Comparison of Early-Life-Stage Sensitivity of Freshwater Fish Exposed as Eggs to 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)

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Abstract

Waterborne exposures of fertilized eggs of fathead minnows (*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*) to TCDD were compared on the basis of TCDD-C_{egg}s (TCDD concentration in eggs). Signs of toxicity in hatched fish, including edema, hemorrhaging and craniofacial malformations were essentially identical to those observed in salmonids following exposure of eggs to TCDD, and preceded or accompanied mortality most often during the period from hatch to shortly after swim-up. The NOECs (no observed effect concentrations) and LOECs (lowest observed effect concentrations), based on significant decreases in survival and growth as compared to the controls at test termination, ranged from 175 and 270 pg/g for lake herring to 424 and 2,000 pg/g for zebrafish, respectively. Concentration-response curves, expressed as TCDD-C_{egg} versus percent mortality, were similar for all species suggesting that the mechanism of action of TCDD is the same among these species. The $LC_{egg}50$s (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 $LC_{egg}50$s indicate that the species tested were approximately 8 to 38 times less sensitive to TCDD than lake trout, the most sensitive species evaluated to date.

Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic chemical of the group of hydrophobic, halogenated aromatic compounds. Both the biochemical and toxic effects of TCDD have been found to be similar in different species indicating that it acts through an aryl hydrocarbon receptor (AhR) mediated mechanism[1,2]. Because of its toxicity to vertebrates and association with aquatic sediments, biota and the organic carbon fraction of ambient waters, TCDD poses a potential risk to fish.

TCDD has been shown to be extremely toxic to newly hatched fish [3,4]. However, comparing the differences in species sensitivity of fish to this compound is difficult because various exposure regimes and life stages have been tested. Within a fish species, sensitivity is highly dependent on the age and size of the organism [5,6] and on exposure time or stage during development [7-9]. In addition, studies that use the same basis for measuring TCDD toxicity (e.g., concentration in eggs) to determine the most sensitive life stages of fish have been available for only a few species. The purpose of this study was to compare the sensitivity of early life stages (embryos-juveniles) of seven freshwater fish species on the basis of TCDD concentration in eggs (TCDD-C_{egg}s) after waterborne exposure of fertilized eggs.

Experimental Methods

Eggs of fathead minnows, medaka and zebrafish were obtained from U.S. EPA, Duluth, MN. Eggs of channel catfish were obtained from Chesapeake State Fish Hatchery, Mt. Vernon, MO, lake herring from Lake Superior at Squirrel Fisheries of Port Wing, WI and northern pike from Lac Court Orielles, WI. 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 mean temperature of the test water was 7.8°C for lake herring, 15-16°C for white sucker and northern pike and 25-26°C for fathead minnows, channel catfish and zebrafish. Mean dissolved oxygen (mg/L) was greater than 80% of saturation in all tests. Other water characteristics such as pH, hardness and alkalinity were similar to those reported for Lake Superior water [10]. Fluorescent lamps provided a 16 h photoperiod with light intensities of 61 to 139 lumens at the water surface.

Fifty to sixty eggs (<24-h post fertilization), from duplicate egg cups, were exposed to a mixed solution of HPLC grade acetone (0.1-2.0 ml/L) and TCDD for different exposure periods (6 to 540 min) to obtain graded TCDD-C_{egg}s. Eggs were concomitantly placed in lake water and in an acetone-lake-water mixture, which served as controls. After the specified period, eggs were removed from TCDD exposure, rinsed with lake water and placed in the solvent control tank to obtain equal solvent exposures for all eggs. When the longest exposure period was completed, all eggs were transferred to test tanks with flowing lake water for further observation. At eye-up, eggs of each species were randomly thinned to 20 to 25 per duplicate. Following hatch, organisms were observed for signs of TCDD toxicity until the end of the tests. Total test length was 32 d for all species except for lake herring, which was 100 d.

Tritiated TCDD ([^{3}H]2,3,7,8-tetrachlorodibenzo-p-dioxin) was obtained from Cambridge Isotope Laboratories, Andover MA (Lot No: AWN-729-87). Large amounts of impurities were detected by GC/MS and the TCDD was re-purified [11]. An acetone-TCDD stock solution (200 ml) 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/mMole TCDD (1.4 Tbq/mMole). Triplicate water, egg and larvae samples were taken from each exposure. All samples were placed into a 20 mL glass scintillation vial and stored at 4°C until 30 min prior to final sample preparation for analysis by LSC (Model 2500TR, Packard Instrument Co., Meriden, CT). The detection limits for water and tissue measurements (wet weight) were 0.0065 ng/L and 0.8 pg/g, respectively.

Data on egg hatchability, survival and growth (length and weight) were analyzed by ANOVA and Dunnett's one-sided comparison of treatment means with combined control (clean-water and solvent) means ($p \leq 0.05$). Response data for species in clean-water and solvent controls were compared using a paired t-test, and percent mortality versus TCDD-C_{egg} was analyzed using probit analysis.

Results and Discussion

Mortality during embryo development was not generally observed in this study; however, some lake herring and medaka with the highest TCDD-C_{egg}s died just prior to and during hatch. The greater tolerance of this life stage to TCDD relative to newly hatched organisms has been shown previously [3,4]. After hatch, organisms showed characteristic signs of early-life-stage TCDD toxicity such as cranial, pericardial and abdominal edema, hemorrhaging and head and spinal deformities, which were always associated with mortality. Other toxic effects observed included lethargy, loss of equilibrium, skin discoloration and reduced growth. Generally, TCDD toxicity decreased in the test species after swim-up, once exogenous feeding began. These signs of toxicity were nearly identical to those observed for TCDD-exposed fish by other investigators [7,9,12-17] and support a generalization that the life stage most sensitive to TCDD-induced mortality is from hatch to swim-up.

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 1. 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. 1). The similarity of the shapes of these curves and concentration-response curves observed for different species of salmonids exposed to TCDD as eggs [13,14,18-20] is consistent with the hypothesis that the mode of action of TCDD is probably the same among fish species. The rank order of species sensitivity based on the lowest to highest $LC_{egg}50$s calculated from the response curves was: fathead minnow, channel catfish, lake herring, medaka, white sucker, northern pike and zebrafish (Table 1).

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. 2). The $LC_{egg}50$ for the fathead minnow, the most sensitive species tested was 8 times greater than that obtained for waterborne-egg-exposures of lake trout [13]. The greater tolerance of the test species compared to salmonids appears to be associated to their shorter development time to swim-up, but limited data exist for interpreting inter-species sensitivity differences on the basis of toxicokinetics or toxicodynamics of TCDD in fish. TCDD concentrations in hatched lake herring, white sucker, and northern pike were similar to TCDD-C_{egg}s measured in these species indicating that TCDD concentrations remained relatively constant in the eggs and larvae until swim-up. Similar results have been reported for lake trout and TCDD [13] and rainbow trout and PCBs [21]. TCDD and PCB concentrations in these species and TCDD concentrations in fathead minnows [9] and zebrafish (T.R. Henry, personal communication) exposed as eggs were shown to decline rapidly in life stages after swim-up. In this study, the incidence of effects also tended to decrease shortly after swim-up. 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 this compound at different times. 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* [17,22]. These findings suggest that inter-species 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 [22].

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 which 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 reproductive effects in fish.

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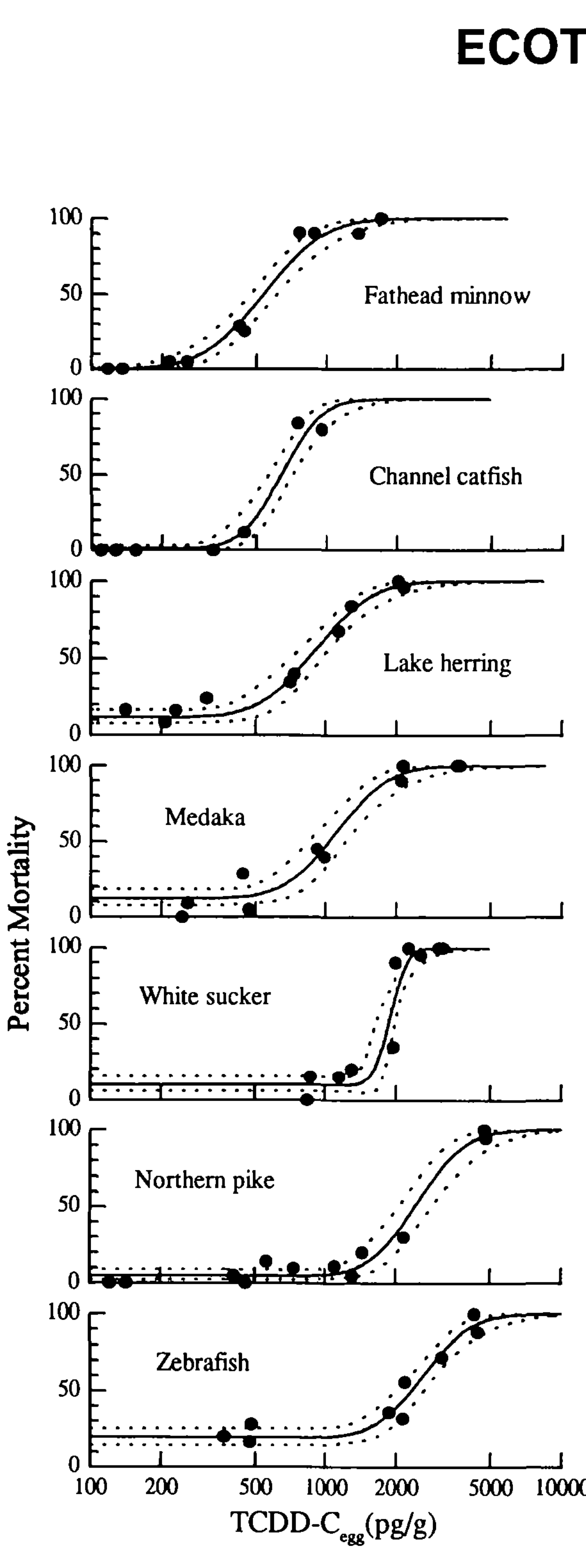

Figure 1. Percent mortality of fish species at test termination plotted as function of TCDD-C$_{egg}$. Solid and dashed lines represent the best-fit probit model and approximate 95% CL, respectively.

Table 1. Effect endpoints based on TCDD concentration in eggs (TCDD-C_{egg}) for seven species of fish (pg/g wet weight).

Species	NOEC[a]	LOEC[b]	SD[c]	$LC_{egg}50$[d]
Fathead minnow	235	435[e]	0.21 (0.16-0.26)	539(476-611)
Channel catfish	385	855[e]	0.14 (0.09-0.18)	644 (576-721)
Lake herring	175	270[e]	0.19 (0.13-0.26)	902 (783-1,040)
Medaka	455	949[e]	0.18 (0.11-0.25)	1,110 (932-1,320)
White sucker	848	1,220[f]	0.06 (0.02-0.09)	1,890 (1,760-2,030)
Northern pike	1,190	1,800[e]	0.16 (0.11-0.22)	2,460 (2,100-2,880)
Zebrafish	424	2,000[e]	0.16 (0.10-0.22)	2,610 (2,310-2,950)

[a] No observed effect concentration.
[b] Lowest observed effect concentration (effect based on survival and growth at test termination).
[c] SD of the distribution of log lethal concentrations (95% CL).
[d] Concentration in eggs causing 50% lethality (95% CL) to fish at test termination.
[e] Significant decrease in survival as compared to controls ($p \leq 0.05$).
[f] Significant decrease in growth as compared to controls ($p \leq 0.05$).

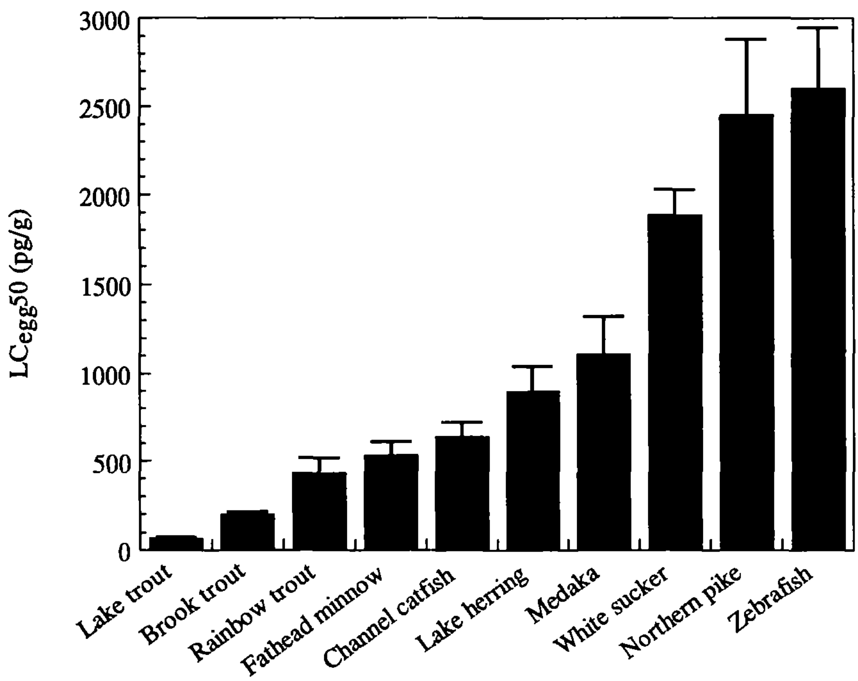

Figure 2. The $LC_{egg}50$s and 95% CLs (vertical lines) for fish exposed to TCDD via waterborne exposure of eggs. Values are from this study except for lake trout[13)], brook trout[14)] and rainbow trout[9)].