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Using Blood Plasma for Monitoring Organochlorine Contaminants in Juvenile White Sturgeon, *Acipenser transmontanus*, from the Lower Columbia River

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Abstract Organochlorine (OC) pesticide concentrations in blood plasma samples from 88 juvenile white sturgeon collected from the lower Columbia River were measured and compared to plasma sex steroid and OC tissue levels previously measured in corresponding fish. Significant squared correlation coefficients between $\sum$ DDT concentrations in sturgeon plasma and gonads and livers were 0.37 and 0.32, respectively. Significant negative correlations between plasma testosterone concentration and plasma $\sum$ DDT concentration in male fish ($r^2 = 0.26$), plasma 17β estradiol concentration and plasma $\sum$ DDT concentration in female fish ($r^2 = 0.38$) and condition factor and plasma $\sum$ DDT concentration in all fish were found ($r^2 = 0.17$). These results suggest that blood plasma may be a suitable nondestructive method for monitoring adult sturgeon population for persistent OC contaminants.

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The Columbia River receives contaminant inputs from a variety of sources, including bleached-kraft pulp mills, aluminum smelters, mining, and agricultural runoff. Many of these contaminants are persistent hydrophobic organic compounds, including polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, dibenzofurans, polycyclic aromatic compounds and organochlorine (OC) pesticides (McCarthy and Gale 2001). Persistent organic pollutants from these sources typically have high sorption coefficients and tend to partition into the sediment organic matter. These contaminated sediments can become trapped behind dams in large-river systems and become a significant source of contaminant exposure for aquatic species, particularly bottom-dwelling species (Seelye et al. 1982).

White sturgeon (*Acipenser transmontanus*) are susceptible to uptake and accumulation of persistent organic pollutants since they are long-lived, late-maturing, benthic species. Feist et al. (2005) found that Lower Columbia River white sturgeon tissues (liver and gonads) contained significant levels of OC pesticides, particularly p,p′-dichlorodiphenyldichloroethylene (DDE). The data from this study indicate that OC contaminants may have deleterious effects on growth and reproduction in white sturgeon, particularly fish collected from the impounded sections of the Lower Columbia River. Monitoring of white sturgeon populations for contaminants in this study was limited to juvenile fish due to the slot limit of 110–137 cm fork length imposed by state fishing regulations. In addition, the number of individuals collected from each site was constrained since analyzing biological tissues for OC pesticides is costly and labor intensive.

A relatively noninvasive, cost-effective method for analyzing OC contaminants in various animal species involves the use of blood plasma. This method has been commonly used as a non-lethal method to monitor persistent OCs in wildlife, particularly endangered or threatened species (Elliott and Shutt 1993; Jensen et al. 1994; Bernhoft et al. 1997; Bishop and Rouse 2000; Keller et al. 2004). Little information exists on the use of blood plasma for monitoring fish populations, particularly large, long-lived freshwater species like white sturgeon.

The objectives of this study were to measure OC pesticide concentrations in blood plasma samples of juvenile white sturgeon collected from 4 sites on the Lower Columbia River and assess the relationship between OC levels in plasma and in corresponding tissues (gonad and liver) from fish that were analyzed as part of a previous study conducted by our research group (Feist et al. 2005). We also looked at the relationship between plasma OC levels and plasma sex steroids that were previously measured in corresponding fish (Feist et al. 2005) to evaluate the effectiveness of utilizing blood plasma as a non-destructive means for monitoring OC contaminants in Columbia River white sturgeon.

Materials and Methods

Tissue samples (liver, and gonad) and blood were collected from white sturgeon during the commercial harvest in February-April of 2000 and 2001 from the estuary and three lower Columbia River reservoirs (Bonneville, The Dalles, and John Day Reservoirs) as described in Feist et al. (2005). A total of 88 fish (41 females and 47 males) were sampled for OC analysis (tissue and plasma) from each of the 4 sites (Estuary 10 females and 10 males, Bonneville 10 females and 9 males, The Dalles 12 females and 12 males, and John Day 9 females and 16 males).

Length (±0.5 cm) and weight (±0.05 kg) were recorded, and condition factor (CF) was determined for each fish. Gonads were removed, weighed (±0.1 g), and gonadosomatic index (GSI) was determined. In addition, blood was collected from the caudal vasculature using a heparinized vacutainer, centrifuged to separate the plasma, and stored at −80 °C for later sex steroid (testosterone, 11-ketotestosterone, and 17β estradiol) and OC analysis. All of the above measurements and related methods were previously published by our research group (Feist et al. 2005), except for the analysis of blood plasma samples for OC pesticides (a total of 88 plasma samples).

Plasma samples were analyzed for 17 OC pesticides (Table 1) based on the methods described by the USEPA (1980).

Plasma samples (2–5 mL) were transferred to 15 mL culture tubes, and extracted with 6 mL of hexane (spectral grade), using a rotary mixer (50 rpm for 2 hours). Samples were centrifuged at 2,000 rpm for 5 minutes, and the organic phase was separated and dried with 10 g anhydrous sodium sulfate and reduced in volume using a warm water bath and a stream of pure nitrogen. Plasma extracts were analyzed using a Varian CP-3800 gas chromatograph equipped with a ^{63}Ni electron capture detector, a CP-8200 AutoSampler, a Star Chromatography Workstation (version 5) and a SPB-608 fused silica capillary column (30 mm × 0.25 mm × 0.25 µm film thickness, Supelco, Bellefonte, PA). Gas chromatographic parameters used were: carrier gas helium (1.5 mL/min), makeup gas nitrogen, detector temperature 300°C, injector temperature 290°C and oven temperature 150°C (4 min) to 290°C (10 min) at 8°C/min. OC pesticides were quantified from individually resolved peak areas with corresponding peak areas of external standards (Supelco). Quality assurance measures included the analysis of reagent blanks, duplicates, and matrix spike samples. Percent recoveries for all of the 17 OC pesticides in matrix spikes ranged between 80% and 97%; therefore, sample extracts were not corrected for percent recovery. Detection limits for individual chlorinated pesticides were 2 ng/mL (wet weight). All plasma OC concentrations are expressed in ppb (ng/mL wet weight).

Table 1 Chlorinated pesticides measured in liver, gonad, and plasma from legal-size white sturgeon captured in commercial fisheries in the lower Columbia River estuary and reservoirs

Aldrin	p,p′-DDD	Endrin	Endosulfan sulfate
α-BHC	p,p′-DDE	Endrin aldehyde	Heptachlor
β-BHC	p,p′-DDT	Endosulfan I	Heptachlor epoxide
γ-BHC	Dieldrin	Endosulfan II	Methoxychlor
δ-BHC			

Comparisons between mean plasma OC levels, and river location, and mean plasma OC levels between sexes were conducted using a one-way ANOVA with the Bonferroni procedure. Correlations between plasma and tissue OC levels were conducted using simple linear regression. Correlations between plasma OC levels and plasma androgen concentrations were conducted using reciprocal-Y regression (reciprocal transformation of Y variable). All other correlations between plasma OC levels and physiologic parameters were conducted using reciprocal-X regression. The accepted level of significance for all tests was $\alpha = 0.05$. All statistical tests were conducted using Statgraphics Plus Version 5 (Manugistics, Inc.).

Results and Discussion

DDT metabolites (DDD and DDE) were the most commonly detected chlorinated pesticides in sturgeon blood

plasma. These metabolites made up on average (±standard error) 81.3% ± 1.5% of the total detectable pesticide burden. DDE plasma levels were always greater than DDD and DDD plasma levels were always greater than DDT. DDE averaged 71.1% ± 1.6% of the total plasma pesticide burden. These findings are consistent with previous results from our research group (Feist et al. 2005), where DDE was the primary chlorinated contaminant detected in Lower Columbia River white sturgeon gonads and livers (the same fish we obtained blood plasma samples from). The greater concentration of DDE over DDD, and DDT in our study and by Feist et al. (2005), suggests that the source of DDE is from agricultural runoff, since environmental degradation of DDT into primarily DDE involves aerobic pathways (Spencer et al. 1996). Although plasma samples were analyzed for 17 OC pesticides, we statistically analyzed only the $\sum$ DDT (DDE + DDD + DDT), since only DDE and DDD were measured in all samples above the detection limit and made up the majority of the total plasma pesticide burden.

The mean $\sum$ DDT concentration was significantly higher in the blood plasma of fish (both males and females) collected from Bonneville pool versus fish collected from the other 3 sites (Fig. 1). This is consistent with the previous findings of our research group (Feist et al. 2005) who found significantly higher $\sum$ DDT levels in the gonads and livers of white sturgeon collected from the Bonneville Reservoir. The Bonneville Dam is the oldest dam of the 3 dams located in our study area, and the elevated pesticide levels found in sturgeon from the Bonneville pool could be due to the longer time for contaminants to accumulate versus the other 2 dams (The Dalles and John Day dams).

There was no significant difference in plasma $\sum$ DDT levels between males and females (Figure 1). This is

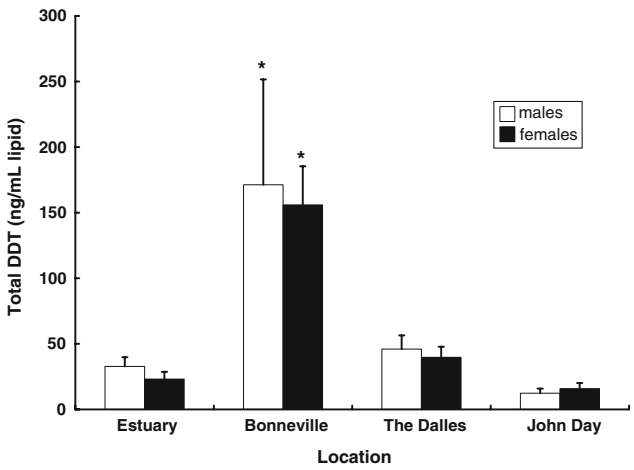

Fig. 1 Concentrations (mean ± SE) of $\sum$ DDT in blood plasma of white sturgeon from four locations on the Columbia River. *Statistically different from other locations (ANOVA, $p < 0.05$)

similar to the findings of Keller et al. (2004), who found no sex-specific differences in plasma OC levels in sea turtles, and Bernhoft et al. (1997) who found no sex-specific differences in plasma DDE levels in juvenile polar bears. The lack of sex-specific differences in plasma OC levels in these studies were attributed to samples being collected from sexually immature individuals, where transfer of contaminants to offspring was not yet possible. White sturgeon collected in our study were of similar size and age and most fish were immature (Feist et al 2004). Previously published results from gonad histology of fish from this study, indicated that all female fish analyzed for plasma OCs were sexually immature and only 5 of the male fish analyzed were mature (Feist et al. 2004). Comparisons between OC levels in blood plasma and tissues in sexually-mature adult sturgeon should be separated by sex since it has been shown in other wildlife species that OC levels are dependent on sex in mature individuals (Gundersen and Pearson 1992; Borrell et al. 1995; Bernhoft et al. 1997).

Since there were no significant sex-specific differences in plasma $\sum$ DDT concentrations, plasma data were pooled when looking at the relationship between plasma $\sum$ DDT concentration and the $\sum$ DDT concentration in tissues (gonad and liver) from corresponding fish. The $\sum$ DDT concentrations in sturgeon tissues (gonads and livers) significantly correlated to blood plasma concentrations for both sexes combined (Figure 2, panels 2A and 2B). Significant squared correlation coefficients between $\sum$ DDT concentrations in white sturgeon plasma and gonads and livers for all fish were 0.37 and 0.32, respectively. Similar relationships have been observed in other species, including humans (Mes 1992; Minh et al. 2005), sea turtles (Keller et el. 2004), marine mammals (Bernhoft et al. 1997), birds (Friend et al. 1979; Elliott and Shutt 1993; Henriksen et al. 1998; Bustnes et al. 2001), and aquatic reptiles (Bishop and Rouse 2000). Keller et al. (2004) observed a significant correlation ($r^2 = 0.66$) between fat and whole blood in loggerhead sea turtles for total DDT. A significant correlation ($r^2 = 0.28$) was seen in polar bears between subcutaneous fat and plasma DDE concentrations (Bernhoft et al. 1997). A significant correlation ($r^2 > 0.7$) was seen between DDE concentrations in blood and liver in glaucous gulls (Henriksen et al. 1998).

Significant negative correlations between plasma testosterone concentration and plasma $\sum$ DDT concentration in male fish, plasma 17β estradiol concentration and plasma $\sum$ DDT concentration in female fish and condition factor and plasma $\sum$ DDT concentration in all fish were found (Table 2). We did not see a significant correlation between plasma 11-ketotestosterone and plasma $\sum$ DDT concentrations in male fish. These results are similar to previous findings reported by our laboratory looking at the relationship between tissue $\sum$ DDT concentrations and plasma sex

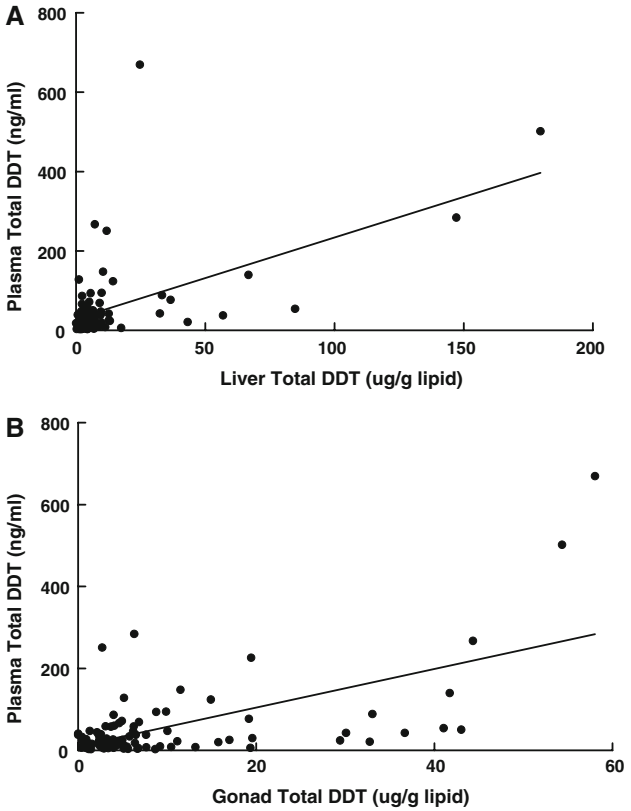

Fig. 2 Relationships between tissue (liver and gonad) and blood plasma concentrations of $\sum$ DDT in white sturgeon collected from the lower Columbia River. Linear regression and correlation statistics for $\sum$ DDT in liver and blood plasma for all fish (**A**) were $y = 2.04x + 29.51$, $r^2 = 0.32$, $p < 0.0001$, for $\sum$ DDT in gonad tissue and blood plasma for all fish (**B**) $y = 4.71x + 10.22$, $r^2 = 0.37$, $p < 0.0001$

steroids and condition factor in the same fish (Feist et al. 2005). Contrary to our results, Feist et al. (2005) did find a significant negative correlation between plasma 11-ketotestosterone and tissue $\sum$ DDT concentration (gonad and liver). However, the r^2 values reported by Feist et al. (2005) for 11-ketotestosterone and tissue $\sum$ DDT concentration was much lower (0.08 and 0.11 for liver and gonad, respectively) than those reported for testosterone and tissue $\sum$ DDT concentration ($r^2 = 0.79$ and 0.85 for liver and gonad respectively) indicating a weak relationship between 11-ketotestosterone and tissue $\sum$ DDT concentrations. We found a significant negative correlation between 17β estradiol and plasma $\sum$ DDT concentration in female sturgeon (Table 2). This was not observed in our previous work (Feist et al. (2005) and is difficult to explain since little information exists on the effects of p,p′-DDE on plasma levels of 17β estradiol, particularly in fish species. It is also difficult to predict that the depressed 17β estradiol levels are directly attributed to DDT metabolites, since other OCs were detected in these fish. It is possible that the low levels of 17β estradiol in sturgeon is the result of contaminants altering

Table 2 Regression analysis of plasma $\sum$ DDT concentration versus plasma testosterone and 11-ketotestosterone concentration in males, plasma 17β estradiol concentration in females, and condition factor in all fish

	Plasma $\sum$ DDT concentration		
	Model	r^2	*p*-Value
Plasma testosterone concentration	Reciprocal-Y	0.26	0.0002
Plasma ketotestosterone concentration	Reciprocal-Y	0.06	NS
Plasma estradiol concentration	Reciprocal-X	0.38	0.0001
Condition factor concentration	Reciprocal-X	0.17	0.0001

NS = Not significant

enzyme pathways responsible for steroid synthesis and metabolism, or disrupting regulation of the hypothalamus-pituitary-gonad axis. Previous work by our laboratory has shown that organochlorine contaminants in sturgeon may cause up-regulation of cytochrome P450 3A (CYP3A), a steroid metabolizing enzyme (Feist et al. 2005). However, the role of this enzyme in the biotransformation of specific sex steroids in fish has not been clearly defined.

The primary goal of this study was to evaluate the effectiveness of using blood plasma as a nondestructive method for monitoring OC pesticides in white sturgeon from the Lower Columbia River. The results of this study produced similar results to a previous study conducted by our laboratory looking at OC pesticides in white sturgeon tissue samples from corresponding fish (Feist et al. 2005). Both studies found that DDT metabolites were the most commonly detected contaminants at the highest levels, fish collected from the Bonneville site had significantly higher levels of DDT metabolites versus the other collection sites, and DDT metabolite levels correlated with suppressed plasma sex steroids and decreased condition factor. Most importantly, there were significant correlations between tissue and blood plasma $\sum$ DDT concentrations from corresponding fish. These results suggest that measuring blood plasma for OCs in juvenile white sturgeon may be an effective nondestructive means for monitoring these contaminants in these fish. Future studies should investigate using this method for monitoring other contaminants in white sturgeon and monitoring contaminants in adult sturgeon.

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References

Bernhoft A, Wiig O, Skaare JU (1997) Organochlorines in polar bears (*Ursus maritimus*) at Svalbard. Environ Pollut 95:159–175

Bishop CA, Rouse JD (2000) Chlorinated hydrocarbon concentrations in plasma of the Lake Erie water snake (*Nerodia sipedon insularum*) and northern water snake (*Nerodia sipedon sipedon*) from the Great Lakes Basin in 1998. Arch Environ Contam Toxicol 39:500–505

Borrell A, Bloch D, Desportes G (1995) Age trends and reproductive transfer of organochlorine compounds in long-finned pilot whales from the Faroe Islands. Environ Pollut 88:283–292

Bustnes JO, Skaare JU, Erikstad KE, Bakken V, Mehlum F (2001) Whole blood concentrations of organochlorines as a dose metric for studies of the glaucous gull (*Larus hyperboreus*). Environ Toxicol Chem 5:1046–1052

Elliott JE, Shutt (1993) Monitoring organochlorines in blood of shrp-shinned hawks (*Accipiter striatus*) migrating through the Great Lakes. Environ Toxicol Chem 12:241–250

Feist GW, Webb MAH, Gundersen DT, Foster EP, Schreck CB, Maule A, Fitzpatrick MS (2005) Evidence of detrimental effects of environmental contaminants on growth and reproductive physiology of white sturgeon in impounded areas of the Columbia River. Environ Health Perspect 113:1675–1682

Friend M, Haegle MA, Meeker DL, Hudson R, Baer HC (1979) Correlations between residues of dichlorodiphenylethane, polychlorinated biphenyl, and dieldrin in the serum and tissues of mallard ducks (*Anas platyrhynchos*). In: Scanlon PF (ed) Animals as Monitors of Environmental Pollutants, National Academy of Sceinces. Washington, DC pp 319–326

Gundersen DG, 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

Henriksen EO, Gabrielsen GW, Skaare JU (1998) Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gull, *Larus hyperboreus*. Chemosphere 13:2627–2643

Jenssen BM, JU Skaare, Ekker M, Vongraven D, Silverstone M (1994) Blood sampling as a non-destructive method for monitoring levels and effects of organochlorines (PCB and DDT) in seals. Chemosphere 28:3–10

Keller JM, Kucklick JR, Harms CA, McClellan-Green PD (2004) Organochlorine contaminants in sea turtles: correlations between whole blood and fat. Environ Toxicol Chem 23:726–738

McCarthy KA, Gale RW (2001) Evaluation of persistent hydrophobic organic compounds in the Columbia River Basin using semi-permeable-membrane devices. Hydrol Process 15:1271–1283

Mes J (1992) Organochlorine residues in human blood and biopsy fat and their relationship. Bull Environ Chem 48:815–820

Minh TB, Watanabe M, Kajiwara N, Iwata H, Takahashi S, Subramanian A, Tanabe S, Watanabe T, Yamada T, Hata J (2005) Human blood monitoring program in Japan: contamination and bioaccumulation of persistent organochlorines in Japanese residents. Arch Environ Contam Toxicol 51:296–313

Seelye JG, Hasselberg RJ, Mac MJ (1982) Accumulation by fish of contaminants released from dredged sediments. Environ Sci Technol 16:459–464

Spencer WF, Singh G, Taylor CD, LeMart RA, Cliath MM, Farmer WJ (1996) DDT persistence and volatility as affected by management practices after 23 years. J Environ Qual 25:815–821

USEPA (1980) Manual of analytical methods for the analysis of pesticides in humans and environmental samples. USEPA No. 600/8-80-038. United States Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, NC