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Toxic Equivalency Factors of Polybrominated Dibenzo-*p*-dioxin, Dibenzofuran, Biphenyl, and Polyhalogenated Diphenyl Ether Congeners Based on Rainbow Trout Early Life Stage Mortality[1]

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Polybrominated and polychlorinated biphenyls (PBBs/PCBs), dibenzo-*p*-dioxins (PBDDs/PCDDs), dibenzofurans (PBDFs/PCDFs), and diphenyl ethers (PBDEs/PCDEs) are persistent, lipophilic environmental contaminants that may pose a risk to fish early life stage survival. To determine this potential risk, a rainbow trout early life stage mortality bioassay was used in which the potency of individual polybrominated chemicals was compared to the potency of the most potent polychlorinated chemical in these classes, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Following injection of newly fertilized rainbow trout eggs, fish-specific toxic equivalency factors (TEFs) were calculated as the molar ratio of TCDD LD50 to brominated compound LD50. Signs of toxicity were identical to those produced by polychlorinated TCDD-like chemicals and included yolk sac edema, pericardial edema, multifocal hemorrhages, reduced growth, and craniofacial malformations. Polybrominated dibenzo-*p*-dioxins, dibenzofurans, and biphenyls exhibited decreased potency with increased bromine substitution. Only 2,3,7,8-TBDD was more potent than 2,3,7,8-TCDD, whereas other polybrominated dibenzo-*p*-dioxins were equipotent or less potent than identically substituted polychlorinated dibenzo-*p*-dioxins in this assay. Although two PBDF congeners were equipotent to identically substituted PCDFs, 2,3,7,8-TBDF was 9-fold more potent than 2,3,7,8-TCDF. Both 3,3′,4,4′-TBB and 3,3′,4,4′,5,5′-HxBB were 10-fold more potent than identically substituted polychlorinated biphenyls. The halogenated diphenyl ethers and di-ortho polybrominated biphenyls were inactive in this assay. Thus, in this *in vivo* assay the polybrominated and polychlorinated TCDD-like chemicals were not always equally potent. To assess the risk posed by mixtures of these chemicals to feral fish populations, fish-specific TEFs for both polybrominated and polychlorinated chemicals should be used. © 1996 Academic Press, Inc.

Polychlorinated and polybrominated dibenzo-*p*-dioxins (PCDDs/PBDDs), dibenzofurans (PCDFs/PBDFs), biphenyls (PCBs/PBBs), and diphenyl ethers (PCDEs/PBDEs) are persistent, lipophilic chemicals that occur as environmental contaminants (reviewed in Safe, 1990; Menear and Lee, 1994). The detection of PCDDs, PCDFs, and PCBs in feral fish tissue and eggs prompted investigations to determine the risk these chemicals pose to fish populations. Lake trout (*Salvelinus namaycush*) early life stages were found to be very sensitive to part per trillion levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent and most studied congener in this class of chemicals. The TCDD dose in lake trout eggs that caused 50% cumulative sac fry mortality was 65 pg/g (Walker *et al.*, 1991). Subsequently, rainbow trout have been used as a model species to determine potencies of PCDD, PCDF, and PCB congeners relative to TCDD in causing fish early life stage mortality (Walker and Peterson, 1991; Zabel *et al.*, 1995). Sensitivity to the lethal effects of TCDD during fish early life stage development is not limited to salmonids. Pike (*Esox lucius*), medaka (*Oryzias latipes*), and zebrafish (*Danio rerio*) embryos and larvae are also sensitive to the toxic effects of TCDD (Helder, 1980; Wisk and Cooper, 1990; Wannemacher *et al.*, 1992). Signs of toxicity produced in lake trout and rainbow trout sac fry after egg exposure to TCDD and related chemicals that act by a putative aryl hydrocarbon (Ah) receptor mechanism are essentially identical to the blue sac disease-induced mortality reported in lake trout sac fry from Lake Ontario between 1977 and 1983 (Simonin, 1991). This latter finding is environmentally significant because concentrations of TCDD and related polychlorinated chemicals measured in sediment core samples can be used to predict the concentration in feral eggs (Cook *et al.*, 1994). Application of this biota–sediment bioaccumulation model to Lake Ontario suggests that these polychlorinated chemicals occurred at high enough concentrations until the late 1970s and early 1980s in eggs of feral

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Lake Ontario lake trout to reduce their populations secondary to early life stage lethality (Cook *et al.,* 1994).

PBDDs, PBDFs, PBBs, PBDEs, and PCDEs that have the potential to act by an Ah receptor mechanism also occur as widespread environmental contaminants and may contribute to TCDD toxic equivalents in feral fish. Thus, the combined toxicity caused by complex mixtures of polybrominated and polychlorinated chemicals in feral fish eggs that act by the same mechanism as TCDD may present a potential threat to exposed fish populations. However, the concentrations of these polybrominated aromatic hydrocarbons are not well characterized in sediments or feral eggs. Therefore, the potential contribution that these polybrominated chemicals make to TCDD-like toxicity in Great Lakes lake trout cannot be determined at this time.

To assess the risk posed to fish early life stage survival by contamination of eggs with these chemicals in the future, information is needed on both exposure and toxicity. Data are limited regarding the concentrations of PBDDs and PBDFs in fish and wildlife in the environment although they have been detected in fly ash from municipal incinerators along with polychlorinated congeners (Sovocool *et al.,* 1988; Schwind *et al.,* 1988). Because both polybrominated and polychlorinated congeners enter the environment through incineration processes, in areas where polychlorinated congeners are of concern, polybrominated congeners may also be present (Buser, 1987). Major precursors of PBDDs and PBDFs are PBDEs, PBBs, and related chemicals that are primarily used as flame-retardant chemicals in many manufactured goods (Buser, 1986; Thoma *et al.,* 1987; Donnelly *et al.,* 1989). As these products are disposed of by incineration, mixtures of polybrominated and polychlorinated TCDD-like chemicals enter the environment. PBBs and PBDEs have been detected in fish and wildlife from Northern Europe, Japan, and the United States (Andersson and Blomkvist, 1981; Jansson *et al.,* 1993; de Boer, 1989; Watanabe *et al.,* 1987; Kuehl *et al.,* 1991). Total PBDE concentrations of 2140 ng/g lipid in osprey (*Pandion haliaetus*) were four times greater than in arctic charr (*Salvelinus alpinus*) and 80 times greater than in whitefish (*Coregonus* sp.) (Jansson *et al.,* 1993), which suggests that biomagnification of these chemicals occurs. Unlike PBDEs, individual PCDE congeners have been more extensively studied and have been shown in mammals to induce aryl hydrocarbon hydroxylase (AHH) and ethoxyresorufin-*o*-deethylase (EROD) activities like PCBs, although *ortho* substitutions may not reduce their activity as shown with PCBs (reviewed in Becker *et al.,* 1991). PCDEs have been detected in human serum (Stanley *et al.,* 1991), fish and wildlife (Paasavirta *et al.,* 1986; Stafford *et al.,* 1983), and in μg/kg levels in lake trout and walleye (*Stizostedeon vitreum vitreum*) from the North American Great Lakes (Niimi *et al.,* 1994).

The toxic equivalency factor (TEF) approach can be used to compare the potencies of polybrominated congeners relative to TCDD, the prototypical congener in this class of chemicals (Safe, 1990). TEF values are determined by comparing the ratio of the molar dose of TCDD to produce a 50% effect to the molar dose of the test chemical required to produce a 50% effect. Thus, the TEF is the congener's fractional potency relative to TCDD for a given toxic or biochemical endpoint. The TEF value for a given congener can be multiplied by the concentration of that congener in a tissue sample to get the TCDD equivalents concentration (TEC) contributed by that congener. TECs can then be added for all TCDD-like congeners in a tissue sample (based on each congener's TEF and its concentration in the sample) to give a total TEC. Therefore, to accurately predict the risk that polybrominated chemical contaminants in fish eggs may pose to feral fish populations, determination of the toxicity of the TCDD-like brominated chemicals to embryo and larval fish is required. This study is the first to report the signs of toxicity and toxic equivalency factors of individual PBDD, PBDF, PBB, and PBDE congeners based on early life stage mortality in rainbow trout.

METHODS

Rainbow trout eggs. Newly fertilized rainbow trout eggs were shipped via commercial overnight carrier from the Ennis National Fish Hatchery (Ennis, MT) to Madison, Wisconsin. Eggs were allowed to warm to 10–12°C at a rate no greater than 1.5°C per hour, after which they were transferred to a perforated Plexiglas tray in a 38-liter aquarium. Erwin, Arlee, Shasta, McConoughy, and Eagle Lake strain rainbow trout were used as they became seasonally available. The LD50 values for TCDD-induced sac fry mortality in these strains ranges from 0.171 ng TCDD/g egg for the Shasta strain to 0.374 ng TCDD/g egg for the Arlee strain (Walker and Peterson, 1991; Zabel *et al.,* 1995).

Chemicals. TCDD (>99% purity) was obtained from Cambridge Isotope Laboratories (Woburn, MA). 1,2,3,7,8-PBDD, 1,2,3,7,8-PBDF, 2,7-Cl-3,8-Br-DD, 2,3,7-Cl-8-Br-DD, 3,3′,4,4′-TBB, and 3,3′,4,4′,5,5′-HxBB of greater than 98% purity were a gift from Dr. Steve Safe (Texas A & M University, College Station, TX) and were synthesized as previously described (Mason *et al.,* 1987; Robertson *et al.,* 1982). 2,3′,4′,6-TCDE, 3,3′,4,4′-TCDE, 2,2′,4,5,6′-PCDE, 2,3′,4,4′,5-PCDE, and 3,3′,4,4′,5-PCDE of greater than 99% purity were a gift from Dr. Ih Chu (Health and Welfare Canada, Ottawa, Canada) and were synthesized as previously described (Chu *et al.,* 1990). 2,2′,4,4′-TBDE, 2,2′,3,4,4′-PBDE, and 2,2′,4,4′,5-PBDE of greater than 98% purity were a gift from Dr. Åke Bergman (Stockholm University, Stockholm, Sweden) and synthesized as described by Örn *et al.* (1995). 2,3,7,8-TBDF was a gift from Dr. Andrew Kende (University of Rochester, Rochester, NY) and determined to be of 98% purity as previously described (Moore *et al.,* 1979). 2,2′-DBB, 2,2′,5,5′-TBB, 2,2′,4,4′,5,5′-HxBB, and 2,3,7,8-TBDD (97–99% purity) were purchased from Ultra Scientific (North Kingstown, RI). 2,3,4,7,8-PBDF, 1,2,3,4,7,8-HxBDF, and 1,2,3,4,7,8-HxBDD (98 or 99% purity) were purchased from Cambridge Isotope Laboratories. 2,7-DBDF, 2,3,7-TrBDD, and 1,3,7,8-TBDD (95–99% purity) were purchased from Chemsyn Science Laboratories (Lenexa, KS). Stock solutions of neat congeners were prepared in 1,4-*p*-dioxane and stored at −20°C. Diphenyl ethers and di-ortho PBBs were weighed as needed and dissolved directly in chloroform the week of rainbow trout egg injections for incorporation into phosphatidylcholine. 2,3,7,8-TBDD, 1,3,7,8-TBDD, 2,3,7-TrBDD, and 1,2,3,4,7,8-

HxBDF were purchased as toluene solutions from which stock solutions were prepared and stored at −20°C. Chicken egg yolk phosphatidylcholine (PC) in chloroform was obtained from Avanti Polar Lipids (Alabaster, AL). Phosphorus content was determined (Bartlett, 1959), diluted to 10 mM with HPLC-grade chloroform, and stored under Argon at −20°C. HPLC-grade chloroform, 1,4-*p*-dioxane, and toluene were obtained from Aldrich (Milwaukee, WI).

Liposome preparation and egg injection. Polybrominated or polychlorinated congeners were incorporated into PC liposomes using the thin-film hydration method (Woodle and Papahadjopoulos, 1989) as described previously (Walker *et al.*, 1992). An aliquot of congener in 1,4-*p*-dioxane or toluene was dried under N_2, redissolved in approximately 0.5 ml chloroform, and quantitatively transferred to a vial containing PC in chloroform. To expose eggs to ppm concentrations of these congeners, both the maximum ratio of congener to PC and the injection volume were increased as described by Zabel *et al.* (1995). Congener concentration in PC was increased from 15 to 30% and injection volumes up to 1 μl were used with PCDEs, PBDEs, 2,2′-DBB, 2,2′,5,5′-TBB, 2,2′,4,4′,5,5′-HxBB, and 3,3′,4,4′,5,5′-HxBB.

Rainbow trout egg injection (Walker *et al.*, 1992) was used to deliver the congener dose into rainbow trout eggs 24–50 hr after fertilization. Thirty eggs per dose were injected with PC or seven graded doses of halogenated congener incorporated into PC liposomes. The injection site was sealed with Superglue immediately after delivery of the dose.

Egg and fry maintenance. Injected rainbow trout eggs were placed in 400-ml glass beakers with nylon mesh screen siliconed approximately 1 cm from the bottom of the beaker. Dechlorinated tap water (11 ± 1°C) entered the beaker below the screen via Tygon tubing that extended from the top of the beaker, through the nylon mesh, to the bottom of the beaker, which allowed the incoming fresh water to circulate past the eggs. Eggs and fry were checked three times per week at which time water temperatures were recorded in each beaker and mortality was scored as either positive (+) or negative (−) for signs of TCDD-like toxicity. Water quality was determined weekly with colorimetric water quality test kits (Lamotte, Chestertown, MA).

Safety. Effluent aquaria water entered a settling tank from which it was pumped through a 1-μm fiber filter and two taste- and odor-type charcoal filters (Ametek, Inc.) to remove polychlorinated or polybrominated dioxin, furan, biphenyl, or diphenyl ether congeners prior to discharge into the municipal sewer system.

Statistical analysis and TEF calculations. Cumulative percentage sac fry mortality (from hatching through swim-up) was used to generate dose–response curves. The SAS statistical program was used to fit the percentage mortality data to a probit model that corrected for background mortality, used χ^2 goodness of fit, and estimated LD50 values and 95% fiducial limits (Finney, 1971; SAS, 1988). TEFs for all congeners were calculated on a molar basis by dividing the TCDD LD50 (pmol/g egg) by the LD50 (pmol/g egg) of the brominated congener.

RESULTS

Signs of early life stage toxicity of PBDDs, PBDFs, PBBs, and PBDEs/PCDEs. All PBDDs and PBDFs tested that had bromine atoms substituted in at least three of the 2, 3, 7, or 8 positions and the non-ortho-substituted PBBs caused mortality in rainbow trout sac fry by a blue sac syndrome (Table 1). On the other hand, the di-ortho PBB, PBDE, and PCDE congeners tested failed to cause sac fry mortality. For the active congeners in this rainbow trout early life stage bioassay, sac fry mortality was preceded by yolk sac edema, pericardial edema, subcutaneous hemorrhages, reduced growth, and craniofacial malformations evidenced by a shortened snout and domed skull. These gross signs of toxicity from exposure to PBDD, PBDF, and non-ortho PBB congeners are identical to those previously reported in rainbow trout (Walker *et al.*, 1991; Zabel *et al.*, 1995), brook trout (Walker and Peterson, 1994), and lake trout (Spitsbergen *et al.*, 1991) after exposure as fertilized eggs to polychlorinated TCDD-like congeners. The TEFs of all congeners tested are also summarized in Table 1.

Figure 1 presents representative dose–response curves of TCDD, 2,3,4,7,8-PBDF, and 3,3′,4,4′,5,5′-HxBB. These three dose–response curves are from TEF determinations conducted in Arlee strain rainbow trout, although not in the same shipment of eggs.

Dibenzo-p-dioxins. PBDDs with at least three bromine atoms or a combination of bromine and chlorine atoms substituted in the 2, 3, 7, or 8 positions all produced early life stage mortality. The rank order potency for these congeners was 2,3,7,8-TBDD > 2,8-Cl-3,7-Br-DD > 2,3,7-Cl-8-Br-DD > 1,2,3,7,8-PBDD > 2,3,7-TrBDD = 1,3,7,8-TBDD > 1,2,3,4,7,8-HxBDD. Comparisons of polychlorinated and polybrominated congener TEFs in the rainbow trout early life stage mortality assay are summarized in Table 2. Among all polychlorinated and polybrominated congeners tested in the rainbow trout sac fry mortality bioassay, 2,3,7,8-TBDD was unique in that it was the only congener more potent than TCDD.

Dibenzofurans. PBDFs with bromine substitutions in the 2, 3, 7, and 8 positions produced TCDD-like toxicity associated with mortality during the sac fry stage. The rank order potency for the congeners with this substitution pattern was 2,3,7,8-TBDF > 2,3,4,7,8-PBDF > 1,2,3,7,8-PBDF > 1,2,3,4,7,8-HxBDF. The only dibenzofuran congener that did not produce TCDD-like toxicity was 2,7-DBDF. This latter congener was injected into fertilized rainbow trout eggs at egg concentrations up to 0.595 μg/g (1831 pmol/g).

Biphenyls. Of the PBBs tested, only the non-ortho-substituted congeners, 3,3′,4,4′-TBB (No. 77) and 3,3′,4,4′,5,5′-HxBB (No. 169), produced sac fry mortality associated with signs of TCDD-like toxicity. TEFs determined for 3,3′,4,4′-TBB in several rainbow trout strains were similar. 3,3′,4,4′-TBB was approximately 10 times more potent than 3,3′,4,4′,5,5′-HxBB for producing sac fry mortality. Di-ortho-substituted biphenyls, 2,2′-DBB (No. 4), 2,2′,5,5′-TBB (No. 52), and 2,2′,4,4′,5,5′-HxBB (No. 153), did not produce TCDD-like toxicity in Arlee strain rainbow trout at concentrations of 366 nmol/g. This corresponds to egg concentrations of these three congeners of 114, 171, and 230 μg/g, respectively.

Diphenyl ethers. Three PBDEs, 2,2′,4,4′-TBDE, 2,2′,3,4,4′-PBDE, and 2,2′,4,4′,5-PBDE, were injected into newly fertilized rainbow trout eggs at egg concentrations up to 12 μg/g without producing any signs of TCDD-like toxic-

TABLE 1

Relative Potencies of PBDD, PBDF, PBB, PCDE, and PBDE for Producing Rainbow Trout Early Life Stage Mortality

Congener	Blue sac syndrome[a]	LD50[b] (ng/g egg)	TEF[c]	Rainbow trout strain
PBDDs				
2,3,7,8-TBDD	+	0.222 (0.118–0.265)	1.14	Eagle Lake
	+	0.264 (0.216–0.322)	1.9[d]	Eagle Lake
	+	0.158 (0.096–0.203)	2.22[d]	Erwin
	+	0.122[e]	2.54	Arlee
2,8-Cl-3,7-Br-DD	+	0.448 (0.304–0.534)	0.68	Erwin
2,3,7-Cl-8-Br-DD	+	0.410 (0.113–0.623)	0.65	Erwin
1,2,3,7,8-PBDD	+	4.16 (2.54–5.73)	0.14	Eagle Lake
	+	4.92 (0.358–16.8)	0.082	Erwin
1,3,7,8-TBDD	+	29 (21–36)	0.013	Erwin
2,3,7-TrBDD	+	18.9 (10.5–21.9)	0.017	Erwin
	+	15.6 (8.42–20.6)	0.018[d]	McConoughy
1,2,3,4,7,8-HxBDD	+	63.7 (41–77.5)	0.0089	Arlee
PBDFs				
2,3,7,8-TBDF	+	1.5 (0.871–1.94)	0.25	Erwin
2,3,4,7,8-PBDF	+	6.19 (1.29–6.75)	0.071	Erwin
1,2,3,7,8-PBDF	+	9.56 (5.12–15.7)	0.041	Erwin
1,2,3,4,7,8-HxBDF	+	247 (10.9–330,000)[f]	0.002	Erwin
2,7-DBDF	–	>597[g]		Erwin
Non-ortho PBBs				
3,3′,4,4′-TBB (No. 77)	+	434 (353–503)[f]	0.0016[d]	Erwin
	+	572[e]	0.0009	Eagle Lake
	+	168[e]	0.0014[d]	Shasta
	+	222[e]	0.0016	Arlee
3,3′,4,4′,5,5′-HxBB (No. 169)	+	3,910 (1,510–6,460)	0.00012	Arlee
Di-ortho PBBs				
2,2′-DBB (No. 4)	–	>114,000[g]		Arlee
2,2′,5,5′-TBB (No. 52)	–	>172,000[g]		Arlee
2,2′,4,4′,5,5′-HxBB (No. 153)	–	>230,000[g]		Arlee
PCDEs				
3,3′,4,4′-TDPE	–	>52,100[g]		Eagle Lake
2′,3,4,6′-TCDE	–	>51,100[g]		Eagle Lake
2,2′,4,5,6-PCDE	–	>51,000[g]		Eagle Lake
2,3′,4,4′,5-PCDE	–	>52,000[g]		Eagle Lake
3,3′,4,4′,5-PCDE	–	>51,000[g]		Eagle Lake
PBDEs				
2,2′,4,4′-TBDE	–	>12,000[g]		Erwin
2,2′,3,4,4′-PBDE	–	>12,000[g]		Erwin
2,2′,4,4′,5-PBDE	–	>12,000[g]		Erwin

[a] TCDD-like toxicity grossly identical to blue sac syndrome was characterized by sac fry mortality that was preceded by yolk sac edema, pericardial edema, multifocal hemorrhages, growth retardation, and craniofacial malformations.

[b] Based on cumulative hatching and sac fry mortality (95% fiducial limits).

[c] Calculated as the ratio of LD50 of TCDD (pmol/g egg) to LD50 of the brominated congener (pmol/g egg).

[d] TEF calculated based on historical TCDD LD50 values in this rainbow trout strain.

[e] Data insufficient to determine 95% fiducial limits using the SAS probit procedure.

[f] LD50 determined from data in which the highest egg dose did not produce 100% mortality.

[g] No signs of toxicity occurred at the highest egg dose tested.

ity or sac fry mortality. Also, five PCDEs, 2,3′,4′,6-TCDE, 3,3′,4,4′-TCDE, 2,2′,4,5,6′-PCDE, 2,3′,4,4′,5-PCDE, and 3,3′,4,4′,5-PCDE, were injected into Eagle Lake strain rainbow trout at egg concentrations near 50 μg/g without producing signs of TCDD-like toxicity or increased early life stage mortality.

Comparison of fish-specific TEFs determined for polychlorinated and polybrominated congeners. The TEFs for the PBDDs, PBDFs, and PBBs were compared to TEFs of PCDDs, PCDFs, and PCBs determined previously by Walker and Peterson (1991) and Zabel *et al.* (1995) in the same rainbow trout early life stage mortality bioassay (Table

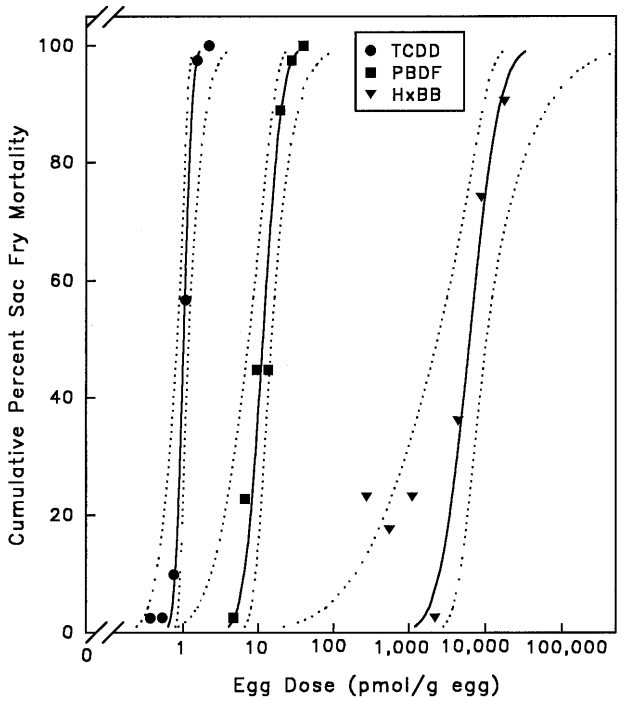

FIG. 1. Representative dose–response curves of TCDD, 2,3,4,7,8-PBDF, and 3,3′,4,4′,5,5′-HxBB. Solid lines are dose–response curves fit by probit model with 95% fiducial limits indicated by the dotted lines. Symbols indicate percentage sac fry mortality corrected for control mortality in vehicle-injected eggs.

2). The relative potencies of 2,3,7,8-TBDD and 1,2,3,7,8-PBDD were within an order of magnitude of TCDD and PCDD, respectively. The PBDDs and PCDDs generally showed the same structure–activity relationships. However, the TEF for 1,2,3,4,7,8-HxBDD was only $\frac{1}{35}$th that of HxCDD. The structure–activity relationship for the PBDFs did not follow the same trend as the PCDFs. The most potent PBDF congener was 2,3,7,8-TBDF for which the TEF was determined to be 0.24 compared to 0.028 for 2,3,7,8-TCDF. Table 2 also shows that the penta-substituted dibenzofurans were similar in potency between the polychlorinated and polybrominated congeners but the HxBDF was significantly less potent than HxCDF. For both the PBBs and the PCBs, the 3,3′,4,4′-substituted congener was more potent than the 3,3′,4,4′,5,5′-substituted congener. The non-ortho PBBs were more potent than the non-ortho PCBs with the same substitution pattern by approximately 10-fold.

DISCUSSION

Polybrominated dibenzo-*p*-dioxins and dibenzofurans produced rainbow trout early life stage mortality associated with signs of toxicity identical to those produced by TCDD. All 2,3,7,8-substituted PBDDs were active in this assay, as well as two PBDDs that had bromine atoms substituted in only three of these four positions. The TEF values of 0.013 and 0.017, respectively, for 1,3,7,8-TBDD and 2,3,7-TrBDD for producing rainbow trout early life stage mortality are not unexpected because both congeners have relatively high affinity for binding to the rat AhR and induce P4501A enzyme activities as measured by AHH and EROD activities (Mason *et al.*, 1987).

Although the structure–activity relationships (SARs) between the PBDDs and PCDDs and the PBBs and PCBs were generally similar for producing rainbow trout early life stage mortality, the SARs were different between the PBDFs and PCDFs. The PBDFs exhibit decreased relative potencies with increased bromine substitution, whereas for the PCDFs the least potent was 2,3,7,8-TCDF (Walker and Peterson, 1991). Comparison of the TEF values for 2,3,7,8-TBDF (TEF = 0.25) and 2,3,7,8-TCDF (TEF = 0.028; Walker and Peterson, 1991) revealed that the polybrominated-substituted congener was ninefold greater in relative potency than 2,3,7,8-TCDF in this rainbow trout early life stage mortality bioassay. Using the induction of cleft palate as the endpoint of toxicity in mice, Birnbaum *et al.* (1991) found that 2,3,7,8-TBDF was twofold more potent than 2,3,7,8-TCDF. The authors of this study hypothesized that the difference in potency may be due to a difference in metabolism. Zabel *et al.* (1995) reported rainbow trout sac fry eliminate 2,3,7,8-TCDF more readily than TCDD and seven related PCDDs, PCDFs, and PCBs after injection as newly fertilized eggs. However, if rainbow trout sac fry eliminate 40% of the original 2,3,7,8-TCDF as determined by Zabel *et al.* (1995) and do not eliminate 2,3,7,8-TBDF, this would still not completely account for the difference between the TEF values of 0.25 for 2,3,7,8-TBDF and 0.028 for 2,3,7,8-TCDF.

The two non-ortho PBBs (PBB 77 and PBB 169) were active and the di-ortho PBBs were inactive in producing TCDD-like toxicity associated with early life stage mortality in rainbow trout. This finding is consistent with the activity of non-ortho and di-ortho PCBs in rainbow trout early life stage mortality (Zabel *et al.*, 1995), P4501A protein induction and EROD activity in adult mirror carp (van der Weiden *et al.*, 1994), and AHH enzyme activity in adult rainbow trout (Janz and Metcalfe, 1990).

The PCDEs did not produce rainbow trout early life stage toxicity at egg doses in the 50 ppm range. Those congeners tested included PCDEs with non-ortho substitution patterns that have been reported to produce TCDD-like effects in medaka (Metcalfe *et al.*, 1994), *in vitro* induction of EROD activity, and *in vitro* Ah receptor binding in rat hepatoma cells (C. Metcalfe, Trent University, personal communication). Because the substitution patterns of two of the PCDEs tested in this study were also identical to 3,3′,4,4′-TCB and 3,3′,4,4′,5-PCB, which were previously shown to be active in producing TCDD-like toxicity in this rainbow trout early life stage bioassay (Walker and Peterson, 1991), we antici-

TABLE 2
Comparison of TEFs Based on Rainbow Trout Early Life Stage Mortality for Polyclorinated and Polybrominated Congeners with the Same Substitution Pattern

Congener	TEF[a]	
	Chlorinated congener	Brominated congener
Dioxins		
2,3,7,8-T(C/B)DD	1	1.5 (0.66–2.9)[b]
1,2,3,7,8-P(C/B)DD	0.73 (0.46–0.88)[c]	0.14 (0.057–0.30)*
1,2,3,4,7,8-Hx(C/B)DD	0.319 (0.12–0.50)[c]	0.0089 (0.0056–0.016)*
Furans		
2,3,4,7,8-P(C/B)DF	0.359 (0.25–0.91)[c]	0.071 (0.057–0.38)
1,2,3,4,7,8-Hx(C/B)DF	0.280 (0.20–0.49)[c]	0.002 (0.000001–0.05)[d,]*
1,2,3,7,8-P(C/B)DF	0.034 (0.023–0.088)[c]	0.041 (0.02–0.089)
2,3,7,8-T(C/B)DF	0.028 (0.021–0.047)[c]	0.24 (0.12–0.49)*
Non-ortho biphenyls		
3,3′,4,4′-T(C/B)BB	0.00016 (0.0001–0.0002)[c]	0.0016 (0.0011–0.0024)*
3,3′,4,4′,5,5′-Hx(C/B)BB	0.000041 (0.000023–0.000062)[e]	0.00012 (0.00004–0.0003)

[a] TEF data were determined on a molar basis for both polychlorinated and polybrominated congeners. The 90% fiducial limits (FL) in parentheses were calculated based on the 95% FL of TCDD LD50 and 95% FL of congener LD50.
[b] Mean of two TEF determinations in Eagle Lake-strain rainbow trout.
[c] Walker and Peterson (1991).
[d] The 90% FLs were calculated based on historical 95% FL of TCDD LD50 in the rainbow trout stain in which the TEF was determined.
[e] Zabel *et al.* (1995).
* The 90% FLs of the polybrominated and polychlorinated TEFs do not overlap.

pated similar results for these PCDEs. The failure in this study to observe signs of TCDD-like toxicity in rainbow trout sac fry exposed as fertilized eggs to PCDEs suggests that differences in sensitivity may exist between medaka and rainbow trout that may be due to the ability of rainbow trout to metabolize and/or eliminate these compounds more effectively than medaka.

The PBDEs were inactive as well when injected into newly fertilized rainbow trout eggs to doses of 12 μg/g. The 2,2′,4,4′-TBDE is an environmentally relevant congener that was found to comprise 70% of the PBDEs in fish samples from the North Sea with various pentabrominated diphenyl ethers making up much of the remaining proportion of the total PBDEs (Andersson and Blomkvist, 1981; de Boer, 1989). Studies using commercial mixtures of PBDEs have also produced little or no TCDD-like effects as measured by effects on cytochrome P450 activity, liver morphology and reproduction in adult three-spined stickleback (*Gasterosteus aculeatus*), and liver morphology and cytochrome P450 activity in rainbow trout early life stages (Holm *et al.*, 1993; Norrgren *et al.*, 1993). Carlson (1980) reported that mixtures of pentabrominated diphenyl ethers were active inducers of xenobiotic metabolism and increased liver weight in rats 2 weeks after exposure. The inactivity of the di-ortho PBDEs examined in the present study does not rule out the possibility that there are active PBDE congeners.

The relative potency of the polybrominated and polychlorinated congeners in this rainbow trout early life stage bioassay are within or near an order of magnitude except for Hx(Cl/Br)DF and Hx(Cl/Br)DD. The recommendation that polybrominated and polychlorinated congeners should be similar for risk assessment purposes (Safe, 1990) is supported by these data, although the risk posed by the more highly brominated congeners would be overestimated. The relative importance of any congener in contributing to the total TECs in animal tissue is based on its potency and tissue concentration. For the polybrominated congeners with low TEFs, they must exist in animal tissue at high concentrations to significantly contribute TCDD equivalents (TEs). However, a congener such as 2,3,7,8-TBDF, which has a relatively high TEF, would contribute more TEs than predicted based on the TEF of 2,3,7,8-TCDF. These results suggest that where the data are available, fish-specific TEFs should be used for assessing the risk posed by elevated egg concentrations of these chemicals to fish early life stages. However, for polybrominated TCDD-like chemicals in the environment for which fish-specific TEF values have not been determined, the fish-specific TEF values for the corresponding polychlorinated dioxin, furan, or biphenyl congener should provide a rough estimate of its potency relative to that of 2,3,7,8-TCDD.

As egg exposure data becomes available for polybrominated congeners in feral fish populations in the future, the TEF approach can be applied to both polychlorinated and polybrominated TCDD-like congeners to determine the risk posed to feral fish populations from egg contamination by

complex mixtures of these embryotoxic Ah receptor agonists. To validate the use of fish-specific TEFs, this egg injection method can be used to determine whether pairs of polybrominated TCDD-like chemicals act in an additive, synergistic, or antagonistic manner for producing fish early life stage mortality. If the additivity assumption is validated, fish-specific TEFs will be useful for ecological risk assessment purposes where both polychlorinated and polybrominated chemicals are detected in feral fish tissue.

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