

## Polychlorinated Biphenyls and Chlordane in the Gonads of Paddlefish, *Polyodon spathula*, from the Ohio River

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The paddlefish (*Polyodon spathula*) is a primitive fish that is found in several large rivers of the Mississippi River drainage. The status of paddlefish populations in these large rivers is debatable due to several factors including habitat loss, contaminants and commercial fishing (Sparrowe 1986; Gundersen and Pearson 1992). The roe of female paddlefish is used to make domestic caviar and commercial fishermen are paid substantial prices (up to \$110/Kg) for this roe (Hoffnagle and Timmons 1989). Paddlefish are classified as a fish "of special concern" in many of these large rivers. Hence, these fish may become threatened or endangered by subtle disturbances to their habitat (Williams et al. 1989).

Another factor that may be influencing the success of paddlefish populations in the Ohio River are organochlorine contaminants. These contaminants may be particularly harmful to paddlefish during their early life-stages. Polychlorinated biphenyls (PCBs) and chlordane are two of the more commonly detected groups of organochlorine contaminants in fish tissues from the Ohio River (Gundersen and Pearson 1992; *Ohio River Valley Water Sanitation Commission, ORSANCO, press* release May, 1996). Female and male paddlefish collected from the Falls of the Ohio River have been found to have mean PCB gonad concentrations of 7.3 and 16.2 ppm respectively (Gundersen and Pearson 1992). These PCB concentrations are higher than those (0.10 - 0.50 ppm) found to cause 5 to 76% mortality in Lake Geneva charr embryos (Monod 1985). Organochlorine contaminants have also been shown to reduce plasma steroid levels in fish (Sivarajah et al. 1978).

The objective of this study was to measure gonad PCB and chlordane levels and plasma sex steroid levels in paddlefish collected from 4 Ohio River sites and a relatively cleaner Ohio River tributary, the Cumberland River. We also evaluated the effects these contaminants may have on reproductive success by transporting mature females to a fish hatchery where they were induced to spawn. Data were analyzed to determine if a correlation existed between gonad contaminant levels and percent hatch.

## MATERIALS AND METHODS

A total of 15 paddlefish were collected between March and September 1996 from four different sites along the Ohio River by use of large mesh gill nets (30 - 60 m in length, 4.8 m deep and 10 - 13 cm bar measure mesh). Three males and 3 females were collected from the Ohio River near Aurora, Indiana, 3 females and 1 male were collected below Meldhal Dam near Bradford, Kentucky, 2 females were collected near Uniontown, Kentucky and 3 females were collected below McAlpine Dam near Louisville, Kentucky. One female and one male paddlefish were also collected from the Cumberland River (control), an Ohio River tributary with fewer pollution sources. The dentary bones were removed from each fish for age determinations and gonads were removed, wrapped in aluminum foil and frozen at 0° C for PCB and chlordane analysis.

Five paddlefish with mature ovaries and 3 mature males were transported to the Aquaculture Research Center of Kentucky State University, Frankfort, Kentucky, and held in 2.5-m diameter circular tanks. Blood samples were taken for plasma testosterone and estradiol determinations. Blood was drawn by use of the vacutainer system with lithium carbonate as an anticoagulant. Samples were kept on ice and then separated by centrifugation. The plasma was drawn off and dispensed into a microcentrifuge tube. Radioimmunoassay was performed on the plasma using a Coat-a-Count Kit specific for the quantitative measurements of plasma testosterone and estradiol (Diagnostic Systems Laboratories, Inc., Webster, Texas). Spawning of female paddlefish and fertilization of eggs were done as described by Shelton et al. (1997). The hatch success of eggs from each female was determined. The age of paddlefish was determined using the dentary bones as described by Gundersen and Pearson (1992).

Extraction and cleanup procedures for all gonad samples were done based on the methods of Gundersen and Pearson (1992). Gonads were homogenized in a Waring blender and a sub-sample was ground in a mortar and pestle with approximately 50 g of anhydrous sodium sulfate. The dried tissue was Soxhlet extracted for 10 hours with 170 mL of 1:1 petroleum ether and hexane (v/v spectral grade). The resulting lipid extract was collected in a tared 250 mL flask and the solvent was evaporated to dryness using a warm water bath and gentle stream of pure nitrogen gas. Lipid weight was determined by reweighing the tared flask until constant weight was achieved. The lipids were separated from the organochlorines using a florisil column and the PCBs and chlordane were eluted with 6% ethyl ether and petroleum ether (v/v). PCBs were separated from chlordane by use of a silica gel column, with the PCBs being eluted with hexane and chlordane eluted with benzene.

Total PCBs and chlordane were determined using a Varian 3700 gas chromatograph equipped with a Ni<sup>63</sup> electron capture detector and Supelco glass megabore column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport. Isothermal gas chromatograph parameters were set as follows; argon

methane (95%/5%) as the carrier gas, 60 cc/min, injector temperature, 240°C; detector temperature, 210° C; and column temperature, 200° C. Gonad residue levels were quantified using Aroclor 1254 and chlordane external standards (Supelco Inc., Bellefonte, Pennsylvania). Quality assurance measures included the analysis of reagent blanks, duplicates and spiked samples. Percent recovery in spiked samples was greater than 90% for total PCBs and chlordane, therefore sample extracts were not corrected for percent recovery. Individual PCB congeners were analyzed by gas chromatography-mass spectrometry (GC-MS) using tissue samples that had PCB concentrations of at least 5.0 ppm. Samples were analyzed by GC-MS using a Varian Saturn II gas chromatograph with a quadrupole ion trap mass spectrometer detector. The gas chromatograph was equipped with a DB-5 capillary column (30 m x 0.25 mm x 0.25 µm) and had a helium flow rate of 1 mL/min. The temperature program used to separate individual PCB congeners started at 90°C for 5 min, increased at 20°C/min to 120° C, then increased to 300° C at 4° C/min and remained at the final temperature for 2.5 min.

Correlations were determined between fish age and organochlorine (PCBs and chlordane) concentrations; percent lipid and organochlorine concentrations; and percent hatch and organochlorine concentrations, from linear regression (least squares) analysis. Differences in mean organochlorine concentrations between males and females were determined by use of a two-tailed, unpaired Student's t test. All calculations were performed using the Statgraphics (1986) statistical software package for the IBM personal computer. Significance level was set at  $p \leq 0.05$  for all analyses.

## RESULTS AND DISCUSSION

Fifteen paddlefish were collected from 4 sites on the Ohio River with the ages of these fish ranging from 7 to 21 years (Tables 1 and 2). Six of the 11 females collected from the Ohio River had mature ovaries and five of these females were taken to the Aquaculture Research Center at Kentucky State University for spawning. Percent hatch of spawned female paddlefish ranged from 73 to 95% (Table 1). There was no correlation between percent hatch and organochlorine (PCB and chlordane) concentration. However, the highest percent hatch (95%) was seen in eggs that had the lowest PCB (0.27 ppm) and chlordane (0.24 ppm) concentrations. The lack of a significant reduction in percent hatch with increasing organochlorine concentration may be due to percent hatch being not as sensitive as an endpoint as are developmental effects. Percent hatch (55 - 96%) of *Pimephales promelas* eggs exposed to Aroclor 1254 (0.23 - 1.80 µg/L) was good when compared to control percent hatch (71 - 76%), although many larvae died after 96 hours of exposure (Nebeker et al. 1974). *Cyprinodon variegatus* fry were more sensitive than embryos when exposed to the PCB mixture Aroclor 1254 (Schimmel et al. 1974). Some studies indicated that yolk sac resorption is the phase where most adverse affects are seen due to the utilization of organochlorine contaminated lipid reserves (Broyles and Noveck 1979; Walker et

**Table 1.** Percent hatch (H), paddlefish age (years), egg PCB and chlordane concentrations (wet weight) egg percent lipid content (% Lipid) and plasma testosterone(T) and estradiol (E<sub>2</sub>) concentrations of female paddlefish collected from the Ohio River spawned at the hatchery.

Site	Age	PCB (ppm)	Chlordane (ppm)	% Lipid	T (ng/mL)	E <sub>2</sub> (pg/mL)	H
Uniontown	18	0.27	0.24	12.2	21.4	342	95
Uniontown	19	0.30	0.30	10.2	10.8	202	85
Louisville	16	0.80	0.56	18.2	11.3	264	80
Louisville	19	0.46	0.39	9.2	24.8	311	87
Louisville	21	0.51	0.42	10.0	16.5	288	73

**Table 2.** Paddlefish age (years), gonad PCB and chlordane concentrations (weight wet) and gonad percent lipid content, of testes, immature ovaries (Im. Ov.) and eggs from paddlefish collected from various Ohio River sites and the Cumberland River.

Site	Age	Tissue	PCB (ppm)	Chlordane (ppm)	% Lipid
Aurora	9	Eggs	1.1	0.69	14.5
Aurora	15	Testes	5.4	0.42	46.0
Aurora	16	Im. Ov.	4.0	0.24	72.9
Aurora	17	Im. Ov.	3.7	0.27	56.5
Aurora	18	Testes	2.0	0.10	68.8
Aurora	19	Testes	5.9	0.14	69.5
Meldhal	7	Testes	2.4	0.26	37.5
Meldhal	8	Im. Ov.	2.9	0.49	72.6
Meldhal	12	Im. Ov.	2.3	0.29	66.6
Meldhal	13	Im. Ov.	6.2	0.10	72.6
Cumberland	15	Eggs	0.12	0.09	12.2
Cumberland	6	Testes	0.73	0.30	75.0

al. 1991). It is also possible that the lack of significant results was due to the small sample size.

There were no significant correlations between egg organochlorine concentrations and plasma steroid levels (Table 1). Previous studies indicated that plasma steroid levels are reduced in fish exposed to PCB mixtures. *Oncorhynchus mykiss* and *Cyprinus carpio* exposed to Aroclor 1254 had reduced plasma oestrogen and androgen levels (Sivarajah et al. 1978). The lack of any significant effects on plasma steroid levels in our study may be due to egg organochlorine levels being below levels that would produce any observable effects.

There was a significant ( $p < 0.05$ ) negative correlation (correlation coefficient = - 0.86;  $R^2 = 73.2\%$ ) between egg PCB concentration and age in female paddlefish collected from the Ohio River (Tables 1 and 2). There was also a negative correlation between egg chlordane concentration and age (correlation coefficient = - 0.79;  $R^2 = 61.7\%$ ) in these fish but it was not significant ( $p = 0.06$ ) based on a probability level of  $p < 0.05$ . Gundersen (1992) saw a similar negative correlation between gonad PCB concentration and age in female paddlefish collected from the Falls of the Ohio River. Female paddlefish are sexually mature at 10 - 12 years (Russell 1986) and probably accumulate persistent organochlorines (e.g. PCBs) prior to their first spawning. Each subsequent spawning would provide a means for elimination of the total organochlorine body burdens. Similar findings of maternal transfer of organochlorines to eggs were reported for striped bass (Westin et al. 1985), and charr (Monod 1985).

PCB concentrations were highest in Ohio River fish testes and immature ovaries, which were also tissues that had the highest lipid content (Tables 1 and 2). A significant positive correlation (correlation coefficient = 0.80,  $R^2 = 64.7\%$ ) was seen between percent lipid and PCB concentration but not between percent lipid and chlordane concentration in Ohio River fish. The correlation between gonad PCB concentration and percent lipid is expected due to the lipophilic nature of organochlorines but the lack of a significant correlation between gonad chlordane concentration and percent lipid is difficult to explain. Perhaps chlordane is more readily metabolized than PCBs in paddlefish, or it accumulates more in other fatty tissues (e.g. liver and adipose tissue). PCB concentrations were lowest in egg samples but this was not the case for chlordane (Tables 1 and 2). PCB and chlordane levels in paddlefish eggs are of particular concern due to human consumption of the eggs as caviar. None of the egg PCB concentrations exceeded the Food and Drug Administration's (FDA) action limit (2 ppm) but 5 out of the seven egg samples exceeded the FDA's action limit for chlordane (0.3 ppm). Egg chlordane concentrations below the FDA's action limit were observed in the one female taken from the Cumberland River and one of the females taken from the Ohio River near Uniontown, Kentucky (Tables 1 and 2). The Cumberland River is an Ohio River tributary that has little industry associated with it and it was not unexpected to find lower gonad PCB and chlordane levels in these fish. The

**Table 3.** PCB congeners detected in the testes of a paddlefish (age 15) collected from Aurora, Indiana.

IUPAC no.	Structure	%
41, 61	2,2',3,4; 2,3,4,5	2.2%
44	2,2',3,5'	2.5%
49	2,2',4,5'	1.6%
56, 60	2,3,3',4'; 2,3,4,4'	1.7%
52	2,2',5,5'	8.4%
66	2,3',4,4'	4.3%
70	2,3',4',5	3.6%
74	2,4,4',5	2.4%
82	2,2',3,3',4	0.7%
84	2,2',3,3',6	1.7%
85	2,2',3,4,4'	3.0%
91	2,2',3,4',6	0.9%
92	2,2',3,5,5'	2.6%
95	2,2',3,5',6	7.4%
97	2,2',3',4,5	2.1%
99	2,2',4,4',5	5.4%
101, 90	2,2',4,5,5'; 2,2',3,4',5	5.5%
104	2,2',4,6,6'	0.5%
105	2,3,3',4,4'	2.3%
110	2,3,3',6	8.4%
115, 87	2,3,4,4',6; 2,2',3,4,5'	2.0%
123, 118	2',3,4,4',5; 2,3',4,4',5	6.2%
134	2,2',3,3',5,6	0.2%
135	2,2',3,3',5,6'	1.6%
136	2,2',3,3',6,6'	0.6%
138	2,2',3,4,4',5'	8.3%
146	2,2',3,4',5,5'	1.3%
149	2,2',3,4',5,6	4.4%
151	2,2',3,5,5',6	2.0%
153	2,2',4,4',5,5'	5.4%
176	2,2',3,3',4,6,6'	0.7%

% = Percent of total PCB peak area.

lower egg PCB/chlordane concentrations seen in fish collected from the lower Ohio River (Uniontown, Ohio River mile 846) is consistent with results from other Ohio River monitoring programs, where higher fish tissue contaminant levels are seen in upper Ohio River fish (*ORSANCO press release 1996*).

The gas chromatography profiles (GC-ECD) of gonad extracts were quite similar and GC-MS analysis indicated that sample extracts contained tetra-, penta-, hexa- and hepta-chlorobiphenyl congeners (Table 3). Greater than 40% of the total detectable PCB congeners were the pentachlorobiphenyls with the tetra- and hexachlorobiphenyls each making up over 20% of the total detectable PCB congeners. Some of the PCB congeners detected in paddlefish gonads have been determined to be potentially toxic, particularly IUPAC numbers 77, 126, 169, 105, 118, 128, 138, 156, 170, 87, 99, 101, 153, 180, 183 and 194 (McFarland and Clarke 1989). Over 50% of the total detectable PCB congeners in the paddlefish gonads we analyzed contained some of these potentially toxic congeners. Some of these congeners are known inducers of cytochrome P-450-dependent mixed-function oxidases (Janz and Metcalfe 1991). Further investigations should examine the effects of these congeners on paddlefish health, and the effects of PCB and chlordane on reproduction and early life stage development.

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