SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 17, No. 3, pp. 472–483, 1998
Printed in the USA
0730-7268/98 $6.00 + .00

COMPARATIVE TOXICITY OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN TO SEVEN FRESHWATER FISH SPECIES DURING EARLY LIFE-STAGE DEVELOPMENT

GREGORY E. ELONEN, ROBERT L. SPEHAR,* GARY W. HOLCOMBE, RODNEY D. JOHNSON, JOSEPH D. FERNANDEZ, RUSSELL J. ERICKSON, JOSEPH E. TIETGE, and PHILIP M. COOK
U.S. Environmental Protection Agency, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, Minnesota 55804

(*Received* 4 *April* 1997; *Accepted* 14 *July* 1997)

Abstract—The toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to fathead minnow (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), lake herring (*Coregonus artedii*), medaka (*Oryzias latipes*), white sucker (*Catastomus commersoni*), northern pike (*Esox lucius*), and zebrafish (*Danio danio*) were observed during early life-stage development after waterborne exposure of fertilized eggs. Species sensitivity based on TCDD-C_{egg} (TCDD concentration in eggs) was determined by effects observed over a 32-d period for all species except lake herring in which a 100-d period was used. Signs of TCDD toxicity, including edema, hemorrhaging, and craniofacial malformations were essentially identical to those observed in salmonids following TCDD egg exposure and preceded or accompanied mortality most often during the period from hatch through swim-up. The no-observed-effect concentrations and lowest-observed-effect concentrations, based on significant decreases in survival and growth as compared to the controls, ranged from 175 and 270 pg/g for lake herring to 424 and 2,000 pg/g for zebrafish, respectively. Shapes of concentration–response curves, expressed as TCDD-C_{egg} versus percent mortality, were similar for all species and were consistently steep suggesting that the mechanism of action of TCDD is the same among these species. The LC_{egg}50s (concentrations in eggs causing 50% lethality to fish at test termination) ranged from 539 pg/g for the fathead minnow to 2,610 pg/g for zebrafish. Comparisons of LC_{egg}50s indicate that the tested species were approximately 8 to 38 times less sensitive to TCDD than lake trout, the most sensitive species evaluated to date. When LC_{egg}50s are normalized to the fraction lipid in eggs (LC_{egg}/50s), the risk to early life stage survival for the species tested ranges from 16- to 180-fold less than for lake trout.

Keywords—2,3,7,8-Tetrachlorodibenzo-*p*-dioxin Fish Early life stage Toxicity

INTRODUCTION

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic chemical of the group of hydrophobic, halogenated aromatic compounds that include similarly structured polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) [1,2]. Because of the toxicity of TCDD and its association with aquatic sediments, biota, and the organic carbon fraction of ambient waters, it poses a potential risk to aquatic organisms. A particular concern is that TCDD and structurally related compounds can bioaccumulate in the food chain [3] and can act additively in causing toxicity [4,5]. Both the biochemical and toxic effects of these compounds have been found to be similar in different species and, therefore, it is proposed that they may act through a common aryl hydrocarbon receptor (AhR)-mediated mechanism [1,2]. Hahn et al. [6] indicate that fish possess this receptor and recent data by Guiney et al. [7] suggest that it may be functional during embryo development. Studies, as reviewed by Guiney et al. [8] and Walker et al. [9], indicate that these compounds are present in both the adults and eggs of wild fish. Their major route of accumulation into the eggs of fish has been shown to be via the adult female during ovary development [9,10].

Effects caused by TCDD in aquatic species occur at various times after exposure depending upon the exposure concentration, life stage of the organism, and endpoint tested. Reviews by Cook et al. [3] and Walker and Peterson [11] show that TCDD is extremely toxic to newly hatched fish, especially salmonids, after the eggs have been exposed. Signs of TCDD toxicity described thus far for salmonids [12–14] resemble the condition called blue-sac disease [15], which consists of edema, hemorrhaging, and craniofacial deformity prior to death. In lake trout (*Salvelinus namaycush*), the most sensitive salmonid tested thus far, significant mortality occurs after hatch when TCDD concentrations in eggs (referred to as TCDD-C_{egg}s in this text) are as low as 40 pg/g egg [12]. Studies with lake trout indicate that the route of exposure of TCDD to the egg does not appreciably affect toxicity. For example, the LC_{egg}50s (concentrations in eggs causing 50% lethality to fish at test termination) for this species exposed via maternal transfer, water, and egg injection are similar at 58, 69, and 80 pg/g, respectively [9].

Comparisons of TCDD toxicity among previously tested fish species are difficult because various test conditions, including exposure regimes (waterborne, intraperitoneal injection, or diet) and life stages (eggs, larvae, juvenile, or adult) have been used [3,11]. Within a fish species, sensitivity to TCDD is highly dependent on the age and size of the organism [16] and on exposure time or stage during development [17–20]. In addition, studies that use the same dose metric for measuring TCDD toxicity (e.g., TCDD-C_{egg}s) are available for only a few species. The purpose of this study was to determine and compare the toxicity of TCDD to early life stages of several freshwater fish species on the basis of TCDD-C_{egg}s from waterborne exposure of fertilized eggs. Subobjectives were to

* To whom correspondence may be addressed (spehar.robert@epamail.epa.gov).

This manuscript has been reviewed in accordance with official U.S. Environmental Protection Agency procedures, however, the content does not reflect EPA policy. Mention of trade names does imply endorsement by the EPA or the U.S. government.

Table 1. Sources of newly fertilized eggs (<24 h old) for TCDD tests with seven species of fish

Species	Source of eggs
Fathead minnow (*Pimephales promelas*)	U.S. EPA, MED—Duluth, MN
Channel catfish (*Ictalurus punctatus*)	Chesapeake State Fish Hatchery, Mt. Vernon, MD
Lake herring (*Coregonus artedii*)	Lake Superior at Squirrel Fisheries of Port Wing, WI
Medaka (*Oryzias latipes*)	U.S. EPA, MED—Duluth, MN
White sucker (*Catastomus commersoni*)	Greenwood Lake, MN
Northern pike (*Esox lucius*)	Lac Court Oreilles, WI
Zebrafish (*Danio rerio*)	U.S. EPA, MED—Duluth, MN

determine if the signs of TCDD toxicity and the shapes of the concentration–response curves were similar among the test species and with those observed for other species exposed to TCDD. Life stages, from eggs to juveniles, were observed for adverse effects over the duration of the tests (32–100 d). Seven fish species with different egg sizes, lipid contents, and embryo development times, and larval and juvenile development stages and feeding regimes were tested. The species included one cold water species, the lake herring (*Coregonus artedii*), and six cool- and warm-water species, the white sucker (*Catastomus commersoni*), northern pike (*Esox lucius*), fathead minnow (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), medaka (*Oryzias latipes*), and zebrafish (*Danio rerio*). Lake herring and the white sucker are important species in the Great Lakes where TCDD bioaccumulation by fish is widespread [21] and northern pike and channel catfish are important game fish that have been shown to be sensitive to TCDD based on measured water concentrations [22,23]. The fathead minnow, medaka, and zebrafish were studied because they belong to two families that are distributed over a wide range of habitats and have been used extensively in aquatic toxicity research.

MATERIALS AND METHODS

Chemical

Tritium-labeled TCDD ([^{3}H]2,3,7,8-tetrachlorodibenzo-*p*-dioxin), hereafter referred to as TCDD in the text, was obtained from Cambridge Isotope Laboratories, Andover, MA, USA (Lot No. AWN-729-87). Impurities were detected by gas chromatography/mass spectrometry (GC/MS) and the TCDD was repurified (B.C. Butterworth, personal communication). An acetone-TCDD stock solution was prepared for use in the experiments and was measured by GC/MS to be 76% chemically pure, 87% radiochemically pure, and had a specific activity of 38.3 Ci/mM TCDD (1.4 Tbq/mM).

Test species

The sources of eggs for the species tested are shown in Table 1. Eggs of northern pike, white sucker, and lake herring were obtained by stripping adult fish and artificially fertilizing the eggs in clean water before transport to the laboratory for testing. Fertilized white sucker eggs were placed in a solution containing lake water and red clay after fertilization to prevent clumping during transport. To provide for a uniform exposure, the mucoid layer surrounding channel catfish eggs was removed before testing using a 1.5% Na_2SO_3 solution as described by Ringle et al. [24]. Eggs from each species were obtained from at least three females (except two female channel catfish were used). Characteristics of eggs and larvae of the fish tested are given in Table 2.

Water characteristics

Tests were conducted in Lake Superior water filtered through sand and treated with ultraviolet light before being adjusted to the desired test-water conditions. The temperature of the test water (Table 2) was measured continuously with a computerized laboratory monitoring system. Dissolved oxygen (DO), conductivity, and pH were measured twice weekly in all tanks and total hardness and alkalinity were measured weekly in one control and one treatment tank. Mean DO (mg/L) and conductivity (μS/cm) values for tests with fathead minnow, channel catfish, lake herring, medaka, white sucker, northern pike, and zebrafish were 7.7 and 100, 7.2 and 80, 10.7 and 62, 7.7 and 97, 9.6 and 63, 9.7 and 68, and 7.6 and 101, respectively. The range of mean values for total hardness, alkalinity, and pH were similar for all tests and were total hardness = 44 to 46 mg/L as $CaCO_3$, alkalinity = 43 to 45 mg/L as $CaCO_3$ and pH 7.8–8.1.

Table 2. Characteristics of eggs and larvae of fish exposed to TCDD in early life stage tests

Species	Initial no. eggs per treatment	Egg weight (mg)[a]	Egg lipid (%)[b]	Time to hatch (d)[c]	Time to first feeding (d)[c,d]	Test temp. (°C)	Test duration (d)[c]
Fathead minnow	130	1.5 (0.1)[e]	2.4 (0.01)[e]	4–6	1–2	24.8 (0.7)[e]	32
Channel catfish	100	35.2 (5.6)	4.8 (0.1)	3–6	2–3	25.5 (0.2)	32
Lake herring	120	5.9 (0.5)	6.6 (0.01)	41–55	13–18	7.8 (0.4)	100
Medaka	110	0.9 (0.1)	2.9 (0.02)	9–14	1–2	25.1 (0.2)	32
White sucker	100	19.0 (2.0)	2.5 (0.2)	8–10	1–2	14.9 (0.2)	32
Northern pike	100	11.9 (1.2)	4.2 (0.1)	5–7	6–7	15.5 (0.2)	32
Zebrafish	100	0.6 (0.1)	1.7 (0.1)	3–12	1–2	26.0 (0.2)	32

[a] Wet weight of individual eggs based on 42 samples (2–5 eggs per sample).
[b] Percentage lipid based on three to six samples consisting of 0.5 g (wet weight) of eggs per sample.
[c] Time in days.
[d] Time is from first hatch.
[e] Mean (SD).

Test system

The exposure system consisted of three 40 × 20 × 26-cm-high glass tanks with water at the appropriate test temperature. One tank contained 5.0 L of lake water and was used for incubating one set of control eggs. The second tank served as a solvent control and contained 5.0 L of clean lake water plus 0.5 to 10 ml of HPLC-grade acetone (0.1–2.0 ml/L) that was added 30 min prior to the start of the exposure. The third tank was used for TCDD exposures and contained 5 L of water with TCDD and the same acetone concentration as the solvent control. As with the solvent controls, the acetone-TCDD solutions were added to the exposure tank 30 min prior to the start of each egg exposure. Each tank was connected to a 1-L overhead glass chamber (16 × 12 × 16 cm high) that had a self-starting siphon to provide water recirculation for eggs that were placed in the tanks. The recirculating flow rate in each of the three tanks was 80 ml/min for all tests.

After exposure, control and exposed eggs and, subsequently, hatched fish were housed in a clean-water system (no TCDD added) that contained 14 glass tanks (23.5 × 14 × 18 cm high). Duplicate tanks for fish from each control (with and without solvent) and the five TCDD exposure treatments were randomly arranged. Tanks contained 4 L of water and had an average flow rate of approximately 90 ml/min. Standpipes with self-starting siphons varied water levels ±2.0 cm to allow for water exchange in and out of suspended egg cups. Standpipe siphons were replaced with stainless steel screens once the hatched fish were released to the tanks. Tanks were immersed in a water bath to maintain uniform temperature. Fluorescent lamps provided a 16-h photoperiod with light intensity that ranged from 61 to 139 lumens at the water surface of the tanks.

Exposure design and test procedures

The number of eggs of each species exposed per treatment are listed in Table 2. Eggs (<24 h postfertilization) for the various treatments were pooled in a round, flat-bottomed glass container that was placed in a 46 × 38 × 12-cm-deep polypropylene pan that contained flowing water at the desired test temperature. Subsequently, groups of 5 to 10 eggs were randomly selected and placed, consecutively, in egg cups until the desired number of eggs was obtained. Egg cups were made from 6.8 cm high glass tubing (5.8 cm outer diameter, 5.0 cm inner diameter) and contained 40-mesh stainless-steel screen bottoms. Two 5.8-cm solid glass rods with a diameter of 1.0 cm were horizontally attached to the bottom of each cup to displace them from the bottom of the exposure tank in order to facilitate water exchange during the exposure period. After the eggs were counted, duplicate cups were randomly placed in both control tanks and the TCDD exposure tank. Five groups of eggs with graded TCDD concentrations were obtained by removing eggs from the tank after different periods of exposure ranging from 6 to 540 min. Exposure periods were selected based on egg size or from preliminary toxicity tests conducted to determine the desired range for TCDD-C_{egg}s. After the specified exposure period, egg cups were removed from the TCDD exposure tank, rinsed with clean water, and placed in the solvent control tank to obtain equal solvent exposures for all eggs. Although this procedure resulted in the detection of TCDD in solvent control eggs of some species, analyses of these eggs showed that TCDD-C_{egg}s were well below those exposed to TCDD from the exposure tank. In addition, significant differences in the responses observed between fish in the clean water and solvent control tanks were not seen with any species. When the longest exposure period was completed, eggs were transferred to the clean-water system test tanks for further observation. Organisms were observed for 32 d postfertilization for all species except for lake herring for which the test lasted 100 d.

Eggs were checked daily during the incubation period for fungus and dead eggs were removed and recorded. At eye-up, eggs were randomly thinned to 20 per duplicate in tests with white sucker, northern pike, fathead minnow, and medaka, and to 25 per duplicate in tests with lake herring, channel catfish, and zebrafish. Following hatch, all organisms were released into the clean-water tanks and were observed daily for signs of TCDD toxicity. Digital images were obtained for some fish to provide a photographic record of toxic effects. Tanks were cleaned as necessary to remove uneaten food and fecal material.

Times from hatch to first feeding for each species are shown in Table 2. All fish except for zebrafish were fed live brine shrimp ad libitum twice per day on Monday through Friday and once per day on weekends. During the last 5 d of the northern pike test, the afternoon feeding of brine shrimp was supplemented with a mixture of approximately 40 (<2-week-old) fathead minnows and white suckers per tank to reduce aggressive behavior and cannibalism. Zebrafish were fed ~0.1 ml of a slurry of Kyowa fish food (Biokyowa Inc., Cape Girardeau, MO, USA) and lake water (1.0 g in 10 ml water) twice per day for the first 4 d of feeding. After 4 d, zebrafish in all treatments were fed live brine shrimp ad libitum and the Kyowa slurry twice per day on Monday through Friday and once per day on weekends. Fish were not fed for 24 h prior to test termination. Tests with warm-water species were terminated by killing surviving fish with ice water, and tests with the cool- and cold-water fish were terminated by killing the fish with MS-222. Fish were then blotted dry, weighed to the nearest mg (wet weight), and measured for total length to the nearest mm for growth determination.

Chemical analyses

GC/MS analysis was used to determine the specific activity and radiopurity of TCDD, as well as for correcting for the influence of tritium decay on TCDD concentrations throughout the study (J.D. Fernandez, personal communication). A 200-ml acetone stock solution of TCDD was prepared and the appropriate dilutions were made for water exposures of the eggs. At the beginning and end of each exposure, triplicate water samples (2 ml) were taken from the control and the exposure tank and were analyzed by liquid scintillation counting (LSC) (Table 3). Water samples were pipetted into a 20-ml glass scintillation vial and stored at 4°C for 30 min prior to the final steps of sample processing. Two to 3 h prior to analysis, 15 ml of an alkyl naphthalene-based counting fluor (Ultima Gold™, Packard Instrument, Meriden, CT, USA) was added to the vial. Vials were capped, shaken vigorously, and placed in the LSC. Radiological analyses were conducted on a model 2500TR LSC using the Alpha/Beta software program (Packard Instrument) operated in tandem with either a 386 Value Point™ PC (IBM, White Plains, NY, USA) or a Proline™ 4/33S PC (Compaq, Houston, TX, USA). The LSC used a ^{133}Ba source as an external standard for sample quench determinations, which was performed by the transformed spectral index of the external standard (tSIE) and subsequent automatic quench corrections (automatic efficiency control—AEC). The 10-vial quench standard set (Packard Instrument) was traceable

Table 3. Measured TCDD concentrations in exposure water (in ng/L) from early life stage tests with seven species of fish[a]

	Fathead minnow	Channel catfish	Lake herring	Medaka	White sucker	Northern pike	Zebrafish
Pre-exposure							
CC[b]	0.01 (0.0)	—[c]	ND[d]	ND	—	ND	0.01 (0.0)
SC[e]	0.07 (0.0)	—	0.05 (0.01)	0.10 (0.0)	—	0.03 (0.0)	0.08 (0.0)
Exp. tank	9 (1)	31[f]	31 (5)	29 (3)	285 (7)	208 (10)	23 (1)
Postexposure							
CC	ND	ND	ND	ND	—	0.02 (0.0)	0.01 (0.0)
SC	0.3 (0.0)	1.7 (0.1)	1.2 (0.1)	0.6 (0.0)	—	3.2 (0.1)	0.4 (0.0)
Exp. tank	7 (2)	21 (2)	16 (1)	17 (2)	105 (8)	51 (1)	9 (1)

[a] Values expressed as mean (SD) of triplicate 2-ml samples.
[b] Clean-water control.
[c] No measurement taken.
[d] No detection (detection limit = 0.0065 ng/L in water).
[e] Solvent control.
[f] Nominal value.

to standards of the National Institute of Standards and Technology. The detection limit, defined by $DPM_{bkg} + 2(\sigma_{bkg} + \sigma_{sample})$ for water measurements, was 0.0065 ng TCDD/L, where DPM = disintegrations per min, bkg = background, and σ = standard deviation (SD).

Immediately after the exposure period, triplicate samples of two to five eggs from each of the treatments and controls were blotted, weighed, and analyzed by LSC to determine TCDD-C_{egg}s (Table 4). Extra eggs that were exposed for the longest duration (highest concentration) were also analyzed by GC/MS to validate LSC measurements of TCDD-C_{egg}s. In addition, triplicate samples of one to eight white suckers and northern pike (≤1 d posthatch) and lake herring (~5 d posthatch) and samples of two to six lake herring, channel catfish, northern pike, medaka, and zebrafish at test termination were measured to determine concentrations of TCDD in organisms for comparisons with TCDD-C_{egg}s. Samples subjected to LSC analysis were placed on the bottom of a 20-ml scintillation vial and stored at 4°C until 30 min prior to final steps of sample processing. After the samples had warmed to room temperature, 2 ml of Soluene® 350 (0.5 N quaternary ammonium hydroxide in toluene solution, Packard Instrument) were added to the vials, which were then placed in a vial rack and set on an orbital shaker (model 3520, Lab-Line Instruments, Melrose Park, IL, USA). The samples were shaken overnight at 100 rpm. Two to 3 h prior to LSC analysis, 15 ml of counting fluor were added to the vials, which were then capped, shaken vigorously, and placed in the LSC. The detection limit for TCDD concentrations measured in wet tissue was 0.8 pg/g based on an average sample size of 20 mg. In addition to TCDD analyses, three to six samples consisting of ~0.5 g of eggs were analyzed for total lipid content (measured as % wet weight of eggs) using the microgravimetric method of Radin [25]. Lipid percentages of the eggs of each species are reported in Table 2.

Statistical analysis

Data on egg hatchability, survival and growth (length and weight) were checked for homogeneity of variances across groups and percentage data were transformed to % arc-sin for variance stabilization before being analyzed by ANOVA and Dunnett's one-sided comparison of treatment means with combined control (clean-water and solvent) means ($p \leq 0.05$) [26]. The NOEC (no-observed-effect concentration) and LOEC (lowest-observed-effect concentration) for each species were based on significant differences ($p \leq 0.05$) in survival and/or growth as compared to the controls at test termination. Survival and growth data were based on the number of eggs thinned at eye-up. Response data for species in clean-water and solvent controls were compared using a paired *t*-test.

Percent mortality versus TCDD-C_{egg} was analyzed using probit analysis. The model assumed that the TCDD-C_{egg} that resulted in the death of individual organisms had a log-normal distribution with mean μ and standard deviation σ and that there existed a background mortality fraction b over the duration of the experiment. The expected percent mortality would therefore be:

$$P = 100\left[b + (1 - b)\int_{-\infty}^{\ln(\text{TCDD-}C_{egg})} \frac{e^{-(x-\mu)^2/2\sigma^2}}{\sigma\sqrt{2\pi}}\,dx\right]$$

Rather than correcting observed mortality using an estimate of background mortality based only on controls, all model parameters (μ, σ, b) were estimated simultaneously by fitting the mortality and TCDD-C_{egg}s to the above model using maximum likelihood analysis [27]. Standard errors of the parameters were estimated using the inverse of the information matrix [27]. Confidence limits (CLs) for parameters were assigned using twice these standard errors. The LC_{egg}50s and CLs were calculated as the antilogs of the estimate for μ and its confidence limits. Standard errors of predicted mortalities at various TCDD-C_{egg}s were estimated by reformulating the model in terms of these mortalities and recomputing the information matrix at the maximum likelihood solution; this allowed CLs on the response curve to be generated. Software used for this probit analysis was written in Fortran using nonlinear search routines based on the Newton-Raphson method [28]. To test the quality of this estimation procedure, simulations were conducted using random samples generated from various specified log-normal distributions and concentration series. For 1,000 simulations with concentrations and "true" parameter values based on the northern pike data set, the proportional bias on μ was <0.1%, on σ was −2.5%, and on b was −6%. Confidence limits were found to be approximately 95%, including the "true" parameter value 94%, 98%, and 94% of the time for μ, σ, and b, respectively.

RESULTS

Concentrations of TCDD in exposure water and tissue

Measured TCDD concentrations in exposure water differed by design, depending upon the projected sensitivity of the

Table 4. Hatchability of eggs and juvenile survival and growth of fish exposed as eggs to TCDD in water at test termination

Species/ exposure period (min)	TCDD-C_{egg}[a] (pg/g wet weight)	Hatch (%)	Survival (%)	Length (mm)	Weight (mg)
Fathead minnow					
0 CC[b]	5 (3)[c]	100 (0)[c]	100 (0)[c]	23 (0.2)[c]	113 (1)[c]
0 SC[d]	32 (11)	100 (0)	100 (0)	24 (0.0)	118 (3)
6	126 (22)	100 (0)	100 (0)	23 (0.6)	120 (5)
15	235 (36)	100 (0)	95 (0)	23 (0.9)	125 (8)
38	435 (62)	100 (0)	73 (3)[c]	24 (1.0)	131 (18)
94	823 (86)	100 (0)	10 (0)[c]	27 (0.4)	181 (19)
234	1,540 (248)	100 (0)	5 (7)[c]	23 (—)[e]	116 (—)[e]
Channel catfish					
0 CC	<0.8[g]	100 (0)	100 (0)	35 (1.5)	329 (35)
0 SC	17 (3)	100 (0)	100 (0)	35 (1.6)	337 (15)
6	41 (10)	100 (0)	94 (8)	35 (1.7)	314 (27)
18	97 (17)	100 (0)	100 (0)	34 (1.0)	310 (21)
52	140 (37)	100 (0)	100 (0)	34 (0.5)	285 (8)
156	385 (84)	100 (0)	94 (8)	35 (0.6)	334 (9)
486	855 (204)	100 (0)	18 (3)[c]	38 (1.9)	523 (13)
Lake herring					
0 CC	<0.8[g]	96 (0)	94 (3)	21 (0.1	38 (2)
0 SC	23 (8)	92 (0)	92 (6)	21 (0.9	40 (10)
7	175 (45)	88 (0)	88 (6)	20 (0.4)	38 (1)
20	270 (46)	84 (6)	80 (6)[c]	20 (0.4)	37 (4)
60	717 (36)	84 (6)	63 (4)[c]	19 (0.1)[c]	31 (1)[c]
180	1,210 (128)	78 (8)	24 (11)[c]	17 (0.6)[c]	25 (4)[c]
540	2,090 (237)	48 (50)[c]	2 (3)[c]	20 (—)[e]	30 (—)[e]
Medaka					
0 CC	9 (2)	90 (0)	83 (11)	15 (0.1)	36 (5)
0 SC	57 (7)	95 (7)	89 (5)	16 (0.9)	39 (9)
6	251 (25)	96 (6)	96 (6)	16 (0.2)	41 (1)
15	455 (32)	93 (10)	83 (17)	15 (0.1)	35 (5)
43	949 (74)	85 (7)	58 (4)[h]	15 (0.7)	39 (7)
114	2,110 (215)	85 (7)	5 (7)[c]	12 (—)[e]	25 (—)[e]
234	3,670 (400)	73 (25)	0[c]	—	—
White sucker					
0 CC	3 (1)	100 (0)	95 (7)	17 (0.1)	20 (1)
0 SC	1 (1)	100 (0)	90 (0)	17 (0.1	21 (1)
30	848 (171)	95 (7)	93 (11)	16 (0.6	19 (1)
60	1,220 (86)	100 (0)	83 (4)	15 (0.3)[c]	19 (1)
120	1,960 (206)	95 (7)	38 (39)[c]	14 (0.2)[c]	14 (2)[c]
240	2,400 (215)	100 (0)	3 (4)[c]	13 (—)[e]	10 (—)[e]
480	3,090 (348)	98 (4)	0[c]	—	—
Northern pike					
0 CC	1 (0)	100 (0)	98 (4)	34 (0.7)	194 (20)
0 SC	132 (14)	100 (0)	100 (0)	34 (1.4)	191 (19)
6	433 (53)	100 (0)	98 (4)	35 (0.7)	206 (10)
15	647 (109)	100 (0)	88 (3)	34 (0.6)	200 (4)
38	1,190 (210)	100 (0)	93 (4)	34 (0.9)	197 (8)
94	1,800 (461)	100 (0)	75 (7)[c]	33 (0.3)	185 (6)
234	4,770 (430)	98 (4)	3 (4)[c]	29 (—)[e]	151 (—)[e]
Zebrafish					
0 CC	7 (6)	98 (3)	84 (6)	20 (0.8)	66 (9)
0 SC	70 (35)	98 (3)	81 (1)	20 (0.4)	76 (8)
6	422 (110)	98 (3)	82 (2)	20 (0.5)	73 (2)
16	424 (109)	96 (6)	76 (6)	20 (0.1)	71 (3)
40	2,000 (1,100)	96 (0)	66 (3)[c]	20 (0.1)	67 (4)
100	2,650 (981)	96 (0)	36 (11)[c]	19 (0.3)	71 (3)
250	4,390 (1,270)	92 (0)	6 (8)[c]	18 (—)[e]	92 (—)[e]

[a] TCDD concentration in eggs from triplicate samples (2–5 eggs per sample).
[b] Clean-water control.
[c] Mean (SD).
[d] Solvent control.
[e] Significant decrease as compared to combined (clean-water and solvent) controls ($p \leq 0.05$).
[f] No SD because fish were alive in only one duplicate
[g] Detection limit
[h] Significant difference at $p = 0.05$ using probit analyses, $p = 0.15$ using Dunnett's procedure

different species, and decreased in all tests during the exposure periods (Table 3). Initial concentrations (pre-exposure) ranged from 9 ng/L in the test with the fathead minnow to 285 ng/L in the test with the white sucker. Losses of TCDD from water ranged from 22% in the fathead minnow test to 75% in the test with northern pike, and were attributed largely to binding of TCDD to surfaces in the exposure apparatus. Mass balance calculations indicated that losses due to accumulation by eggs were small and ranged from 0.3 to 7% in tests with zebrafish and the fathead minnow, respectively.

Measured TCDD-C_{egg}s increased with length of the exposure period (Table 4). After the maximum exposure periods, TCDD-C_{egg}s (in pg/g) for fathead minnows (1,540), channel catfish (855), lake herring (2,090), medaka (3,670), white sucker (3,090), northern pike (4,770), and zebrafish (4,390) and resulted in TCDD-C_{egg} to TCDD–water concentration ratios of 223, 41, 132, 219, 30, 93, and 493 for these species, respectively. These ratios were inversely correlated with total egg mass (wet weight); however, this relationship was not linear. Previous studies have provided evidence that concentrations of TCDD and structurally related compounds in the most sensitive life stages of lake trout and rainbow trout (from hatch to swim-up) remain relatively constant [13,29]. In this study, TCDD concentrations measured in white suckers, northern pike, and lake herring during this time period (data not shown) were not significantly different from TCDD-C_{egg}s. Therefore, TCDD-C_{egg} was used as the basis for determining adverse-effect concentrations in this study.

TCDD toxicity to embryos

Hatchability of eggs of the test fish exposed to TCDD are shown in Table 4. Percent hatch of control eggs ranged from 90 to 100%. In general, embryo mortality was not observed in this study except that some lake herring and medaka eggs with the greatest TCDD-C_{egg}s died just prior to or during hatch (some lake herring died partially emerged from the chorion). The highest TCDD-C_{egg} of 2,090 pg/g significantly decreased lake herring hatch as compared to the controls (mean of clean-water and solvent controls) by ~47%. The hatchability of medaka eggs was decreased by 20% in the highest TCDD-C_{egg} of 3,670 pg/g as compared to the controls. Hatchability of eggs of the other species exposed to the greatest TCDD-C_{egg}s ranged from 92 to 100%. Delays in hatch were not observed for any species.

Signs of TCDD toxicity after hatch

After hatch, all species showed characteristic signs of early life stage TCDD toxicity, such as edema, hemorrhaging, and head and spinal deformities. Cranial, pericardial, and abdominal (yolk-sac) edema and jaw deformities in lake herring, white sucker, and northern pike are depicted in Figure 1. Other toxic effects observed for some species included lethargy, loss of equilibrium, and skin discoloration. Although signs of TCDD toxicity increased with increased TCDD-C_{egg}, the timing and incidence varied among species. For example, fathead minnow, zebrafish, and medaka exposed to TCDD-C_{egg}s of $\geq$435, 4,390, and $\geq$2,110 pg/g, respectively, developed edema at or immediately after hatch, whereas the occurrence and severity of such lesions in lake herring, white sucker, channel catfish, and northern pike, exposed to TCDD-C_{egg}s of $\geq$270, 3,090, 855, and $\geq$1,190 pg/g, respectively, were delayed from a few days to ~2 weeks posthatch. Specific lesions included craniofacial malformations (e.g., domed skulls and deformed jaws), subcutaneous hemorrhaging, and spinal deformities (spines bent upward). However, other effects such as irregular-shaped or bulging eyes were observed only in some lake herring, northern pike, and fathead minnows. In addition, all fish except for northern pike were lethargic and stayed near the bottom of the tanks, appeared disoriented, or had difficulty maintaining equilibrium at various times from hatch to several days posthatch, but skin discoloration was observed only in some fathead minnows and medaka.

Mortality and growth effects after hatch

The signs of TCDD toxicity described above usually preceded or accompanied mortality of the test species after hatch. Decreased survival of all species at test termination was correlated directly with increased TCDD-C_{egg}s (Table 4). In all species, the greatest mortality occurred between the period immediately after hatch until shortly after swim-up when feeding began. Mortality of channel catfish, northern pike, and the fathead minnow, essentially ceased within 7 to 10 d posthatch, whereas the incidence of mortality of lake herring, white sucker, zebrafish, and medaka continued until the end of the tests. The TCDD-C_{egg}s, which caused significant decreases ($p \leq 0.05$) in survival as compared to the controls by the end of the tests, ranged from 270 pg/g for lake herring to 2,000 pg/g for zebrafish. Decreased survival was generally the most sensitive measure of effect observed across species, although growth also was decreased at similar TCDD-C_{egg}s in some species at test termination. The exception was the white sucker, for which growth was significantly decreased at lower TCDD-C_{egg}s than survival (Table 4). White suckers with a TCDD-C_{egg} of 3,090 pg/g were severely stunted within 1 week posthatch and subsequently developed edema and cranial and spinal deformities prior to death. Growth effects in white suckers with lower TCDD-C_{egg}s and in other species were more subtle and were observed at later periods of development. In general, decreased growth was directly correlated with increased TCDD-C_{egg}, although some fish with the greatest TCDD-C_{egg} were larger than the controls due to the reduced numbers of fish per tank, which was caused by high mortality. The TCDD-C_{egg}s that caused significant decreases ($p \leq 0.05$) in growth (length and weight) as compared to the controls at test termination ranged from 717 pg/g for lake herring to 4,770 pg/g for northern pike.

Effect endpoints and concentrations

The NOEC and LOEC for each species, based on significant decreases in survival and/or growth as compared to the controls at test termination (32–100 d) are listed in Table 5. The NOECs and LOECs ranged from TCDD-C_{egg}s of 175 and 270 pg/g for lake herring to 424 and 2,000 pg/g for zebrafish, respectively. Shapes of the concentration–response curves, modeled as a function of TCDD-C_{egg} versus percent mortality, were similar for all species and were characterized by a steep increase in mortality with an increase in TCDD-C_{egg} (Fig. 2). The steepness of these curves was similar (i.e., 95% CLs of the SD overlapped for all species, Table 5). Although 95% CLs for the white sucker distribution overlapped with those for channel catfish, the white sucker data set indicated that the concentration–response curve for this species was steeper (smaller SD) than those for the other test species. The LC_{egg} 10s and LC_{egg}50s calculated from these curves also are shown in Table 5. The LC_{egg}10s were similar to the range of NOECs and LOECs for most species. The rank order of species sensitivity based on

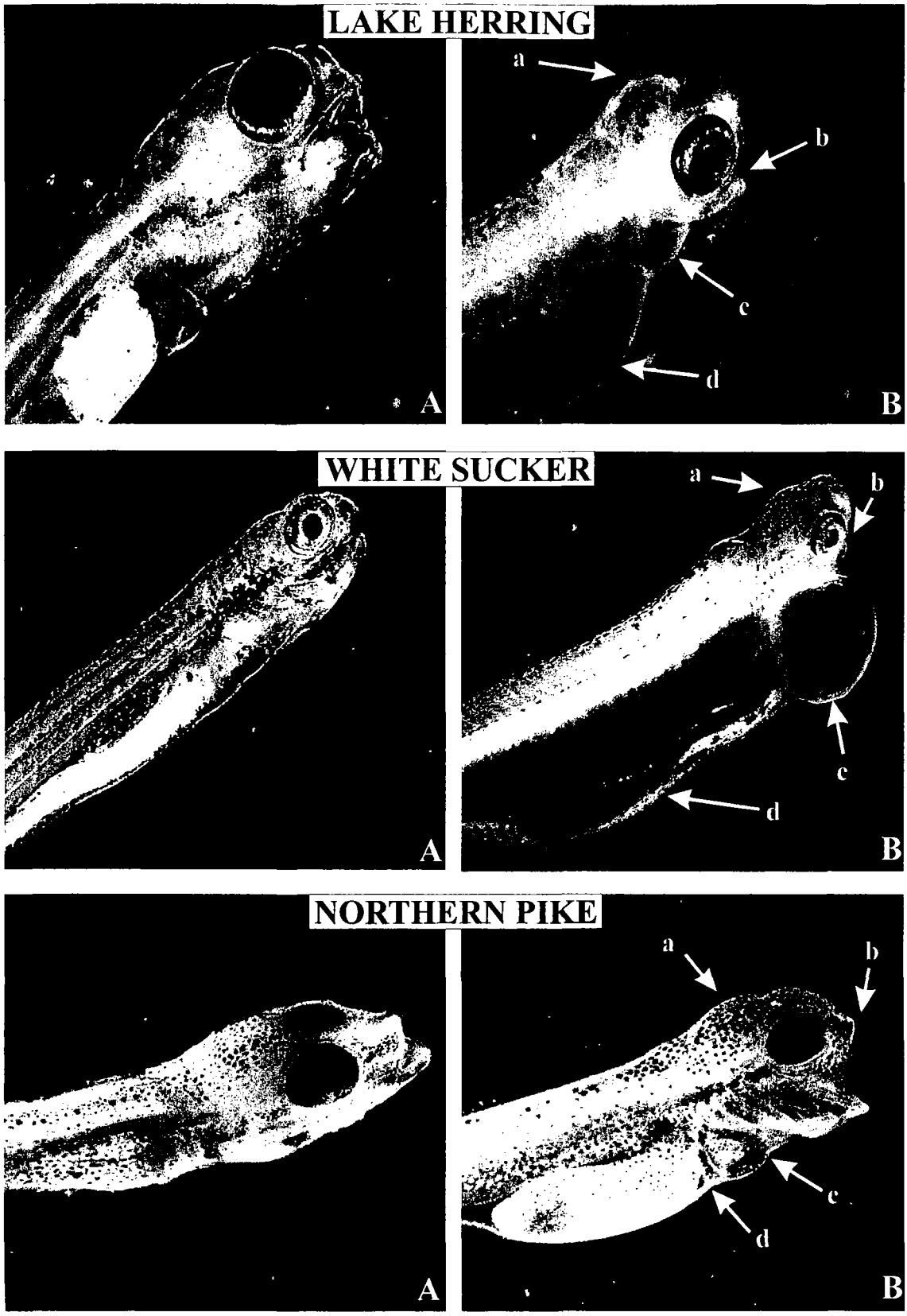

Fig. 1. Signs of TCDD toxicity including (a) cranial edema, (b) jaw deformity, (c) pericardial edema, and (d) abdominal (yolk-sac) edema in lake herring, white sucker, and northern pike at various exposure times and TCDD-C_{egg}s. Lake herring at 46 d posthatch: (**A**) Control, (**B**) 2,090 pg/g; white sucker at 10 d posthatch: (**A**) Control, (**B**) 1,960 pg/g; northern pike at 6 d posthatch: (**A**) Control, (**B**) 4,770 pg/g.

Table 5. Effect endpoints based on TCDD concentration in eggs (TCDD-C_{egg}) for seven species of fish (pg/g wet weight)

Species	NOEC[a]	LOEC[a]	$LC_{egg}10$[b]	$LC_{egg}50$[b]	SD[c]
Fathead minnow	235	435[d]	293 (242–355)	539 (476–611)	0.21 (0.16–0.26)
Channel catfish	385	855[d]	429 (355–518)	644 (576–721)	0.14 (0.09–0.18)
Lake herring	175	270[d]	509 (382–678)	902 (783–1,040)	0.19 (0.13–0.26)
Medaka	455	949[d]	656 (484–889)	1,110 (932–1,320)	0.18 (0.11–0.25)
White sucker	848	1,220[e]	1,590 (1,350–1,880)	1,890 (1,760–2,030)	0.06 (0.02–0.09)
Northern pike	1,190	1,800[d]	1,530 (1,210–1,920)	2,460 (2,100–2,880)	0.16 (0.11–0.22)
Zebrafish	424	2,000[d]	1,610 (1,270–2,050)	2,610 (2,310–2,950)	0.16 (0.10–0.22)

[a] NOEC = no observed effect concentration; LOEC = lowest observed effect concentration.
[b] Concentration in eggs causing 10 and 50% lethality (95% CL), respectively, to fish at test termination.
[c] SD of the distribution of log lethal concentrations (95% CL).
[d] Significant decrease in survival as compared to controls ($p \leq 0.05$).
[e] Significant decrease in growth as compared to controls ($p \leq 0.05$).

the lowest to highest $LC_{egg}50$s was fathead minnow, channel catfish, lake herring, medaka, white sucker, northern pike, and zebrafish.

DISCUSSION

Concentrations of TCDD in eggs

Measured TCDD-C_{egg}s increased with length of exposure but at a rate that decreased with exposure time. This result was attributable, in part, to the decrease in TCDD concentrations in water during the exposure period. Comparisons of TCDD uptake rates and TCDD-C_{egg} were further complicated because eggs were placed in acetone solvent after different exposure times to insure that they received the same solvent exposure. The additional, varied exposure to this solvent may have caused transport of some TCDD out of the eggs. Regardless of this complication, the successful attainment of TCDD-C_{egg}s of all species that spanned the range of no observable effects to 100% lethality, was achieved (Table 4). Ratios of TCDD-C_{egg}s to TCDD concentrations in water were inversely correlated with egg mass, probably because smaller eggs have a higher surface-to-volume ratio than larger eggs. The resulting TCDD-C_{egg}s did not appear to be related to water temperature or egg lipid concentration.

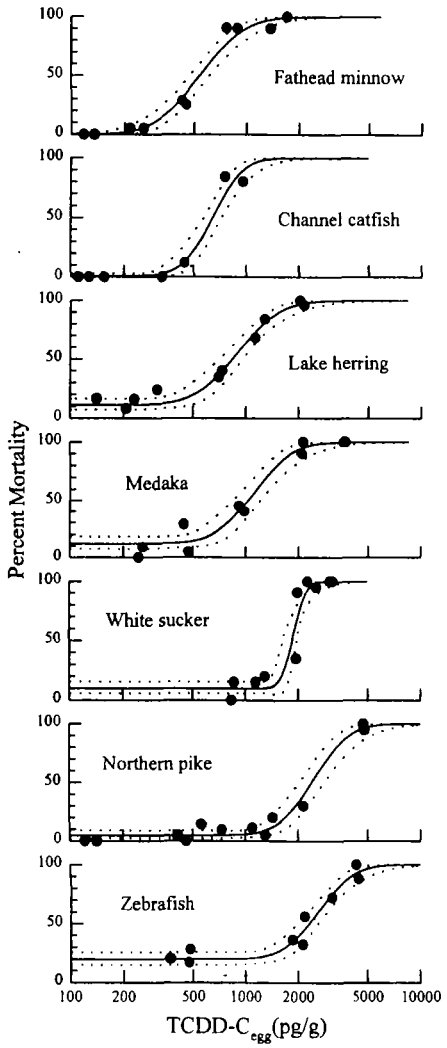

Fig. 2. Percent mortality of fish species at test termination plotted as a function of TCDD-C_{egg}. Solid and dashed lines represent the best-fit probit model and approximate 95% CL, respectively.

TCDD toxicity during embryo development

Toxicity of TCDD during embryo development was not generally observed in this study; however, mortality just prior to and during hatch occurred in some lake herring and medaka. The greater tolerance of this life stage to TCDD relative to newly hatched organisms has been shown previously in the literature [3,11]. Observations of lake herring embryos dying partially emerged from the chorion were similar to those observed in TCDD exposures with other fish species such as lake trout [8,12], brook trout [30], rainbow trout [14], and northern pike [23]. The condition of partial hatching in lake trout was attributed to neuromuscular weakness that prevented the hatching embryo from breaking through the chorion [12]. Ruptures of the yolk sac (yolk material protruding from the yolk sac) accompanied partial hatching in lake herring tests also have been reported for lake trout [12]. It is unclear why these signs of toxicity were not observed in any of the other species tested in this study. The present results and those from other studies [9,12–14,30] indicate that TCDD does not delay egg hatchability.

TCDD toxicity after hatch

The present test results and comparable results observed for several fish species support a generalization that the life stage most sensitive to TCDD-induced mortality is from hatch to swim-up. The signs of TCDD toxicity that were associated with mortality during this period, including edema, hemorrhaging, and head and spinal deformities, were nearly identical to those observed in TCDD-exposed salmonids [9,12–14,30,31], northern pike [23], fathead minnows [20], zebrafish [32], and medaka [18]. Generally, TCDD toxicity decreased

in the test species after swim-up, once exogenous feeding began.

Other effects attributable to TCDD consisted of disequilibrium, lethargy, loss of skin pigmentation, and decreased growth. Although histological examinations were not conducted in this study, loss of equilibrium and the lethargic response of fish with higher TCDD-C_{egg}s could be related to TCDD effects on the swim bladder. The absence of swim bladders or underdeveloped, deflated, and/or partially inflated swim bladders have been reported in larvae of medaka [33] and zebrafish [32] after egg exposure to TCDD. The observed skin discoloration of fathead minnows and medaka was similar to that observed for these and other TCDD-exposed fish by several investigators [16,20,34]. Growth, as measured by length or weight, was reduced at different times and in varying degrees in most of the test species and often was associated with edema and mortality in fish with the highest TCDD-C_{egg}s. Similarly, TCDD-induced growth effects of newly hatched lake trout [9,13], rainbow trout [31,35], and northern pike [23] have been associated with mortality and edema, which sometimes has been quantified by increased weight but also, in some individuals, by shortened body length. Weight gain associated with edema is apparently related to water intake, while reduced length appears to be related to edema by preventing blood flow through the vitelline vasculature resulting in decreased absorption of yolk nutrients to the body [12]. Reduced blood flow to several regions of the body appears to be coincidental with the onset of pericardial and yolk-sac edema in TCDD-exposed fish [18,32].

Comparative toxicity

The AhR is a ligand-activated transcription factor that mediates many of the biological effects of TCDD and structurally related halogenated aromatic compounds. The mechanism of action underlying the toxicity of TCDD and related compounds is only partially understood, but is believed to involve changes in the expression of genes that control cell growth and differentiation [1]. Evidence suggests that these effects require functional AhRs [1,2] and that both teleost and elasmobranch fish possess these receptors [6]. The similarity of toxic effects of TCDD and related compounds in different fish species indicates that the AhR-mediated mechanism is probably responsible for toxicity in these organisms.

The exposures reported here involved the uptake of TCDD by eggs just after fertilization. Recent results by Guiney et al. [7] suggest that the AhR is functional in very early life stages of fish, as indicated by the induction of cytochrome P4501A in the cardiovascular endothelium of lake trout embryos at least 1 week before hatch. These results indicate that the vascular endothelium is a sensitive cell type for TCDD activity, and the initiation of adverse effects that can result in mortality. The cardiovascular system appears to be affected early in development by TCDD, leading to yolk-sac, pericardial, and meningeal edema, and hemorrhaging associated with death of posthatched salmonids [12–14], medaka [18], fathead minnows [20], and zebrafish [32]. Dose-related decreases in regional blood flow of zebrafish indicate that TCDD affects cardiovascular function through interference with the maintenance of peripheral vascular beds after their formation [32]. Observation of DNA degradation in cells of vasculature in medaka embryos exposed to TCDD has led to a hypothesis that TCDD-induced DNA degradation and subsequent apoptypic cell death, possibly due to oxidative stress, contributes to embryo toxicity [36]. Regardless of the specific mechanism responsible for the signs of TCDD toxicity observed in each fish species, differences in sensitivity may be related to interspecies differences in early life stage development patterns, TCDD dose to vulnerable tissues, and physiology.

Concentration–response curves calculated from probit analyses showed that the relationship between percent mortality at test termination and TCDD-C_{egg} was similar across test species (Fig. 2). The similarity of the shapes of these curves and concentration–response curves observed for different species of salmonids exposed to TCDD as eggs [8,9,13,14,30] is consistent with the hypothesis that the mode of action of TCDD is probably the same among fish species. Direct comparisons of $LC_{egg}50$s calculated from these curves and those reported by other investigators for the present test species are limited because few TCDD exposures have been conducted in which TCDD-C_{egg} has been used as the dose metric. The $LC_{egg}50$ of 2,610 pg/g for zebrafish was similar to a 10-d $LC_{egg}50$ of 2,500 pg/g egg observed for this species by Henry et al. [32]. However, the $LC_{egg}50$ of 539 pg/g calculated for the fathead minnow was ~48 times lower than the 7-d 50% lethal concentration of 25,710 pg/g measured in larvae after a continuous embryo–larval exposure of this species by Olivieri and Cooper [20]. The large difference between the lethal concentrations for fathead minnows may have been due to differences in the time at which the TCDD concentrations were measured in larvae [20]; the 7-d (48-h posthatch) exposure period was ~43 times longer than the exposure period used to measure the $LC_{egg}50$ in the present study. Because the difference in exposure periods was approximately the same as the 48-fold difference in lethal doses associated with the two exposure regimes, the lethal TCDD doses to the embryos during early development were probably similar. This suggests that early TCDD dose to the embryo is primarily responsible for mortality that occurs later during larval development and that the AhR is functional during early life stages of embryonic development. If so, the $LC_{egg}50$, based on TCDD in the egg shortly after fertilization would be the most accurate measure of dose–response for fathead minnow early life stage mortality, regardless of subsequent, additional TCDD accumulation during further embryo and early larval development. Similar tendencies for decreased sensitivity as a result of delayed exposure of embryos to TCDD have been observed for medaka [18] and lake trout [19]. Severe lesions that resulted in death of medaka were specifically associated with exposure of embryos between days 4 and 6 of the 11- to 14-d period from fertilization to hatch [18]. Posthatch exposures of fish, however, remain a concern because continued accumulation of TCDD during larval and juvenile stages may result in adverse effects on sexual development and reproduction.

Early life stages of all seven fish species in this study were found to be less sensitive to TCDD, on the basis of $LC_{egg}50$s, than salmonids (Fig. 3). The $LC_{egg}50$s for the fathead minnow, the most sensitive species tested were three to eight times greater than those obtained for waterborne egg exposures of brook trout [30] and lake trout [9,13], respectively. Although the $LC_{egg}50$s for the fathead minnow and the Fish Lake strain of rainbow trout [14] were not significantly different, rainbow trout sensitivity to TCDD has been shown to be strain dependent [35,37], and the lowest $LC_{egg}50$ of 171 pg/g for the Shasta strain (exposed via egg injection) [37] was approximately three times lower than that determined for the fathead minnow in this study.

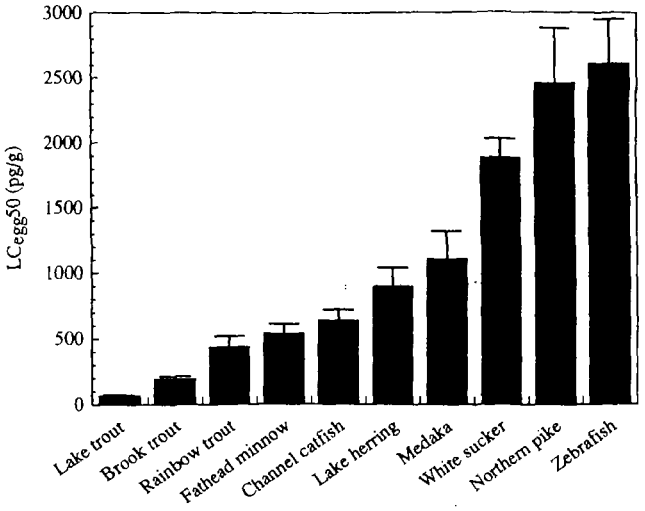

Fig. 3. The $LC_{egg}50$s and 95% CLs (vertical lines) for fish exposed to TCDD via waterborne exposure of eggs. Values for lake trout, brook trout, and rainbow trout are from Walker et al. [9,13], Walker and Peterson [30], and Walker et al. [14], respectively. Values for all other species are from this study.

The greater tolerance of the test species to TCDD compared to salmonids appears to be associated with their shorter development time to swim-up. The period from hatch to swim-up and first feeding ranged from 1 to 18 d for the test species compared to approximately 30 to 70 d for salmonids. Among the test species, however, the fathead minnow and channel catfish, both with very short development times to swim-up, were more sensitive (Fig. 3) than lake herring and northern pike that have longer development times. Also, fathead minnow sensitivity was much greater than that observed for zebrafish, a species from the same family and having similar developmental stages. Limited data exist for interspecies differences in early life stage toxicokinetics and toxicodynamics that may contribute to the sensitivity differences observed following egg exposures to TCDD. TCDD masses in lake herring, white sucker, and northern pike shortly after hatch (~1–5 d) were similar to TCDD masses measured in the eggs of these species. Similar results have been reported for posthatched lake trout and TCDD [13] and rainbow trout and PCBs [29] following egg exposure, although TCDD and PCB concentrations in these species declined more rapidly after swim-up. Decreases in TCDD masses in fish after swim-up (at test termination) also were observed for lake herring (83%), channel catfish (88%), northern pike (94%), medaka (98–100%), and zebrafish (98–99%) and by other investigators for zebrafish (T.R. Henry, personal communication) and fathead minnows [20]. Comparison of post-swim-up elimination rates among these species and with lake trout [13] suggest that fish with longer development times retain TCDD longer than smaller fish with relatively short development times. In this study, the incidence of effects tended to decrease shortly after swim-up when decreases in TCDD concentrations in fish may have occurred in association with the onset of exogenous feeding and the absence of continuing exposure through the food or water. Therefore, species sensitivity differences could have been associated, in part, with the duration and magnitude of TCDD accumulation in vulnerable tissues as a result of the fish's ability to eliminate such compounds. However, recent studies indicate that the difference in sensitivity between zebrafish and rainbow trout to TCDD is approximately the same, regardless of whether the effect is mortality following in vivo exposure or a biochemical response in vitro [32,38]. This suggests that interspecies differences in AhR-related toxicodynamics may be equally or more important than toxicokinetic differences in predicting the sensitivities of different fish species when exposed to TCDD [38].

Test results relative to assessing TCDD risk to fish

Comparisons of $LC_{egg}50$s (Fig. 3) for waterborne egg exposures indicate that the species examined in the present study were approximately 8 (for fathead minnow) to 38 (for zebrafish) times less sensitive to TCDD than lake trout [9,13], the most sensitive species evaluated to date. However, because of differences in TCDD exposure and bioaccumulation, $LC_{egg}50$s (Table 5) may not accurately predict relative risks. In aquatic ecosystems with sediments contaminated with TCDD, fish species with benthic food chain connections will experience greater exposure than fish species whose diets are not linked to such sediments. Although such exposure differences may be site specific, other exposure differences exist that are related to species characteristics in general. A useful first approximation for eliminating the influence of species-specific TCDD bioaccumulation in interpreting relative risks of AhR agonists can be made by normalizing $LC_{egg}50$s (Table 5) to the fraction lipid in eggs (f_l)(Table 2): $LC_{egg}50(f_l) = LC_{egg,l}50$. The rank order of species at risk to TCDD, based on lowest to highest $LC_{egg,l}50$s (in pg TCDD/g lipid) is channel catfish (13,400), lake herring (13,700), fathead minnow (22,500), medaka (38,300), northern pike (58,600), white sucker (75,600), and zebrafish (153,500). Because lake trout eggs have greater f_l (~0.08) than eggs of the species tested in the present study, comparisons of $LC_{egg,l}50$s indicate that the relative risk of TCDD to early life stage survival for these species are from 16- to 180-fold less than that for lake trout.

The $LC_{egg,l}50$s determined in this study may be applied to fish bioaccumulation data to estimate risks for early life stage mortality. The lake trout $LC_{egg,l}50$ of 860 pg/g [9] was probably exceeded in Lake Ontario in the 1970s, resulting in an absence of recruitment by stocked lake trout due to early life stage mortality [39]. Similar problems for other species in Lake Ontario have not been documented. Although lake herring, with an $LC_{egg,l}50$ of 13,700 pg/g, are shown in this study to be approximately 16 times less sensitive than lake trout, the decline in lake herring populations in Lake Ontario prior to 1970 may have been influenced by bioaccumulation of TCDD and related chemicals. Maximum TCDD toxicity equivalence concentrations in lake herring eggs are predicted to have been 7,500 pg/g egg lipid (P.M. Cook, personal communication). Maximum concentrations of TCDD or TCDD toxicity equivalents (for PCDDs and PCDFs but not PCBs) in white suckers, channel catfish, or northern pike measured for a survey of chemical residues in fish throughout the United States [40] did not exceed 100 pg/g whole fish or 2,000 to 4,000 pg/g egg lipid in these species. Thus, maximum exposures recorded for these species indicate there was a low probability for early life stage mortality, at the locations sampled, due to the exposure of embryos to PCDDs and PCDFs. White suckers exposed to pulp and paper mill effluents in Canada accumulated up to 124 pg TCDD toxicity equivalents/g liver but exposures to PCDDs and PCDFs did not correlate to mixed function oxidase activity, gonadosomatic index, or circulating plasma 11-ketotestosterone [41]. Because eggs of these white suckers probably did not exceed 600 pg TCDD toxicity equivalents/g egg

lipid and the $LC_{egg,i}50$ for white suckers in this study is 75,600 pg/g, the probability of early life stage mortality from exposure to PCDDs and PCDFs alone is very low.

This study involved a single TCDD exposure of the eggs just after fertilization. To date, early life stage and partial life-cycle tests with TCDD have not been designed to provide continuous exposure to the organisms past swim-up when fish are actively feeding and developing secondary sexual characteristics. Experiments that extend TCDD exposure periods from the egg, past swim-up through to the spawning adult stage would help to determine the nature and extent to which TCDD may elicit additional developmental or reproductive effects in fish.

Acknowledgement—We are grateful to R. Erickson for assistance with data analysis; the Wisconsin and Minnesota Departments of Natural Resources for assistance in obtaining wild collected eggs and to Chesapeake State Fish Hatchery for providing channel catfish eggs; B. Butter worth, J. Libal, and D. Nessa for assistance with chemical analyses; C. West for assistance with egg collection and in conducting the tests; K. Jensen for assistance with data management; D. Lothenbach for photo imagery; G. Ankley and S. Broderius for manuscript review; and M. Johnson for assistance with table preparation.

REFERENCES

1. Poland A, Knutson JC. 1982. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related halogenated aromatic hydrocarbons: Examination of the mechanism of toxicity. *Annu Rev Pharmacol Toxicol* 22:517–524.
2. Safe SH. 1986. Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Annu Rev Pharmacol Toxicol* 26:371–399.
3. Cook PM, Erickson RJ, Spehar RL, Bradbury SP, Ankley GT. 1993. Interim report on data and methods for assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin risks to aquatic life and associated wildlife. EPA/600/R-93/055. U.S. Environmental Protection Agency. Washington, DC.
4. Zabel EW, Cook PM, Peterson RE. 1995. Potency of 3,3',4,4',5-pentachlorobiphenyl (PCB 126), alone and in combination with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), to produce lake trout early life-stage morality. *Environ Toxicol Chem* 14:2175–2179.
5. Cook PM, Zabel EW, Peterson RE. 1997. The TCDD toxicity equivalence approach for characterizing risks for early life stage mortality in trout. In Rolland R, Gilbertson M, Peterson RM, eds, *Chemically Induced Alterations in the Functional Development and Reproduction of Fishes,* SETAC, Pensacola, FL, USA, pp 9–27.
6. Hahn ME, Poland A, Glover E, Stegeman JJ. 1994. Photoaffinity labeling of the Ah receptor: Phylogenetic survey of diverse vertebrate and invertebrate species. *Arch Biochem Biophys* 310:218–228.
7. Guiney PD, Smolowitz RM, Peterson RE, Stegeman JJ. 1997. Correlation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induction of cytochrome P4501A in vascular endothelium with toxicity in early life stages of lake trout. *Toxicol Appl Pharmacol* 143:256–273.
8. Guiney PD, Cook PM, Casselman JM, Fitzsimmons JM, Simonin HA, Zabel EW, Peterson RE. 1996. Assessment of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin induced sac fry mortality in lake trout (*Salvelinus namaycush*) from different regions of the Great Lakes. *Can J Fish Aquat Sci* 53:2080–2092.
9. Walker MK, Cook PM, Batterman AR, Butterworth BC, Berini C, Libal JJ, Hufnagle LC, Peterson RE. 1994. Translocation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin from adult female lake trout (*Salvelinus namaycush*) to oocytes: Effects on early life stage development and sac fry survival. *Can J Fish Aquat Sci* 51:1410–1419.
10. Guiney PD, Melancon MJ Jr, Lech JJ, Peterson RE. 1979. Effects of egg and sperm maturation and spawning on the distribution and elimination of a polychlorinated biphenyl in rainbow trout (*Salmo gairdneri*). *Toxicol Appl Pharmacol* 47:261–272.
11. Walker MK, Peterson RE. 1994. Aquatic toxicity of dioxins and related chemicals. In Schecter A, ed, *Dioxins and Health,* Plenum, New York, NY, USA, pp 347–387.
12. Spitsbergen JM, Walker MK, Olson JR, Peterson RE. 1991. Pathological 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.
13. Walker MK, Spitsbergen JM, Olson JR, Peterson RE. 1991. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity during early life stage development of lake trout (*Salvelinus namaycush*). *Can J Fish Aquat Sci* 48:875–883.
14. Walker MK, Hufnagle LC Jr, Clayton MK, Peterson RE. 1992. An egg injection method for assessing early life stage mortality of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls in rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 22:15–38.
15. Wolf K. 1956. Experimental induction of blue-sac disease. *Trans Am Fish Soc* 86:61–70.
16. Miller RA, Norris LA, Hawkes CL. 1973. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in aquatic organisms. *Environ Health Perspect* 5:177–186.
17. Adams WJ, DeGraeve GM, Sabourin TD, Coopey JD, Mosher GM. 1986. Toxicity and bioconcentration of 2,3,7,8-TCDD to fathead minnows (*Pimephales promelas*). *Chemosphere* 15: 1503–1511.
18. Wisk JD, Cooper KR. 1990. The stage specific toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in embryos of the Japanese medaka (*Oryzias latipes*). *Environ Toxicol Chem* 9:1159–1169.
19. Zabel EW, Peterson RE. 1994. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) toxicity at three stages of lake trout egg development. *Abstracts,* 15th Annual Meeting, Society of Environmental Toxicology and Chemistry, Denver, CO, USA, October 30 to November 3, p 153.
20. Olivieri CE, Cooper KR. 1996. Toxicity of the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in embryos and larvae of the fathead minnow (*Pimephales promelas*). *Chemosphere* 34:1139–1150.
21. Zacharewski T, Safe L, Safe S, Chittim B, DeVault D, Wiberg K, Bergquist PA, Rappe C. 1989. Comparative analysis of polychlorinated dibenzo-*p*-dioxin and dibenzofuran congeners in Great Lakes fish extracts by gas chromatography–mass spectrometry and in vitro enzyme induction activities. *Environ Sci Technol* 23:730–735.
22. Yockim RS, Isensee AR, Jones GE. 1978. Distribution and toxicity of TCDD and 2,4,5-T in an aquatic model ecosystem. *Chemosphere* 7:215–220.
23. Helder T. 1980. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of the pike (*Esox lucius L.*). *Sci Total Environ* 14:255–264.
24. Ringle JP, Nickum JG, Moore A. 1992. Chemical separation of channel catfish egg masses. *Prog Fish Cult* 54:73–80.
25. Radin NS. 1981. Extraction of lipids with a solvent of low toxicity. *Methods Enzymol* 72:5–7.
26. Steel RGD, Torrie JH. 1960. *Principles Procedures of Statistics with Special Reference to the Biological Sciences, A Biometrical Approach.* McGraw-Hill, New York, NY, USA.
27. Breiman L. 1973. *Statistics: With a View Toward Applications.* Houghton-Mifflin, Boston, MA, USA.
28. Dahlquist G, Björck A. 1974. *Numerical Methods.* Prentice-Hall, Englewood Cliffs, NJ, USA.
29. Guiney PD, Lech JJ, Peterson RE. 1980. Distribution and elimination of a polychlorinated biphenyl during early life stages of rainbow trout (*Salmo gairdneri*). *Toxicol Appl Pharmacol* 53: 521–529.
30. Walker MK, Peterson RE. 1994. Toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin to brook trout (*Salvelinus fontinalis*) during early development. *Environ Toxicol Chem* 13:817–820.
31. Helder T. 1981. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on early life stages of the rainbow trout (*Salmo gairdneri* Richardson). *Toxicology* 19:101–112.
32. Henry TR, Spitsbergen JM, Hornung MW, Abnet CC, Peterson RE. 1997. Early life stage toxicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in zebrafish (*Danio rerio*). *Toxicol Appl Pharmacol* 142:56–68.
33. Harris GE, Kiparissis Y, Metcalfe CD. 1994. Assessment of the toxic potential of PCB congener 81 (3,4,4',5-tetrachorobiphenyl) to fish in relation to other non-ortho-substituted PCB congeners. *Environ Toxicol Chem* 13:1405–1413.

34. Wannemacher RA, Rebstock A, Kulzer E, Schrenck D, Bock KW. 1992. Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on reproduction and oogenesis in zebrafish (*Brachydanio rerio*). *Chemosphere* 24:1361–1368.
35. Walker MK, Peterson RE. 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 (*Oncorhynchus mykiss*). *Aquat Toxicol* 21:219–238.
36. Cantrell SM, Lutz LH, Tillett DE, Hannink M. 1996. Embryo toxicity of 2,3,7,8 tetrachlorodibenzo-*p*-dioxin (TCDD): The embryonic vasculature is a physiological target for TCDD-induced DNA damage and apoptotic cell death in medaka (*Oryzias latipes*). *Toxicol Appl Pharmacol* 141:23–34.
37. Zabel EW, Cook PM, Peterson RE. 1995. Toxic equivalency factors of polychlorinated dibenzo-*p*-dioxin, dibenzofuran and biphenyl congeners based on early life stage mortality in rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 31:315–328.
38. Henry TR, Nesbit DJ, Peterson RE. 1996. Toxic equivalency factors for polychlorinated dibenzo-*p*-dioxins, dibenzofurans and biphenyls in zebrafish liver cells. *Abstracts,* 17th Annual Meeting, Society of Environmental Toxicology and Chemistry, Washington, DC, USA, November 17–21, p 270.
39. Cook PM, Butterworth BC, Walker MK, Hornung MW, Zabel EW, Peterson RE. 1994. Lake trout recruitment in the Great Lakes: Relative risks for chemically induced early life stage mortality. *Abstracts,* 15th Annual Meeting, Society of Environmental Toxicology and Chemistry, Denver, CO, USA, October 30 to November 3, p 58.
40. U.S. Environmental Protection Agency. 1992. National study of chemical residues in fish, Vol. II. EPA 823-R-92-008b. Office of Science and Technology, Washington, DC.
41. Servos MR, Huestis SY, Whittle DM, Van Der Kraak GJ, Munkittrick KR. 1994. Survey of receiving-water environmental discharges from pulp mills. 3. Polychlorinated dioxins and furans in muscle and liver of white sucker (*Catostomus commersoni*). *Environ Toxicol Chem* 13:1103–1115.