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Blood Plasma Levels of Sex Steroid Hormones and Vitellogenin in Striped Bass (*Morone saxatilis*) Exposed to 3,3',4,4'-Tetrachlorobiphenyl (TCB)

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Exposure to polychlorinated biphenyls (PCB) can impair reproductive processes in fish. Laboratory studies have demonstrated adverse effects in several different fish species (Thomas 1988; Black and McElroy 1991; Monosson et al. 1994). Evidence also exits for an association between exposure to PCBs and related compounds and impaired reproduction in wild fish (Johnson et al. 1988). Although the mechanism of reproductive toxicity of PCBs is unclear, it appears that PCBs act at several different levels of the hypothalamus-pituitary-gonadal axis (HPG) (Thomas 1988). Because of their structural similarity to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin), planar PCB congeners (e.g. 3,3',4,4'-tetrachlorobiphenyl (TCB)) are among the most toxic PCBs. Both TCB and dioxin are reproductive toxicants in fish (Black and McElroy 1991; Wannamacher et al. 1992; Monosson et al. 1994). TCB exposure (via intraperitoneal injections) impaired maturation in adult female white perch (*Morone americana*) (Monosson et al. 1994) and reduced egg deposition in killifish (*Fundulus heteroclitus*) (Black and McElroy 1991). Larval or fry survival was also reduced following either maternal exposure to TCB for white perch (Monosson et al. 1994) or injections of TCB into fertilized eggs of rainbow trout (Walker and Peterson 1991).

TCB has been measured in several wild fish populations including Hudson River striped bass (Morone saxatilis) (Hong and Bush 1990). The present studies were designed to investigate the effects of exposure to TCB on reproductive processes in female striped bass. Specifically we were interested in its effects on circulating levels of the sex steroid hormones, estradiol-17β (E_2) and testosterone (T), and of the egg yolk precursor, vitellogenin (VTG). The dose of TCB (1.0 mg/kg) we used was selected based on published values for TCB burdens in striped bass (Hong and Bush 1990), coupled with the results of a prior study in which the relationship between injected doses and resulting tissue levels of TCB was examined in white perch (Monosson et al. 1994).

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MATERIALS AND METHODS

Striped bass used in these studies were raised from larvae to adulthood (5 yr) at the Pamlico Aquaculture Field Laboratory (PAFL), Aurora, North Carolina. All fish at the PAFL were maintained outdoors in a large 2000-gal(7600 L) pool with flow-through well water (salinity = 0.0 ppt, hardness = 250-300 mg/L $CaCO_3$, and alkalinity = 200-250 mg/L $CaCO_3$). Fish in all studies were fed commercial trout chow to satiation (Ziegler Brothers, Inc., Gardners, Pennsylvania) once daily.

Beginning in January 1992, fish were anesthetized (2 mg/L quinaldine sulfate) and injected (i.p.) with TCB (Ultra Scientific, Providence, Rhode Island) suspended in corn oil (n=10) for a final dose of 1.0 mg TCB/kg body weight. Vehicle controls were injected with an equivalent volume of corn oil (1 mL/kg body weight) (n=9). All fish were injected once every 3 wk for a total of three injections.

Blood samples were collected every 3 wk, just prior to giving the TCB injections, beginning in January (week 0) and ending in late March (week 9) when the fish were spawned. Blood was drawn from the caudal vessels and transferred to microfuge tubes containing heparin and aprotinin to prevent clotting and proteolysis of VTG (Tao et al. 1993). Samples were centrifuged 5 min at 10,000 x g and the plasma was aliquoted into 400 µl microfuge tubes and stored at -80°C for later analysis of steroid hormones and VTG.

The females' ovaries were biopsied beginning on week 3 of the experiment using a polished plastic tube inserted 1-2 cm into the urogenital pore. Several of the largest oocytes from each fish were measured immediately using a stereo-microscope fitted with a calibrated ocular micrometer.

Females with fully grown oocytes (≥ 750 µm diameter) were induced to spawn in tanks either by implantation with cholesterol/cellulose pellets containing D-Ala^6 Des-Gly^{10}-LHRHa (GnRHa) (Woods and Sullivan 1993) followed by injection with human chorionic gonadotropin (hCG) (Hodson and Sullivan 1993) (Experiment I), or by hCG injection(s) only (one or two injections; fish that were injected twice were injected for the second time 6 d after the first injection) (Experiment II). Each female was placed in a separate 600 gal (2280 L) spawning tank with two males (also injected with hCG). The fish spawned within 24 hr, and fertilized eggs were collected from the tanks and placed in McDonald hatching jars supplied with well water.

Blood plasma levels of E_2 and T were measured by highly specific radioimmunoassays validated for striped bass (Woods and Sullivan 1993). VTG was measured using a single radial immunodiffusion assay for striped bass with purified striped bass VTG as the standard (Tao et al. 1993).

TCB was extracted from fertilized eggs for chemical analysis according to the methods of J. Lake (personal communication, United States Environmental

Protection Agency, Narragansett, Rhode Island). Solvents used for extraction and gas-chromatography (using electron capture detection) (GC-ECD) were purchased from Baxter Scientific (McGaw Park, Illinois). Samples (1 g) and blanks (sodium sulfate only) were homogenized with a mortar and pestle in anhydrous sodium sulfate (5 g) (dry weights were determined for each sample by drying a preweighed portion in a drying oven for 24 hours). After addition of PCB 198 as an internal standard, PCBs were twice extracted with acetone (5 mL) by vortexing the acetone sodium sulfate mixture. The acetone supernatant was decanted into a 20 mL glass vial and samples were transferred to heptane (1 mL). The heptane fraction was treated with H_2SO_4 to eliminate lipids, and analyzed using GC-ECD. Data were corrected for recovery. Chemical analysis (and QA/QC) were conducted at the USEPA-ERL in Narragansett, Rhode Island.

A least squares means comparison test in PC-SAS (SAS Institute, Cary, North Carolina) was used to determine significant differences in sample means.

RESULTS AND DISCUSSION

Plasma concentrations of E_2 increased during the study period for both control and TCB exposed fish. Plasma concentrations of VTG and oocyte diameter also tended to increase throughout the study, although the only significant changes were observed in the oocyte diameters of the TCB exposed fish (Tables 1 and 2). Testosterone concentrations did not change throughout the study period. We were able to induce spawning in 6 of 6 control female striped bass and in 5 of 6 females exposed to TCB in experiment I, and 3 of 3 control females and 2 of 4 TCB exposed females in experiment II. We observed no deleterious effects on the reproductive endpoints measured in adult female striped bass following exposure to 1.0 mg/kg TCB. This is in concurrence with previous studies in white perch, in which exposure to 1.0 mg/kg TCB did not affect maturation, although exposure to 5.0 mg/kg reduced the number of females that matured and the gonadal somatic index in fish that did mature (Monosson et al. 1994). The concentrations of E_2, T and VTG measured throughout this study were similar to concentrations measured in captive striped bass during the same months in other studies (Woods and Sullivan 1993; Tao et al. 1993). The lack of effect of TCB on steroid hormone and vitellogenin concentrations in the mature females is also in agreement with previous studies in white perch (Monosson et al. 1994). Both the white perch and striped bass studies were initiated when the fish were already vitellogenic; it is possible that timing of exposure may result in quite different effects. Further studies are needed to address this issue.

Exposure to TCB did not affect fertility or number of eggs spawned following GnRH implants and hCG injections in either experiment I or II. The percent of eggs fertilized in Experiment I ranged from 6% (observed in a control animal) to 94%, and averaged 45 ± 15 % in controls and 56 ± 18 % in TCB treated fish. The number of eggs spawned (normalized for female body weight) ranged from 5,000 eggs/kg (observed in the control with 6% fertilization) to 88,000 eggs /kg

with a combined mean of 37,909 ± 25,311. Fertility was lower in Experiment II and ranged from 9% to 24%. The number of eggs spawned in Experiment II ranged from 18,800 eggs/kg to 127,500 eggs/kg for both TCB and control animals, with a combined mean of 50,352 ± 40,000. The range of fertility for eggs spawned by the various females in this study falls within that reported previously for hatchery spawned (wild or domestic) striped bass (Woods and Sullivan 1993; Hodson and Sullivan 1993). Using the volitional tank spawning method, variation in fertility may be largely attributable to performance of males or failure of the fish to engage in normal courtship and mating behavior within the confines of the tank.

Table 1. Plasma concentrations of estradiol , testosterone and vitellogenin in female striped bass exposed to 3,3',4,4'-tetrachlorobiphenyl (TCB).

Steroid	Week[a]	Treatment[b]	
		Control	TCB
Estradiol (ng/mL)			
	0	0.14±0.019 A	0.15±0.018 A
	3	0.12±0.016 A	0.17±0.027 AB
	6	0.24±0.018 B	0.21±0.047 AB
	9	0.19±0.029 AB	0.26±0.066 B
Testosterone (ng/mL)			
	0	0.31±0.027 A	0.34±0.043 A
	3	0.32±0.024 A	0.36±0.042 A
	6	0.37±0.038 A	0.43±0.094 A
	9	0.46±0.121 A	0.34±0.063 A
Vitellogenin (mg/mL)			
	0	0.75±0.39 A	0.91±0.43 A
	3	1.30±0.32 A	1.41±0.44 A
	6	1.76±0.35 A	1.45±0.57 A

[a]Exposure to TCB was initiated in January (week 0), with injections every 3 wk until week six. All fish were spawned during week 9 (mid-March).
[b]Fish were injected (i.p.) with either 1.0 mg/kg TCB (n=10) suspended in corn oil, or with an equal volume of corn oil (n=9) (controls) every 3 wk, on weeks 0,3 and 6. n=6 for both treatments for week 9. Data shown are mean(±)SEM. Different letters are significantly different (p=0.05) within groups, there were no differences between treatments.

The mean concentration of TCB in the eggs spawned from females exposed to 1.0 mg/kg for 9 wk (eggs were analyzed from n=6 females) was 0.96 mg/kg wet weight, and ranged from 0.69 to 1.41 mg/kg. Concentrations of TCB ranging from 0.002 to 0.068 ug/g wet weight have been measured in the white muscle of striped bass from the Hudson River (Hong and Bush 1990). Lipophilic compounds, such as PCBs tend to accumulate to a greater degree in tissues with high lipid contents such as the ovaries or liver (Vodicnik and Peterson 1985). PCB concentrations measured in eggs obtained from Hudson River striped bass were 10-20 fold greater than in the white muscle (Westin et al. 1983). Similarly, concentrations of TCB were approximately 10 times greater in mature ovaries of white perch injected with TCB as compared with the white muscle (Monosson et al. 1934). It is possible, therefore that striped bass collected from highly contaminated sites may be exposed to TCB concentrations similar to those attained in this study.

The mean TCB concentration measured in the striped bass eggs from this study (0.96 mg/kg) was within range of concentrations measured in the ovaries of white perch associated with reduced larval survival (Monosson et al. 1994). These TCB concentrations were also similar to those reported to cause toxicity in rainbow trout fry following injection of TCB into fertilized eggs (Walker and Peterson 1991). Thus, although exposure to 1.0 mg/kg TCB did not affect maturation and spawning in striped bass females exposed for 9 wk prior to spawning in this study, it is possible that the concentration we used may be lethal to larval striped bass. Further studies with eggs from exposed females are warranted.

Table 2. Oocyte diameters in female striped bass exposed to 3,3',4,4'-tetrachlorobiphenyl (TCB).

Sample week[a]	Oocyte diameter (μm)	
	Treatment[b]	
	Control	TCB[c]
3	672±29 A	724±35 A
6	681±30 A	754±29 AB
9	783±33 A	841±58 B

[a]Exposure to TCB was initiated in January. Individual fish were biopsied and oocytes were measured immediately using a dissecting scope fitted with an ocular micrometer starting in February (week 3).
[b]Fish were injected (i.p) with either 1.0 mg/kg TCB (n=10) suspended in corn oil, or with an equal volume of corn oil (n=9) (controls) every 3 wk, on weeks 0,3 and 6. n=6 for both treatments for week 9.
Data shown are mean(±)SEM. Different letters are significantly different (p=0.05) within groups, there were no differences between treatments.

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