Dioxin-like Embryotoxicity of a Lake Michigan Lake Trout Extract to Developing Lake Trout

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Abstract

Planar halogenated hydrocarbons (PHHs), such as polychlorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls, are known to be present in the Great Lakes ecosystem in sufficient concentrations to have the potential of causing adverse effects on reproduction in certain species of fish. Previously, we demonstrated the toxicity of a PHH mixture through the nanoinjection of environmental extracts into newly fertilized eggs from two strains of rainbow trout. The current study was designed to investigate the embryotoxicity of a complex organic extract on developing lake trout embryos. The objectives of the study were: 1) to determine the accuracy of our predictions about lake trout made from the previous studies in rainbow trout; and 2) to examine the chemical composition of this extract in relation to an additive model of toxicity; and 3) estimate the hazard these chemicals may represent toward lake trout populations in the Great Lakes. An organic extract was made from whole adult lake trout collected from Lake Michigan in 1988. Graded doses of the final extract were injected into eggs of hatchery reared lake trout. The doses used for the injections were quantified as 2,3,7,8-tetrachlorodibenzo-p-dioxin toxic-equivalents (TEQs) based on the concentrations of dioxins, furans and non-o-PCBs in the extract, and as equivalent amounts found in the eggs of the original lake trout (eggEQ). Total TEQs in the Lake Michigan lake trout sample were 14.7 pg TEQ/g. The extract of the Lake Michigan lake trout was embryotoxic to lake trout embryos in the laboratory with an LD50 value of 7 eggEQ (4-11, 95% F.L.) in lake trout. The LD50 of the Lake Michigan extract in terms of TEQs was 103 TEQs/g of lake trout egg. The estimated ED50 values for sublethal responses were: 7.4 eggEQ for craniofacial anomalies, 3.0 eggEQ for yolk sac edema (YSE), and 0.4 eggEQ for hemorrhage in the lake trout embryos. The LD50 of TCDD in lake trout was 81 pg/g egg and was similar to previous reports. Our previous studies with rainbow trout embryos injected with this same extract predicted an LD50 in lake trout of 4 eggEQ based on the Erwin strain of rainbow trout or 7 eggEQ based on the Arlee strain of rainbow trout. The mixture of chemicals present in this extract in all cases acted to cause embryotoxicity nearly in an additive fashion in these trout. The composition of the extract was portioned equally on a TEQ basis among non-ortho-substituted PCBs, dioxins, and furans. This study confirms our earlier conclusions that PHHs in lake trout from Lake Michigan are above a threshold for adverse effects and these compounds may have implications on the lack of recruitment in certain Great Lakes lake trout populations.

Introduction

The lake trout fishery in the Great Lakes is hatchery-stocked, with no natural reproduction in the lower lakes (Michigan and Ontario) and few signs of natural reproduction in northern Lake Huron.[1-3] The exact cause of the lack of recruitment observed in Great Lakes lake trout populations is not known at this time; however, it is known that lake trout are particularly sensitive to the effects of planar halogenated hydrocarbons (PHHs).[4] In a recent study, we tested the hypothesis that the

chemical mixture that exists in lake trout is embryotoxic to salmonids.[5] Graded doses of extracts from lake trout were injected into newly fertilized eggs of rainbow trout. We observed dioxin-like symptoms of toxicity in the developing rainbow trout embryos that increased with dose.[5] The mixture of PHHs in that study followed an additive model of toxicity with respect to early life stage mortality. The results of that study with the surrogate species, rainbow trout, were used to make predictions about the potential for PHHs to cause effects on lake trout, based on the relative sensitivity differences among the two species.[6] The predictions were that the existing concentrations of PHHs in Lake Mcihigan lake trout are below a concentration to cause overt mortality, but above a threshold for the sublethal effects of yolk sac edema and hemorrhaging.[5]

Therefore, the objectives of this study were to: 1) to determine the accuracy of our predictions about lake trout made from the previous studies in rainbow trout; and 2) examine the chemical composition of this extract in relation to an additive model of toxicity; and 3) estimate the hazard these chemicals may represent toward lake trout populations in the Great Lakes.

Experimental Methods

Extraction and Clean-up of lake trout tissue

Lake trout collected in 1988 from Sheboygan, WI on Lake Michigan was the source of the complex environmental mixture used for the extraction. The extraction and cleanup procedures of the lake trout were described previously [5] and followed the methods of Meadows et al.[7] Briefly, the lake trout tissue (whole fish) was ground and mixed with 4X Na_2SO_4 (sodium sulfate) and extracted in large, 4-cm i.d. glass extraction columns with methylene chloride (CH_2Cl_2). The extracted lipid was dialyzed against 80:20 hexane:CH_2Cl_2 utilizing polyethylene membranes. The sample was then subjected to a two-step reactive/destructive column cleanup on sulfuric acid-impregnated silica gel and potassium silicate, and final clean-up by adsorption chromatography. The final sample was then subject to high performance gel permeation chromatography (HPGPC). Dosing solutions were prepared by evaporation of the extract in methylene chloride, and then dissolution of the residues into triolein as a carrier for dosing.

Analytical Methods

Triplicate aliquots (25 g) of the Lake Michigan lake trout sample were prepared for analysis according to the methods of Feltz et al. [8] and analyzed as in Peterman et al.[9] Each sample was spiked with 5 ng of ^{13}C-labeled non-*o*-PCBs (#77, 126, and 169) and 50-500 pg of ^{13}C-labeled PCDDs or PCDFs and column-extracted with CH_2Cl_2. All extracts were then treated by a two-stage reactive cleanup; and were further purified using HP-GPC. PCDDs, PCDFs, and PCBs were separated on PX-21 activated carbon dispersed on C_{18} HPLC packing material and analyzed as previously described.[8] The analytes were then separated by HPLC-C, isolating four fraction: fraction 1, bulk and di-*ortho*-PCB congeners; fraction 2, mono-*ortho*-PCB congeners; fraction 3, non-*ortho*-PCB congeners; and fraction 4, PCDDs/PCDFs. Mono-*ortho*-PCB congeners were determined by GC/ECD, while non-o-PCBs, PCDDs and PCDFs were determined by Gas Chromatography/High Resolution Mass Spectrometry (GC/HRMS).[9] Dioxin toxic-equivalents (TEQs) in the samples were calculated with toxic equivalency factors (TEFs) developed for early life stage mortality in rainbow trout where available[6,10] or TEFs from the PLHC-1 bioassay.[11]

Injection of Lake trout eggs

Lake trout eggs were air shipped at ~4°C from the U.S. Fish and Wildlife Service and arrived unfertilized. Eggs were fertilized after several hours of slow warming to within one degree of the incubator water temperature (10°C ±1). After water-hardening, eggs were placed in preformed

agarose plates in preparation for injection. Two separate dose-response experiments were conducted with the Lake Michigan lake trout extract. Doses, measured as egg-equivalents (eggEQ) were based on gram tissue/gram egg values normalized to the lipid content of lake trout eggs. Doses used were control (triolein), 0.02, 0.10, 0.20, 1.0, 2.0, 4.0, 10.0, and 20.0 eggEQ. Injections were conducted with glass micropipettes, a regulated gas pressure system, and a digital control device.[12] The injection volume delivered to each egg (~50 nl, or 0.1% of egg volume) was quantified by measuring the size of a triolein droplet which forms at the tip of the needle during injection.

Statistical Analysis

Mortality data and gross pathological lesions were analyzed by probit analysis, corrected for control responses.[13] Probit models were tested for goodness-of-fit (p-values >0.05). The no observable adverse effect level (NOAEL) and the lowest observable adverse effect level (LOAEL) for mortality data and pathological lesion data were analyzed using Dunnett's test ($p < 0.05$) comparing treatment and control groups.[13]

Results

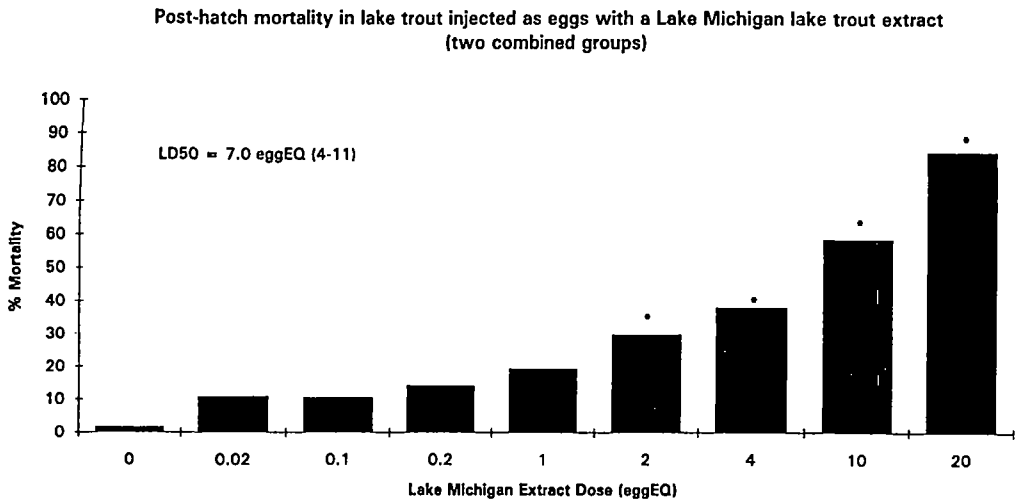
Contaminant Exposure Assessment

The concentrations of PCDDs, PCDFs, and non-*o*-PCBs were determined in the Lake Michigan lake trout tissue (Table 1). The concentration of 1,2,3,7,8-PCDD was the greatest of the PCDDs (except for OCDD) and had the greatest contribution to 2,3,7,8-TCDD-equivalents (TEQs). 2,3,7,8-TCDF had the greatest concentration, while 2,3,4,7,8-PeCDF had the greatest contribution to TEQs among PCDF congeners (Table 1). The concentrations of the planar PCB congeners were 59-2600 pg/g (Table 1). PCB congener 3,3',4,4'-TCB (#77) had the greatest concentration of the non-ortho-substituted PCBs (2600 pg/g), while 3,3',4,4',5-PCB (#126) resulted in the greatest TEQ contribution among any of the measured PHHs (Table 1). Concentrations of mono-*o*-chlorosubstituted PCB congeners were approximately 5-75 ng/g in the tissue of the lake trout used for extraction. Total TEQs based on the concentrations of dioxins, furans and non-o-chlorosubstituted PCBs in the lake trout sample were 14.7 pg TEQ/g (Table 1). The relative contribution of PCDDs, PCDFs, and non-o-chlorosubstituted PCBs were nearly equivalent, each of these classes contributed approximately 5 pg TEQ/g of lake trout.

Table 1. Concentrations of PCDDs, PCDFs, and planar PCBs and resultant TEQs (pg/g, wet wt.) in the lake trout tissue.

	TEF	Conc. (pg/g)	TEQs (pg/g)		TEF	Conc. (pg/g)	TEQs (pg/g)
Dioxins				**Furans**			
2,3,7,8-TCDD	1	1.7	1.7	2,3,7,8-TCDF	0.028	32.3	0.9
1,2,3,7,8-PECDD	0.73	4.1	3.0	1,2,3,7,8-PECDF	0.034	3.8	0.1
1,2,3,4,7,8-HXCDD	0.319	0.5	0.2	2,3,4,7,8-PECDF	0.359	9.2	3.3
1,2,3,6,7,8-HXCDD	0.024	2.1	0.1	1,2,3,4,7,8-HXCDF	0.28	0.9	0.3
1,2,3,7,8,9-HXCDD	0.1	0.5	0.1	1,2,3,6,7,8-HXCDF	0.04	0.8	<0.1
1,2,3,4,6,7,8-HPCDD	0.002	0.6	<0.1	1,2,3,7,8,9-HXCDF	0.09	ND	0
OCDD	0.001	7.5	<0.1	2,3,4,6,7,8-HXCDF	0.1	0.9	0.1
	Total Dioxins		5.0	1,2,3,4,6,7,8-HPCDF	0.1	0.3	<0.1
Planar PCBs				1,2,3,4,7,8,9-HPCDF	0.1	ND	0
3,4,4',5-TCB	0.00056	291	0.2	OCDF	0.001	2.8	<0.1
3,3',4,4'-TCB	0.00016	2600	0.4			**Total Furans**	4.7
3,3',4,4',5-PECB	0.005	883	4.4				
3,3',4,4',5,5'-HXCB	0.000041	59	<0.1	**Grand Total TEQs**	**14.7 pg/g (wet wt.)**		
	Total Planar PCBs		5.0				

1) Limit of detection 0.1 pg/g.
2) TEF values from Zabel et al. 1995 (10) or Tillitt and Cantrell 1992 (11).



Embryotoxicity of the Lake Michigan lake trout extract

The extract of the Lake Michigan lake trout was embryotoxic to lake trout, based on elevated mortality from fertilization through swim-up (Figure 1). The LD50 values based on the probit model was 7 eggEQ (4-11, 95% F.L.). Egg mortality (%) increased in a dose-dependent fashion and was significantly elevated over sham-injected controls at a dose of 2 eggEQ (Figure 1). Gross lesions characteristic of exposure to PHHs were present, and also increased in a dose-related manner in the exposed embryos. These gross pathological abnormalities were classified into three main categories: yolk-sac edema, craniofacial deformities, and hemorrhaging. The effective dose in which 50% of the organisms exhibited hemorrhaging (ED50), was 0.4 eggEQ and the LOAEC was 0.1 eggEQ. Subcutaneous edema of the yolk-sac increased in a dose-related manner with an ED50 of 3.0 eggEQ and a LOAEC of 1.0 eggEQ. Craniofacial deformities, including domed skull, foreshortened maxillae, and deformed jaw structures, also increased in a dose-dependent manner, but were less sensitive biological markers of toxicity as compared to hemorrhaging or yolk-sac edema. The ED50 and LOAEC estimates for craniofacial deformities were 7.4 eggEQ and 2 eggEQ, respectively.

Discussion

The TEF concept for PHHs assumes additivity among congeners in order to assess the risk of complex mixtures of chemicals. The LD50 for the Lake Michigan lake trout extract injected into lake trout was 7 eggEQ (4-11, 95% F.L.). The LD50 of the extract in terms of TEQs was predicted to be 103 pg TEQ/g of egg, based on the TEQs in the original lake trout. We measured an LD50 of 81 pg/g in lake trout eggs injected with 2,3,7,8-TCDD, which is virtually identical to the LD50 value of TCDD (80 pg/g) measured by others.[14] The difference between the LD50 of the extract (103 pg TEQ/g) and the actual LD50 of TCDD (81 pg/g) is relatively small. This indicates that the complex mixture of PHHs in the extract taken from Great Lakes lake trout have additive effects on embryonic toxicity in lake trout. Thus, an additive model for PHHs appears to be appropriate to predict effects and conduct hazard assessments. This also indicates that PCDDs, PCDFs, and PCBs appear to comprise almost all of the dioxin-like potency in Lake Michigan lake trout, while other PHH chemicals (ie. chlorinated naphthalenes) are of limited importance toward lake trout embryo mortality.

The concentrations of PHHs currently present in Great Lakes lake trout are below the threshold for lethality. However, based on the gross pathological lesions observed in this study, the concentrations of dioxin-like chemicals in Lake Michigan lake trout are above a threshold for these sublethal effects.

The significance of the various lesions and the relative importance of such untoward effects might have on the surviving fry are unknown at this point. It is not unreasonable to speculate that these pathological lesions, that are observed in the same patterns as seen in fish exposed to TCDD in the laboratory,[15] may result in a decreased ability of the fry to forage, avoid predation, or compete with other species for habitat or resources in the wild. This study confirms our earlier conclusions that PHHs in lake trout from Lake Michigan are above a threshold for adverse effects and these compounds may have implications on the lack of recruitment in certain Great Lakes lake trout populations.

Acknowledgements
We wish to thank the staff at the Midwest Science Center for their cooperation and support, especially John Meadows, Dennis Schroeder, and Mark Alexander for their technical assistance with the extract preparation, and Kathy Echols, Paul Peterman and Robert Gale for chemical analyses. We also thank Susannah Cantrell, Pam Alt, and Diane Nicks for their assistance in the laboratory.

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