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Environmental Contamination and Toxicology

Maternal Transfer of Organochlorine Compounds in Lake Superior Siscowet (*Salvelinus namaycush siscowet*) to Their Eggs

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While a concerted effort has been made to curtail the use of organochlorine (OC) pesticides and industrial compounds such as polychlorinated biphenyls (PCBs) within North America, continued use elsewhere and vast oceanic reservoirs of these atmospherically-transported compounds indicate they will persist in the North American environment well into the future (Murphy 1988; Tateya et al. 1988). Lake Superior's large surface area (82,100 km^2), drainage basin (127,700 km^2), and long hydraulic retention time (191 yr), makes the lake a prominent receptacle of anthropogenic compounds. Understanding of the sources, sinks, and kinetics of environmental contaminants within Lake Superior's aquatic environment is needed to assess potential environmental impacts, and provide guidance for monitoring and managing this resource.

Various studies provide evidence of harmful effects of OC contamination on reproduction in Great Lakes salmonines (Walker et al. 1991; Mac et al. 1993). Miller (1993) provides evidence that indicates the concentrations of OCs in the eggs of chinook salmon (Oncorhynchus tshawytcha) and lake trout (Salvelinus namaycush namaycush) are influenced by and can be predicted from the concentrations of these compounds in the muscle tissue of the gravid fish. In a continuation of this study, we examined relationships between the concentrations of lipids and OC compounds in the muscle tissue and eggs of siscowet (S. n. siscowet), a subspecies of lake trout, endemic to Lake Superior, that attains the greatest somatic lipid concentration of the Laurentian Great Lakes salmonines. Results of the siscowet analyses were compared with the findings for lipid and OC concentration dynamics of lean lake trout from Lake Superior and Lake Michigan (Miller 1993).

MATERIALS AND METHODS

Wisconsin Department of Natural Resources personnel collected gravid

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siscowet (n = 5) from the western waters of Lake Superior in October 1991. The fish were wrapped whole in aluminum foil and frozen. The fish were thawed at room temperature for approximately 16 hr prior to tissue sample preparation.

One skin-on fillet was removed from each fish and ground with a commercial food grinder, following U.S. Food and Drug Administration procedures (McMahon 1968). The roe were removed, and ground with a commercial food blender. Homogeneous subsamples of approximately 100 g of ground muscle tissue and eggs from each fish were frozen in glass jars sealed with aluminum foil-lined lids for not more than 5 mon prior to laboratory analysis. All chemical analyses were conducted at the Wisconsin State Laboratory of Hygiene, Madison, Wisconsin.

The tissue sub-samples were ground with dry ice to form a free-flowing powder. After sublimation of the dry ice, 10 g of tissue were mixed with 60 g of anhydrous sodium sulfate and column-extracted with dichloromethane at an elution rate of 5 mL/min. The extract was concentrated to less than 5 mL using rotary evaporation and combined with 5 mL of cyclohexane and diluted to 10 mL with dichloromethane. A 2 mL aliquot of the extract was taken and the solvent was evaporated to determine the percent lipid. Automated gel permeation chromatography (GPC) was used to separate lipids from the PCBs and chlorinated pesticides. A 60 g bed of SX-3 Bio-Beads gel resin (Bio Rad), in a 1:1 mixture of cyclohexane and dichloromethane, was packed in a 2.5 cm i.d. X 48 cm glass column fitted with two adjustable-end plungers. The column was placed on an automated low-pressure GPC Autoprep 1001 chromatograph (ABC Labs), and the solvent was pumped through the column at 5 mL/min.

Florisil® and silica gel column chromatography were used to separate PCBs from as many chlorinated pesticides as possible before electron-capture gas chromatography (EC-GC) analysis. The Florisil® columns were prepared by filling with pesticide grade hexane and adding 1 cm of anhydrous sodium sulfate in a 1 cm i.d. X 30 cm chromatography column. Eight grams of 60/100 mesh Florisil®, activated at 130^{o} C for 16 hrs was then added and topped with another 1-cm layer of sodium sulfate. The GPC concentrate was then transferred to the column and further fractionated. The first Florisil® fraction was cleaned and fractionated with silica gel to separate the PCBs and chlorinated pesticides. The silica gel was prepared by heating overnight at 130^{o} C and deactivated before use by equilibrating for one hr with 4% distilled water. The silica gel columns (1 cm i.d. X 30 cm) were first filled with hexane, then one cm of anhydrous sodium sulfate, then five g of deactivated silica gel and topped with

another 1-cm layer of sodium sulfate. The first Florisil® fraction was then added to the silica column. The PCBs were eluted with 50 mL of hexane, followed by the addition of 60 mL 25% ethyl-ether in hexane to elute the chlorinated pesticides. The second Florisil® fraction, eluted with 100 mL 50/50% hexane/ethyl-ether was concentrated and analyzed for dieldrin (1,2,3,4,10,10-hexachloro-exo-5,8-dimethanonapthelene) by EC-GC using a 1.8 m X 4 mm column packed with 4% SE30/6% OV210, at 230° C with 90/10% argon/methane carrier gas at 40ml/min.

The first silica gel fraction was then analyzed for PCBs and p,p'DDE (a metabolite of dichlorodiphenyltrichloroethane) with the packed column method used in the dieldrin analysis. The PCBs were quantified by comparing the sample chromatograms with Aroclor® standards. Florisil® and silica gel column chromatography were used to separate PCBs from as many of the chlorinated pesticides as possible before EC-GC analysis. The PCBs were quantified by comparing the sample chromatograms with Aroclor® standards. The Aroclor® standards used were 1242, 1248, 1254, and 1260. The peak heights of the PCB components present in each sample were summed and compared to the sum of the same peaks in the proper Aroclor® standard. A complete description of methods used for the tissue sample preparation and analyses are described in Laboratory Analytical Methods (Wisconsin State Laboratory of Hygiene, 1989).

All OC concentrations are reported on a milligram per kilogram wet weight basis and not corrected for extraction efficiency. Mean recovery efficiencies and standard errors of laboratory-spiked fish tissue samples were 92.0 ± 1.2 for PCBs; 91.4 ± 2.6 for DDT and metabolites; 90.3 ± 1.2 for 1,2,4,5,6,7,8,8-octachloro-2,3,3a,4,7,7a-hexahydro-4,7-methanoindene (chlordane) and constituents; and 92.6 ± 1.2 for dieldrin, respectively (D. Degenhardt, Wisconsin Laboratory of Hygiene, unpubl. data).

One of two statistical treatments was used to derive numeric values for analytical results of samples below the laboratory detection limits (Table 1). If less than three laboratory samples analyzed for a given OC compound in a sample group were at or above the laboratory detection limit, a random number between zero and one was multiplied by the detection limit value for that compound. This value was assigned to each below-detection sample. The random number was drawn from a uniform distribution. If three or more sample determinations for a given sample group were equal to or greater than the detection limit, then the lognormal distribution method described by Gilliom and Helsel (1986) was used. To use the lognormal method, the logarithm values of the OC concentrations are regressed upon the normal scores of the OC concentration values that

were equal to, or greater than, the laboratory detection limit. The regression line is then extrapolated to determine values for below-detection observations. These techniques provided more accurate estimates of the mean and standard deviation of OC values, rather than simply replacing all sample observations below the detection limit with a single value, such as the detection limit, one half of the detection limit, or zero (Gilliom and Helsel 1986). These methods were only used on the data presented in Table 1.

All samples used in regression analyses had OC concentrations above the laboratory detection limits. ANOVA for unequal sample sizes was used to compare muscle tissue and egg lipid concentrations following procedures outlined in Sokal and Rohlf (1981). Regression analyses comparing the relationships between the logarithm concentrations of OCs in the eggs and muscle tissue samples were compared using procedures outlined in Draper and Smith (1981). Siscowet age estimates were calculated from length at age data from siscowet (n = 459) collected in 1987 (Stephen Schram, Wisconsin Department of Natural Resources unpubl. data). The relationship of siscowet length and age is described by the regression equation: $Y = 1.440e^{(0.00373\ X)}$, where Y represents the estimated age of the fish in years, and X equals the total length of the fish in millimeters. Statistical significance levels were set equal to 0.01, and were met by all tests performed in this study, unless otherwise stated in the text.

RESULTS AND DISCUSSION

The total length of the five siscowet collected from Lake Superior in 1991 ranged between 58 and 75 cm, with a sample mean of 65.9 ± 3.0 cm. The weights of these fish ranged between 2.1 and 4.0 kg, with a sample mean of 3.0 ± 0.4 kg. The estimated age of the siscowet ranged between 13 and 24 yr, with a sample mean of 16.8 ± 1.6 yr.

The lipid concentrations in the muscle tissue of siscowet ranged between 24 and 37 percent, while the lipid concentration in the eggs of these fish ranged between 5.8 and 7.9 percent (Fig. 1). Although the mean lipid concentration in the muscle tissue of siscowet was significantly greater than that of Lake Superior or Lake Michigan lean trout, there were no significant differences between the lipid concentrations in the eggs of these fish (P = 0.53).

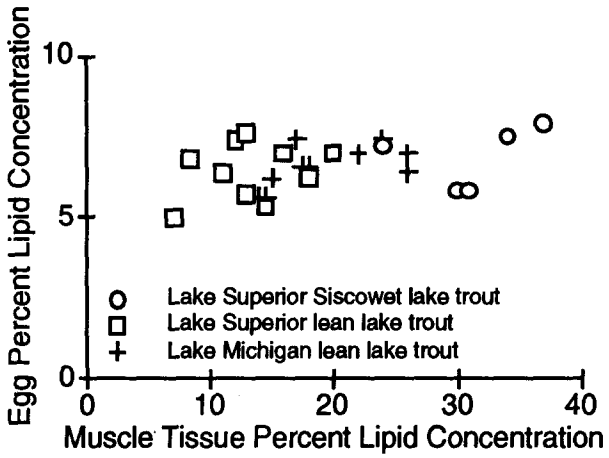

Figure 1. Percent lipid concentrations in the muscle tissue and eggs of Lake Superior siscowet, and Lake Superior and Lake Michigan lean lake trout. Lean lake trout data from: Miller 1993.

Results of the analyses for OC concentrations in siscowet muscle tissue and eggs are presented in Table 1. The total concentrations of PCBs and the concentrations of p,p'DDE in the muscle tissue of the five gravid siscowet, attained the greatest concentrations of the OC compounds quantified. The concentrations of these compounds in the eggs of the gravid fish were positively correlated with the concentrations of these compounds in the muscle tissue of the fish (Fig. 2).

Table 1. Descriptive statistics for organochlorine concentrations in the muscle tissue and eggs of siscowet lake trout collected from Lake Superior in 1991 (n = 5). Values represent the sample mean ± 1 SE. Laboratory detection limits are in square brackets, and the number of samples at or above the detection limit are in parentheses. ND denotes that all samples were below the laboratory detection limit.

	Substance mg • kg $^{-1}$						
	ΣPCB [0.20]	cis-chlord. [0.05]	cis-nonachlord. [0.05]	trans-nonachlord. [0.05]	p,p' DDE [0.05]	p,p' DDT [0.05]	Dieldrin [0.02]
Muscle	2.7±0.6	0.06±0.01	0.11±0.03	0.25±0.05	0.48±0.13	0.14±0.04	0.10±0.0
	(5)	(4)	(5)	(5)	(5)	(4)	(5)
Eggs	0.45±0.09	ND	ND	0.03±0.01	0.09±0.02	ND	0.02±0.01
	(5)			(1)	(4)		(2)

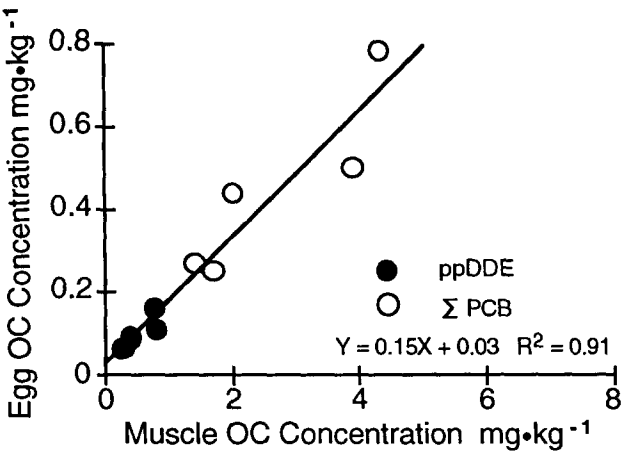

Figure 2. Total concentrations of PCBs (open symbols) and p,p'DDE concentrations (solid symbols) in the muscle tissue and eggs of Lake Superior siscowet.

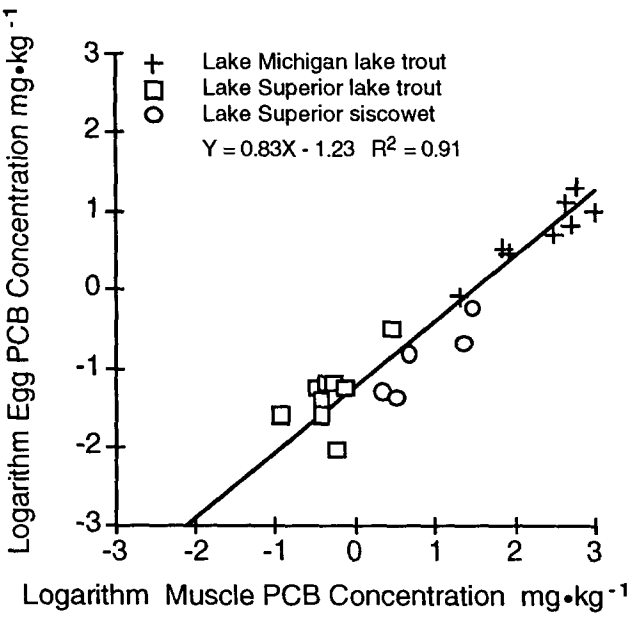

Figure 3. Natural logarithm values of the total concentrations of PCBs in the muscle tissue and eggs of Lake Superior siscowet, and Lake Superior and Lake Michigan lean lake trout. Lean lake trout data from: Miller 1993.

Logarithm values of total concentrations of PCBs in the eggs of Lake Superior siscowet, and lean trout from Lake Michigan and Lake Superior were positively correlated with the total concentrations of PCBs in the

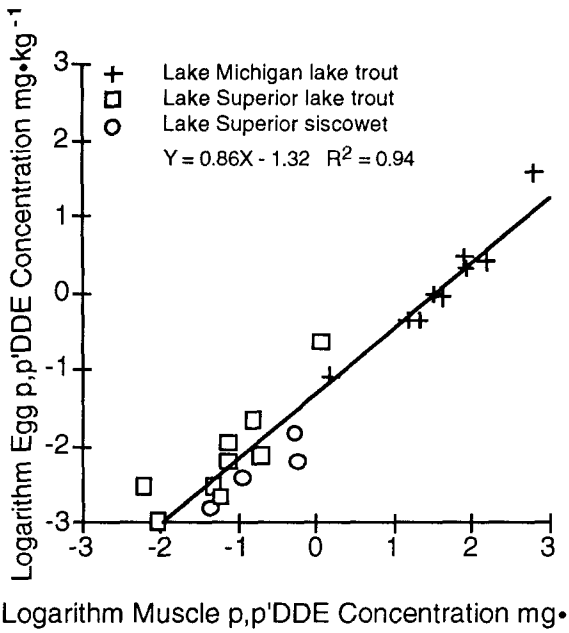

Figure 4. Natural logarithm values of the concentrations of p,p'DDE in the muscle tissue and eggs of Lake Superior siscowet, and Lake Superior and Lake Michigan lean lake trout. Lean lake trout data from: Miller 1993.

muscle tissue of the fish (Fig. 3). The concentrations of p,p'DDE in the muscle tissue and eggs were also positively correlated (Fig. 4). There was no significant difference between the slopes of the PCB and p,p'DDE regression plots (P = 0.62) or the intercepts of these plots (P = 0.51).

Lake Superior siscowet differ markedly from lean lake trout in bathymetric distribution, diet, growth rate, and caloric density (Becker 1983; Miller et al. 1992). In spite of these behavioral and physiological differences, the results of this study reveal that the concentrations of lipids in the eggs of siscowet, and the dynamics of OC concentrations in siscowet muscle tissue and eggs are similar to that of lean trout from Lake Superior and Lake Michigan. This study further strengthens the hypothesis that OC concentrations in salmonine eggs are influenced by and can be predicted from the OC concentrations in the somatic tissue of the gravid fish.

Lake trout currently do not reproduce in Lake Michigan and organochlorine contamination is a suspected cause (Walker et al. 1991; Mac et al. 1993).

Understanding the dynamics of OC compounds in this species may prove to be important in the re-establishment of self-sustaining populations in Lake Michigan. Lake Superior lean lake trout and siscowet have significantly lower OC burdens relative to Lake Michigan lake trout (Miller et al. 1992), and reproduce. However, understanding the processes that influence the concentrations of OCs in the bodies and eggs of these ecologically and economically important species is of value in the environmental surveillance and monitoring of Lake Superior, as lean lake trout and siscowet are biological indicators of the dynamics of OC concentrations in lower trophic levels and ultimately in the water column.

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