Bull. Environ. Contam. Toxicol. (1992) 49:455–462

Environmental Contamination and Toxicology

Partitioning of PCBs in the Muscle and Reproductive Tissues of Paddlefish, *Polyodon spathula*, at the Falls of the Ohio River

Deke T. Gundersen[1] and William D. Pearson[2]

[1]Department of Fisheries and Wildlife, Oak Creek Laboratory of Biology, Oregon State University, Corvallis, Oregon 97330, USA and [2]Water Resources Laboratory, University of Louisville, Louisville, Kentucky 40292, USA

The paddlefish, Polyodon spathula, is a primitive fish characterized by a predominantly cartilaginous skeleton, a virtually scaleless body, and a rostrum or paddle nearly one-third of the body length. Presently the American Fisheries Society presents the paddlefish on their 1989 list of rare North American fishes as a fish "of special concern" (Williams et al. 1989). At the Falls of the Ohio River near Louisville, Kentucky, paddlefish are harvested for their valuable roe which is used to produce domestic caviar. Over the years prices paid to fishermen for paddlefish roe have ranged from $26-110/kg (Bronte and Johnson 1985; Hoffnagle and Timmons 1989). Pearson and Pearson (1989) have suggested that although paddlefish populations in the Ohio River appear to have increased since 1970, the persistence of this small but intensive fishery for the roe of female paddlefish may be slowing or even reversing the recovery of populations in the Falls area of the Ohio River.

Residues of toxic substances that are harmful to humans are being detected in many fishes in the Ohio River. Organochlorine pollutants such as chlordane and polychlorinated biphenyls (PCBs) are being detected at levels as high as those of 10 yr ago. PCBs have been quantified in excess of the Food and Drug Administration's (FDA) action limit of 2 ug/g in 31% of the channel catfish samples reported by the Ohio River Valley Water Sanitation Commission (ORSANCO) between 1978 and 1986 (ORSANCO 1987). Levels of PCBs exceeding the FDA's action limit are still being consistently detected along the Ohio River. Recent data from studies conducted in the Missouri River on PCB levels in the tissues of the shovelnose sturgeon, a species closely related to the paddlefish, indicate that the concentration of PCBs in sturgeon roe is several times that of concentrations in flesh (Jerry J. Presley, Personal Communication).

Send reprint requests to William D. Pearson at above address.

Therefore we decided to examine the PCB content of the roe of paddlefish in the Ohio River at a location where a commercial fishery for these species existed. For this study we collected male and female paddlefish from the Falls of the Ohio River (38° 17'N, 85° 47'W) over a 2 yr period. The study was designed 1) to investigate the partitioning of PCBs into the muscle and reproductive tissues of paddlefish, 2) to determine possible correlations between PCB concentration and age, and PCB concentration and percent lipid, and 3) to determine the existence of any differences in mean PCB concentrations between male and female paddlefish.

MATERIALS AND METHODS

Paddlefish were collected from the Falls of the Ohio River between May and June of 1988, and June through August 1989. In 1988, 16 paddlefish were collected (10 males and 6 females). In 1989, 17 paddlefish were collected (9 males and 8 females). Immediately after capture all fish were measured for standard and total lengths. Fillets and gonads were removed, wrapped in aluminum foil and frozen at -5°C for later PCB residue analysis.

Age determinations using dentary bones were carried out in a manner similar to the procedure described by Adams (1942). Approximately 15 cross sections were made from each dentary bone at the point where it bends mesially. They were mounted on glass slides with epoxy resin and ground with various polishing stones, until growth rings could be seen clearly. Sections were examined under a dissection microscope with xylene added to enhance the definition of growth rings.

Extraction and cleanup procedures for all tissue samples were based on those described by Ruckriegel (1979). Frozen samples (skinless white and red muscle, and gonads) were homogenized separately in a Waring blender. Ten gram sub-samples of homogenate were combined with 50 g of anhydrous sodium sulfate and ground into a fine powder using a mortar and pestle. The dried samples were extracted for 10 hr in a Soxhlet extraction apparatus using 170 mL of a 1:1 solvent of petroleum ether and hexane (v/v spectral grade). The extract was collected in a tared 250 mL flask and evaporated to dryness on top of a moderately heated water bath under a gentle stream of pure nitrogen gas. Lipid weight was determined by re-weighing the tared flask until a constant weight was obtained. The extract (120 mg lipid) was subjected to liquid chromatography cleanup and fractionation using

columns layered with partially deactivated silica (bottom layer) and alumina (top layer). PCBs were eluted with 170 mL of spectral grade hexane.

Gas chromatography analysis was performed using a Varian 3700 gas chromatograph equipped with a Ni^{63} electron capture detector and a Hewlett-Packard integrator model HP 3394A. Glass megabore columns were supplied by Supelco and packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport. The carrier gas was argon methane (95%/5%) with a flow rate of 60 mL/min. The temperatures of the injector and detector were 260 ^{o}C and 220 ^{o}C. For PCB determinations, a standard containing the PCB mixture Aroclor 1260, which most resembled tissue extract chromatographs, was used for quantitation. Quality assurance measures included the analysis of reagent blanks, duplicates, and spiked samples. Average percent recovery in spiked samples ranged between 90-100%, so sample extracts were not corrected for percent recovery.

Data were analyzed statistically by using a PC-SAS computing system. Pearson's correlation coefficient was used to determine closeness of linear relationships between age and PCB concentration and percent lipid and PCB concentration. Differences in sample means were computed using a students t-test (SAS Institute Inc. 1986).

RESULTS AND DISCUSSION

A total of 33 paddlefish were collected from the Falls of the Ohio River, the majority of which ranged between 6 and 12 yr of age. This reported range appears to be somewhat low. Russell (1986), reported that where paddlefish populations are sustained by sexually mature adults, the majority of the fish harvested ranged from 7-18 yr of age. Bronte and Johnson (1985) also reported a high frequency of younger fish being collected from an exploited population, suggesting that through commercial and sport fishing, many individuals are harvested before being able to contribute to reproduction. This could certainly be the case for paddlefish populations in the Falls area of the Ohio River, where commercial fishermen have been observed fishing for paddlefish with large mesh gill nets. In addition to this, some paddlefish and paddlefish eggs have been stranded during rapid closures of McAlpine Dam at the head of the Falls area of the Ohio River, particularly when these closures occur during the spawning season (Pearson and Froedge 1989).

PCB levels (expressed on a wet weight basis) for paddlefish fillets ranged from 0.05-3.35 ug/g (Table 1). These findings are comparable to those of other studies on other species of Ohio River fishes by Ruckriegel (1979) and ORSANCO (1987) who reported ranges between 0.20-2.80 ug/g and 0.01-4.60 ug/g, respectively. The FDA action limit was exceeded in just one paddlefish fillet. However, all fillets analyzed were carefully trimmed of skin, excess fat, and red muscle, all tissues which are high in lipids and might, therefore, contain relatively high concentrations of PCBs. It was determined that the red muscle of paddlefish, when analyzed separately, had a statistically significant ($p<0.05$) higher mean PCB concentration (4.24 ug/g) than the mean PCB concentration (0.70 ug/g) of white muscle tissue analyzed from the same fish (Table 1). The lipophilic nature of PCBs probably accounted for these high PCB concentrations since the red muscle of paddlefish also had a high percent lipid content (mean = 22.07%).

Table 1. Summary statistics of PCB concentrations (ug/g wet weight, as Aroclor 1260), and percent lipid in all tissue analyses of paddlefish from the Falls of the Ohio River near Louisville, Kentucky

			Age	PCB concentration ug/g wet weight		% Lipid	
Tissue	Sex	N	(mean/range)	(mean/range)	SD	(mean/range)	SD
Gonads	F	12	11.0 3.0-17.0	7.3 0.05-18.70	4.66	43.6 1.07-72.00	24.84
	M	5	9.0 6.0-17.0	16.2 5.63-23.00	7.57	54.8 26.00-49.00	21.54
White Muscle	F	13	11.5 3.0-17.0	0.4 0.05-1.03	0.36	3.2 0.20-7.50	2.86
	M	19	9.6 6.0-18.0	0.7 0.05-3.35	0.80	2.9 0.29-8.70	2.85
Red Muscle	F	4	11.2 7.0-16.0	4.2 1.98-6.30	11.47	22.1 8.50-36.40	11.47

Sheridan (1988), reported that of the two muscle types in fish (red and white), red muscle stores more lipid than white. Sanders and Haynes (1988), found that trimming fillets of all excess fat can reduce PCB concentrations by as much as 49%. Studies done on PCB concentrations in the fillets of shovelnose sturgeon Scaphirhynchus platorynchus in the Missouri River report a range between 0.05-1.80 ug/g (total PCBs as

Aroclor 1260), with none of the PCB levels exceeding the FDA's action limit (Presley Personal Communication). These results are similar to ours, and are of special interest due to the similarities and close relationship between paddlefish and shovelnose sturgeon.

The highest PCB concentrations in fish are usually found in tissues of high lipid content (Smith 1988). This is also true for Ohio River paddlefish since the high mean percent lipid value found in reproductive tissues (46.88%), was accompanied by equally high mean PCB concentrations (Table 1). Presley (Personal Communication), found the same to be true in shovelnose sturgeon where both mean percent lipid values and PCB concentrations in reproductive tissues were more than two times the values in muscle tissues.

The immature ovaries removed from paddlefish for PCB analysis were highly convoluted organs containing numerous fat bodies. Mature ovaries (roe) removed for PCB analysis had no visible fat bodies and the majority of the ovarian tissue consisted of eggs. Findings on PCB levels in the ovaries of Ohio River paddlefish (Table 1), appear to be somewhat high compared to other studies. Presley (Personal Communication) reported mean PCB levels in the roe of shovelnose sturgeon of 1.93 ug/g, substantially lower than our findings.

This may be due in part to the fact that the female paddlefish we collected from the Ohio River were much larger and older than the female shovelnose sturgeon collected by Presley in the Missouri River. This would allow a longer time for paddlefish to collect PCBs from their environment. In addition, the spawning interval of female paddlefish is 2 yr or more, versus a presumed annual spawning period for shovelnose sturgeon.

Three of the female paddlefish collected contained mature ovaries (roe). PCB levels in the mature roe of paddlefish were lower than those in immature ovaries. This appears to be due to a change in lipid concentration, in which ovaries are substantially larger, and percent lipid values are considerably lower compared to immature ovaries, which are small but contain higher concentrations of lipids. The mean value for PCB levels in the gonads of male paddlefish (Table 1), was also high. Again these high PCB levels appear to be due to the high percent lipid values (range 26.00-81.00%) and the age of the fish analyzed (6-17 yr old).

Students t-test ($p<0.05$) performed on mean PCB concentrations in the gonads of male and female paddlefish revealed that mean PCB concentrations in the testes of males were significantly higher than mean concentrations found in ovaries of females. In addition to this, a significant positive correlation ($p<0.05$, $r^2=0.42$) was found between age and PCB concentration in the white muscle tissue of males. These results may be explained by considering that the weight of milt passed by spawning males is substantially less than the weight of roe produced by similar-sized females. Bengtsson (1978) reported a similar finding, stating that spawning in male fish is a less important factor in reducing PCB body burdens, based on the relative weights of milt and egg production. Monod and Keck (1982) found that the influence of age on PCB accumulation was more important in male fish and attributed this to the above differences between male and female fish in the amount of PCBs eliminated during spawning.

A positive correlation (Figure 1) was found between percent lipid and PCB concentration for all paddlefish tissues analyzed. Due to the lipophilic nature of PCBs one would expect to see a positive correlation between percent lipid and PCB concentration. Smith (1988) pointed out that significant PCB-lipid correlations are generally found in fish from large, highly-industrialized river systems similar to the Ohio River. Accumulation of PCBs in individual fish occupying a habitat downstream from the source is driven by the fairly homogeneous and unidirectional pulse of PCBs which can produce concentrations that are closely correlated with the lipid content of the individual fish.

Findings of high PCB levels (>4.0 ug/g), in all mature roe samples from paddlefish would certainly warrant advisories to the general public about consuming this domestic caviar. These high PCB levels in the reproductive tissue of paddlefish not only pose a human health risk but could also be potentially dangerous for the continued existence of paddlefish in the Ohio River. Elevated levels of PCBs in the gonads of various species of fish may result in poor reproductive success. Monod (1985) found PCB levels varying from 7.70 to 34.00 ug/g (lipid weight) in the eggs of Lake Geneva char correlated with a mortality rate of 29 to 100%. Our findings of PCBs in the roe of paddlefish ranged from 15.97 to 42.99 ug/g (lipid weight), which is higher than Monod's reported values. Paddlefish may take up PCBs either directly from the water through the gills and other body surfaces, or from food consumed. Since paddlefish are filter

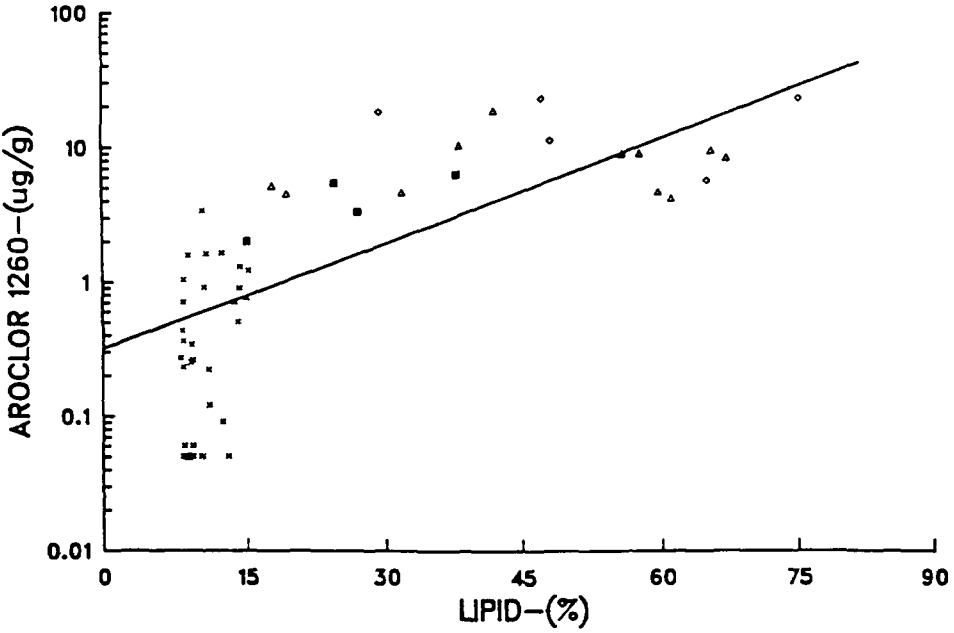

Figure 1. Correlation between PCB concentration and percent lipid in the muscle and reproductive tissues of paddlefish from the Falls area of the Ohio River near Louisville Kentucky (x = white muscle, squares = red muscle, diamond = testes, and triangles = ovaries; $r^2=0.53$, $p<0.05$).

feeders and pass relatively large amounts of water across their gills in search of food (zooplankton) one might expect water to be the main uptake route of PCBs. Rosen and Hales (1981) found detritus and sand averaging over 50% of the stomach contents of paddlefish from the Missouri River. Since PCBs tend to adsorb onto sediment organic matter, it is possible that this is one major uptake route of PCBs in paddlefish. Although stomach contents of Ohio River paddlefish were not examined it is probable that the accumulation of PCBs is from both the water and the diet. Future investigations should determine the relative importance these three pathways, as well as the source of PCBs in paddlefish.

REFERENCES

Adams LA (1942) Age determination and rate of growth in Polyodon spathula, by means of the growth rings of the otoliths and dentary bone. Amer Midl Nat 28:617-630

Bengtsson BE (1978) Long-term effects of PCB (Clophen A50) on growth, reproduction and swimming performance in the minnow, Phoxinus phoxinus. Wat Res 14:681-687

Bronte CR, Johnson DW (1985) Growth of paddlefish in two mainstream reservoirs with reference to commercial harvest. Trans KY Acad Sci 46:28-32
Hoffnagle TL, Timmons TJ (1989) Age growth and catch analysis of the commercially exploited paddlefish population in Kentucky Lake, Kentucky-Tennessee. N Amer J Fish Mngt 9:316-326
Monod G, Keck G (1982) PCBs in Lake Geneva (Lake Leman) fish. Bull Environ Contam Toxicol 29:570-576
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
ORSANCO (1987) The presence of toxic substances in the Ohio River, Executive summary. Tech Comm Meeting 13 May, 1987. Cincinnati, Ohio 5pp
Pearson WD, Froedge MA (1989) Stranding of fishes below McCalpine Dam on the Ohio River. Trans KY Acad Sci 50:183-201
Pearson WD, Pearson J (1989) Fishes of the Ohio River. Ohio J Sci 89:181-187
Rosen RA, Hales DC (1981) Feeding of paddlefish Polyodon spathula. Copeia 2:441-455
Ruckriegel MJ (1979) Bioaccumulation of PCBs in Ohio River fish. Unpubl M Eng Thesis, Univ of Louisville. Louisville, Kentucky 48 pp
Russell TR (1986) Biology and life history of the paddlefish-a review. In: Dillard JG, Graham LK, Russell TR (eds) The paddlefish: status, management and propagation. Amer Fish Soc spec publ 7:2-21
Sanders M, Haynes BL (1988) Distribution pattern and reduction of polychlorinated biphynels (PCB) in bluefish Pomatomus saltatrix (Linnaeus) fillets through adipose tissue removal. Bull Environ Contam Toxicol 41:670-677
SAS Institute (1986). SAS/STAT Guide for personal computers, version 6 Cary North Carolina: SAS Institute Inc. 378 pp
Sheridan MA (1988) Lipid dynamics in fish: aspects of adsorption, transportation, deposition, and mobilization. Comp Biochem Physiol 90B:679-690
Smith CL ed. (1988) Fisheries research in the Hudson River: Hudson River Environmental Society. State University of New York Press, Albany, N.Y.
Williams JE, Johnson JE, Hendrickson DA, Contreras Balderas S, Williams JD, Navarro-Mendoza M, McAllister DE, Deacon JE (1989) Fishes of North America endangered, threatened, or of special concern. Fisheries 14:2-20

Received July 9, 1991; accepted May 1, 1992.