare EROD induction to the results of the present study. It appears from the results of these studies and the current study that

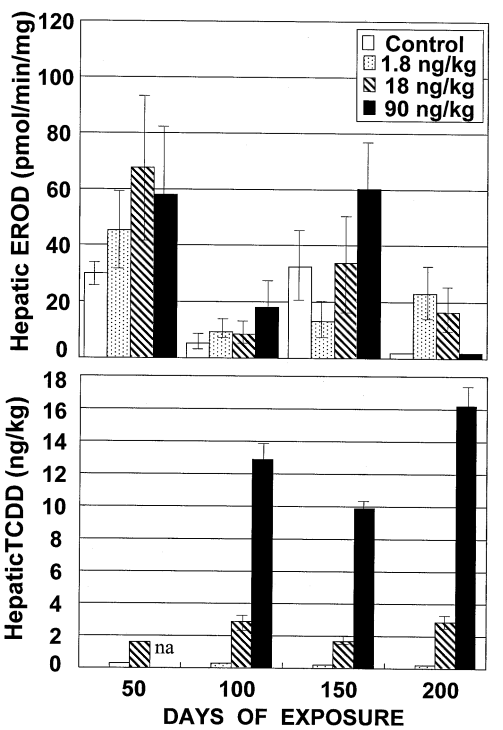

Fig. 3. Ethoxyresorufin-*O*-deethylase (EROD) activity (Top) and concentrations of 2,3,7,8-tetrachloro-*p*-dioxin (TCDD) (Bottom) in the livers of adult females at each of the sampling periods.

small, long-term exposure to TCDD results in only limited induction of EROD activity. While the mechanism for this phenomenon is unknown, several possibilities exist. For example, some studies demonstrate that relatively great doses of HDHs, such as PCBs, inhibit the activity of CYP1A isozymes (Gooch et al., 1989; Schlezinger and Stegeman, 2001). However, these effects are only observed at concentrations several orders of magnitude greater than those used in the current study. Duration and magnitude of TCDD exposure are also important considerations in the interpretation of EROD activity in fish. In an oral gavage study with rainbow trout, Parrott et al. (1995b) observed that a single dose of TCDD was rapidly distributed to the liver (<2 day) this corresponded with a significant increase in hepatic EROD activity. There were no dramatic differences in hepatic EROD activity over the 16 day study for fish given oral doses of 0.2, 0.6 or 2.0 μg TCDD/kg. The exception was the least dose, 0.06 μg TCDD/kg, where hepatic EROD activity increased between 2 and 16 day. In addition, the authors also observed that for liver, there was a trend of decreasing percent of nominal dose in the liver as oral dose was decreased. This decrease in partitioning to the liver was attributed to several potential factors. They include less absorption from the gastrointestinal tract and excretion of TCDD from the fish, absorption to the walls of the gastrointestinal tract, or distribution and partitioning into tissues other than liver. Therefore, when fish are exposed to small concentrations of TCDD in the diet over a long period of time, TCDD is accumulated in a dose- and time-dependent manner but may not accumulate into critical tissues to the same extent as that observed at greater doses (Fig. 3). Finally, a lack of dramatic EROD response in fish exposed to low environmental TCDD concentrations may be due to decreased internal bioavailability. For instance, TCDD is known to bind with high affinity to the CYP1A enzyme in liver and may result in a reduction of bioavailable TCDD (Hahn et al., 1993). Based on the results of the present study and that found in the literature, it appears that long-term exposure to small concentrations of TCDD can result in dose-dependent accumulation of TCDD in the liver without a concomitant functional response, such as induction of EROD activity. The lack of an observed EROD response most likely is due to several of these factors acting simultaneously. Thus, based on the results of this study, induction of EROD activity was not a good predictor of total concentrations of TCDD in the liver, nor was it a good predictor of adult toxicity observed during the study. This phenomenon will be discussed further when the relationships between responsiveness of various endpoints, including reproduction and biochemical responses are discussed.

3.1.2. Hematology

No statistically significant, TCDD-related alterations in any serum parameters, including chemistry, hematology or circulating enzymes were observed at any exposure time (data not shown). The total number of leukocytes was decreased in the 90 ng TCDD/kg treatment group fish when compared with controls. This was attributed to a decrease in both lymphocytes and neutrophils at all time points except 100 day, in which only the number of lymphocytes was decreased. There were no significant differences between control and any of the TCDD treatments for the following parameters: sodium (Na), potassium (K), Na to K ratio, calcium (Ca), magnesium (Mg), iron (Fe), phosphorus (PO_4), total anions, carbon dioxide (CO_2), albumin, globulins, the albumin to globulin ratio, cholesterol, total billirubin, creatine, total protein, urea nitrogen, glucose, osmolality, chloride (Cl) or, gap charge balance. There were no significant differences in plasma enzymes between any of the doses at any of the sampling times. The enzymes measured included: alkaline phosphatase, amylase, creatine kinase, G-glucuronyl transferase (GGT), sorbitol dehydrogenase (SDH), alanine aminotransferase (ALT) or aspartate aminotransferase (AST). This indicated that there were no tissue-level lesions caused by the long-term, chronic exposure to TCDD (Folmar 1993).

3.1.3. Behavior

Adult females in all TCDD treatment groups exhibited behavioral effects. However, since this

was not an anticipated endpoint in the initial design of the study, these effects could not be assessed quantitatively throughout the exposure. The behavioral effects were dose-dependent with the greatest effects observed in fish fed 90 ng TCDD/kg. These fish were listless and did not move around the tank while the control fish were constantly moving. The treated fish were unresponsive to tactile stimulation and did not become active when food was provided. The control fish actively responded to the presence of food and avoided tactile stimulation. Fish treated with TCDD also did not struggle when netted and removed from the water. However, these behavioral effects were not the readily quantified alterations in behavior that have been reported for studies with other species, particularly birds (Kubiak et al., 1989; Peakall and Peakall, 1973). While some previous studies have reported similar behavioral changes in trout (van der Weiden et al., 1992) and carp (van der Weiden et al., 1994) exposed at greater doses, only minor behavioral changes were observed. In another long-term feeding study (Tietge et al., 1998), only minor behavioral effects were also observed. While these behavioral alterations are of interest in laboratory studies, quantifying these effects in field populations would be very difficult as would be assessing their impact on the survival of fish in the wild. While the behavioral effects could not be quantified in such a way as to statistically determine NOEL or LOEL values, the effects were observed in all treatment groups. Thus, the LOEL was the least dose of 1.8 ng TCDD/kg.

3.1.4. Reproductive outcomes

There were no significant treatment-related effects on any of the measures of egg quality, total number of eggs produced, egg diameter, egg weight, egg density, lipid content or caloric content of eggs (data not shown). The fecundity (number of eggs produced) was not affected by any of the TCDD doses used in this study (Table 4). However, there were significant effects on the survival of eggs and fry (Figs. 4 and 5). There was a statistically significant ($P < 0.0001$) decrease in survival of eggs as a function of exposure and although there were no significant differences among the three exposure groups, survival was less at greater exposure concentrations (Table 4, Fig. 4). Survival of fry from unexposed adults was approximately 95%. Due to a pump failure that resulted in the loss of the remaining control fish before spawning 'control' egg survival was determined in additional control fish maintained in an associated identical facility. Reproductive outcomes of these additional controls were not monitored past hatch. Survival of eggs from females

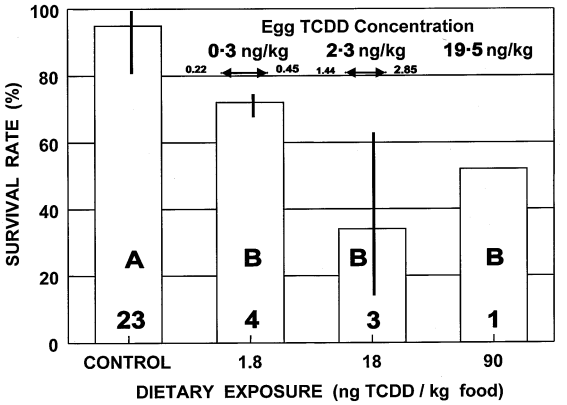

Fig. 4. Rate of survival of eggs to hatching. Number of females included in the sample are given on the bottom of the bars. Vertical bars represent 95% confidence intervals. Bars identified with the same letter (A or B) are not significantly different (Kruskal–Wallace, $P < 0.05$). The average concentration of TCDD in the eggs (with ranges given below) is given at the top of the figure.

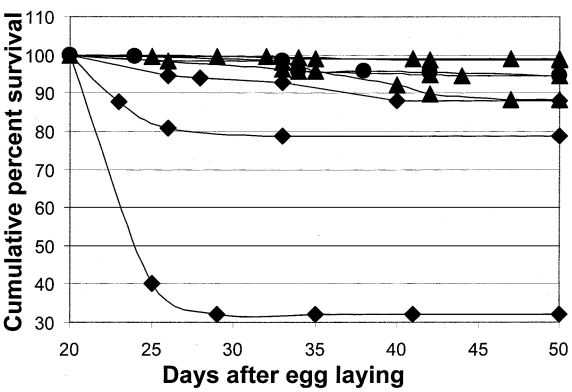

Fig. 5. Cumulative survival of fry from hatch at day 20 to swim—up. Each line represents data for a single female. Circles = 90 pg/g dose group; diamonds = 18 pg/g dose group; triangles = 1.8 pg/g dose group.

Table 4
Fecundity, fertility and survival of eggs from individual female rainbow trout after exposure to ^{3}H-2,3,7,8-TCDD

Dose	Egg TCDD (ng TCDD/kg)	Total eggs	% viable eggs	Total # fry	% dead fry	Total # Blue sac fry	% blue sac fry	Total # tail deformity	% tail deformity
C		1005	84.9	135	12.6	1	0.7	6	4.4
C		1050	65.2	349	4.6	7	2.0	4	1.2
1.8	0.70	1252	24.9	931	1.0	0	0.0	3	0.3
1.8	0.94	1398	15.5	1089	12.1	26	2.4	8	0.7
1.8	0.81	1144	40.7	679	5.5	8	1.2	3	0.4
1.8	0.59	2015	34.2	1326	1.9	6	0.5	9	0.7
18	6.35	1731	86.7	231	29.9	0	0.0	14	6.1
18	8.86	1271	16.4	1062	12.2	31	2.9	15	1.4
18	5.13	1170	88.3	137	24.1	0	0.0	1	0.7
90	35.1	1652	48.9	844	5.6	10	1.2	18	2.1

exposed to 18 or 90 ng TCDD/kg was 30 and 50%, respectively. While these decreases in survival were less than the control and least dose, the difference was not statistically significant compared with that of fish fed the 1.8 ng TCDD/kg diet. Thus, the LOEL, based on dietary exposure was 1.8 ng TCDD/kg. This LOEL corresponded with a concentration of 0.3 ng TCDD/kg in the egg.

Survival of fry decreased as a function of time for all three TCDD dose (Fig. 5). However, there was no statistically significant difference between the cumulative survival of the least and greatest dose, both of which were significantly greater than that of the 18 ng TCDD/kg dose group. The greatest decrease in survival occurred between the egg and fry stages. There was little cumulative mortality during the fry stage, with only a slight increase in mortality at swim-up.

3.1.5. Deformities

A number of deformities were observed in fry (Fig. 6, Table 4). These deformities included a preponderance of deformities of the spine and tail (Table 4) as well as deformities of the craniofacial area. The proportion of the fry that exhibited tail deformities ranged from 0.3 to 6.1% per individual spawning female. The greatest number of fry with tail deformities was observed in the greatest exposure dose (Table 4). The average proportion of fry exhibiting deformities for the control, low, medium and high dose groups were 2.8, 0.52, 2.7 and 2.1, respectively. There was no dose-dependent trend in the rates of deformities in the fish.

3.1.6. Edema

Edema or 'blue sac' disease was observed in the fry (Table 4). The proportion of fry exhibiting blue sac disease for the control, low, medium and high dietary exposures was 1.4, 1.0, 0.97 and 1.2, respectively. The rates of blue sac disease were rather low and there was no dose-dependent trend in the proportions of blue sac disease observed. 'Blue sac' disease has been observed in wild Great Lakes salmonids (Mac et al., 1993) and in salmonid eggs that have been injected with extracts of organochlorines from Great Lakes fish (Wright and Tillitt, 1999). Edema was also reported to occur in brook trout as a result of long-term parenteral exposure to TCDD. However, prevalence was not determined in that study (Johnson et al., 1998). The incidence of blue sac disease in the current study (0–2.9%) is within the range of values reported for the incidence in wild and laboratory 'control' populations (Wright and Tillitt, 1999; Mac et al., 1993). The lack of a dose-dependent increase in blue sac disease in the current study can be attributed to the relatively small concentrations of TCDD in the eggs (Table 4) that were less than the range of LOAEL values, 15–34 ng/kg TCDD or dioxin equivalents, for salmonids (Walker and Peterson, 1991; Johnson et al., 1998).

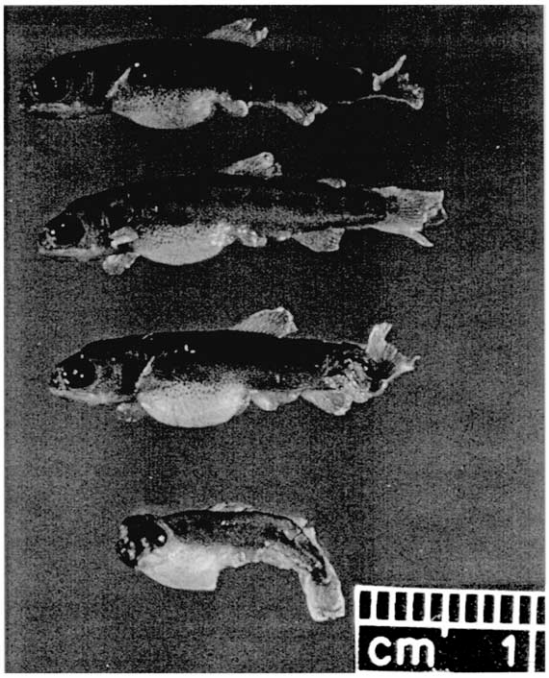

Fig. 6. Representative abnormalities in fry from female trout fed 18 ng ^{3}H-TCDD/kg in diet for 250 + day. The individuals shown were samples at day 42 post-spawning. Deformities include deformed and eroded fins, deformities of the spinal column and deformities of the jaw and craniofacial area.

3.1.7. TCDD toxicant reference values (TRVs)

Based on the results of this study, a set of toxicant reference values (TRVs) based on concentrations of TCDD in the diet, tissues and

water were developed. The most sensitive endpoint measured in this study was survival of the adult females. Behavioral effects were observed at the same treatment level. Thus, the LOEL based on either survival or behavioral effects was 1.8 ng TCDD/kg food. The next most sensitive endpoint was survival of eggs and fry. However, there was not a consistent dose-response relationship between treatments. Thus, the best estimate of the LOEL for reproduction was the same as that for long-term survival of adults. No treatment related effects were observed on blood chemistries or hematology. Thus, the LOEL was greater than the greatest dose for these parameters. The poor relationship between hepatic EROD activity and TCDD concentrations precluded the establishment of a LOEL. In addition, no relationship with any ecologically relevant endpoints measured in this study could be established based on EROD activity. The LOEL and NOEL based on concentrations in tissues were based on concentrations at the 150 day sampling, except for that based on concentrations of TCDD in eggs, which was from the time when fish were spawned. A detailed discussion of rates of uptake and disposition in tissues of the fish on which we report here, is presented elsewhere (Jones et al., 2001). The most appropriate, ecologically relevant endpoint is survival of adult fish. Based on this endpoint, the LOEL values were 1.8, 0.22, 0.21, 2.32 and 0.11 ng TCDD/kg, ww for diet, liver, muscle, adipose and egg tissue, respectively. In each case, the NOEL would be less than these values.

The food used in this experiment contained small, but measurable concentrations of TCDD and PCBs. Environmental contamination with TCDD and other dioxin-like compounds is ubiquitous. Therefore, it is virtually impossible to obtain commercial fish food that does not contain some of these compounds. Thus, the control diet was checked to determine the concentration of TCDD-like activities that could be present. Untreated (control) food contained < 0.2 ng TCDD/kg, moist weight (mw) and < 0.2 mg/PCB/kg, mw. Based on this concentration of PCBs, assuming the total TCDD-like activity (TCDD-TEQ) contained in Aroclor 1254, which has been measured to be 10.83 pg TCDD-TEQ/ug PCBs (unpublished data), the total concentration of TCDD-TEQ that would have been contributed by the PCBs would be 2.3 ng TCDD-TEQ/kg, ww of diet. This dioxin-like activity is contributed both by trace concentrations of polychlorinated dibenzo-*p*-dioxins (PCDD) and furans (PCDF) as well as the dioxin-like or co-planar PCB congeners. Other technical PCB mixtures contain lesser concentrations of TEQ. Thus, this would be an upper estimate of the concentrations of TEQ in the control diet. For this reason, there is some uncertainty in the actual LOEC that can be calculated. The actual, absolute value is at least 2.3-fold greater than the concentration of TCDD in the diet. While this effectively doubles the least dose, it has little effect on the greater doses.

3.1.8. Comparison to other studies

The results of this long-term, dietary exposure were compared with the results of similar studies of the effects of TCDD in salmonids, where the route of exposure and/or duration of exposure were different (Table 5). In a study in which adult rainbow trout were injected intra-peritoneally with TCDD, the LD50 was found to be 10 000 ng TCDD/kg, bw, while the LD20 was found to be 5000 ng TCDD/kg, bw (Spitsbergen et al., 1988). In addition, significant hematological changes were observed. While the concentration based on body weight cannot be directly compared with tissue concentrations, if the LC20 is assumed to be a LOEL based on mortality, the ratios between the LOEL observed in the i.p. injection study to those observed in the long-term feeding study, on which we report here, would be 2.4×10^{-4} for both liver and muscle. This indicates that the chronic LOEL is 24 000-fold less than the acute LOEL.

The LOEL for effects on the developing egg observed in this study was < 90 ng/kg. Using microinjection the LD50 for survival to swim-up was determined to be between 230 and 488 ng TCDD/kg, ww of egg (Walker and Peterson, 1991). Since some reduction in survival of fry was observed at the least concentration in our long-term feeding study, the LOEL was approximately 0.1 ng/kg, ww of egg, which is as much as 2300-fold less than that observed in the egg injection study.

Table 5
Effects of TCDD in rainbow trout exposed through different vectors and for different exposure duration

Species/Life stage	Vector	Concentration	Exposure	Whole body concentration	Effects	Reference
Eggs	Water	0.1	96 h		Growth retardation at day 72	Helder, 1981
Eggs	Water	25, 50, 75, 100, 150 ng/l	48 h	0.28,0.43, 0.47, 0.72, 1.62 g/kg	Sac fry mortality, LOAEL = 0.279 μg/kg in egg	Walker et al., 1990
Juvenile	Diet	0.0023, 2.3, 2300 μg/kg	15 week	0.06, 1.6, 1380 μg/kg	Reduced feed intake, delayed mortality, fin necrosis, LOAEL = 1380 g/kg	Hawkes and Norris, 1977
Juveniles	Water	10	96 h		Growth retardation at day 72	Branson et al., 1985
Juveniles	Oral	630	33 day		Some deaths at day 33	Miller et al., 1973
Swim-up fry	Water	0.038–0.789	28 day	0.99	Growth retard., mortality, fin necrosis, LOAEL = 0.99 ng/g	Mehrle et al., 1988
Juvenile	i.p. injection		80 day	1, 5, 25, 125 μg/kg	Delayed mort., wasting, fin necrosis, LOAEL = 5 μg/kg	Kleeman et al., 1988
Juvenile	i.p. injection		12 week	0.01, 0.05, 0.1, 0.5, 1, 5 μg/kg	Delayed mort, wasting, fin necrosis. LOAEL = 5 μg/kg	van der Weiden et al., 1990
Adult	i.p injection		80 day		Mortality and hematological changes. LD50 = 10 μg/kg, LOAEL = 1 ng/kg	Spitsbergen et al., 1988

In a study of swim-up fry exposed to TCDD in water, the tissue-based LOEL (28 day LD45) was found to be 765 ng/kg while the NOEL (28 day) was found to be 25 ng/kg, ww (Mehrle et al., 1988). Thus, the NOEL observed in the long-term feeding study was approximately 120-fold less than the value determined when fry accumulated TCDD directly from water, as it was when it was maternally deposited into the fry.

The most significant difference in the current study to those previously reported is that adult female fish exhibited effects, including mortality. Thus, the adults were as are sensitive as early life-stages. This sensitivity can be attributed to the extended duration of the exposure compared with the other studies that exposed trout to relatively great doses for a maximum of 16 week (Tietge et al., 1998). Therefore, it is not unexpected that no mortality was observed in brook trout with whole body concentrations of up to 1200 ng TCDD/kg after 105 day of exposure (Tietge et al., 1998) given that in the present study dose-dependent mortality was not observed until after 150–200 day of exposure. In the current study histological alterations were also noted in adult fish with a LOAEL of 5.7 ng TCDD/kg in food equivalent to a liver concentration of 0.9 ng TCDD/kg (Walter et al., 2000). The current concentrations of dioxin-like chemicals in food species likely to be consumed by salmonids range between 5 and 15 ng TCDD equivalents/kg (Jones et al., 1993), therefore it is possible that fish currently resident in the North American Great Lakes are being adversely affected by exposure to TCDD and TCDD-like compounds.

4. Conclusions

In this and the accompanying papers from this study we have demonstrated significant effects on the biochemistry, physiology and reproduction of rainbow tout exposed for an extended period to environmentally relevant TCDD concentrations. The effects observed in this study occurred at concentrations considerably less than those previously known to cause adverse effects in this or similar species. We attribute this decrease in the response threshold to the long-term effects of the exposure. Some of the key conclusions from this study are:

1. A statistically significant increase in the mortality of adult rainbow trout exposed to 1.8 ng TCDD/kg, ww in the diet.
2. Survival of eggs was not the most sensitive endpoint. Survival of eggs and adults were equally sensitive endpoints.
3. The LOEL dose was the least dietary dose studied. Thus, the threshold dose might be less. The dietary LOEL was 1.8 ng TCDD/kg, mw. This was equivalent to a daily intake of approximately 0.027 ng TCDD/kg, bw per day. These doses are equivalent to concentrations in the adipose tissue, muscle liver of 2.0, 0.043 and 0.43 ng TCDD/kg, mw. On a lipid weight basis the LOEL was 14 ng TCDD/kg lipid in muscle tissue.
4. The LOEL, based on survival of adult females or eggs, occurred at a concentration that caused little induction of hepatic EROD. This indicates that monitoring of EROD induction may not be protective of effects on survival or reproduction.
5. Long-term feeding results in a LOEL that is approximately 24 000-fold less than that observed for IP injections.
6. The LOEL is estimated to be approximately 1 ng TCDD/kg, ww of whole body.

Acknowledgements

This research was supported by a grant from the Biology Panel of the Exploratory Research Program on the US-EPA (R814850). The assistance of R. Crawford, is greatly appreciated. The helpful comments of two anonymous reviewers are also acknowledged.

References

Anderson, T., Forlin, L., 1992. Regulation of the cytochrome P450 enzyme system in fish. Aquat. Toxicol. 24, 1–20.

Asplund, L., Athanasiadou, M., Sjödin, A., Bergman, Å., Börjeson, H., 1999. Organohalogen substances in muscle,

egg and blood from healthy Baltic salmon (*Salmo salar*) and Baltic salmon that produced offspring with the M74 syndrome. Ambio 28, 67–76.

Batterman, A.R., Cook, P.M., Lodge, K.B., Lothenbach, D.B., Butterworth, B.C., 1989. Methodology used for a laboratory determination of relative contribution of water, sediment and food chain routes of uptake for 2,3,7,8-TCDD bioaccumulation by lake trout in Lake Ontario. Chemosphere 19, 451–458.

Bengtsson, B.-E., Hill, C., Bergman, Å., Brandt, I., Johansson, N., Magnhagen, C., Södergren, A., Thulin, J., 1999. Reproductive disturbances in Baltic fish: A synopsis of the FiRe Project. Ambio 28, 2–8.

Blaxhall, P.C., Daisley, K.W., 1973. Routine hematological methods for use with fish blood. J. Fish Biol. 5, 771–781.

Branson, D.R., Takahashi, I.T., Parker, W.M., Blau, G.E., 1985. Bioconcentration kinetics of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in rainbow trout. Environ. Toxicol. Chem. 4, 779–788.

Campbell, T.W., 1988a. Avian Hematology and Cytology. Iowa State University Press, Ames, IO, p. 101.

Campbell, T.W., 1988b. Fish cytology and hematology. Vet. Clin. North Am. 18, 349–364.

Cormier, S.M., Millward, M.R., Mueller, C., Subramanian, B., Johnson, R.D., Tietge, J.E., 2000. Temporal trends in ethoxyresorufin-O-deethylase activity of brook trout (*Salvelinus fontinalis*) fed 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Environ. Toxicol. Chem. 19, 462–471.

Fisk, A.T., Yarechewski, A.L., Metner, D.A., Evans, R.E., Lockhart, W.L., Muir, D.C.G., 1997. 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. Aquat. Toxicol. 37, 201–220.

Fitzsimons, J.D., Brown, S.B., Honeyfield, D.C., Hnath, J.G., 1999. A review of early mortality syndrome (EMS) in Great Lakes salmonids: Relationship with thiamine deficiency. Ambio 28, 9–15.

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.

Giesy, J.P., Snyder, E.M, 1998. Xenobiotic modulation of endocrine function in fishes. In: Principles and Processes for evaluating endocrine disruption, Anonymous Pensacola: SETAC Press, pp. 155–237.

Giesy, J.P., Newsted, J.L., Garling, D.L., 1986. Relationship between chlorinated hydrocarbon concentrations and rearing mortality of chinook salmon (*Onchorhynchus tshawytscha*) eggs from Lake Michigan. J. Great Lakes Res. 12, 82–98.

Gobas, F.A.P.C., Schrap, S.M., 1990. Bioaccumulation of some polychlorinated dibenzo-p-dioxins and octachlorodibenzofuran in the guppy (*Poecilia reticulata*). Chemosphere 20, 495–512.

Goldstein, J.A., Graham, M.J., Sloop, T., Maronpot, R., Goodrow, T., Lucier, G.W., 1989. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on estradiol metabolism and enzyme-altered foci in a two stage hepatocarcinogenesis model in rats. Chemosphere 18, 695–700.

Gooch, J.W., Elskus, A.A., Kloepper-Sams, P.J., Hahn, 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.

Guiney, P.D., Cook, P.M., Casselman, J.M., Fitzsimmons, J.D., Simonin, H.A., Zabel, E.W., Peterson, R.E., 1996. Assessment of 2,3,7,8-TCDD- Induced sac fry mortality in Lake Trout (*Salvelinus namaycush*) from different regions of the Great Lakes. Can. J. Fish. Aquat. Sci 53, 2080–2092.

Hahn, M.E., Lamb, T.M., Schultz, M.E., Smolowitz, R.M., Stegeman, J.J., 1993. Cytochrome P4501A induction and inhibition by 3,3′,4,4′-tetrachlorobiphenyl in an Ah receptor-containing fish hepatoma cell line (PLHC-1). Aquat. Toxicol. 26, 185–208.

Hawkes, C.L., Norris, L.A., 1977. Chronic Oral Toxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD) to Rainbow Trout. Trans. Am. Fish. Soc. 106, 641–647.

Helder, T., 1981. Effects of 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD) on early life stages of rainbow trout (*Salmo gairdneri*, Richardson). Toxicology 19, 101–112.

Jiminez, B.D., Stegeman, J.J., 1990. Detoxification enzymes as indicators of environmental stress on fish. Am. Fish. Soc. Symp. 8, 67–79.

Johnson, R.D., Tietge, J.E., Jensen, K.M., Fernandez, J.D., Linnum, A.L., Lothenbach, D.B., Holcombe, G.W., Cook, P.M., Christ, S.A., Lattier, D.L., Gordon, D.A., 1998. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to early life stage brook trout (*Salvelinus fontinalis*) following parental dietary exposure. Environ. Toxicol. Chem. 17, 2408–2421.

Jones, P.D., Ankley, G.T., Best, D.A., Crawford, R., DeGalan, N., Giesy, J.P., Kubiak, T.J., Ludwig, J.P., Newsted, J.L., Tillitt, D.E., Verbrugge, D.A., 1993. Biomagnification of bioassay derived 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents. Chemosphere 26, 1203–1212.

Jones, P.D., Kannan, K., Newsted, J.L., Tillitt, D.E., Williams, L.L., Giesy, J.P., 2001. Accumulation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by rainbow trout (*Onchorhynchus mykiss*) at environmentally relevant dietary concentrations. Environ. Toxicol. Chem. 20, 344–350.

Kleeman, J.M., Olson, J.R., Peterson, R.E., 1988. Species differences in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin toxicity and biotransformation in fish. Fundam. Appl. Toxicol. 10, 206–213.

Kubiak, T.J., Harris, H.J., Smith, L.M., Schwartz, T.R., Stalling, D.L., Trick, J.A., Sileo, L., Docherty, D.E., Erdman, T.C., 1989. Microcontaminants and reproductive impairment of the Forster's Tern on Green Bay, Lake Michigan-1983. Arch. Environ. Contam. Toxicol. 18, 706–727.

Mac, M.J., Schwartz, T.R., Edsall, C.C., Frank, A.M., 1993. Polychlorinated biphenyls in great lakes lake trout and

their eggs: Relationships to survival and congener composition 1979–1988. J. Great Lakes Res. 19, 752–765.

Mehrle, P.M., Buckler, D.R., Little, E.E., Smith, L.M., Petty, J.D., Peterman, P.H., Stalling, D.L., De Graeve, G.M., Coyle, J.J., Adams, W.J., 1988. Toxicity and Bioconcentration of 2, 3, 7, 8-tetrachlorodibenzodioxin and 2,3,7,8-Tetrachlorodibenzofuran in Rainbow Trout. Environ. Toxicol. Chem. 7, 47–62.

Miller, R.A., Norris, L.A., Hawkes, C.L., 1973. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin aquatic organisms. Environ. Health Perspect. 5, 177–186.

Monosson, E., Stegeman, J.J., 1991. Cytochrome P450E(P450Ia) Induction and Inhibition in Winter Flounder By 3,3′,4,4′-Tetrachlorobiphenyl: Comparison Response in Fish from Georges Bank and Narragansett Bay. Environ. Toxicol. Chem. 10, 765–774.

Muir, D.C.G., Yarechewski, A.L., Knoll, A., Webster, G.R.B., 1986. Bioconcentration and disposition of 1,3,6,8-tetrachlorodibenzo-*p*-dioxin and octachlorodibenzo-*p*-dioxin by rainbow trout and fathead minnows. Environ. Toxicol. Chem. 5, 261–272.

Muir, D.C.G., Yarechewski, A.L., Metner, D.A., Lockhart, W.L., Webster, G.R.B., Friesen, K.J., 1990. Dietary accumulation and sustained hepatic mixed function oxidase enzyme induction by 2,3,4,7,8-pentachlorodibenzofuran in rainbow trout. Environ. Toxicol. Chem. 9, 1463–1472.

Munkittrick, K.R., Van Der Kraak, G.J., McMaster, M.E., Portt, C.B., 1992. Response of hepatic MFO activity and plasma sex steroids to secondary treatment of bleached kraft mill effluent and mill shutdown. Environ. Toxicol. Chem. 11, 1427–1439.

Newsted, 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.

Parrott, J.L., Hodson, P.V., Servos, M.R., Huestis, S.L., Dixon, D.G., 1995a. Relative potency of polychlorinated dibenzo-*p*-dioxins and dibenzofurans for inducing mixed function oxygenase activity in rainbow trout. Environ. Chem. Toxicol. 6, 1041–1050.

Parrott, J.L., Hodson, P.V., Dixon, D.G., 1995b. Rainbow trout hepatic mixed-function oxygenase induction by polychlorinated dibenzo-*p*-dioxins (PCDDs) as a function of time and tissue concentration. J. Toxicol. Environ. Health 46, 301–316.

Peakall, D.B., Peakall, M.L., 1973. Effect of a chlorinated biphenyl on the reproduction of artificially and naturally incubated dove eggs. J. Appl. Ecol. 10, 863–868.

Rifkind, A.B., Sassa, S., Reyes, J., Muschick, H., 1985. Polychlorinated aromatic hydrocarbon lethality, mixed-function oxidase induction, and uroporphyrinogen decarboxylase inhibition in the chick embryo: dissociation of dose-response relationships. Toxicol. Appl. Pharmacol. 78, 268–279.

Schlezinger, J.J., Stegeman, J.J., 2001. Induction and suppression of cytochrome P450 1A by 3,3′,4,4′,5- pentachlorobiphenyl and its relationship to oxidative stress in the marine fish scup (*Stenotomus chrysops*). Aquat. Toxicol. 52, 101–115.

Sivarajah, K., Franklin, C.S., Williams, W.P., 1978. The effects of polychlorinated biphenyls on plasma steroid levels and hepatic microsomal enzymes in fish. J. Fish Biol. 13, 401–409.

Sleiderink, H.M., Breraarts, J.M., Goksoyr, A., Boon, J.P., 1995. Hepatic cytochrome P4501A induction in Dab (*Limanda limanda*) after oral dosing with the PCB mixture Clophen A40. Environ. Toxicol. Chem. 14, 679–687.

Spitsbergen, J.M., Kleeman, J.M., Peterson, R.E., 1988. Morphologic lesions and acute toxicity in rainbow trout (*Salmo gairdneri*) treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. J. Toxicol. Environ. Health 23, 333–358.

Spitsbergen, J.M., Walker, M.K., Olson, J.R., Peterson, R.E., 1991. Pathologic alterations in early life stages of lake trout, *Salvelinus namaycush*, exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin as fertilized eggs. Aquat. Toxicol. 19, 41–72.

Stegeman, J.J., Brouwer, M., Di Giulio, R.T., Förlin, L., Fowler, B.A., Sanders, B.M., Van Veld, P.A., 1992. Molecular responses to environmental contamination: Enzyme and protein systems as indicators of chemical exposure and effect. In: Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress. Lewis Publishers, Chelsea, MI, pp. 235–335.

Stegeman, J.J., Woodin, B.R., Smolowitz, R.M., 1990. Structure, function and regulation of cytochrome P-450 forms in fish. Biochem. Soc. Trans. 18, 19–22.

Sweeney, G.D., Jones, K.G., Cole, F.M., Basford, D., Krestynski, F., 1979. Iron deficiency prevents liver toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Science 204, 332–335.

Tietge, J.E., Johnson, R.D., Jensen, K.M., Cook, P.M., Elonen, G.E., Fernandez, J.D., Holcombe, G.W., Lothenbach, D.B., Nichols, J.W., 1998. Reproductive toxicity and disposition of 2,3,7,8-tetrachlorodibenzo-pdioxin in adult brook trout (*Salvelinus fontinalis*) following a dietary exposure. Environ. Toxicol. Chem. 17, 2395–2407.

Umbreit, T.H., Gallo, M.A., 1988. Physiological implications of estrogen receptor modulation by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Tox. Lett. 42, 5–14.

van der Weiden, M.E.J., van der Kolk, J., Penninks, A.H., Seinen, W., Van den Berg, M.K., 1990. A dose/response study with 2,3,7,8-TCDD in the rainbow trout (*Oncorhynchus mykiss*). Chemosphere 20, 1053–1058.

van der Weiden, M.E.J., van der Kolk, J., Bleumink, R., Seinen, W., Van den Berg, M., 1992. Concurrence of P450 1A1 induction and toxic effects after administration of a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in the rainbow trout (*Onchorhynchus mykiss*). Aquat. Toxicol. 24, 123–142.

van der Weiden, M.E.J., Bleumink, J.R., Seinen, W., Van den Berg, M., 1994. Concurrence of P450 1A induction and toxic effects in the mirror carp (*Cyprinus carpio*), after administration of a low dose of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Aquat. Toxicol. 29, 147–162.

Walker, M.K., Spitsbergen, J.M., Olson, J.R., Peterson, R.E., 1990. 2,3,7,8-tetrachlorodibenz-*p*-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 48, 875–883.

Walker, M.K., Peterson, R.E., 1991. Potencies of polychlorinated dibenzo-*p*-dioxin, dibenzofuran and biphenyl congeners, relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin for producing early life stage mortality in rainbow trout (*Onchorhynchus mykiss*). Aquat. Toxicol. 21, 219–238.

Walker, M.K., Peterson, R.E., 1994. Aquatic toxicity of dioxins and related chemicals. In: Schecter, A. (Ed.), Dioxins and Health. Plenum Press, New York, pp. 347–387.

Walter, G.L., Jones, P.D., Giesy, J.P., 2000. Pathologic alterations in adult rainbow trout, *Oncorhynchus mykiss*, exposed to dietary 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Aquat. Toxicol. 50, 287–299.

Wright, P.H., Tillitt, D.E., 1999. Embryotoxicity of a Great Lakes Lake Trout Extract to Developing Rainbow Trout. Aquat. Toxicol. 47, 77–92.

Zabel, E.W., Cook, P.M., Peterson, R.E., 1995. Potency of 3,3′,4,4′,5-Pentachlorobiphenyl (PCB126), Alone and in Combination with 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (TCDD), To Produce Lake Trout Early Life-Stage Mortality. Environ. Toxicol. Chem. 14, 2175–2179.