SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 19, No. 9, pp. 2275–2285, 2000
Printed in the USA
0730-7268/00 $9.00 + .00

BIOMARKER RESPONSE AND HEALTH OF POLYCHLORINATED BIPHENYL- AND CHLORDANE-CONTAMINATED PADDLEFISH FROM THE OHIO RIVER BASIN, USA

DEKE T. GUNDERSEN,*† RUTHELLEN MILLER,† AMY MISCHLER,† KRISTA ELPERS,† STEVE D. MIMS,‡ JODY G. MILLAR,§ and VICKI BLAZER‖

†Environmental Science Program, Pacific University, 2043 College Way, Forrest Grove, Oregon 97116, USA
‡Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky 40601, USA
§U.S. Fish and Wildlife Service, Rock Island Field Office, 4469 48th Avenue Court, Rock Island, Illinois 61201
‖National Fish Health Laboratory, U.S. Geological Survey, Biochemical Resources Division, Leetown Science Center, Kearneyville, West Virginia 25430

(*Received* 29 *July* 1999; *Accepted* 27 *January* 2000)

Abstract—Fifty paddlefish (*Polyodon spathula*) collected from two sites on the Ohio River and from one site on the Cumberland River, USA, were examined to determine gonad polychlorinated biphenyl (PCB) and chlordane concentrations, amounts of plasma sex steroids (testosterone and estradiol), hepatic microsomal ethoxyresorufin-*O*-deethylase (EROD) activity, and the presence of immunoreactive cytochrome P450 1A (CYP1A) protein. Percent hatch and liver, spleen, and kidney histology were also determined. Gonad PCB and chlordane concentrations were significantly higher in Ohio River paddlefish than in Cumberland River paddlefish. Gonad PCB and chlordane concentration and gonad percent lipid were correlated in Ohio River paddlefish. Five of 10 Ohio River egg samples exceeded the Food and Drug Administration's action limit for chlordane (0.30 μg/g). Polychlorinated biphenyl congener-specific analysis detected predominantly the tetra-, penta-, and hexachlorobiphenyls in paddlefish testes. Plasma testosterone levels were significantly lower in males collected from the upper Ohio River site than those collected from the lower part of the river. There was no measurable hepatic microsomal EROD activity in any of the 50 paddlefish collected from the three sites. Western blotting analysis confirmed that a rabbit antitrout CYP1A1 IgG antibody did not recognize a CYP1A protein in paddlefish liver microsomes. Percent hatch was not significantly different in eggs collected from the Cumberland (88–96%) and Ohio Rivers (90–95%). Histological analysis of liver, spleen, and kidney detected the presence of hepatic steatosis and hemosiderosis, splenic lymphoid cell depletion, and hyperplasia of interrenal and chromaffin tissues. Immunosuppression, hepatic metabolic disorders, and altered neuroendocrine function may be occurring in Ohio River paddlefish. Results presented here suggest that organochlorine exposure may be jeopardizing the long-term health of Ohio River paddlefish and that additional investigation of contaminant effects on immune system function and hormone levels in paddlefish is warranted.

Keywords—Paddlefish Polychlorinated biphenyls Chlordane Reproduction Biomarkers

INTRODUCTION

The paddlefish, because of its virtually scaleless body, long paddle-like rostrum, and heterocercal tail, is a unique fish. Only one other species is known from the family Polydontidae, the Chinese paddlefish *Psephurus gladius*, which inhabits the Yangtze-Kiang River in the Chinese lowlands [1]. The paddlefish is a large, long-lived freshwater fish that can attain weights of more than 45 kg, and individuals exceeding more than 15 years of age are not uncommon [2]. Typically, most male paddlefish are not sexually mature until age 8 and most females do not mature until age 10 [2]. Paddlefish were historically abundant in most of the large rivers of the Mississippi River and Gulf Coast drainage, USA. Since the beginning of the 20th century, significant declines in numbers have occurred. The decline of paddlefish populations is likely due to degradation of water quality and habitat destruction because of the use of the Ohio River as a transportation corridor, industrial development, and dam construction. Recently, the paddlefish has become a commercially valuable species due to the processing of its roe into caviar. The depression of sturgeon stocks in eastern Europe has significantly increased the price of caviar and has in turn increased the pressure on both paddlefish and sturgeon in North America [2]. In 1992, paddlefish were added to the Appendix II list of the United Nation's Convention on International Trade of Endangered Species of Wild Fauna and Flora [3]. This protection was designed to monitor the global import and export of products from paddlefish and to curtail the illegal trafficking of caviar, which can be detrimental to wild populations of paddlefish. Further, the World Conservation Union/Species Survival Commission declared paddlefish as a vulnerable species because of the decrease in population numbers and the decline in habitat quality. Currently, the Mississippi Interstate Cooperative Resource Association, USA, which includes 22 states within the endemic range of the paddlefish, has coordinated several paddlefish studies among these states. Ohio River paddlefish have been the focus of conservation efforts by adjacent states and by the U.S. Fish and Wildlife Service, Ohio River Islands National Wildlife Refuge. Established in 1990, the Ohio River Island National Wildlife Refuge protects over 1,100 acres of habitat, including 19 islands located along 362 miles of river. Refuge areas serve as potential safe harbors for paddlefish and other threatened species and as such are serving an increasingly important function.

However, Ohio River water quality may be limiting pad-

* To whom correspondence may be addressed (dgunders@usi.edu).

The conclusions represent the views of the authors and do not necessarily represent the opinions, policies, or recommendations of the U.S. Fish and Wildlife Service. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the authors or the U.S. Fish and Wildlife Service.

dlefish productivity. One indicator of poor water quality is fish advisories. Fish consumption advisories based on polychlorinated biphenyls (PCBs) and chlordane are in place for the entire length of the Ohio River. Kentucky, USA, is the only state that specifically identifies fish consumption advisories for paddlefish. Fish containing organochlorines at concentrations that require the establishment of fish advisories may be physiologically impaired by that contaminant. The U.S. Fish and Wildlife Service found whole-body PCB concentrations in channel catfish at 3.5 μg/g in the vicinity of the Ohio River Islands National Wildlife Refuge [4]. Gundersen and Pearson [5] found PCB concentrations in the roe of paddlefish at about two times the U.S. Food and Drug Administration action limit (>4 μg/g). These concentrations exceed those where reproductive effects in similar species have been found [6]. Certain PCB congeners are also structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and have the ability to induce bioactivating enzyme systems [7], and some chemical components of these organochlorine mixtures are also suspected environmental endocrine disruptors [8].

Due to the rising concern for the protection of paddlefish populations and the habitat required to sustain these populations, we began an investigation to examine the extent of gonad PCB and chlordane contamination in paddlefish from two areas on the Ohio River, the Ohio River Islands National Wildlife Refuge and the Falls of the Ohio near Louisville, Kentucky, and one site on the Cumberland River (a relatively less industrialized Ohio River tributary). This study was an attempt to determine if there was a relationship between gonad organochlorine contamination and certain biomarkers that could be used to assess the long-term health and viability of Ohio River paddlefish populations. The biomarkers examined included observations of liver, kidney, and spleen histology; quantitation of ethoxyresorufin-*O*-deethylase (EROD) activity; immunoquantitation of cytochrome P-450 1A; hatchability of fertilized eggs taken from contaminated fish; and measurements of plasma sex steroids. Establishing the validity of these biomarkers would permit a broad assessment of paddlefish health to be done at minimal cost.

MATERIALS AND METHODS

Paddlefish collections

Paddlefish, *Polyodon spathula,* were collected from three sites in the Ohio River Basin, USA, during the 1997 spawning season (April–October). Two of the sites were located on the Ohio River and the other site was located on the Cumberland River, an Ohio River tributary. A total of 50 paddlefish were collected from the three sites using large mesh gill nets (30–60 m in length, 4.8-m deep, and 10–13-cm bar measure mesh). Nineteen paddlefish (8 males and 11 females) were collected from the Falls of the Ohio River, near Louisville, Kentucky, in the tail waters of the McAlpine Dam (Ohio River mile 606.8). Eleven paddlefish (10 males and 1 female) were collected from the Ohio River Islands National Wildlife Refuge, near Hendersen, West Virginia, USA, in the tail waters of Robert Byrd Dam (Ohio River mile 279.2). Twenty paddlefish (8 males and 12 females) were collected from the Cumberland River, near Aaron, Kentucky, in the tail waters of Wolf Creek Dam. Captured fish (alive) were weighed and measured for total length. Paddlefish not used for spawning experiments were killed by a blow to the head, and the dentary bone was removed for age determinations as described by Gundersen and Pearson [5]. The gonads were removed and wrapped in aluminum foil and put on ice for transport to the laboratory, where they were stored at −20°C. The liver, kidney, and spleen were removed from each fish and pieces fixed in 10% buffered formalin. The remaining liver portions from each fish were frozen in liquid nitrogen and stored on dry ice for transport to the laboratory, where they were kept at −80°C for later EROD analysis. Blood was drawn using the vacutainer system (Franklin Lakes, NJ, USA) with lithium heparin as an anticoagulant. Blood samples (approximately 2 ml) were placed on dry ice for transport to the laboratory. At the laboratory, blood samples were centrifuged and the plasma was pipetted into a microcentrifuge tube for storage at −80°C.

Paddlefish spawning

Of the 50 fish collected, 7 mature female paddlefish and 12 mature male paddlefish were transported to the Aquaculture Research Center at Kentucky State University, Frankfort, Kentucky, USA, for spawning. Plasma was collected from each fish prior to transport. Spawning of paddlefish and fertilization of eggs were done by injecting male and female paddlefish with a luteinizing hormone-releasing hormone analog (LHRH-A; des-Gly[D-Ala6]-LHRH) at a dose of 0.05 mg/kg and 0.1 mg/kg, respectively. Paddlefish ovulated 12 to 14 h after injection and males were actively spermiating 12 to 18 h after injection. The ovulated eggs from each female were stripped into a dry pan, and the milt from males collected from the same site as the female was added and mixed. An aqueous suspension of Fuller's earth was added to the fertilized eggs in order to activate the spermatozoa and to prevent egg adhesion. The eggs were mixed and incubated at a water temperature of 18°C for 10 min. Several hundred eggs were then loaded into screened incubation units and maintained at 18 ± 0.3°C in an aerated water bath. The hatch success of eggs from each female was determined.

PCB and chlordane analysis

Extraction and cleanup procedures for paddlefish gonads were done based on the methods described by Gundersen et al. [9]. Subsamples of gonad homogenates (5–10 g) were combined with sodium sulfate (~50 g) and ground to a fine powder using a mortar and pestle. Dried tissues were Soxhlet extracted (10 h) with 170 ml of 1:1 petroleum ether/hexane (v/v spectral grade; Sigma-Aldrich, St. Louis, MO, USA). Extracts were concentrated to less than 15 ml with a rotary evaporator and transferred to tared vials, where the remaining solvent was evaporated to dryness using a warm water bath and a stream of pure nitrogen (N_2). Lipid extracts were cleaned up using florisil columns (400 × 19 mm), and PCBs and chlordane were eluted with 6% ethyl ether/petroleum ether (v/v). Polychlorinated biphenlys and chlordane were separated using silica gel columns (10.5 × 300 mm); polychlorinated biphenlys were eluted with hexane, and chlordane was eluted with benzene.

The cleaned extracts were analyzed for total PCBs and chlordane by gas chromatography using a Varian 3700 (Palo Alto, CA, USA) gas chromatograph equipped with a ^{63}Ni electron-capture detector and a glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport (Supelco, Bellefonte, PA, USA). Isothermal gas chromatographic parameters used were carrier gas, argon/methane (95%/5%), 60 ml/min; injector temperature, 240°C; detector temperature, 210°C; and column temperature, 200°C. Quantification of cleaned-up PCB fractions involved the use of an Aroclor® 1254 external standard (Supelco) that most resembled PCB mixtures in tissue

extracts as determined by gas chromatography-mass spectrometry (GC-MS) analysis. A technical chlordane (Supelco) external standard was used for quantifiying cleaned-up chlordane fractions. Quality assurance measures included the analysis of reagent blanks, duplicates, and spiked samples. Percent recovery in spiked samples was greater than 94% for total PCBs and 91% for chlordane; therefore, sample extracts were not corrected for percent recovery. The Patuxent Analytical Control Facility (Laurel, MD, USA) analyzed two tissue homogenates for total PCBs (for interlaboratory comparison) and other organochlorines. Total PCBs (reported as Aroclor 1254 by both laboratories) reported by the two laboratories differed by an average of less than 8%.

Some gonad extracts were analyzed for individual PCB congeners by GC-MS using a Varian Saturn II gas chromatograph coupled to a quadrapole ion trap mass spectrometer. The gas chromatograph was equipped with a Supelco SPB-5 capillary column (30 m × 0.25-mm i.d., 0.25 μm film) and had a carrier gas (He) flow rate of 1 ml/min. The column temperature program started at 90°C for 5 min, raised at 20°C/min to 120°C, then raised to 300°C at 4°C/min, and remained at the final temperature for 2.5 min. An Aroclor 1254 standard and individual PCB congeners (AccuStandard, New Haven, CT, USA) were used to identify PCB congeners in gonad extracts. The GC-MS analysis indicated that the Aroclor 1254 standard was a close match to PCB mixtures in field samples.

Steroid hormone radioimmunoassays

Unextracted, unfractionated plasma from each fish was assayed for testosterone and estradiol with Diagnostic Systems Laboratories (Webster, TX, USA) Active Testosterone (DSL-4000) and Ultra-sensitive Estradiol (DSL-4800) Radioimmunoassay Kits. Subsequent procedures were performed according to the guidelines provided by the kit manufacturer. Results were reported as nanograms of testosterone per milliliter of plasma (ng/ml) and picograms of estradiol per milliliter of plasma (pg/ml).

EROD assays and Western blotting

Liver sections were homogenized in four volumes (w/v) of ice-cold buffer (0.1 M tris-acetate, 0.1 M KCl, 1.0 M ethylenediaminetetraacetic acid (EDTA), 20.0 μM butylated hydroxytoluene, and 0.1 mM phenylmethylsulphonyl fluoride; pH 7.4) and were centrifuged at 10,000 *g* for 30 min followed by centrifugation of the supernatant at 100,000 *g* for 90 min. Microsomal pellets were resuspended in two volumes (w/v) of buffer (0.1 M K_2HP0_4, 1.0 mM EDTA, 20 μM butylated hydroxytoluene, 0.1 mM phenylmethylsulphonyl fluoride, and 20% glycerol) and were stored at −80°C until use in EROD assays and Western blotting.

Ethoxyresorufin-*O*-deethylase was measured using the technique of Prough et al. [10] with 500 μg of microsomal protein. Enzyme activities were assayed on a Hitachi MPF-2A fluorometer using resorufin standards (Sigma, St. Louis, MO, USA). Cytochrome P450 1A (CYP1A) protein was analyzed by Western blotting using a polyclonal rabbit antitrout CYP1A-IgG (gift of D. R. Buhler). Microsomes were separated by sodium dodecyl sulfate-polyacrylamide gel elecrophoresis using 8% minigels (Jule Biotechnologies, New Haven, CT, USA), and the resolved proteins were transferred electrophoretically onto microporous polyvinylidene difluoride membranes (Boehringer Mannheim GmbH, Mannheim, Germany) using a Hoefer transfer unit (model TE 70, San Francisco, CA, USA). Membranes were incubated with primary antibody (with 2% bovine serum albumin) for 1 h followed by incubation in secondary antibody (rabbit Ig, horseradish peroxidase-linked antibody; Amersham, Arlington Heights, IL, USA) for 1 h. Cross-reactions were visualized with enhanced chemiluminescence reagents (Amersham) detected by exposure to autoradiography film (Hyperfilm; Amersham). Protein concentrations were determined by the method of Lowry et al. [11]. Measuring EROD activity and cytochrome P450 1A protein in a female shovelnose sturgeon, a closely related species to the paddlefish, validated these methods. Hepatic microsomal EROD activity in this fish was 12 pmol/min/mg protein, and there was cross-reactivity between a rabbit antitrout antibody and CYP1A protein in this species.

Histology

Pieces of liver, spleen, and anterior and posterior kidney were fixed in 10% neutral buffered formalin. Tissues were embedded in paraffin (two samples/tissue) and sectioned at 5 μm. Sections were stained with hematoxylin and eosin and Perl's method for hemosiderin [12]. Sections were examined for tissue changes indicative of the effects of contaminant exposure or histologic biomarkers [13]. Tissue changes were classified as to type, and observations of Perl's positive material (hemosiderin) within hepatocytes and macrophage aggregates, concentration of eosinophils within splenic white pulp, and hepatocyte vacuolization were rated on a scale of 0 to 4 (0 representing tissues that had no observable lesions or changes, 1 representing minimal lesions/changes, and 2, 3, and 4 representing mild, moderate, and severe lesions/changes, respectively). Mean ratings were compared among sites.

Statistics

Linear regression (least squares) analysis was used to determine correlation between paddlefish age and organochlorine (PCBs and chlordane) concentration, percent lipid and organochlorine concentration, and percent hatch and organochlorine concentration. Analysis of variance (comparison of all three sites) and a two-tailed, unpaired Student's *t* test (comparison between two sites) were used to determine site differences in organochlorine accumulation and plasma sex steroid concentration. Multiple linear regression analysis was used to determine which independent variables (mean gonad PCB and chlordane concentrations) were involved in the best-fitting model for predicting plasma testosterone levels in Ohio River males. Significance level was $p \leq 0.05$ for all analyses. Mean values were reported ± SD (standard deviation). All statistics were performed using the Statgraphics® (Statistical Graphics, Rockville, MD, USA) statistical software package.

RESULTS

Paddlefish condition factors

Body condition factors were calculated for all 50 fish collected from the three sites. There was no significant difference in condition factors between Falls, Refuge, and Cumberland fish (1.28 ± 0.20, 1.33 ± 0.10, and 1.47 ± 0.19 mm/g, respectively).

Gonad PCB and chlordane analysis

Gonad percent lipid, PCB and chlordane concentrations, and age of paddlefish collected from the three sites are shown in Table 1. Gonad PCB and chlordane concentrations were higher in fish collected from the Ohio River (Refuge and Falls)

Table 1. Age, gonad percent lipid content (% lipid), gonad PCB and chlordane concentration, plasma testosterone (T, ng/ml) and estradiol (/E_2, pg/ml) concentration of paddlefish collected from the Falls (F), Refuge (R), and Cumberland (C) sites

Sample no., tissue	Age	% Lipid	Tissue PCB and chlordane concentrations: Lipid (μg/g) PCBs	Lipid (μg/g) Chlordane	Wet tissue (μg/g) PCBs	Wet tissue (μg/g) Chlordane	T	E_2	%H[a]
F4, eggs	13	14.0	5.70	3.00	0.80	0.42[b]	20.1	394.4	—
F5, eggs	14	11.1	6.82	2.69	0.76	0.30	19.8	398.6	—
F6, eggs	15	24.0	6.39	2.46	1.53	0.59[b]	20.8	339.8	—
F7, eggs	10	12.8	7.16	3.50	0.92	0.45[b]	22.9	387.9	—
F8, eggs	13	11.5	5.15	3.14	0.59	0.36[b]	19.1	390.1	—
F11, eggs	10	5.8	5.51	4.65	0.32	0.27	20.8	399.2	—
F13, eggs	16	7.8	4.84	2.29	0.38	0.18	20.4	377.9	92
F15, eggs	16	7.8	5.35	2.68	0.42	0.21	23.7	384.4	95
F19, eggs	9	12.1	8.07	3.30	0.93	0.38[b]	18.4	375.1	90
F2, testes	12	20.0	18.66	7.69	3.74	1.54	17.9	391.1	—
F3, testes	17	70.8	12.25	3.01	8.68	2.13	10.2	402.5	—
F9, testes[c]	12	70.9	4.08	1.38	2.90	0.98	10.0	393.8	—
F10, testes[c]	11	38.4	9.91	2.24	3.81	0.86	20.2	381.1	—
F12, testes[c]	11	47.4	7.66	3.08	3.63	1.46	18.7	385.2	—
F16, testes[c]	16	11.2	12.6	5.29	1.43	0.60	22.6	357.4	—
F17, testes[c]	12	39.0	14.01	4.48	5.47	1.75	21.1	365.7	—
F18, testes[c]	13	58.5	1.61	0.84	0.94	0.49	18.3	358.8	—
F20, testes[c]	14	43.2	4.93	2.20	2.13	0.95	10.1	354.9	—
F14, IMO[d]	11	5.8	5.51	4.65	3.57	1.24	23.3	351.4	—
R1, testes	11	70.9	2.85	1.55	2.02	1.10	3.9	398.7	—
R2, testes	16	44.9	9.18	4.01	4.12	1.80	8.4	183.9	—
R3, testes	11	42.9	8.58	3.29	3.68	1.41	15.6	251.1	—
R4, testes	10	49.2	8.23	2.80	4.05	1.38	14.1	383.7	—
R5, testes	9	73.0	3.31	1.75	2.42	1.28	14.8	330.5	—
R6, testes	8	72.0	1.74	1.10	1.24	0.78	8.4	384.7	—
R7, testes	17	44.0	7.82	3.23	3.44	1.42	15.9	53.6	—
R8, testes	17	28.3	9.01	4.20	2.55	1.19	14.6	373.9	—
R9, testes	9	63.8	5.50	2.15	3.51	1.37	2.8	389.4	—
R10, testes	10	68.6	12.10	3.911	8.30	2.92	3.1	365.0	—
R11, eggs	10	12.6	2.94	1.98	0.37	0.25	5.6	361.4	—
C8, eggs	9	7.4	4.30	1.61	0.32	0.12	16.8	376.3	—
C10, eggs	15	21.5	<0.05	0.33	<0.05	0.07	23.9	372.4	88
C13, eggs	12	10.5	0.89	0.43	0.07	0.03	24.9	357.3	—
C15, eggs	12	18.3	<0.05	0.27	<0.05	0.05	23.9	363.5	—
C17, eggs	9	12.0	<0.05	0.50	<0.05	0.06	16.4	377.9	—
C18, eggs	11	11.6	<0.05	<0.05	<0.05	<0.05	17.1	379.5	—
C19, eggs	11	11.3	<0.05	0.70	<0.05	0.08	16.9	383.4	96
C20, eggs	8	10.4	<0.05	<0.05	<0.05	<0.05	17.3	385.9	93
C3, IMO	13	71.5	0.49	0.08	0.35	0.06	14.6	295.3	—
C4, IMO	7	71.6	0.31	0.22	0.22	0.16	15.4	368.2	—
C5, IMO	7	74.3	0.08	0.40	0.06	0.30	14.6	366.5	—
C7, IMO	9	76.9	0.17	0.31	0.13	0.24	16.9	24.5	—
C1, testes	6	81.0	0.63	0.12	0.51	0.10	17.8	161.3	—
C2, testes	9	64.7	0.40	0.08	0.26	0.05	19.8	60.9	—
C6, testes	11	52.7	0.61	0.21	0.32	0.11	20.9	364.8	—
C9, testes[c]	7	70.0	0.58	0.19	0.41	0.13	15.8	372.9	—
C11, testes[c]	7	66.3	0.48	0.57	0.32	0.38	21.6	370.9	—
C12, testes[c]	8	72.0	<0.05	0.29	<0.05	0.21	24.1	356.3	—
C14, testes[c]	6	10.4	5.01	2.80	0.52	0.29	12.0	371.2	—
C16, testes[c]	8	61.9	<0.05	0.24	<0.05	0.15	20.0	368.5	—

[a] %H = percent egg hatchability from corresponding female.
[b] Exceeds Food and Drug Administration's action limit of 0.30 μg/g, for edible tissues.
[c] The milt of these males was combined and used to fertilize eggs of females from the same site.
[d] IMO = immature ovary.

compared with those collected from the Cumberland River. Mean PCB and chlordane concentrations in the testes of Cumberland fish were significantly lower than those seen in the testes of Refuge and Falls fish (Table 2). Mean egg PCB and chlordane concentrations in Cumberland fish were also significantly lower than those seen in Falls fish. Comparison of immature ovaries could not be made since only one fish was collected from the Ohio River that had immature ovaries. Chlordane and PCB concentrations (wet wt basis) were highest in testes and immature ovaries, i.e., in tissues that had a higher percent lipid content than eggs (Tables 1 and 2). However, when PCB and chlordane tissue concentrations were reported on a lipid-adjusted basis, tissue concentrations (eggs, immature ovaries, and testes) were similar. None of the 16 egg samples exceeded the Food and Drug Administration's action limit for PCBs in edible tissues (2 μg/g), but five egg samples did exceed the action limit for chlordane (0.30 μg/g). Egg samples that exceeded the Food and Drug Administration's action limit for chlordane came from paddlefish collected from the Ohio River (Table 1). There was no significant correlation between

Table 2. Mean (±SD) values from tissue analyses; mean age and plasma testosterone (T) and estradiol (E_2) concentrations of paddlefish collected from the Falls (F), Refuge (R), and Cumberland (C) sites

				Mean PCB and chlordane concentrations					
				Lipid (μg/g)		Wet tissue (μg/g)			
Site	Tissue	Age	% Lipid	PCBs	Chlordane	PCBs	Chlordane	T (ng/ml)	E_2 (pg/ml)
F	Eggs	13 ± 3	11.9 ± 5.3	6.08 ± 0.99	3.07 ± 0.70	0.74 ± 0.38	0.35 ± 0.13	20.9 ± 1.8	383.0 ± 18.2
	Testes	13 ± 2	44.4 ± 20.5	9.54 ± 5.46	3.19 ± 1.80	3.64 ± 2.34	1.19 ± 0.55	16.9 ± 4.9	372.5 ± 21.4
R	Testes	12 ± 3	55.8 ± 15.8	6.83 ± 3.33	2.80 ± 1.11	3.53 ± 1.92	1.46 ± 0.57	9.7 ± 5.4	306.9 ± 106.1
C	Eggs	11 ± 2	12.9 ± 4.6	0.92 ± 1.38[a]	0.53 ± 0.47[a]	0.09 ± 0.09[a]	0.06 ± 0.03[a]	18.2 ± 3.7[b]	374.5 ± 9.8
	IMO[c]	9 ± 3	73.6 ± 2.6	0.26 ± 0.18	0.25 ± 0.14	0.19 ± 0.12	0.19 ± 0.10	15.4 ± 0.9	263.6 ± 141.2
	Testes	8 ± 2	59.9 ± 21.6	0.98 ± 1.64[a]	0.56 ± 0.91[a]	0.30 ± 0.18[a]	0.18 ± 0.11[a]	19.0 ± 3.8	311.5 ± 116.5

[a] Mean gonad PCB and chlordane concentrations in Cumberland fish are significantly different from corresponding tissue concentrations in Falls and Refuge fish.
[b] Mean plasma testosterone levels in Refuge fish are significantly different from plasma testosterone levels in Cumberland and Falls fish.
[c] IMO = Immature ovaries.

age and gonad organochlorine concentration when using wet weight tissue concentrations. However, there was a significant correlation between age and lipid-adjusted gonad chlordane concentrations in Falls fish with eggs ($r^2 = 0.58$; correlation coefficient = −0.76; $p = 0.02$).

Three female and seven male paddlefish from the Falls of the Ohio River and three female and five male paddlefish from the Cumberland River were used for spawning experiments at the fish hatchery. The percent hatch of the fertilized eggs from each female is shown in Table 1. Percent hatch of fertilized eggs collected from both sites ranged from 90 to 95% for eggs from Falls fish and from 88 to 96% for eggs from Cumberland River fish. Mean PCB levels (±SD) in the eggs of spawned Falls fish (0.58 ± 0.30 μg/g wet tissue) were significantly higher than mean PCB levels in spawned Cumberland River eggs (<0.05 μg/g). Mean egg chlordane levels (±SD) were not significantly different between spawned eggs from Falls fish (0.26 ± 0.11 μg/g wet tissue) and spawned eggs from Cumberland River fish (0.06 ± 0.03 μg/g). Mean gonad PCB and chlordane concentrations (±SD) were significantly higher in spawned males from the Falls (2.90 ± 1.56 and 1.01 ± 0.45 μg/g wet tissue, respectively) versus mean PCB and chlordane concentrations in spawned males collected from the Cumberland River (0.27 ± 0.21 and 0.23 ± 0.10 μg/g, respectively). There were no significant differences in mean plasma testosterone and estradiol concentrations between spawned males or females collected from the Falls and the Cumberland River.

Thirty-five different PCB congeners were detected in the testes of paddlefish collected from the three sites (Table 3). Testes contained tetra-, penta-, and hexachlorobiphenyls in the highest concentrations. Dominant PCB congeners (>5% of total) found in samples from all three sites were 66, 118+123, 138, and 153 (Table 3). The pattern of chlorine substitution in the testes of all fish was quite similar to the pattern of substitution seen in an Aroclor 1254 standard.

Plasma sex steroids

Mean (±SD) plasma hormone levels (testosterone and estradiol) for male and female paddlefish collected from the three sites are shown in Table 2. Mean plasma estradiol levels were not significantly different between females collected from the Falls and Cumberland sites. There was no significant difference in mean plasma estradiol levels between males collected from all three sites. The only significant difference in plasma testosterone levels was seen in Refuge males. Mean plasma testosterone levels in Refuge males were significantly lower than those seen in males or females from the Falls or the Cumberland River (Table 2). There was a significant negative correlation between mean plasma testosterone levels and gonad chlordane and PCB concentration in Refuge males ($r^2 = 0.29$ and 0.20; correlation coefficient = −0.54 and −0.45; $p = 0.004$ and 0.02, respectively). Of the variables studied, multiple regression analysis indicated that gonad chlordane concentration was the most important independent variable affecting plasma testosterone levels in male Refuge fish.

Cytochrome P450 analysis

There was no measurable hepatic microsomal EROD activity in the 50 paddlefish collected from the three sites. We were unable to detect CYP1A protein in paddlefish in a Western blotting analysis using rabbit antitrout CYP1A1 (LM_{4b}) IgG antibody.

Histology

Many observations were noted and rated during the histologic examination of the liver, spleen, and anterior/posterior kidney. Only those for which differences among sites were noted will be presented.

Liver

Liver tissue of paddlefish from Cumberland River was composed of hepatocytes that were vacuolated (10 received a 4 rating, 3 received a 3, and 6 received a 2) with eccentric nuclei (Fig. 1a). Liver tissue from one fish had focal areas in which the cells were highly vacuolated and other areas with only +2 vacuolization. The hepatocytes contained varying amounts of Perl's positive material or hemosiderin (mean ± SD: 1.9 ± 0.9). Two of the fish contained large amounts of fat around vessels of the liver. Macrophage aggregates were also present in the liver of paddlefish. In fish from the Cumberland River, there were many nonpigmented macrophages within these aggregates (Fig. 1b). However, three pigments—hemosiderin, melanin, and ceroid/lipofuscin—could also be observed using the Perl's stain. Rating means for hemosiderin within macrophage aggregates was 1.9 ± 0.7.

Liver sections from Ohio River sites were more difficult to rate for vacuolization. The majority of the sections had an uneven distribution with foci of highly vacuolized cells whereas other areas were rated only 2 or 3. At the Refuge site, 7 of 11, and at the Falls of Ohio River site, 10 of 20 fish had focal areas of vacuolized cells. The less vacuolated areas had greater amounts of hemosiderin within hepatocytes, while the vacu-

Table 3. Polychorinated biphenyl congener analysis (percent of total detectable PCBs) of paddlefish testes collected from the Ohio River Islands National Wildlife Refuge (R), the Cumberland River (C), and the Falls of the Ohio river (F)

IUPAC no.	Structure	R3	R4	C2	F20
41, 64	2,2′,3,4′; 2,3,4′,6	4.0	1.0	3.4	0.8
44	2,2′,3,5′	2.4	1.3	2.7	0.8
49	2,2′,4,5′	2.8	0.7	2.0	<LOD[a]
52	2,2′,5,5′	6.0	4.1	7.4	2.0
56, 60	2,3,3′,4′; 2,3,4,4′	1.3	1.6	6.2	<LOD
66	2,3′,4,4′	5.4	5.6	12.7	6.4
70	2,3′,4′,5	4.2	3.5	5.2	2.1
74	2,4,4′,5	2.4	3.2	6.3	2.9
82	2,2′,3′,3′,4	<LOD	<LOD	0.8	<LOD
84	2,2′,3,3′,6	1.1	1.0	1.1	<LOD
85	2,2′,3,4,4′	1.7	2.4	3.1	<LOD
87, 115	2,2′,3,4,5′; 2,3,4,4′,6	3.2	1.3	2.8	3.0
90, 101	2,2′,3,4′,5; 2,2′,4,5,5′	7.9	3.8	4.6	2.6
91	2,2′,3,4′,6	0.9	<LOD	<LOD	<LOD
92	2,2′,3,5,5′	2.1	2.2	1.6	2.5
95	2,2′,3,5′,6	5.8	4.6	4.5	3.4
97	2,2′,3′,4,5	2.4	1.1	1.1	<LOD
99	2,2′,4,4′,5	4.8	5.0	<LOD	6.2
105	2,3,3′,4,4′	3.1	3.0	11.6	2.9
110	2,3,3′,4′,6	10.4	6.8	<LOD	3.0
118, 123	2,3′,4,4′,5; 2′,3,4,4′,5	6.5	7.4	9.6	8.9
132	2,2′,3,3′,4,6	1.8	<LOD	1.1	<LOD
135	2,2′,3,3′,5,6′	1.3	1.8	<LOD	<LOD
136	2,2′,3,3′,6,6′	<LOD	0.6	<LOD	<LOD
138	2,2′,3,4,4′,5′	5.5	14.1	5.0	18.7
146	2,2′,3,4′,5,5′	2.1	2.6	0.1	3.8
149	2,2′,3,4′,5′,6	3.6	5.6	1.3	5.4
151	2,2′,3,5,5′,6	1.7	<LOD	<LOD	2.9
153	2,2′,4,4′,5,5′	5.6	15.1	5.6	20.7
176	2,2′,3,3′,4,6,6′	<LOD	0.6	<LOD	0.9

[a] LOD = level of detection.

olated areas contained little or no hemosiderin (Fig. 1b). Mean ratings for hepatocyte hemosiderin were 3.1 ± 0.6 for Refuge fish and 3.3 ± 0.6 for Falls fish. Macrophage aggregates were more numerous, larger, and contained more pigmented cells at both of these sites (Fig. 1b) when compared to those collected from fish in the Cumberland River (Fig. 1a). Mean ratings for macrophage aggregate hemosiderin were 3.2 ± 0.7 for Refuge fish and 3.1 ± 0.9 for Falls fish. Ten livers from the Falls site and two from the Refuge site contained large fat deposits around vessels.

Spleen

Splenic tissue in paddlefish was composed of both white and red pulp. The white pulp formed follicle-like masses around vessels and, in most spleens from Cumberland River fish, were composed primarily of lymphocytes, with some macrophages and eosinophils (Fig. 2a). Three fish from this site had mild lymphocyte depletion of the white pulp. In contrast, 15 of 20 Falls paddlefish and 10 of 11 Refuge fish had lymphocyte depletion ranging from mild to severe (Fig. 2b). This lymphocyte depletion was often accompanied by an increase in the number of eosinophils within the white pulp (Fig. 2b).

Anterior kidney

Anterior kidney of paddlefish contained both interrenal and chromaffin tissue, the functional equivalents of adrenal tissue in higher vertebrates. In paddlefish from the Cumberland River, small clusters of interrenal cells were noted in two fish and a large cluster in one fish. The cytoplasm of these cells was eosinophilic, finely granular, and nuclei contained prominent nucleoli (Fig. 3a). Seven fish from the Falls site had moderate to large clusters of interrenal cells, while four fish from the Refuge had large clusters and one had small clusters of interrenal cells. Interrenal cells from Ohio River fish were more vacuolated, ranging from slightly to highly vacuolated (Fig. 3b). Nuclei were larger and stained more indistinctly.

Small clusters of chromaffin tissue (Fig. 4a) were noted in five fish from the Cumberland River. In contrast, there appeared to be a hyperplasia of chromaffin tissue in fish from the Ohio River. At the Refuge site, one fish had a small amount of chromaffin tissue, while five had moderate to large clusters of cells (Fig. 4b). At the Falls site, three had small clusters, while seven had the moderate to large clusters.

DISCUSSION

Gonad PCB and chlordane levels

The elevated gonad PCB and chlordane concentrations observed in Ohio River paddlefish compared with Cumberland River paddlefish were not unexpected since the Cumberland River is an Ohio River tributary that has little industry and urban development associated with it. These results are consistent with previous findings of low PCB levels in Cumberland River paddlefish [9]. Because Cumberland River fish had lower gonad PCB and chlordane concentrations, this area was deemed an appropriate reference site.

The elevated PCB and chlordane concentrations seen in the testes and immature ovaries of Ohio River paddlefish probably were the result of the high lipid content of these tissues, while the lower organochlorine levels seen in paddlefish eggs can

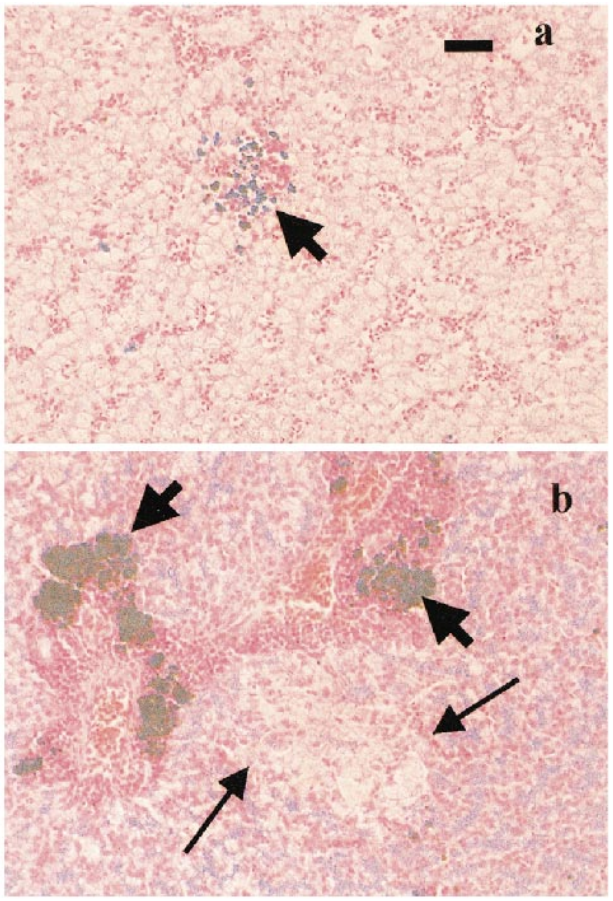

Fig. 1. Paddlefish liver tissue. (**a**) Typical liver of paddlefish from the Cumberland River. Hepatocytes are of uniform size, highly vacuolated, contain low to moderate amounts of hemosiderin, and often have eccentric nuclei. Macrophage aggregates (thick arrow) were composed of macrophages, occasionally other inflammatory cells, and varying amounts of pigment. (**b**) Liver from Falls of the Ohio River paddlefish. Hepatocytes are less vacuolated and contain moderate to large amounts of hemosiderin. Foci of highly vacuolated cells that contained little or no hemosiderin (thin arrows) were common. Scale bar = 50 μm. Perl's stain.

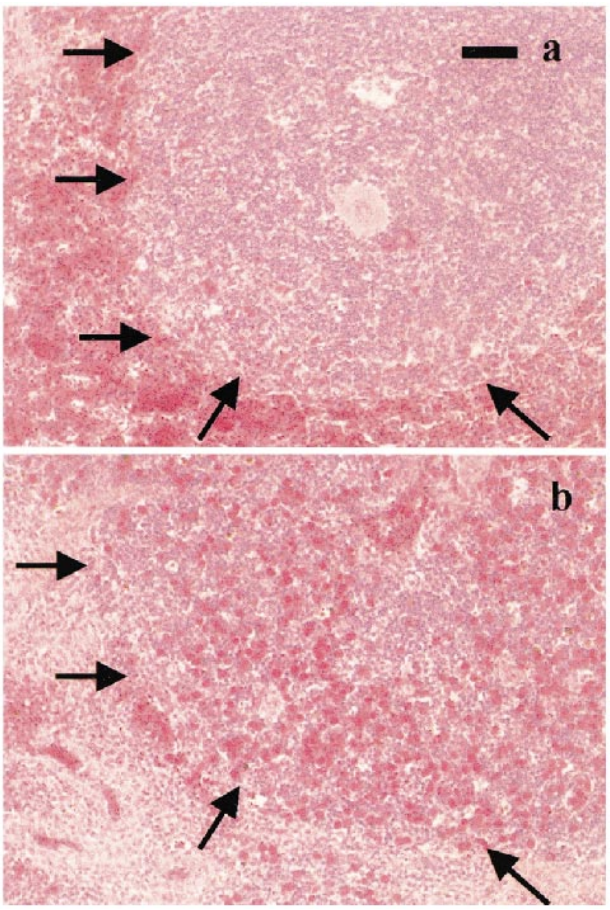

Fig. 2. Splenic tissue of paddlefish. (**a**) Typical region of white pulp (outlined by arrows) in spleen of Cumberland River fish. This region is composed primarily of lymphocytes with a few macrophages and eosinophils. (**b**) White pulp of a Refuge paddlefish. This region is filled with larger, darker staining eosinophils and is depleted of lymphocytes. Scale bar = 50 μm. Hematoxylin and eosin stain.

be attributed to the lower lipid content of these tissues. This was apparent when tissues were compared on a lipid-adjusted basis. Chlordane and PCB concentrations were similar when comparing the different reproductive tissues from each site on a lipid-adjusted basis. The female paddlefish reproductive cycle begins with a relatively small immature ovary that is associated with large fat bodies. This lipid-rich tissue likely has a high organochlorine content that becomes diluted as an egg mass develops and increases in size (1.5–4.5 kg). This large lipid reserve is used to produce an egg mass that may comprise 15 to 25% of the fish's body weight [2], resulting in lower organochlorine levels versus immature ovaries when reported on a wet weight basis. Gonad PCB and chlordane concentrations in our study are similar to levels seen in Ohio River paddlefish gonads from a previous study done in 1998 [9]. Ohio River paddlefish collected by Gundersen and Pearson in 1991 [5] had much higher egg (4.5–5.1 μg/g) and testis (5.6–23.0 μg/g) PCB concentrations than those presented here and those collected by Gundersen et al. [9]. These combined results indicate that PCB levels in the Ohio River may be declining.

The high gonad PCB and chlordane concentrations in Ohio River paddlefish were likely the result of several years of accumulation of these persistent compounds. Paddlefish examined in our study ranged from 6 to 17 years old. Paddlefish probably bioaccumulate these organochlorines, with most residue uptake resulting from their feeding strategy. Paddlefish are filter feeders, trapping zooplankton in their large close-set gill rakers. As they ingest their food, they also ingest detritus and suspended sediments, which can make up a large portion of the stomach content. We did not look at stomach contents of Ohio River paddlefish, but one study found that detritus and fine sediment made up over 50% of the stomach content of Missouri River paddlefish [14]. Since lipophilic compounds like PCBs and chlordane bind to particulate matter [15], it is possible that substantial levels of these contaminants are taken up by ingesting contaminated sediment. Another major source of contaminant uptake would be from ingestion of contaminated zooplankton. Little information exists on zooplankton surveys in the Ohio River. Previous research by Thorpe et al. [16] indicated that zooplankton richness and seasonal variation were not different among pools along the Ohio River, including Cannelton pool, which is within our study area. In addition, since paddlefish migrate in open rivers and filter feed on zooplankton indiscriminately [2], it is difficult to make comparisons on contaminant uptake between sites.

Female paddlefish may exhibit lower total organochlorine body burdens compared to males because these compounds

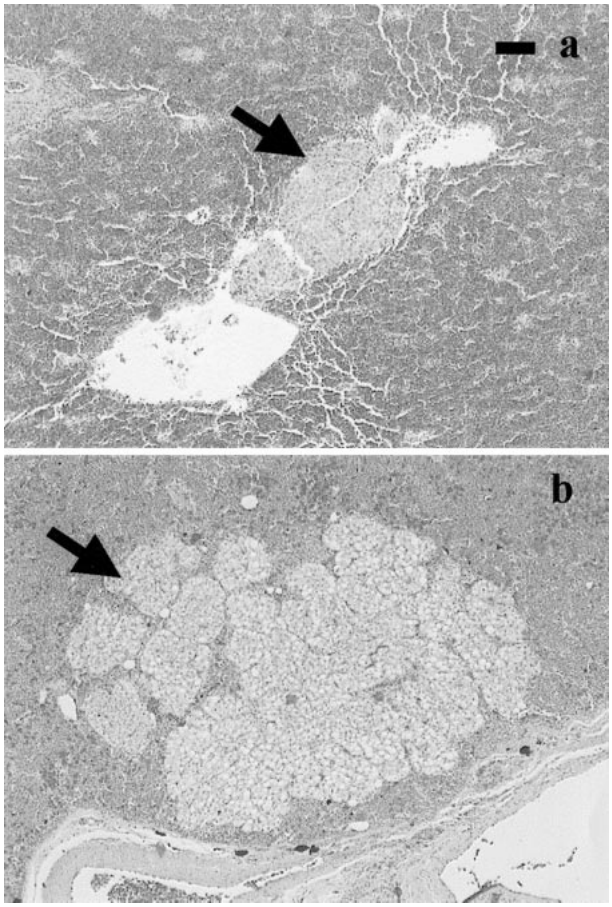

Fig. 3. Interrenal tissue of paddlefish. (**a**) Typical small foci (arrow) of interrenal tissue from Cumberland River paddlefish. (**b**) Large foci (arrow) of interrenal tissue of Refuge paddlefish. Cells are enlarged and vacuolated. Scale bar = 100 μm. Hematoxylin and eosin stain.

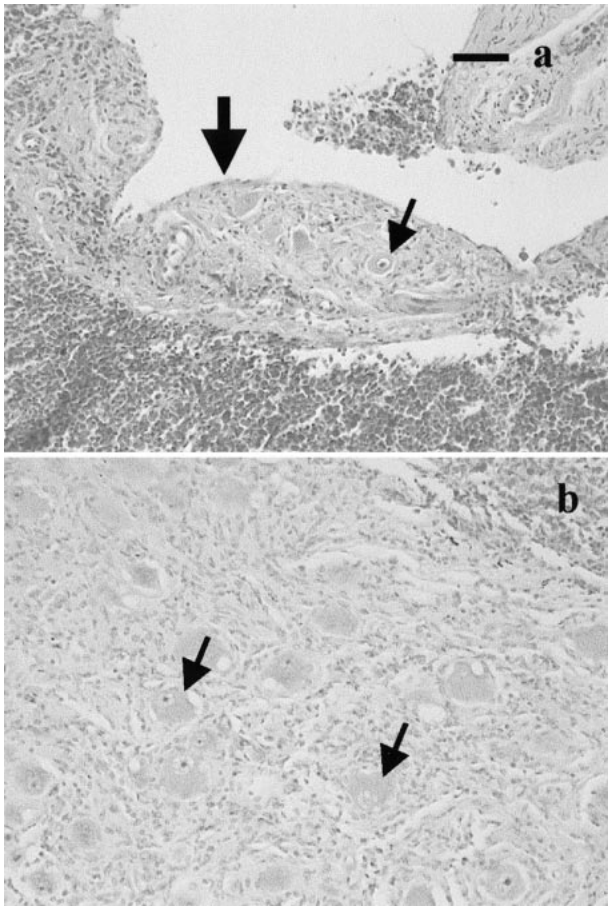

Fig. 4. Chromaffin tissue of paddlefish. (**a**) Typical chromaffin tissue (large arrow) of Cumberland River fish, composed of chromaffin cells (small arrow) within nervous tissue (large arrow). (**b**) A portion of a large nodule of chromaffin tissue from Falls fish, composed of hypertrophied chromaffin cells (arrows) and nervous tissue. Scale bar = 50 μm. Hematoxylin and eosin stain.

depurate during spawning, particularly when a female paddlefish can release between 6 to 10 lb of eggs. It is likely that a significant amount of their organochlorine body burden is passed on to their offspring. Gundersen et al. [9] reported a negative correlation between gonad PCB concentration and age in female paddlefish collected from the Falls of the Ohio River. A negative correlation was seen in our study between gonad chlordane concentration and age in Falls females when using lipid-adjusted values. Clearance of organochlorines via spawning was reported by Bengtsson [17] for minnows (*Phoxinus phoxinus*) and by Monod [6] for Lake Geneva charr (*Salvelinus alpinus*) when lipid-adjusted values were used. This depuration of organochlorines is probably insignificant in male paddlefish because there is a relatively small amount of milt released at spawning.

The PCB congeners were analyzed in male paddlefish testes alone because, at the Ohio River Islands National Wildlife Refuge, the primary emphasis of the study, only one female was collected. Paddlefish testes contained primarily tetra-, penta-, and hexachlorobiphenyls, which likely reflects the availability and persistence of these higher chlorinated congeners. Analysis of Lake Ontario smelt, *Osmerus mordax,* and Alewives, *Alosa pseudoharengus* (whole fish), showed they contained mainly tetra-, penta-, and hexachlorobiphenyls [18]. These fish feed on a zooplankton diet similar to paddlefish.

Some of the PCB congeners (87, 99, 101, 118, 138, and 153) detected in Ohio River paddlefish testes made up a high percentage of the total detectable PCBs. These congeners are considered to be potentially toxic based on their structural similarities to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and/or their ability to induce bioactivating enzyme systems [7,19]. Some of these congeners induce cytochrome P450-dependent mixed-function oxidases [20]; however, no EROD activity was detected in any of the 50 paddlefish (discussed later). Additional research should examine the effects of these congeners on paddlefish physiology.

The high concentrations of chlordane seen in the eggs of Ohio River paddlefish may pose some concern for human consumption. Since paddlefish eggs are used to produce domestic caviar and 6 out of the 10 Ohio River egg samples exceeded the Food and Drug Administration's action limit of 0.30 μg/g for chlordane, public advisories may be warranted. These results are consistent with Gundersen et al. [9], who reported that five out of six Ohio River paddlefish eggs samples exceeded the Food and Drug Administration's action limit for chlordane. These findings certainly suggest the need for inclusion of paddlefish roe in the various monitoring programs.

Paddlefish spawning

Previous studies in other species have shown that PCBs and chlordane have the ability to decrease egg hatchability [6,21–25]. Here, however, fertilization and percent hatch of

paddlefish eggs from the Falls and Cumberland River were not significantly affected by the presence of PCBs and chlordane. Falls eggs had significantly higher PCB concentrations yet percent hatch was comparable to percent hatch in Cumberland River eggs (Table 2). These results are consistent with findings by Gundersen et al. [9], who showed that there were no affects on percent hatch in Ohio River paddlefish eggs contaminated with PCB (0.27–0.80 μg/g) and chlordane (0.24–0.56 μg/g) concentrations similar to those seen in our study. Polychlorinated biphenyl egg concentrations in Falls paddlefish are lower than PCB egg concentrations of 78 and 170 μg/g (wet wt) reported to adversely affect percent hatch in minnow (*Phoxinus phoxinus*) and brook trout (*Salvelinus fomtinalis*) eggs, respectively [17,22]. Nebeker et al. [26] found that female fathead minnows (*Pimephales promelas*) containing greater than 400 μg/g of aroclor 1254 had egg hatchability similar to controls. In addition, the effects of PCBs and chlordane on egg hatchability at the concentrations observed in our study are difficult to assess because limited information exists on the combined effects of these contaminants. The lack of a significant affect on hatchability may be due in part to this not being a sensitive toxicity endpoint in paddlefish.

Plasma sex steriods

Testosterone levels seen in Refuge males were lower than in other fish examined, and there was a negative correlation between mean plasma testosterone levels and gonad chlordane and PCB concentration in Refuge males. However, it would be inaccurate to conclude that the gonad PCB and chlordane levels in Refuge fish were the only factors responsible for the low plasma testosterone levels. Nevertheless, some components of these complex chemical mixtures are suspected environmental endocrine disruptors [8]. Polychlorinated biphenyl-induced hepatic hydroxylation of testosterone and inhibition of testicular steroidogenesis has been suggested as a major mechanism leading to depression of plasma androgen levels [27]. Brook trout, *Salvelinus fontinalis*, exposed to PCB (Aroclor 1254) contaminated water for 21 d had stimulated in vitro 11 β-hydroxylation of testosterone by testicular tissue [22]. Carp, *Cyprinus carpio*, and rainbow trout, *Oncorhynchus mykiss*, injected with 25 mg/kg PCB (Aroclor 1254) showed a significant decrease in plasma androgen levels after 4 weeks [28]. Refuge males had significantly lower plasma testosterone levels than Falls males, yet fish from both sites had similar gonad PCB and chlordane levels. All Refuge and Falls males were determined to be sexually mature based on gross observations of the testes and the estimated age of the collected fish (Tables 1 and 2), but it is possible that males from the two sites were in different reproductive cycle stages. It is also possible that other contaminants may be involved. Typically, contaminant levels are highest in the upper part of the Ohio River. This is likely due to dilution by Ohio River tributaries. Polychlorinated biphenyl suspended sediment levels in the Ohio River were higher than those in all the major Ohio River tributaries from May 1988 to June 1990 [29]. Dichlorodiphenyltrichloroethane (DDT) metabolites were over 3.5-fold higher in the one Refuge fish analyzed for other organochlorines compared with a sample taken from a Falls fish. The persistent DDT metabolite *p,p′*-dichlorodiphenyldichloroethylene (DDE) has been identified as a potent environmental antiandrogen in mammals [30]. Since only one sample from each site (Falls and Refuge) was screened for other organochlorines, we cannot conclude that contaminants caused the lower testosterone levels seen in Refuge fish, particularly since several other factors may be involved.

Cytochrome P450

There was no measurable hepatic microsomal EROD activity in any of the fish collected from the Ohio and Cumberland Rivers. In addition, a Western blot analysis with rabbit antitrout antibody failed to detect a CYP1A protein in paddlefish. These findings indicated that this biomarker may not be useful for the monitoring of exposure of this species to dioxins, organochlorines, and polyaromatic hydrocarbons. Sex steroids, particularly estradiol, have been reported to influence monooxygenase activity in fish [31,32]. Since most fish collected were in prespawning conditions, it is possible that this could have contributed to the lack of expression of EROD activity. However, it is difficult to conclude that plasma sex steroids were responsible for the lack of EROD activity in all 50 fish. Some males and females had considerably lower estradiol levels (54–184 pg/ml) than the other fish (250–402 pg/ml).

In an ongoing study in our laboratory (D.G. Gundersen, personal communication), juvenile paddlefish (second year class) were injected with 5, 10, 20, and 40 mg/kg β-naphthoflavone, a known inducer of EROD in a number of species, and EROD activity was measured after 48 h. None of the treated fish had measurable EROD activity. Similarly, in a study that examined 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-contaminated Northern squawfish (*Ptychoceilus oregonensis*), Curtis et al. [33] reported little or no EROD activity in this species.

Histology

A number of microscopic differences were observed between paddlefish collected in the Cumberland River and those collected in the Ohio River. Unfortunately, there is very little information available on the normal histologic appearance of paddlefish organs and almost no information available on the response of various organs to toxicant exposure. The microscopic anatomy of paddlefish differed from most teleosts in a number of ways. First, the spleen had well-defined white and red pulp, as described for sturgeons [34], but no macrophage aggregates, which are usually found in the splenic tissue of teleosts. Rather, foci of macrophages, lymphocytes, and sometimes eosinophils were found in the liver in association with clusters of pigment-containing macrophages. The three pigments—hemosiderin, ceroid/lipofuscin, and melanin—seen in other fish macrophage aggregates were observed within these pigmented macrophages. In teleosts, the interrenal and chromaffin tissues are concentrated mainly in the anterior portion of the kidney, while in paddlefish, it was dispersed throughout the length of the kidney. These findings were in agreement with those of Rahn [35], who reported interrenal tissue throughout the kidney of paddlefish.

Many of the histologic changes we observed in paddlefish from the Ohio River have been previously documented as responses to contaminants in other fishes. The liver is important in normal digestion and storage of lipid and glycogen, xenobiotic metabolism and excretion, and production of yolk protein. For these reasons, histopathologic changes that serve as biomarkers in the liver have received much attention. In higher teleosts, two types of hepatic changes, hepatocellular steatosis or fatty change and hepatocellular hemosiderosis [36–

38], are found in fish collected from contaminated sites. These intracytoplasmic storage disorders suggest altered metabolism in these fish. Hepatocytes of Cumberland River fish were highly vacuolated, probably as a consequence of the liver being very important in lipid and glycogen storage. Livers of Ohio River paddlefish had less vacuolization and greater amounts of hemosiderin. There were focal areas of more vacuolated cells, which resembled the clear or vacuolated altered cell foci in other fishes. As in altered cell foci from English sole [36], the foci in paddlefish showed a marked decrease in hemosiderin (Fig. 3b). Steatosis/hemosiderosis of sole have shown consistent, statistically significant associations with polycyclic aromatic hydrocarbons in bottom sediments [39]. The histopathologic changes seen in Ohio River paddlefish liver may be associated with the higher levels of organochlorines seen in these fish versus Cumberland River paddlefish.

Increases in macrophage aggregate number/density have been noted in numerous fish species collected at contaminated sites versus those collected at reference sites [40]. Splenic and hepatic macrophage aggregates of centrarchids were more prominent at sites in Tennessee containing high levels of PCBs in sediment and biota when compared with a reference site [41]. Because this lymphoid depletion was noted only at the contaminated sites, it was believed to be related to contaminant exposure [41]. Both findings have previously been reported to relate to immunosuppression or potential defects in disease resistance [42]. However, further studies on infectious disease prevalence, functional assessment of lymphocytes, macrophages, and the specific immune response would be necessary to determine if paddlefish from the Ohio River are indeed immunosupressed.

Although the stress response, specifically circulating cortisol, has been examined in contaminant-related studies, few investigators have examined interrenal or chromaffin tissue histologically. Interrenal cells are homologous to the adrenal cortex of higher vertebrates and are responsible for the synthesis of corticosteroids, primarily cortisol. Chromaffin tissue is equivalent to adrenal medulla and contains the catecholamines epinephrine (adrenaline), norepinephrine (noradrenalin), and dopamine [43]. Bromage and Fuchs [44] found an increase in cell size, nuclear diameter, and nucleolar size and number in goldfish exposed to sodium lauryl sulphate. Degenerative changes were found after exposure to higher concentrations (10 and 15 mg/L) and to two levels (2 and 3 mg/L) of zinc sulfate. They also report an apparent reduction in the thickness of the layers of interrenal cells around veins and the number of areas of interrenal cells [44]. Ram and Singh [45] found initial hypertrophy of cells followed by degeneration in interrenal and chromaffin cells of *Channa punctatus* exposed to ammonium sulfate. Donaldson et al. [46] reported an increased interrenal nuclear diameter in salmonids exposed to a variety of contaminants. In paddlefish, we observed some hypertrophy of interrenal cells. More importantly, however, there was an apparent hyperplasia or increased cell number in interrenal tissue and to a greater extent in chromaffin tissue. There appeared to be more foci of these cells and the foci were larger in Ohio River paddlefish. This is interesting in that PCBs have been shown to lead to dopamine depletion and neurotoxicity in other animals [47]. We were concerned that this response may have occurred in response to transport of selected fish for spawning. However, there was no correlation between increased interrenal or chromaffin tissue and transport. In addition, Barton et al. [48] found that paddlefish exhibit a much lower physiological stress response to physical disturbances than those documented for many teleost fishes. Effects of contaminants on endocrine systems in fish have primarily centered on reproductive hormone disorders; however, perhaps more research should be directed toward examining the ability of these chemicals to exert neuroendocrine effects.

In summary, gonad PCB and chlordane levels were relatively high in Ohio River paddlefish, particularly in the testes of male fish, which was correlated with the higher lipid content of these tissues. Egg chlordane levels were higher than the Food and Drug Administration's action limit for chlordane in 5 out of 10 Ohio River egg samples. Polychlorinated biphenyl congener-specific analysis of Ohio River paddlefish testes showed that these tissues largely consisted of the tetra-, penta-, and hexachlorobiphenyls. Some of these congeners have been shown to induce hepatic microsomal EROD activity in other fish species, but we did not detect EROD activity in any of the 50 fish collected. This finding was substantiated by the lack of cross-reactivity between a rabbit antitrout CYP1A1 IgG antibody and a CYP1A protein in paddlefish microsomes. CYP1A does not appear to be a useful biomarker for contaminant exposure in this species. Percent hatch of fertilized paddlefish eggs from the Ohio River was good (90–95%), indicating that the PCB (0.38–0.93 μg/g) and chlordane (0.18–0.38 μg/g) levels in these eggs did not significantly affect this reproductive parameter. Plasma testosterone levels were significantly lower in males collected from the Refuge site compared with males collected from the lower part of the river. These significantly lower testosterone levels may be due to the presence of other contaminants in the upper part of the Ohio River. Histologic examination of paddlefish tissues (liver, spleen, and kidney) suggests that Ohio River paddlefish could experience chronic health problems due to immunosuppression and altered hepatic and neuroendocrine (adrenal) function. Continued investigations on Ohio River paddlefish health are warranted, particularly since the status of Ohio River paddlefish populations is questionable. Continued monitoring of edible tissues (roe and fillets) for persistent contaminants to establish health-based consumption advisories is also recommended since paddlefish are a long-lived species that have the potential to accumulate significant levels of organochlorine contaminants.

Acknowledgement—This project was funded by the U.S. Fish and Wildlife Service, grant 14-48-0003-97-966. We thank E. Hermel, J.J. Brokaw, and H. Carpenter for their critical comments of the manuscript.

REFERENCES

1. Vasetskiy SG. 1971. Fishes of the family Polyodontidae. *J Ichthyol* 11:18–31.
2. Russell TR. 1986. Biology and life history of the paddlefish—A review. In Dillard JG, Graham LK, Russel TR, eds, *The Paddlefish: Status, Management and Propagation.* Special Publication 7. American Fisheries Society, Bethesda, MD, USA, pp 2–21.
3. Graham KL, Rasmussen JL. 1998. A MICRA perspective on closing paddlefish and sturgeon commercial fisheries. *Proceedings,* Harvest, Trade and Conservation of North American Paddlefish and Sturgeon, North American World Wildlife Fund, Chattanooga, TN, USA, May 7–8, pp 130–142.
4. U.S. Fish and Wildlife Service. 1993. Concentrations of dioxin, organochlorines, and trace elements in freshwater mussels and fish from the Ohio River at Apple Grove, West Virginia. Special Project Report 93-3. West Virginia Field Office, Elkins, WV.
5. Gundersen DT, Pearson WD. 1992. Partitioning of PCBs in the muscle and reproductive tissues of paddlefish, *Polyodon spathula,*

at the Falls of the Ohio River. *Bull Environ Contam Toxicol* 49: 455–462.
6. Monod G. 1985. Egg mortality of Lake Geneva charr, *Salvelinus alpinus* L., contaminated by PCB and DDT Derivatives. *Bull Environ Contam Toxicol* 35:531–536.
7. McFarland VA, Clarke JU. 1989. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: Considerations for a congener-specific analysis. *Environ Health Perspect* 81:225–239.
8. Keith LH. 1997. *Environmental Endocrine Disrupters: A Handbook of Property Data.* John Wiley & Sons, New York, NY, USA.
9. Gundersen DT, Krahling MD, Donosky JJ, Cable RG, Mims SD. 1998. Polychlorinated biphenyls and chlordane in the gonads of paddlefish, *Polyodon spathula,* from the Ohio River. *Bull Environ Contam Toxicol* 61:650–652.
10. Prough RA, Borke MD, Mayer RT. 1978. Direct fluorometric methods for measuring mixed-function oxidase activity. *Methods Enzymol* 52:372–377.
11. Lowry OH, Rosebrough NJ, Fan AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275.
12. Luna LG. 1992. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. American Histolabs, Gaithersburg, MD, USA.
13. Hinton DE, Baumann PC, Gardner GR, Hawkins WE, Hendricks JD, Murchelano RA, Okihiro MS. 1992. Histopathological biomarkers. In Huggett RJ, Kimerle RA, Mehrle PM, Bergman HL, eds, *Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress*. Lewis, Boca Raton, FL, USA, pp 155–209.
14. Rosen RA, Hales DC. 1981. Feeding of paddlefish, *Polyodon spathula*. *Copeia* 2:441–455.
15. Hansen LG. 1994. Halogenated aromatic compounds. In Cockerham LG, Shane BS, eds, *Basic Environmental Toxicology*. CRC, Boca Raton, FL, USA, pp 199–230.
16. Thorpe JH, Black AR, Haag KH, Wehr JD. 1994. Zooplankton assemblages in the Ohio River: Seasonal, tributary, and navigation dam effects. *Can J Fish Aquat Sci* 51:1634–1643.
17. Bengtsson BE. 1980. Long-term effects of PCB (Clophen A50) on growth, reproduction and swimming performance in the minnow, *Phoxinus phoxinus. Water Res* 14:681–687.
18. Oliver BG, Niimi AJ. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. *Environ Sci Technol* 22: 388–397.
19. Da Costa EG, Curtis LR. 1995. Bioaccumulation of dietary 2,2′, 4,4′,5,5′-hexachlorobiphenyl and induction of hepatic arylhydrocarbon hydroxylase in rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 14:1711–1717.
20. Janz DM, Metcalfe DC. 1991. Nonadditive interactions of mixtures of 2,3,7,8-TCDD and 3,3′,4,4′-tetrachlorobiphenyl on aryl hydrocarbon hydroxylase induction in rainbow trout (*Oncorhynchus mykiss*). *Chemosphere* 23:467–472.
21. Örn S, Andersson PL, Förlin L, Tysklind M, Norrgren L. 1998. The impact on reproduction of an orally administered mixture of selected PCBs in zebrafish (*Danio rerio*). *Arch Environ Contam Toxicol* 35:52–57.
22. Freeman HC, Ider DR. 1975. The effect of polychlorinated biphenyls on steroidogenesis and reproduction in the brook trout, *Salvelinus fontinalis*. *Can J Biochem* 53:666–670.
23. Broyles RH, Noveck MI. 1979. Uptake and distribution of 2,5,2′,5′-tetrachlorobiphenyl in developing lake trout. *Toxicol Appl Pharmacol* 50:291–298.
24. Parrish PR, Dyar EE, Enos JM, Wilson WG. 1978. Chronic toxicity of chlordane, trifluralin and pentachlorophenol to sheepshead minnows, *Cyprinodon variegatus.* EPA/600/13-78-010. U.S. Environmental Protection Agency, Cincinnati, OH.
25. Cardwell RD, Foreman DG, Payne TR, Wilbur DJ. 1977. Acute and chronic toxicity of chlordane to fish and invertebrates. EPA/600/13-77-019. U.S. Environmental Protection Agency, Duluth, MN.
26. Nebeker AV, Puglishi FA, DeFoe DL. 1974. Effect of polychlorinated biphenyl compounds on survival and reproduction of the fathead minnow and flagfish. *Trans Am Fish Soc* 3:562–568.
27. Machala M, Neca J, Drabek P, Ulrich R, Sabatova V, Nezveda K, Raszyk J, Gajduskova V. 1998. Effects of chronic exposure to PCBs on cytochrome P450 systems and steroidogenesis in liver and testis of bulls (*Bos taurus*). *Comp Biochem Physiol A* 120: 65–70.
28. Sivarajah K, Frankling CS, Williams WP. 1978. The effects of polychlorinated biphenyls on plasma steroid levels and hepatic microsomal enzymes in fish. *J Fish Biol* 13:401–409.
29. Rostad CE, Bishop LM, Ellis GS, Leiker TJ, Monsterleet SG, Pereira WP. 1995. Organic contaminant data for suspended sediment from the Mississippi River and some of its tributaries. Open-File Report 93-360. U.S. Geological Survey, Reston, VA.
30. Kelce WR, Stone CR, Laws SC, Gray LE, Kemppainen JA, Wilson EM. 1995. Persistent DDT metabolite *p,p′*-DDE is a potent androgen receptor agonist. *Nature* 375:581–585.
31. Förlin L, Lidman U. 1981. Effects of Clophen A50 and 3-methylcholanthrene on the hepatic mixed function oxidases in rainbow trout. *J Endocrinol* 95:245–252.
32. Förlin L, Andersson T, Koivusaari U, Hansson T. 1984. Influence of biological and environmental factors on hepatic steroid and xenobiotic metabolism in fish: Interaction with PCB and β-naphthoflavone. *Mar Environ Res* 14:47–58.
33. Curtis LR, et al. 1993. Sensitivity of cytochrome P450-1A1 induction in fish as a biomarker for distribution of TCDD and TCDF in the Willamette River, Oregon. *Environ Sci Technol* 27:2149–2157.
34. Fange R. 1986. Lymphoid organs in sturgeon (*Acipenseridae*). *Vet Immunol Immunopathol* 12:153–161.
35. Rahn AB. 1997. The distribution, ultrastructure, and steriodogenic activity of interrenal tissue of paddlefish, *Polyodon spathula.* MA thesis. Department of Biology, University of South Dakota, Vermillion, SD, USA.
36. Myers MS, Rhodes LD, McCain BB. 1987. Pathologic anatomy and patterns of occurrence of hepatic neoplasms, putative preneoplastic lesions, and other idiopathic hepatic conditions in English sole (*Parophyrs vetulus*) from Puget Sound, Washington. *J Natl Cancer Inst* 78:333–351.
37. Bucke D, Feist SW. 1993. Histopathological changes in the livers of dab, *Limanda limanda* (L). *J Fish Dis* 16:281–296.
38. Thiyagarajah A, Hartley WR, Abdelghani A. 1998. Hepatic hemosiderosis in buffalo fish (*Ictiobus* spp.). *Mar Environ Res* 46: 203–207.
39. Landahl JT, McCain BB, Myeres MS, Rhodes LO, Brown DW. 1990. Consistent association between lesions in English sole (*Parophrys vetulus*) and polycyclic aromatic hydrocarbons in bottom sediment. *Environ Health Perspect* 89:195–203.
40. Blazer VS, Fournie JW, Weeks-Perkins BA. 1997. Macrophage aggregates: Biomarker for immune function? In Dwyer FJ, Doane TR, Hinman ML, eds, *Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment,* Vol 6. STP 1317. American Society for Testing and Materials, Conshohocken, PA, pp 360–375.
41. Teh SJ, Adams SM, Hinton DE. 1997. Histopathologic biomarkers in feral freshwater fish populations exposed to different types of contaminant stress. *Aquat Toxicol* 37:51–70.
42. Weeks BA, Anderson DP, Dufour AP, Fairbrother A, Govern AJ, Lahvis GP, Peters G. 1992. Immunological biomarkers to assess environmental stress. In Huggett RJ, Kimerle RA, Mehrle PM, Bergman HL, eds, *Biomarkers: Biochemical, Physiological and Histological Markers of Anthropogenic Stress.* Lewis, Boca Raton, FL, USA, pp 211–234.
43. Pickering AD. 1993. Endocrine-induced pathology in stressed salmonid fish. *Fish Res* 17:35–50.
44. Bromage NR, Fuchs A. 1976. A histologic study of the response of the interrenal cells of the goldfish (*Carassius auratus*) to treatment with sodium lauryl sulphate. *J Fish Biol* 9:529–535.
45. Ram RN, Singh SK. 1988. Long-term effect of ammonium sulfate fertilizer on histopathology of adrenal in the teleost *Channa punctatus* (Bloch). *Bull Environ Contam Toxicol* 41:880–887.
46. Donaldson EM, Fagerlund UHM, McBride JR. 1984. Aspects of the endocrine stress response to pollutants in salmonids. In Cairns VW, Hodson PV, Nriagu JR, eds, *Contaminant Effects in Fisheries.* John Wiley & Sons, New York, NY, USA, pp 213–221.
47. Shain W, Bush B, Seegal R. 1991. Neurotoxicity of polychlorinated biphenyls: Structure–activity relationships of individual congeners. *Toxicol Appl Pharmacol* 111:33–42.
48. Barton BA, Rahn AB, Feist G, Bollig H, Schreck CB. 1998. Physiological stress responses of the freshwater chondrostean paddlefish (*Polyodon spathula*) to acute physical disturbances. *Comp Biochem Physiol A* 120:355–363.