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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[1]

Patrick D. Guiney, Philip M. Cook, John M. Casselman, John D. Fitzsimmons, Howard A. Simonin, Erik W. Zabel, and Richard E. Peterson

Abstract: Background levels of TCDD toxic equivalents (TEs) in lake trout (*Salvelinus namaycush*) eggs (calculated using fish-specific toxicity equivalency factors (TEFs) for polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs)) were higher for two locations in Lake Ontario than Lake Superior and a hatchery (10.5–10.9 versus 0.3 and 0.05 pg TE/g egg). Despite the higher contamination of Lake Ontario eggs, sac fry mortality was uniformly low for all eggs. This is consistent with TCDD toxicity equivalence concentrations (TECs) of all eggs being less than the TCDD no observable adverse effect level (NOAEL) for Lake Superior lake trout sac fry mortality (34 pg TCDD/g egg). Eggs exposed in the laboratory to [^{3}H]TCDD and maintained at either 8 or 8–3–8°C had similar sac fry mortalities. For all sources of eggs, [^{3}H]TCDD-induced sac fry mortality was associated with blue sac disease, which was not affected by water temperature, and resulted in similar LD_{50} values (42–72 pg [^{3}H]TCDD/g egg). Thus, lack of sac fry mortality in wild Lake Ontario lake trout eggs was not caused by lack of responsiveness to TCDD but rather was attributable to egg TECs being below the TCDD NOAEL for sac fry mortality.

Résumé : La concentration naturelle exprimée en équivalents de toxicité (ÉT) de la TCDD dans les oeufs de touladi (*Salvelinus namaycush*) (déterminés à partir des facteurs caractéristiques aux poissons d'équivalence de la toxicité des dibenzo-*p*-dioxines et dibenzofuranes polychlorés (PCDD et PCDF) ainsi que des biphényles polychlorés (BPC), était plus élevée à deux stations du lac Ontario qu'au lac Supérieur et dans une écloserie (10,5–10,9 contre 0,3 et 0,05 pg ÉT/g d'oeufs). Malgré la contamination plus marquée des oeufs du lac Ontario, la mortalité des alevins vésiculés était uniformément faible peu importe la provenance des oeufs. Cette constatation est conforme au fait que la concentration exprimée en équivalents de toxicité (ÉT) de la TCDD dans tous les oeufs est inférieure à la concentration sans effet nocif observé (NOAEL) de cette substance (soit 34 pg TCDD/g d'oeufs) déterminée en termes de mortalité des alevins vésiculés du touladi du lac Supérieur. Le taux de mortalité d'alevins vésiculés issus d'oeufs exposés à la [^{3}H]TCDD au laboratoire et conservés à une température de 8 ou de 8–3–8°C, était similaire. Peu importe l'origine des oeufs, la mortalité causée par la [^{3}H]TCDD chez les alevins vésiculés était associée à la maladie du sac bleu; celle-ci n'était pas soumise à l'influence de la température de l'eau. Cette mortalité correspondait à une CL_{50} comparable, soit de 42–72 pg [^{3}H]TCDD/g d'oeufs. Ainsi, la mortalité réduite des alevins vésiculés issus d'oeufs pondus par des génitrices sauvages du lac Ontario n'est pas attribuable à une réaction peu marqeé à la TCDD, mais plutôt au fait que la concentration des équivalents de toxicité est inférieure à la NOAEL de la TCDD mesurée par la mortalité des alevins vésiculés.
[Traduit par la Rédaction]

Introduction

The Lake Ontario ecosystem has been used extensively as an archetype for studying problems associated with natural reproduction of lake trout (*Salvelinus namaycush*) and for measuring and modeling levels of 2,3,7,8-tetrachlorodibenzo-

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p-dioxin (TCDD) and other persistent halogenated aromatic chemicals in the Great Lakes. Lake Ontario has the most widespread TCDD contamination of all the Great Lakes (U.S. EPA 1990; Cook et al. 1991). Several investigators (O'Keefe et al. 1983; Stalling et al. 1983; DeVault et al. 1989; Zacharewski et al. 1989) have reported the presence of TCDD in several Lake Ontario fish species, with greatest concentrations present in lake trout and other salmonids having a high lipid content. In addition, concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) have been measured in salmonid eggs from the Great Lakes (Niimi 1983; Mac et al. 1985; Smith et al. 1990; Cook et al. 1994). The bioaccumulation of these persistent chemicals in fish early life stages is critical to the characterization of risks of TCDD and related compounds in Lake Ontario for two reasons. First, early life stages of fish are generally more sensitive than adults to toxicity from exposure to TCDD and other Ah receptor agonists (Walker and Peterson 1994). Among all fish species studied thus far lake trout are the most sensitive to sac fry mortality caused by such exposure of eggs. This implies that environmental concentrations of TCDD and related compounds will probably pose the greatest risk to recruitment of sac fry to wild lake trout populations through lethal effects on early life stages. Second, the major route of accumulation of halogenated aromatic chemicals in wild salmonid eggs is through the transfer from maternal tissue during vitellogenesis (Guiney et al. 1979; Niimi 1983; Vodicnik and Peterson 1985; Walker et al. 1994) rather than through uptake from water.

After approximately two decades of progressive lake trout population decline, lake trout in Lake Ontario were essentially extinct by 1960 (Hartman 1988), while contamination by TCDD and various other anthropogenic organochlorine chemicals increased significantly beginning in the 1940s. This initial decline of the lake trout population was attributed to a combination of commercial overfishing, sea lamprey predation, and habitat degradation including eutrophication (Christie 1974). Concentrations of TCDD and related chemicals in wild lake trout eggs, predicted on the basis of the Lake Ontario sediment record, probably caused extensive sac fry mortality during the period of lake trout decline (Cook et al. 1994). Thus, reduced exposure of lake trout to these chemicals is an important requirement for re-establishment of natural reproduction. A lamprey control program was initiated in 1971, and successful stocking of lake trout began in 1973. In 1978, the restoration of a naturally reproducing lake trout population became a Lake Ontario management objective under the Great Lakes Water Quality Agreement (Edwards et al. 1990). Despite extensive restocking (in excess of 2 million lake trout annually since 1985) and the successful re-establishment of adult lake trout in Lake Ontario, the evidence for a naturally reproducing population remains limited. Since 1984, Lake Ontario lake trout eggs have been successfully propagated in U.S. Fish and Wildlife Service hatcheries for the subsequent stocking of yearlings (Marsden et al. 1988; Elrod et al. 1988). Although the data on the percentage of blue sac disease and other early life stage mortality are not reported in these studies, they suggest that at least since 1984, the contaminant load contained within these eggs does not appear to directly affect egg viability. The first report of natural reproduction since stocking began in 1973 was by Marsden et al. (1988). Since 1986, only a few other investigators have reported observing fry emergence (Marsden and Krueger 1991; Grewe et al. 1994) or collecting wild indigenous yearling lake trout (Casselman 1991). This suggests that naturally produced lake trout sac fry or fry have experienced mortality that contributed to recruitment failure of lake trout in Lake Ontario since 1984.

Early life stage mortality due to blue sac disease, a naturally occurring edematous condition observed in hatchery-raised salmonid sac fry (Wolf 1969; Roberts and Shepherd 1986), has been documented in Lake Ontario lake trout (Symula et al. 1990; Simonin et al. 1990). A strong relationship has also been established between TCDD exposure of lake trout eggs via waterborne exposure (Spitsbergen et al. 1991; Walker et al. 1991), injection (Walker et al. 1992), and maternal transfer (Walker et al. 1994), and blue sac syndrome associated mortality of sac fry. Significant mortality during lake trout early life stage development has also been documented from various other regions of the Great Lakes, and the presence of PCDDs, PCDFs, and (or) PCBs in wild lake trout eggs has been proposed as one explanation for this mortality and natural recruitment failure (Willford et al. 1981; Eschenroder et al. 1984; Mac et al. 1985, 1988; Symula et al. 1990; Walker and Peterson 1990).

Cook et al. (1994) have estimated that TCDD concentrations in Lake Ontario lake trout eggs declined approximately 65% between 1978 and 1988. This is consistent with the relatively high (48%) incidence of blue sac disease and associated mortality reported by Symula et al. (1990) for hatchery reared lake trout eggs collected in 1979 from Lake Ontario. Even at reduced egg concentrations, TCDD in combination with other TCDD-like PCDDs, PCDFs, and PCBs may still contribute sufficient toxicity to act in concert with other environmental stressors to impede the re-establishment of naturally reproducing lake trout populations.

This study had three major objectives. The first was to determine if the hallmark sign of TCDD-induced early life stage toxicity, blue sac syndrome, was occurring in fertilized lake trout eggs obtained from Lake Ontario in 1991. Secondly, this study sought to determine if Lake Ontario lake trout sac fry, which were naturally exposed to TCDD and related chemicals, would display a similar sensitivity to [^{3}H]TCDD induced early life stage mortality compared with hatchery-reared and wild Lake Superior lake trout sac fry contaminated with lower levels of these chemicals. To this end, background concentrations of PCDD, PCDF, and PCB congeners that have been shown to produce sac fry mortality associated with a blue sac syndrome were determined in lake trout eggs from eastern and western Lake Ontario, Lake Superior, and from a hatchery, using high resolution gas chromatography – high resolution mass spectrometry (HRGC–HRMS). Background mortality due to blue sac syndrome was determined in the wild and hatchery lake trout sac fry along with their response to graded egg doses of [^{3}H]TCDD. In addition, sac fry from Lake Ontario were observed for a period of 1 month past swim-up to assess the incidence of "swim-up syndrome," an effect not known to be associated with TCDD exposure in salmonids but which occurs in lake trout sac fry from Lake Michigan (Mac et al. 1993) and Lake Ontario (Fitzsimons et al. 1995). Because water temperature

may modulate the occurrence and severity of blue sac disease in salmonids (Ostergaard, 1987), our third objective was to determine if the sensitivity of lake trout sac fry to TCDD-induced mortality by blue sac syndrome would be affected if the study were conducted at a constant water temperature of 8°C versus a water temperature profile that lake trout eggs, embryos, sac fry, and fry might typically encounter in the environment (i.e., a variable temperature of 8–3–8°C). The 8–3–8°C temperature profile was designed to resemble the seasonal water temperature fluctuations anticipated at lake trout spawning reefs in the Great Lakes (Martin and Olver 1980; Bronte 1993; Casselman 1995).

Materials and methods

[^{3}H]TCDD

[1,6-^{3}H]TCDD was obtained from Chemsyn Science (Lenexa, Kan.) and purified to a final radiochemical purity of >99% (specific activity 33.6 Ci/mmol (1 Ci = 37 GBq)) by reverse-phase high performance liquid chromatography (HPLC) as described by Olson (1986). TCDD was stored in HPLC-grade acetone (Aldrich, Milwaukee, Wis.) at −20°C prior to dilution as stock solutions in anhydrous 1,4-*p*-dioxane (Aldrich).

Lake trout egg collection

Lake trout eggs were obtained from wild populations collected by gill nets at two distinct Lake Ontario spawning shoals during October–November 1991. Eggs were stripped from 6 females from Fifty Point in western Lake Ontario and 12 females from Stony Island in eastern Lake Ontario and fertilized with the milt of multiple males at each respective location. Eggs were similarly collected from 28 females and fertilized with milt from multiple males off Gull Island Shoal in southwestern Lake Superior. These eggs were used as a reference site for comparing sensitivity to TCDD-induced sac fry mortality. In addition, fertilized lake trout eggs were also obtained from Crystal Springs hatchery (Altura, Minn.) to serve as a control group. After water hardening and within 24 h of fertilization the eggs from each of the locations were transported to Madison, Wis.

Background contaminant concentrations in lake trout eggs and calculation of fish-specific TECs in lake trout eggs

Concentrations of individual PCB, PCDF, and PCDD congeners in fertilized lake trout eggs from all sources were analyzed. Egg samples (10.1 ± 0.1 g wet weight; mean ± SD) were ground, homogenized, and blended with anhydrous sodium sulfate (50 g). After addition of $^{13}C_{12}$-labeled surrogate standards for PCDDs and PCDFs (Marquis et al. 1994) and PCBs (Kuehl et al. 1991), the samples were Soxhlet extracted with hexane – methylene chloride (1:1, v:v) for 12 h. Lipids, cholesterol, fatty acids, and other interferences were removed through gel permeation chromatography followed by 1% deactivated silica gel column chromatography. PCDDs, PCDFs, and PCBs in the purified extracts were isolated from more polar chemicals through high pressure liquid chromatography (HPLC) using a cyanopropyl (Phase-Sep, UK) semipreparative column in normal phase with a hexane – methylene chloride solvent gradient. Further HPLC treatment of the nonpolar fraction containing the analytes of interest was accomplished with a pyrenyl column (Nacalai Tesque, Japan) in normal phase with a hexane–toluene gradient, which resulted in three fractions for HRGC–HRMS analysis: PCDDs and PCDFs, non-ortho-chlorinated biphenyls, and ortho-chlorinated biphenyls. Concentrations of individual PCB, PCDF, and PCDD congeners in lake trout eggs were analyzed with a Finnigan-Mat 8230 double-focusing HRGC–HRMS instrument using a 30-m DB-5 gas chromatography column. The HRGC–HRMS conditions, quantification procedures, and quality assurance criteria have been described previously (Marquis et al. 1994), with the exception that minimum levels of detection for this study were calculated on the basis of a 3:1 signal to noise criteria for each analyte in each sample.

The "background" TCDD toxicity equivalence concentrations (TECs) due to PCBs, PCDDs, and PCDFs in eggs from each source was estimated. For Lake Superior lake trout eggs from Gull Island Shoal, concentrations of each chemical measured in three annual samples of eggs collected during 1989–1991 were averaged for the TEC calculation. Trout-specific TCDD toxicity equivalence factors (TEFs) for each chemical based on the molar concentration of each chemical in the egg that causes 50% mortality in rainbow trout (*Oncorhynchus mykiss*) sac fry (Walker and Peterson 1991; Zabel et al. 1995*a*) were used. The TCDD toxicity equivalents (TE_x) due to each chemical (x) in the eggs was calculated as

$$TE_x = TEF_x(MW_{TCDD}/MW_x)\ Cegg_x$$

where MW is the molecular weights used to convert TEFs from molar to mass concentration units and Cegg is the concentration of chemical measured in eggs as picograms per gram wet mass of eggs. The sum of TEs for each chemical in an egg sample equals the TEC. The units of TEs and TEC are picograms TCDD equivalent per gram wet mass of eggs. The additive TE model has been validated for trout early life stage mortality with binary mixtures of congeners in rainbow trout eggs (Zabel et al. 1995*b*), TCDD and PCB 126 in lake trout eggs (Zabel et al. 1995*c*), and a complex mixture of 14 congeners in lake trout and rainbow trout eggs (Walker et al. 1996).

Static water exposure of lake trout eggs to [^{3}H]TCDD

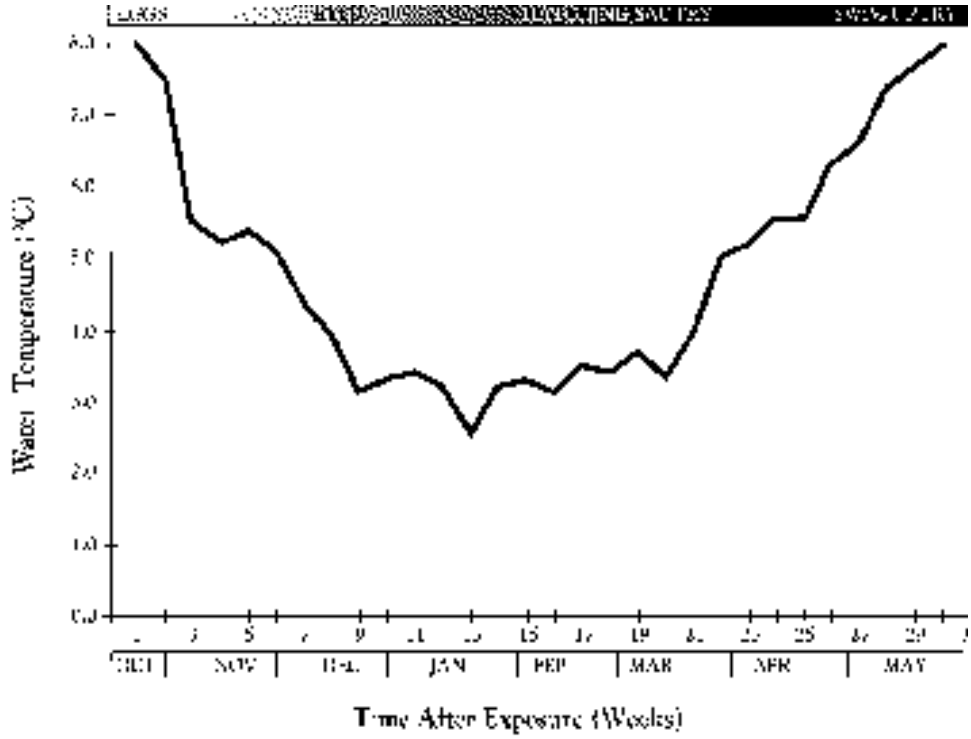
Eggs from each source were treated prophylactically with 100 ppm Argentyne disinfectant (Argent Laboratories, Redmond, Wash.) for 10 min before they were divided into subgroups; egg numbers were estimated by volume displacement. Lake Ontario and hatchery eggs were divided into 32 subgroups (200 eggs per subgroup), which were randomly assigned to 1 of 16 treatment groups. The 16 groups consisted of 2 vehicle control groups and 14 graded [^{3}H]TCDD dosage groups. Fertilized Lake Superior eggs were divided into 24 subgroups (1300 eggs per subgroup) that were assigned to 1 of 6 treatment groups, which consisted of a vehicle control and 5 [^{3}H]TCDD dosage groups.

Within 24 h after fertilization, each subgroup of eggs was statically exposed for 48 h to water containing 4 mL acetone/L (solvent control) or [^{3}H]TCDD dissolved in 4 mL acetone/L. For Lake Ontario and hatchery eggs, 2 vehicle controls and 14 nominal water concentrations of [^{3}H]TCDD were used: 2, 4, 6, 8, 10, 15, 20, 30, 40, 60, 80, 100, 120, and 150 ng [^{3}H]TCDD/L water. For Lake Superior eggs, one vehicle control and five nominal water concentrations of [^{3}H]TCDD were used: 10, 20, 40, 62 and 100 ng [^{3}H]TCDD/L water. Throughout the static exposure, the exposure water (6–8°C) was aerated and renewed three times at approximately 12-h intervals with water containing the appropriate concentration of acetone or [^{3}H]TCDD in acetone. Following the 48 h exposure, each group was transferred to an individual tank supplied with flowing [^{3}H]TCDD-free water.

Determination of TCDD-derived [^{3}H] in eggs

To determine the concentration of [^{3}H]TCDD accumulated in lake trout eggs exposed to waterborne [^{3}H]TCDD, five eggs per group were sampled 10 days postexposure (to allow for desorption of [^{3}H]TCDD from the outer surface of the chorionic membrane). Previous work has shown that during the egg and sac fry stages of lake trout development, the absolute amount of [^{3}H]TCDD in eggs and sac fry remains essentially constant with no significant elimination until after swim-up (Walker et al. 1991; Zabel et al. 1995). Lake trout eggs (140–160 mg) were blotted to remove excess water, weighed, and digested and dissolved with 2 mL Soluene 350 (Packard Instrument Co., Downers Grove, IL) at 50–60°C overnight. The

Fig. 1. Average weekly (measured 4 days/week) water temperatures used to maintain lake trout early life stages over the course of this study for comparison with lake trout reared at a constant 8°C.



digested and dissolved samples were cooled, 10 mL of Hionic (Packard) liquid scintillation medium was added, and low-energy chemiluminescence was allowed to decay prior to determining counts per minute (cpm) of TCDD-derived ^{3}H by liquid scintillation counting (Packard, model 2000 CA). Using appropriate quench corrections, disintegrations per minute (dpm) were calculated from cpm and counting efficiency.

Maintenance and assessment of eggs, sac fry, and fry

Eggs, sac fry, and fry were maintained in filtered (rust and sediment type filter), dechlorinated tap water (8–12 replacements/day, dissolved oxygen 12.0 ppm, pH 7.5, hardness 304 ppm as $CaCO_3$, alkalinity 374 ppm as $CaCO_3$). Each group of lake trout eggs was maintained in water kept at 8.0 ± 1.0°C. In addition, duplicate sets of vehicle and [^{3}H]TCDD-exposed eggs from Fifty Point and Stony Island, Lake Ontario, and a hatchery were also maintained under a variable cold water temperature regime (8–3–8°C) designed to more closely mimic seasonal variations in water temperatures on Lake Ontario spawning reefs (Fig. 1). The original experiment included a temperature profile based on expert input from the joint authors of this research. Subsequent findings by Casselman (1995) confirmed the appropriateness of the selected natural water temperature simulation regime. To ensure the appropriate water temperatures, beakers containing each of the egg dose groups were individually supplied with flow-through water and placed in a 38-L glass aquarium (two aquaria/experiment), which contained flow-through water at either a constant 8°C or the 8–3–8°C spawning reef water temperature appropriate for that time of year.

Beginning at swim-up, control and TCDD-treated fry derived from Lake Ontario eggs and maintained at water temperatures of 8–3–8°C were fed No. 1 Rangen trout pellets daily for 14–20 days and then switched to No. 5 Rangen trout pellets ad libitum until termination of the experiment, 1 month after swim-up. The purpose of this experiment was to assess the remaining fry for evidence of "swim-up" or "drop-out" syndrome, which is separate and distinct from blue sac syndrome caused by TCDD, and is reported to occur in a female-specific fashion in Lake Michigan and Lake Ontario lake trout fry (Symula et al. 1990; Simonin et al. 1990). In this study we used the following signs associated with drop-out – swim-up mortality: loss of equilibrium, erratic swimming, fry lying on their sides on the bottom of the tank, and hyperexcitability followed by lethargy prior to death to measure the incidence of this effect.

To assess cumulative mortality throughout these experiments, dead sac fry or fry from each treatment group were removed and recorded every other day. At the termination of each experiment (swim-up or 1 month after swim-up), surviving fry in each treatment group were counted and killed. Cumulative mortality was calculated excluding the viable eggs that were sampled for accumulated [^{3}H]TCDD.

Statistical procedures

Cumulative mortality data at swim-up for each [^{3}H]TCDD treatment group were analyzed by chi-square (Wonnacott and Wonnacott 1985) to determine TCDD-related effects. A continuous dose–response curve was generated for mortality data from hatching onset to swim-up as a function of the egg [^{3}H]TCDD dose using a probit analysis procedure, which accounted for control mortality, used chi-square goodness of fit, estimated the slope and intercept, and was based on the assumption that mortality was independent for fish within a treatment group and among treatment groups (Finney 1971; SAS 1988). The LD_{50} of TCDD (50% mortality above vehicle control group from hatching onset to swim-up) and 95% fiducial limits were calculated based upon the [^{3}H]TCDD concentration accumulated in the eggs.

Results

Background concentrations of PCBs, PCDFs, and PCDDs and calculation of TCDD toxic equivalence concentrations (TECs) in lake trout eggs

Concentrations of PCDD and PCDF congeners in both Lake

Table 1. Concentrations of toxicologically important PCDD, PCDF, and PCB congeners measured in feral Lake Superior lake trout eggs (1989–1991) and in hatchery and feral Lake Ontario lake trout eggs collected in 1991.

	Egg congener concentration (pg/g)			
	Southwestern Lake Superior (Gull Island)[a]	Hatchery (Crystal Springs)	Western Lake Ontario (Fifty Point)	Eastern Lake Ontario (Stony Island)
PCDDs				
2,3,7,8-TCDD	nd (0.2)[b,c]	nd (0.2)	6.9 (1.2)	6.0 (0.7)
1,2,3,7,8-PeCDD	nd (1.1)	nd (0.9)	nd (1.3)	nd (0.8)
1,2,3,4,7,8-HxCDD	nd (0.7)	nd (0.8)	nd (1.3)	nd (0.8)
1,2,3,6,7,8-HxCDD	nd (0.7)	nd (0.8)	nd (1.3)	nd (0.8)
1,2,3,4,6,7,8-HpCDD	nd (0.7)	nd (0.8)	nd (1.1)	nd (1.0)
PCDFs				
2,3,7,8-TCDF	2.1 (0.5)	nd (0.7)	3.9 (0.5)	4.4 (0.8)
1,2,3,7,8-PeCDF	nd (0.6)	nd (0.3)	1.1(1.0)	1.4 (1.1)
2,3,4,7,8-PeCDF	nd (0.6)	nd (0.3)	4.6(1.1)	5.0 (1.2)
1,2,3,4,7,8-HxCDF	nd (0.6)	nd (0.3)	nd (2.2)	1.7 (0.7)
Non-Ortho PCBs				
3,4,4′,5-TCB (No. 81)	na[d]	na[d]	50[e]	50[e]
3,3′4,4′-TCB (No. 77)	54	30	1 000	960
3,3′,4,4′,5-PeCB (No. 126)	52	8	400	380
3,3′,4,4′,5,5′-HxCB (No. 169)	nd (10)	nd (10)	20	16
Mono-Ortho PCBs				
2,3,3′,4,4′-PeCB (No. 105)	800	200	11 000	12 000
2,3,4,4′,5-PeCB (No. 118)	8 000	2 200	39 000[e]	39 000[e]

[a]Concentrations of congeners in Lake Superior, Gull Island Shoal lake trout eggs are mean values from samples collected over the period 1989–1991.
[b]nd, concentration below the limit of detection.
[c]Numbers in parentheses are minimum levels of detection for each congener in the sample.
[d]na, PCB congener was not analyzed when the sample set was processed.
[e]PCB Nos. 81, 105, and 118 concentrations in Lake Ontario lake trout eggs in 1991 have been extrapolated from HRGC–HRMS analysis of 1988 samples.

Superior and hatchery samples were below the HRGC–HRMS limits of detection with the exception of detectable 2,3,7,8-tetrachlorodibenzofuran in the Lake Superior eggs (Table 1). These results are consistent with PCDD and PCDF concentrations previously reported for 1984 Lake Superior lake trout (DeVault et al. 1989) if one considers the approximately 3:1 ratio observed between concentrations in Great Lakes wild lake trout as compared with their eggs (Miller 1993; Cook et al. 1994) and the expected decline in residues between 1984 and 1990. Concentrations of PCDD and PCDF congeners in the lake trout eggs from Lake Ontario are similarly consistent with the concentrations reported for 1984 Lake Ontario lake trout by DeVault et al. (1989).

PCB congeners were detectable in all egg samples, but much larger concentrations were found in those from Lake Ontario. Concentrations of PCB No. 126, the most toxic PCB congener, were 6-fold greater in Lake Superior eggs and 50-fold greater in Lake Ontario eggs than in hatchery eggs. The other PCB congeners were similarly distributed with the exception of PCB No. 77 concentrations, which seemed greater in the hatchery eggs and less in the Lake Superior eggs than expected from the PCB No. 126 distribution.

The TEC for hatchery lake trout eggs was ≥0.05 pg TCDD toxicity equivalent/g wet egg and two PCB congeners (Nos. 118 and 126) contributed >83% of the TECs (Table 2). TECs in lake trout eggs from Lake Superior was ≥0.3 pg TCDD toxicity equivalent/g of which 2,3,7,8-TCDF plus PCBs No. 118 and No. 126 accounted for >96%. TECs were significantly higher in lake trout eggs collected from Lake Ontario. For eggs from Fifty Point and Stony Island, Lake Ontario, TEs contributed by 2,3,7,8-TCDD were predominant, making up 62 and 56% of the TECs, respectively. For lake trout eggs from Fifty Point, PCDFs contributed 16% and PCBs, 22% of the TECs. For Stony Island eggs, PCDFs accounted for 23% and PCBs, 21% of the total TEC.

Sac fry mortality

Sac fry mortality due to blue sac syndrome in vehicle-exposed groups was less than 20% in eggs from all sources (Table 3). For eggs obtained from Lake Ontario, sac fry mortality tended to be higher at 8°C than at 8–3–8°C, whereas the opposite was observed for background sac fry mortality in eggs obtained from the hatchery.

The cumulative mortality of lake trout embryos was unaffected by [^{3}H]TCDD exposure prior to the onset of hatching (data not shown). There was in fact no significant difference in mean egg mortalities between the vehicle control groups and the combined TCDD treatment groups for each source of

Table 2. TCDD toxicity equivalents (TEs) derived from fish-specific toxic equivalency factors (TEFs) based on early life stage mortality and the chemical concentrations measured in hatchery and Great Lakes lake trout eggs as shown in Table 1.

		Egg TEs (pg TCDD equivalents/g wet egg) contributed by individual congeners			
Congener	Fish-specific TEF[a]	Southwestern Lake Superior (Gull Island Shoal)	Hatchery (Crystal Springs)	Western Lake Ontario (Fifty Point)	Eastern Lake Ontario (Stony Island)
PCDDs					
2,3,7,8-TCDD	1.0	nd (0.2)[b,c]	nd (0.2)	6.90	6.00
1,2,3,7,8-PeCDD	0.730	nd (1.1)	nd (0.9)	nd (1.3)	nd (0.8)
1,2,3,4,7,8-HxCDD	0.319	nd (0.7)	nd (0.8)	nd (1.3)	nd (0.8)
1,2,3,6,7,8-HxCDD	0.024	nd (0.7)	nd (0.8)	nd (1.3)	nd (0.8)
1,2,3,4,6,7,8-HpCDD	0.002	nd (0.7)	nd (0.8)	nd (1.1)	nd (1.0)
PCDFs					
2,3,7,8-TCDF	0.028	0.05	nd (0.7)	0.11	0.12
1,2,3,7,8-PeCDF	0.034	nd (0.6)	nd (0.3)	0.04	0.05
2,3,4,7,8-PeCDF	0.359	nd (0.6)	nd (0.3)	1.65	1.80
1,2,3,4,7,8-HxCDF	0.280	nd (0.6)	nd (0.3)	nd (2.2)	0.48
Non-Ortho PCBs					
3,4,4′,5-TCB (No. 81)	0.000 56	na[d]	na[d]	0.03	0.03
3,3′4,4′-TCB (No. 77)	0.000 16	0.009	0.005	0.16	0.15
3,3′,4,4′,5-PeCB (No. 126)	0.005	0.260	0.040	2.000	1.900
3,3′,4,4′,5,5′-HxCB (No. 169)	0.000 041	nd (10)	nd (10)	0.001	0.001
Mono-Ortho PCBs					
2,3,3′,4,4′-PeCB (No. 105)	<0.000 002 6	<0.002	<0.001	<0.029	<0.031
2,3,4,4′,5-PeCB (No. 118)	<0.000 004 5	<0.036	<0.010	<0.176	<0.176
TEC[e]		≥0.32	≥0.05	≥10.89	≥10.53

[a]TEF was calculated by dividing the LD_{50} of 2,3,7,8-TCDD (pmol TCDD/g egg) by the LD_{50} of the congener (pmol congener/g egg) based on early life stage mortality in the same strain of rainbow trout (Walker and Peterson 1991; Zabel et al. 1995*a*).

[b]nd, concentration below the limit of detection.

[c]Numbers in parentheses are minimum limits of analytical detection (pg/g sample) for each congener in the sample.

[d]na, PCB congener was not analyzed when the sample set was processed.

[e]Toxicity equivalence concentrations (TECs) were obtained for each location by summing the individual TCDD toxicity equivalents (TEs) of each PCDD, PCDF, and PCB congener using fish-specific TEF values for each congener that were based on the endpoint of sac fry mortality in rainbow trout (Walker and Peterson 1991; Zabel et al. 1995*a*). The TEs listed for the mono-ortho-PCBs were not included in the final TEC calculations based upon a lack of early life stage mortality as indicated by their TEF values. The ≥ sign in front of each TEC value indicates that the TEC may be greater because of either undetected chemicals or undetected toxicity for some of the chemicals tested.

Table 3. Background sac fry mortality for lake trout eggs collected from various Great Lakes locations and a hatchery source maintained under two different water temperature regimes.

	Lake trout sac fry mortality (%)[a]	
Egg source	8°C	8–3–8°C
Southwestern Lake Superior (Gull Island)	11	nc[b]
Hatchery (Crystal Springs, Altura, Minn.)	6	18
Western Lake Ontario (Fifty Point)	11	7
Eastern Lake Ontario (Stony Island)	8	1

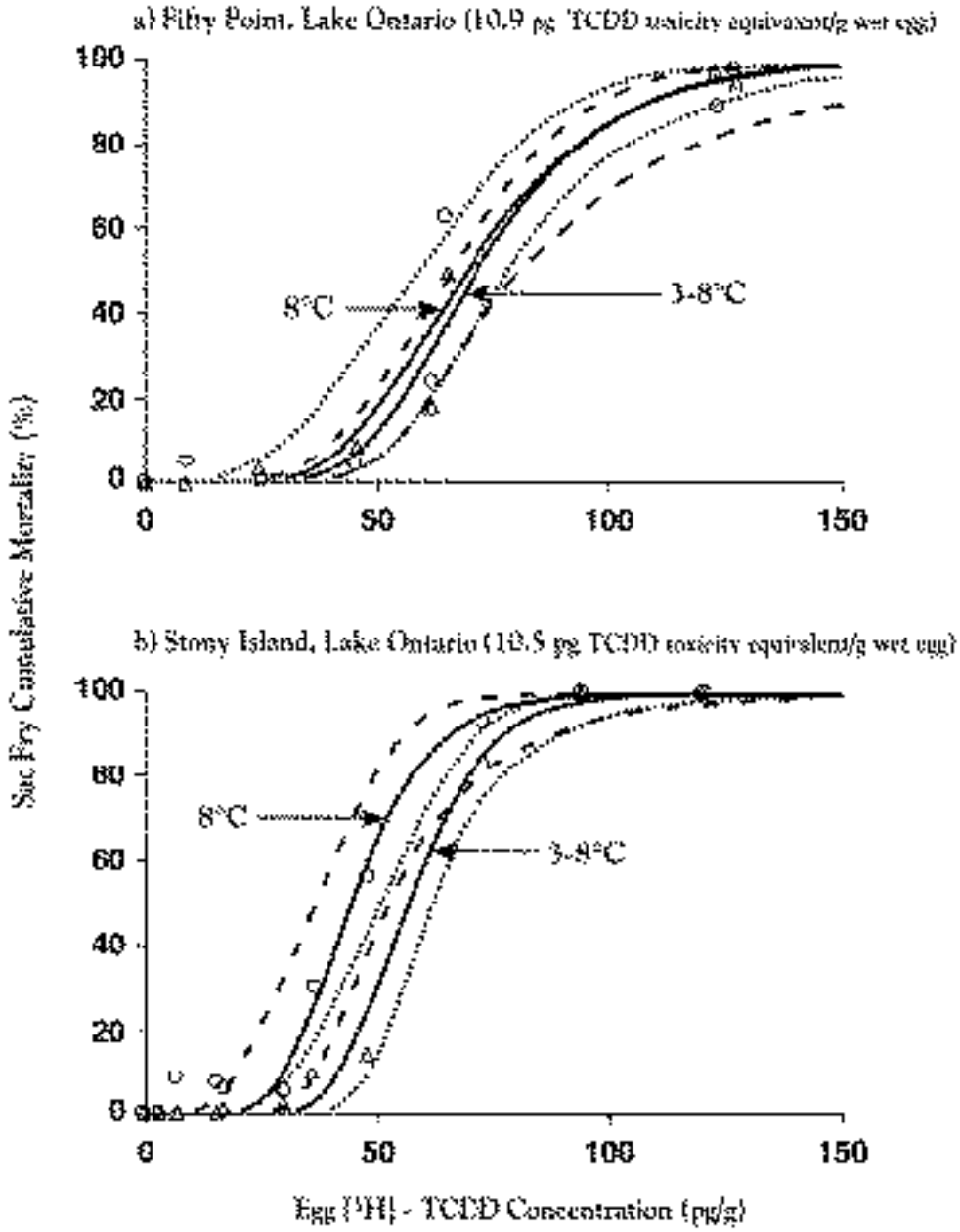
[a]Mean percent cumulative mortality (half hatched plus blue sac syndrome) determined from the onset of hatching to swim-up for two vehicle treatment groups (i.e., non-[^{3}H]TCDD exposed controls).

[b]nc, not conducted.

eggs. Furthermore, reducing the incubation temperature from a constant 8°C to the variable 8–3–8°C temperature profile had no effect on egg mortality prior to the onset of hatching.

Waterborne exposure of fertilized lake trout eggs to [^{3}H]TCDD resulted in egg [^{3}H]TCDD dose-related increases in cumulative mortality of larvae from the onset of hatching to swim-up. In general, the greatest mortality occurred as sac fry, with less mortality associated with embryos dying partially emerged from the chorion (half-hatched). In sac fry from all sources the signs of TCDD toxicity during the sac fry stage of development were the same. They consisted of subcutaneous hemorrhages, subcutaneous edema of the yolk sac, pericardial and meningeal edema, craniofacial malformations, regional ischemia, and growth retardation, culminating eventually in death during the sac fry stage of development (Spitsbergen et al. 1991; Walker et al. 1991). These signs are essentially identical to those of blue sac disease (Wolf 1969;

Fig. 2. Cumulative mortality of lake trout sac fry from (*a*) Fifty Point and (*b*) Stony Island from hatching onset to swim-up as a function of [^{3}H]TCDD concentrations in eggs incubated at a constant 8°C or 8–3–8°C. Egg [^{3}H]TCDD concentrations shown are based on the accumulated TCDD-derived [^{3}H] measured in eggs 10 days postexposure. The values in parentheses are the background TECs determined for eggs from each location. Cumulative mortality in TCDD groups has been corrected for mean cumulative mortality in the acetone vehicle control group ((○), 8°C mortality data points; (Δ), 8–3–8°C data). Broken lines (8°C) and dotted lines (8–3–8°C) represent the probit model's upper and lower 95% fiducial limits for each probit generated dose–response curve.

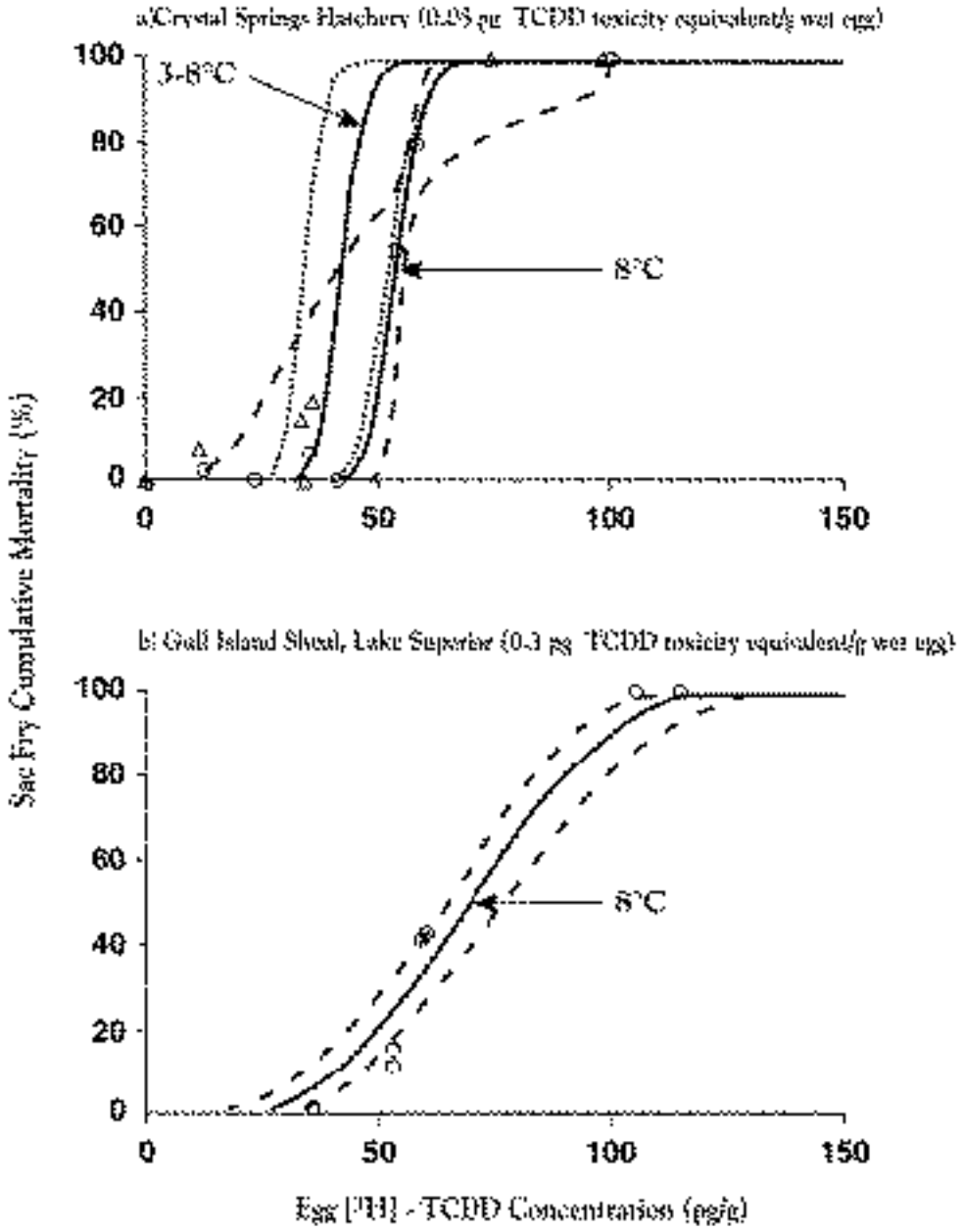


Roberts and Shepherd 1986), which has been reported in the past in Lake Ontario sac fry (Symula et al. 1990; Simonin et al. 1990). For eggs collected from Fifty Point and Stony Island locations and incubated using the 8–3–8°C water temperature profile for up to 1 month after swim-up, the incidence of swim-up syndrome characteristics and associated mortality was low. The average occurrence of swim-up syndrome across all groups ([^{3}H]TCDD exposure groups and control) was 5–8% and was unaffected by [^{3}H]TCDD egg dose or incubation temperature.

TCDD dose response relationships for sac fry mortality

There was no significant effect of water temperature on [^{3}H]TCDD-induced sac fry mortality in eggs obtained from either Fifty Point (Fig. 2*a*) or Stony Island, Lake Ontario (Fig. 2*b*). At both sites and at both temperatures there was a

Fig. 3. Probit-generated dose–response curves for percent cumulative sac fry mortality from hatching onset to swim-up for (*a*) Crystal Springs Hatchery lake trout eggs, exposed to waterborne [^{3}H]TCDD and incubated under two different temperature regimes. Individual mortality data points for 8°C (○) and 8–3–8°C (Δ) have been corrected for control mortality. The probit model's 95% fiduciary limits for each curve are shown by broken lines (8°C) or dotted lines (8–3–8°C). (*b*) Gull Island Shoal lake trout eggs incubated at a constant 8°C (adapted with permission from Walker et al. 1991). The probit model (solid line) is illustrated with the 95% fiducial limits shown as broken lines on either side.



steep [^{3}H]TCDD dose-related increase in lake trout sac fry mortality. The 95% confidence intervals for the egg [^{3}H]TCDD doses at all levels of sac fry mortality at the constant temperature (8°C) overlapped those for the spawning reef temperatures (8–3–8°C).

Essentially the same profile of [^{3}H]TCDD dose-related effects was observed for lake trout reared from hatchery eggs (Fig. 3*a*). In this case the dose–response curve was even steeper than for wild eggs, but again there was no effect of water temperature on the slope of the curve. The dose–response curve for cumulative mortality of Lake Superior Gull Island Shoal sac fry as a function of [^{3}H]TCDD dosage in the egg at a constant 8°C water temperature (Fig. 3*b*) was generally very similar to those observed for wild Lake Ontario lake trout (Fig. 2).

For all sources of eggs the background TECs, which ranged from 0.05 to 10.9 pg TE/g egg, were below the NOAEL for lake trout sac fry mortality (Table 4). The

Table 4. Calculated early life stage toxicity endpoints based on sac fry mortality of lake trout exposed as fertilized eggs to graded concentrations of [^{3}H]2,3,7,8-TCDD and incubated under two different water temperature regimes.

Egg source	Background egg TEC (pg TE/g)	Egg [^{3}H]TCDD concentration (pg [^{3}H]TCDD/g) NOAEL[a] 8°C	NOAEL[a] 8–3–8°C	LD_{50}[b] 8°C	LD_{50}[b] 8–3–8°C
Western Lake Ontario (Fifty Point)	10.9	45 (31–55)	45 (37–51)	69 (58–80)	72 (65–78)
Eastern Lake Ontario (Stony Island)	10.5	30 (20–37)	36 (25–43)	44 (36–52)	57 (50–61)
Southwestern Lake Superior (Gull Island)	0.3	34 (26–40)	nc[c]	65 (60–71)	nc[c]
Hatchery (Crystal Springs, Altura, Minn.)	0.05	41 (8–47)	35 (28–44)	53 (41-55)	42 (33-52)

[a]The no observable adverse effect level (NOAEL) was the whole egg [^{3}H]TCDD concentration that did not significantly increase cumulative sac fry mortality compared with control. Values in parentheses are 95% confidence intervals.

[b]LD_{50} values represent TCDD concentrations in eggs that caused 50% mortality above control from hatching onset to swim-up as determined using the probit procedure (Finney 1971; SAS Institute Inc. 1988). Values in parentheses are 95% confidence intervals.

[c]nc, not conducted.

background TEC was, however, higher for eggs from Lake Ontario than from Lake Superior or a hatchery (Table 4). The background TEC in the most heavily contaminated eggs (Fifty Point Lake Ontario) was less than the 95% confidence interval of the LD_{50} determined for the lake trout sac fry in this study. Therefore, it was not surprising that the LD_{50} for sac fry mortality, based on egg [^{3}H]TCDD dose, was generally similar for eggs with low (Lake Superior and hatchery) and higher (western and eastern Lake Ontario) background TECs.

The LD_{50s}, based on [^{3}H]TCDD egg dose, ranged from 44 to 69 pg/g for Lake Ontario, Lake Superior, and hatchery sac fry reared at 8°C. For sac fry from those same sources but reared at a water temperature of 8–3–8°C, the range was essentially the same (42–72 pg/g). The range in no observable adverse effect levels (NOAELs) for sac fry mortality, based on egg [^{3}H]TCDD dose, for the same four sources of eggs, was relatively narrow. For sac fry reared at 8°C the NOAEL ranged from 30 to 45 pg/g. At a water temperature of 8–3–8°C, the NOAELs ranged from 35 to 45 pg/g. Taken together, these results demonstrate that water temperatures that would be encountered by lake trout embryos and sac fry at spawning reefs in Lake Ontario would not result in a change in the potency of TCDD in causing sac fry mortality.

Discussion

Relationships between background TECs in Lake Ontario lake trout eggs and background sac fry mortality

Several studies have documented that lake trout eggs collected from Lake Ontario females exhibited high incidences of blue sac disease and associated mortality between 1977 and 1983. In 1977, Simonin et al. (1990) reported a range from 7 to 100% with a mean incidence of 70%. Lake Ontario lake trout eggs collected in 1979 displayed a range of blue sac disease from 2 to 98% with a mean incidence of 48% (Symula et al. 1990). By 1983 these same researchers were reporting a range of blue sac disease incidence for Lake Ontario lake trout of 16–23%. In these studies, lake trout sac fry edema and mortality was female dependent, occurred at a constant 8°C holding temperature, and was not correlated with concentrations of DDT, DDE, total PCBs, dieldrin, or mirex in the eggs. A few years later, Ostergaard (1987) reported a rough estimate of 60% loss of Stony Island Lake Ontario lake trout sac fry due to blue sac disease from eggs collected and incubated during 1984–1985 at ambient hatchery temperatures of 8.3–10.5°C. Although this 60% estimate of blue sac disease was not well quantified, it appears high based on the trend for reduced incidences of blue sac reported from 1977 to 1983 by Simonin et al. (1990) and Symula et al. (1990). Use of chilled water (3.7–7.7°C) by Ostergaard significantly enhanced the survival of these Lake Ontario lake trout and decreased the incidence of blue sac disease, although the exact percent reduction in blue sac disease related mortality was not reported.

Because adult lake trout from Lake Ontario have greater concentrations of TCDD and related aryl hydrocarbon (Ah) receptor agonists than lake trout from any of the other Great Lakes (Zacharewski et al. 1989; DeVault et al. 1989; U.S. EPA 1993), we hypothesized that the TECs in wild Lake

Ontario lake trout eggs would be elevated compared with lake trout eggs from the other Great Lakes. The major route for accumulation of halogenated aromatic chemicals in salmonid eggs is not from contaminated water but rather as translocated lipophilic contaminants, deposited directly from the sexually mature adult females into eggs prior to spawning (Guiney et al. 1979; Niimi 1983; Vodicnik and Peterson 1985). These halogentated aromatic chemicals are then taken up by the developing embryo and sac fry from yolk lipid globules and lipotropic fluid contained inside salmonid eggs (Guiney et al. 1980; Zabel et al. 1995*a*). Recently Walker et al. (1994) reported that maternally derived TCDD resulted in the same signs of toxicity and lethal potency during lake trout sac fry development as exposure of fertilized lake trout eggs to waterborne or injected TCDD.

In this study, background TECs were higher for Lake Ontario eggs than for lake trout eggs obtained from the Crystal Springs hatchery or from Gull Island Shoal, Lake Superior, from 1989 to 1991. Despite the greater TECs in Lake Ontario lake trout eggs in 1991 compared with other Great Lakes, sac fry mortality was uniformly low. The incidence of blue sac disease in the vehicle-exposed lake trout eggs from these two wild Lake Ontario populations (1–11%) is consistent with the finding that total TECs in these eggs were about one third of the TCDD NOAEL for lake trout sac fry mortality for fertilized eggs from Lake Superior (Walker et al. 1991) and Lake Ontario eggs (Table 4). Thus, results of this study show that it is unlikely that lake trout reproduction is currently at risk in Lake Ontario due solely to the effects of background egg TECs on blue sac syndrome-related mortality. Results of a retrospective risk assessment (Cook et al. 1994), however, suggest that, in the period from the mid 1940s to the mid 1970s, the egg TEC exceeded the LD_{100} for causing blue sac syndrome in Lake Ontario lake trout, which would have precluded successful recruitment of lake trout sac fry into the population.

Sensitivity of wild Lake Ontario lake trout to [^{3}H]TCDD-induced early life stage mortality

Our results clearly demonstrate that Lake Ontario lake trout are extremely sensitive and equally responsive when exposed as eggs to waterborne [^{3}H]TCDD as Lake Superior and hatchery derived lake trout sac fry (Table 4). This confirms the strong association that has now been established between TCDD exposure of lake trout eggs via waterborne exposure (Spitsbergen et al. 1991; Walker et al. 1991), injection (Walker et al. 1992), and maternal transfer (Walker et al. 1994) with TCDD-induced blue sac disease. TCDD-induced lesions observed in this study were essentially identical to those induced in the above studies by various routes of exposure.

The failure of wild Lake Ontario lake trout sac fry that are not exposed as eggs to [^{3}H]TCDD to manifest a blue sac disease type of early life stage mortality (Table 3) is because their background egg burdens of TCDD and related chemicals (Table 1) are below the NOAEL for sac fry mortality. When egg concentrations of [^{3}H]TCDD exceed the NOAEL, then dose-related increases in sac fry mortality are seen in Lake Ontario lake trout (Fig. 2), just as is the case in lake trout from Lake Superior and a hatchery source (Fig. 3).

Effect of water temperature on TCDD-induced lake trout early life stage mortality

No significant relationships were established between water temperature and mean egg mortalities or sac fry mortality due to blue sac disease. Eggs from each location, maintained at either 8°C or a 8–3–8°C temperature profile had similar egg mortality and uniformly low sac fry mortality. In addition, for all sources of eggs, exposure to [^{3}H]TCDD resulted in sac fry mortality associated with blue sac syndrome, which was not reduced by lowering the water temperature. The LD_{50} values were all similar, ranging from 42 to 72 pg [^{3}H]TCDD/g egg. Similar results have recently been reported by Fitzsimons et al. (1995) who studied the occurrence of swim-up syndrome in Lake Ontario lake trout and its relationship with rearing temperatures. The incidence of swim-up syndrome (an effect not known to be associated with TCDD exposure in salmonids) was found to be unrelated to incubation temperatures (4 vs. 8°C).

Symula et al. (1990), however, found that reduction in water temperature below 8.3°C for incubation of eyed eggs resulted in a decrease in mortality of sac fry from the same group of Lake Ontario female lake trout in 1982–1984. The reduction in the incidence of mortality associated with blue sac syndrome was from 20% at 8.3°C to 6% at 3.0°C and from 24% at 8.3°C to 9% at 1.7°C. Ostergard (1987) also reported reduced mortality of sac fry when eggs collected in 1984–1985 from Seneca strain lake trout from both Lake Ontario and Seneca Lake were incubated under colder water temperatures (mean 5.3°C as compared with 9.4°C). Water temperatures on shallow lake trout spawning reefs in Lake Ontario fall below 3.0°C in late winter (Casselman 1996), but water temperatures below 3.0°C have not been shown to result in further reduction of sac fry mortality. Symula et al. (1990) reduced water temperatures throughout the incubation period following eying of the eggs, unlike this study in which water temperatures were varied over time (Fig. 1) in a pattern similar to the spawning reef water temperature profile reported by Casselman (1996). The mechanism by which reduced incubation temperature provides partial protection against blue sac disease in some studies but not in others remains to be elucidated. Casselman (1996) associated low sac fry survival on a shallow reef in eastern Lake Ontario with high water tempeatures at the beginning of the egg incubation period. Symula et al. (1990) suggested that temperature may influence blue sac syndrome mortality induced by fungal or bacterial infections. The myoxobacteria *Cytophaga psychrophilia* was isolated from the 1984 lake trout eggs studied by Symula et al. (1990), but the roles of such potential pathogens and possible interactions with chemicals to increase blue sac syndrome mortality remain unknown.

This study was designed to evaluate whether seasonal water temperature fluctuations expected at lake trout spawning reefs in the Great Lakes might affect the sensitivity of lake trout sac fry to TCDD-induced mortality. A constant low temperature effect such as that observed by Symula et al. (1990) could reduce TCDD-induced sac fry mortality. Conversely, it is unknown if the beneficial effect observed by Symula et al. (1990) would occur under the water temperature conditions experienced by lake trout eggs in Lake Ontario. Based on our findings and the water temperature data of Casselman (1996), water temperatures encountered in typical

lake trout spawning reefs in Lake Ontario would not be expected to reduce the potency of TCDD to induce lake trout sac fry mortality.

Implications for lake trout reproduction in Lake Ontario

Despite greater TECs (10.5–10.9 pg TCDD equivalent/g egg) in Lake Ontario eggs, lake trout sac fry mortality due to blue sac disease was uniformly low for all control eggs. The LD_{50} and NOAEL values for [^{3}H]TCDD-induced sac fry mortality in Lake Ontario lake trout (Table 4) are very similar to the values Walker et al. (1991) reported for Lake Superior lake trout (65 and 34 pg TCDD/g egg, respectively). Background TECs in Lake Ontario eggs (present at about one third the NOAEL) also did not cause a significant shift to the left of the [^{3}H]TCDD dose–response curve for percent cumulative mortality in comparison with the Lake Superior or hatchery eggs. We believe a fundamental reason for this observation is that the 95% confidence interval for the LD_{50} was generally greater than the background egg TEC. In other words, the bioassay for [^{3}H]TCDD-induced sac fry mortality is not sensitive enough for a background egg TEC of 10.5–10.9 pg/g to cause a statistically significant shift in an LD_{50} that is based on exogenously administered [^{3}H]TCDD. Also, our recent experience indicates that placement of contaminated eggs in an acetone–water exposure medium without chemicals will result in loss of TCDD and related chemicals from the eggs as a [^{3}H]TCDD mass distribution of approximately 1:4 between the eggs and acetone–water is approached. The magnitude of chemical loss from the wild eggs in this study during the 48-h exposure to [^{3}H]TCDD was not measured, so the amount of the background TEC remaining in the eggs for an additive contribution to the observed toxicity is uncertain.

Prince and Cooper (1995) recently reported that killifish (*Fundulus heteroclitus*) embryos collected from a heavily TCDD-impacted estuary of Newark Bay, New Jersey, were resistant to the toxicity of [^{3}H]TCDD when compared with killifish embryos collected from a site reported to be nonimpacted by TCDD. Their study also exposed eggs to [^{3}H]TCDD via acetone–water so Newark Bay contaminants may have been eliminated. More important by comparison to this study, however, is the lack of evidence for a similar difference in the responsiveness of Lake Ontario lake trout and control sac fry to [^{3}H]TCDD exposure of eggs. We conclude that lake trout are unlikely to develop resistance to early life stage toxicity of TCDD and related chemicals under exposure conditions experienced in the Great Lakes.

The slope of the dose response curve for TCDD-induced mortality in Lake Ontario lake trout is steep (Figs. 2 and 3). Previous studies (Walker et al. 1991; Walker and Peterson 1991, 1994) have reported steep slopes for dose–response curves for TCDD and similar compounds in lake trout, rainbow trout, and brook trout (*Salvelinus fontinalis*). Thus, given the statistical power of our assay, strain variability, and the observed steepness in the dose–response curve, TECs in Lake Ontario lake trout eggs from 1991 may be just below a threshold for significant adverse impact. Although PCDD, PCDF, and PCB concentrations appear to be declining in lake trout eggs from the Great Lakes (Cook et al. 1994), a spill or significant non-point-source contamination of TCDD and other related Ah receptor agonists in Lake Ontario could push the current TECs over the critical early life stage mortality threshold for lake trout. In addition, TECs below the threshold for sac fry mortality may cause sublethal effects or enhance nonchemical stressor effects, which may contribute to the lack of lake trout recruitment in Lake Ontario.

In this study, HRGC–HRMS was used to provide sensitive and reliable measurements of PCDDs, PCDFs, and PCBs in egg sample extracts. In addition, we used salmonid-specific TEFs to calculate total TECs present in Lake Ontario lake trout eggs. The TECs present in these eggs in 1991 are below the estimated NOAEL for lake trout sac fry mortality. Lake trout recruitment is probably not currently at risk in Lake Ontario solely because of the effects of TCDD and related chemicals on blue sac syndrome related mortality. The reproductive failure however, of Lake Ontario lake trout in the past, when TCDD and related chemical residues were much higher in eggs, could have occurred because of the bioaccumulation of these chemicals by lake trout and their transfer to the eggs, causing sac fry mortality by a blue sac syndrome.

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References

Bronte, C.R. 1993. Evidence of spring spawning lake trout in Lake Superior. J. Great Lakes Res. **19**: 625–629.

Casselman, J.M. 1991. Research project: lake trout rehabilitation studies. *In* Lake Ontario Fisheries Unit, 1990 Annual Report, LOA 91.1. Ontario Ministry of Natural Resources, Glenora, Ont. pp. 24-1 – 24-12.

Casselman, J.M. 1995. Survival and development of lake trout eggs and fry in eastern Lake Ontario—*in situ* incubation, Yorkshire Bar, 1989–1993. J. Great Lakes Res. **21**(Suppl. 1): 384–399.

Christie, W.J. 1974. Changes in the fish species composition of the Great Lakes. J. Fish. Res. Board Can. **31**: 827–854.

Cook, P.M., Walker, M.K., Kuehl, D.W., and Peterson, R.E. 1991. Bioaccumulation and toxicity of TCDD and related compounds in aquatic ecosystems. *In* Biological basis for risk assessment of dioxins and related compounds. *Edited by* M.A. Gallo, R.J. Scheuplein, and C.A. van der Heijden. Banbury Rep. No. 35. Cold Spring Harbor Laboratory Press, Plainview, N.Y. pp. 143–167.

Cook, P.M., Butterworth, B.C., Walker, M.K., Hornung, M.W., Zabel, E.W., and Peterson, R.E. 1994. Lake trout recruitment in the Great Lakes: relative risks for chemical-induced early life stage mortality. Soc. Environ. Toxicol. Chem. Abstr. **15**: 58.

DeVault, D., Dunn, W., Bergqvist, P., Wiberg, K., and Rappe, C. 1989. Polychlorinated dibenzofurans and polychlorinated dibenzo-*p*-dioxins in Great Lakes fish: a baseline and interlake comparison. Environ. Toxicol. Chem. **8**: 1013–1022.

Edwards, C.J., Ryder, R.A., and Marshall, T.R. 1990. Using lake trout as a surrogate of ecosystem health for oligotrophic waters of the Great Lakes. J. Great Lakes Res. **16**: 591–608.

Elrod, J.H., Ostergaard, D.E., and Schneider, C.P. 1988. Comparison of hatchery-reared lake trout stocked as fall fingerlings and as spring yearlings in Lake Ontario. North Am. J. Fish. Manage. **8**: 455–462.

Eshenroder, R.L., Poe, T.P., and Olver, C.H. (*Editors*). 1984. Strategies for rehabilitation of lake trout in the Great Lakes: Proceedings of a Conference on Lake Trout Research, Aug. 1983. Great Lakes Fish. Comm. Tech. Rep. No. 40.

Finney, D.J. 1971. Probit analysis. 3rd ed. Cambridge University Press, Cambridge, London.

Fitzsimons, J.D., Huestis, S., and Williston, B. 1995. Occurrence of a swim-up syndrome in Lake Ontario lake trout in relation to contaminants and cultural practices. J. Great Lakes Res. **21**(Suppl. 1): 277–285.

Grewe, P.M., Krueger, C.C., Marsden, J.E., Aquadro, C.F., and May, B. 1994. Hatchery origins of naturally produced lake trout fry captured in Lake Ontario: temporal and spatial variability based on allozyme and mitochondrial DNA data. Trans. Am. Fish. Soc. **123**: 309–320.

Guiney, P.D., Melancon, M.J., Lech, J.J., and Peterson, R.E. 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.

Guiney, P.D., Lech, J.J., and Peterson, R.E. 1980. Distribution and elimination of a polychlorinated biphenyl during early life stages of rainbow trout (*Salmo gairdneri*). Toxicol. Appl. Pharmacol. **53**: 521–529.

Hartman, W.L. 1988. Historical changes in the major fish resources of the Great Lakes. Adv. Environ. Sci. Technol. **21**: 103–131.

Kuehl, D.W., Butterworth, B.C., Libal, J.J., and Marquis, P.J. 1991. An isotope dilution high resolution gas chromatographic – high resolution mass spectrometric method for the determination of coplanar polychlorinated biphenyls: applications to fish and marine mammals. Chemosphere, **22**: 849–858.

Mac, M.J., Edsall, C.C., and Seelye, J.G. 1985. Survival of lake trout eggs and fry reared in water from the upper Great Lakes. J. Great Lakes Res. **11**: 520–529.

Mac, M.J., Schwartz, T.R., and Edsall, C.C. 1988. Correlating PCB effects on fish reproduction using dioxin equivalents. Soc. Environ. Toxicol. Chem. Abstr. **33**: 116.

Mac, M.J., Schwartz, T.R., Edsall, C.C., and Frank, A.M. 1993. Polychlorinated biphenyls in Great Lakes lake trout and their eggs: relations to survival and congener composition 1979–1988. J. Great Lakes Res. **19**: 752–765.

Marquis, P.J., Hackett, M., Holland, L.G., Larsen, M.L., Butterworth, B., and Kuehl, D.W. 1994. Analytical methods for a national study of chemical residues in fish. I. Polychlorinated dibenzo-*p*-dioxins/dibenzofurans. Chemosphere, **29**: 495–508.

Marsden, J.E., and Krueger, C.C. 1991. Spawning by hatchery-origin lake trout (*Salvelinus namaycush*) in Lake Ontario: data from egg collections, substrate analysis, and diver observations. Can. J. Fish. Aquat. Sci. **48**: 2377–2384.

Marsden, J.E., Krueger, C.C., and Schneider, C.P. 1988. Evidence of natural reproduction by stocked lake trout in Lake Ontario. J. Great Lakes Res. **14**: 3–8.

Martin, N.V., and Olver, C.H. 1980. The lake charr, *Salvelinus namaycus*. *In* Charrs: salmonid fishes of the genus *Salvelinus*. *Edited by* E.K Balon. W. Junk, The Hague, the Netherlands. pp. 205–277.

Miller, M.A. 1993. Maternal transfer of organochlorine compounds in salmonines to their eggs. Can. J. Fish. Aquat. Sci. **50**: 1405–1413.

Niimi, A.J. 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. Can. J. Fish. Aquat. Sci. **40**: 306–312.

O'Keefe, P., Meyer, C., Hilker, D., Aldous, K., Jelus-Tyror, B., Dillon, K., Donnelly, R., Horn, E., and Sloan, R. 1983. Analysis of 2,3,7,8-terachlorodibenzo-*p*-dioxin in Great Lakes fish. Chemosphere, **12**: 325–332.

Olson, J.R. 1986. Metabolism and disposition of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in guinea pigs. Toxicol. Appl. Pharmacol. **85**: 263–273.

Ostergaard, D.E. 1987. Effects of water temperature on survival of eggs and fry of lake trout. Prog. Fish-Cult. **49**: 115–116.

Prince, R., and Cooper, K.R. 1995. Comparisons of the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on chemically impacted and nonimpacted subpopulations of *Fundulus heteroclitus*: I. TCDD toxicity. Environ. Toxicol. Chem. **14**: 579–587.

Roberts, R.J., and Shepherd, C.J. 1986. Diseases of eggs and sac fry alevins. *In* Handbook of trout and salmon diseases. Fishing New Books, Blackwell Scientific Publications, Ltd., Oxford, U.K. p. 94.

SAS Institute Inc. 1988. Additional SAS/STAT^{TM} procedures, release 6.03 edition. SaS^{R} Tech. Rep. No. P-179. SAS Institute Inc, Cary, N.C.

Simonin, H.A., Skea, J.C., Dean, H.J., Symula, J., and Colquhoun, J.R. 1990. Summary of reproductive studies of Lake Ontario salmonids. *In* Proceedings of the Roundtable on Contaminant-Caused Reproductive Problems in Salmonids. *Edited by* M. Mac and M. Gilbertson. International Joint Commission, Windsor, Ont. pp. 15–18.

Smith, L.M., Schwartz, T.R., Feltz, K., and Kubiak, T.J. 1990. Determination and occurrence of AHH-active polychlorinated biphenyls, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and 2,3,7,8-tetrachlorodibenzofuran in Lake Michgan sediment and biota. The question of their relative toxicological significance. Chemosphere, **21**: 1063–1085.

Spitsbergen, J.M., Walker, M.K., Olson, J.R., and Peterson, R.E. 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.

Stalling, D.L., Smith, L.M., Petty, J.D., Hogan, J.W., Johnson, J.L., Rappe, C., and Buser, H.R. 1983. Residues of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in Laurentian Great Lakes fish. *In* Human and environmental risks of chlorinated dioxins and related compounds. *Edited by* R.E. Tucker, A.L. Young, and A.P. Gray. Plenum Publishing Corporation, New York, N.Y. pp. 221–240.

Symula, J., Meade, J., Skea, J.C., Cummings, L., Colquhoun, J.R., Dean, H.J., and Miccoli, J. 1990. Blue-sac disease in Lake Ontario lake trout. J. Great Lakes Res. **16**: 41–52.

U.S. Environmental Protection Agency (EPA). 1990. Assessment of risks from exposure of humans, terrestrial and avian wildlife, and aquatic life to dioxins and furans from disposal and use of sludge from bleached kraft and sulfite pulp and paper mills. EPA-560/5-90-013. U.S. EPA, Office of Pesticides and Toxic Substances, Washington, D.C.

U.S. Environmental Protection Agency (EPA). 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. EPA, Office of Research and Development, Washington, D.C.

Vodicnik, M.J., and Peterson, R.E. 1985. The enhancing effect of spawning on elimination of a persistent polychlorinated biphenyl from female yellow perch. Fundam. Appl. Toxicol. **5**: 770–776.

Walker, M.K., and Peterson, R.E. 1990. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin produces a blue-sac syndrome during early life stage development of lake trout (*Salvelinus namaycush*). *In* Proceedings of the Roundtable on Contaminant-Caused Reproductive Problems in Salmonids. *Edited by* M. Mac and M. Gilbertson. International Joint Commission, Windsor, Ont. pp. 21–26.

Walker, M.K., and 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 (*Oncorhynchus mykiss*). Aquat. Toxicol. **21**: 219–238.

Walker, M.K., and Peterson, R.E. 1994. Aquatic toxicity of dioxins and related chemicals. *In* Dioxins and health. *Edited by* A. Schecter. Plenum Press, New York, N.Y. pp. 309–346.

Walker, M.K., Spitsbergen, J.M., Olson, J.R., and Peterson, R.E. 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.

Walker, M.K., Hufnagle, L.C., Jr., Clayton, M.K., and Peterson, R.E. 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.

Walker, M.K., Cook, P.M., Batterman, A.R., Butterworth, B.C., Berini, C., Libal, J.J., Hufnagle, L.C., and Peterson, R.E. 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.

Walker, M.K., Cook, P.M., Butterworth, B.C., Zabel, E.W., and Peterson, R.E. 1996. Potency of a complex mixture of polychlorinated dibenzo-*p*-dioxin, dibenzofuran and biphenyl congeners compared to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in causing fish early life stage mortality. Fundam. Appl. Toxicol. **30**: 178–186.

Willford, W.A., Bergstedt, R.A., Berlin, W.H., Foster, N.R., Hesselberg, R.J., Moe, M.J., Passino, D.R.M., Reinert, R.E., and Rottiers, D.V. 1981. Introduction and summary. *In* Chlorinated hydrocarbons as a factor in the reproduction and survival of lake trout (*Salvelinus namaycush*) in Lake Michigan. Tech. Pap. No. 105. U.S. Fish and Wildlife Service, Ann Arbor, Mich. pp. 1–7.

Wolf, K. 1969. Blue-sac disease of fish. U.S. Fish Wildl. Serv. Fish Dis. Leafl. No. 15.

Wonnacott, R.J., and Wonnacott, T.H. 1985. Introductory statistics. 4th ed. Wiley, New York, N.Y.

Zabel, E.W., Cook, P.M., and Peterson, R.E. 1995*a*. 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.

Zabel, E.W., Walker, M.K., Hornung, M.W., Clayton, M.K., and Peterson, R.E. 1995*b*. Interactions of polychlorinated dibenzo-*p*-dioxin, dibenzofuran and biphenyl congeners for producing rainbow trout early life stage mortality. Toxicol. Appl. Pharmacol. **134**: 204–213.

Zabel, E.W., Cook, P.M., and Peterson, R.E. 1995*c*. 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 mortality. Environ. Toxicol. Chem. **14**: 2175–2179.

Zacharewski, T., Safe, L., Safe, S., Chittim, B., DeVault, D., Wiberg, K., Bergquist, P.A., and 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.