Bull. Environ. Contam. Toxicol. (1996) 56:866-872

Bulletin of Environmental Contamination and Toxicology

Environmental Contaminants and Cholinesterase Activity in the Brain of Fisher (*Martes pennanti*) Harvested in Northern Wisconsin

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Received: 26 July 1995/Accepted: 8 February 1996

The fisher (Martes pennanti), a member of Mustelidae, was once common in the northern forested region of the upper Great Lakes, including Wisconsin. Fishers are medium sized carnivores (2.5 kg for females; 6.5 kg for males) which feed on a wide range of food items (Powell and Zielinski 1994; Powell 1993; Gilbert and Keith, unpublished data) and require closed canopy forests with large diameter trees for den sites (Powell 1994, Thomasma et al 1991, 1994, Wright and Gilbert in prep). Fishers were extirpated from Wisconsin in the 1920's due to a combination of over-harvest and habitat destruction (Pils 1983, Powell 1994). From 1956 to 1963 fishers were translocated from New York and Minnesota into Wisconsin in order to reestablish a self sustaining population and to achieve porcupine (Erethizon dorsatum) control. Kohn and Creed (1983) concluded that this reestablishment was successful. In 1985 the fisher was designated a game species and the first trapping season in 50 years was established. Harvests initially occurred over a portion of northern Wisconsin but now include the entire northern part of the state.

Subsequent research efforts have focused on population monitoring and the role fishers play in northern forest ecosystems. Parameters such as food habits, habitat use, home range area and reproductive rates are being investigated. Fishers are also suitable species for bioaccumulation studies, including the effects of contaminants on fisher reproduction and survival, because of their role as top level predators.

One area of concern is the possible central nervous system effects associated with low level exposure to polychlorinated biphenyls (PCBs), mercury (Hg), and other organochlorine (OC) compounds. Species similar to the fisher, such as mink (Mustela vison), are highly sensitive to the effects of PCBs, Hg, and OCs (Wren 1991; Wren et al 1987a,b). Mink exposed chronically to fish diets containing 2.5 ppm Aroclor 1254 showed significant elevations in cerebellar and hypothalamic norepinephrine concentrations, as well as elevations in hypothalamic dopamine concentrations. Individual PCB congeners such as 3,4,5,3',4',5' hexachlorobiphenyl cause significant changes in biogenic amine concentrations at dietary doses as low as 0.1 ppm (Aulerich et al 1995). Despite these neurochemical alterations, limited information regarding the contaminant concentrations in fisher or related species could be located.

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Therefore, our objectives in this study were to test fisher brain tissue for total Hg, cholinesterase activity, selected OCs, and PCB congeners, as limited data exists regarding the ability of fishers to accumulate environmental toxicants in brain tissue. Low concentrations of contaminants are expected in the fisher brain tissue, as these animals are a representation of a wild, healthy population existing in northern Wisconsin.

MATERIALS AND METHODS

Fourteen fisher brains were obtained from the Great Lakes Indian Fish and Wildlife Commission during the routine monitoring of fisher harvest during the 1992-1993 trapping seasons in northern Wisconsin. Since the fishers were harvested for their pelts, the only portions of the fishers available after processing were the brain and feet.

The Wisconsin deer management unit in which the fishers were harvested and the sex of the animals were recorded. Brain tissue was removed, cut into pieces, and frozen for later chemical analysis. Upon thawing, the tissue pieces were combined, homogenized and placed in plastic sample bags. The fisher brain tissue was analyzed for 12 organochlorine compounds including B-BHC, Aldrin, Endosulfan I, 4,4'-DDE, Endosulfan II, 4,4'-DDT, Alpha-BHC, Gamma-BHC, Heptachlor, Heptachlor epoxide, Dieldrin, Endrin, and 4,4'-DDD.

Additionally, PCB congeners 8, 18, 28, 47, 48, 49, 52, 66, 70, 74, 78, 84, 95, 101, 105, 110, 118, 126, 153, total mercury concentrations, and cholinesterase values were determined on each fisher brain. Standards for the OC compounds and PCBs were purchased from Ultra Scientific, while $HgCl_2$ (Mercuric Chloride) was purchased from J.T. Baker.

PCB and OC concentrations were determined using modifications of the methods developed by Burse, et al, 1983 and Loutamo, et al, 1985. Approximately 0.5 g of homogenous brain tissue was weighed and placed in a glass beaker and the exact weight recorded. Each sample was spiked with 500 uL PCB 14 and 100 uL of PCB 166 at 100 ng/mL each. Sodium sulfate and hexane are added and the mixture and ground with a glass rod until homogenous. The sample was sonicated for 15 min and quantitatively transferred to a silica gel column. The silica gel column was rinsed with 15 mL of hexane and the eluant collected as the silica gel one (SG-1) fraction. Twenty mL of hexane was added to the column, followed by 20 mL of benzene. The volume of hexane (28 mL) collected just prior to the benzene passing through the column was the silica gel two (SG-2) fraction. A final fraction, approximately 15 mL, was allowed to pass through the column and collected as the silica gel three (SG-3) fraction. The SG-2 fraction was evaporated to < 1 ml under nitrogen gas and the internal standards, 100 uL of PCB 30 at 100 ng/mL and 1 mL of octochloronapthalene at 49 ng/mL are added. The sample volume was adjusted to exactly 1 mL and transferred to an

autoinjection vial. Similarly, the SG-1 and SG-3 fractions were dried under nitrogen gas and adjusted to 1 mL volumes for organochlorine analysis.

Gas chromatographic analysis was performed using a Hewlett Packard model 5790A series gas chromatograph equipped with a DB-5 60 meter column and electron capture detector. The carrier gas was hydrogen and the make-up gas to the detector was argon/methane. Oven temperature programming was as follows, initial temperatures 90°C hold for one min, increase to 175°C at 20° per min, increase temperature from 175°C to 275°C at 3° per min, and hold for 5 min. Detection temperature was 300°C, while the injection temperature was 250°C.

Recoveries for the OCs and PCB congeners ranged from 65-114%, with an average of 83%. Data were corrected for recoveries. Limits of quantitation for the OCs and PCBs were 0.5 ppb, with the highly chlorinated PCB congeners having slightly lower limits of detection.

Cholinesterase activity was determined using a method similar to that of Ellman, et al, 1961. Approximately 0.5g of brain tissue was placed into a 50mL glass tube. Twenty-five mL of 1% Triton x-100 phosphate buffer (pH 8) was added and homogenized with a bio-homogenizer for 30 sec at low speed, followed by 30 sec at the high speed. Two hundred uL of the homogenized mixture and 2.5mL of pH 8.0 1% Triton X-100 phosphate buffer were combined and placed into each of three 1 cm cuvettes. Two cuvettes are sample cuvettes are sample cuvettes, while one is used for the reference cell. The cuvettes were placed in a Shimadzu UV visible recording double beam spectrophotometer and monitored at a wavelength of 412 nm. Fifty uL of 0.01 M dithiobisnitrobenzoic acid (DTNB) was added to each cuvette, and the baseline set to zero. Twenty uL of 0.075 M Acetylthiocholine iodide (ATCI) was added to the sample cuvettes only, and the spectrophotometer was started. Samples were run in duplicate and cholinesterase activity was calculated as the average of two trials.

Total Hg in brain tissue was determined following a method similar to that of Lobring and Potter, 1991. Approximately 0.5g of brain tissue was placed in a 125 mL bottle and the exact weight recorded. Four mL of concentrated H_2SO_4 and 0.5 mL of concentrated HNO_3 was added to each sample. The samples were then placed in a covered water bath and heated at 100°C for no less than 15 min, removed and allowed to cool to room temperature. Five mL of 5% potassium permanganate solution was added, turning the sample purple. If the purple color did not persist, additional potassium permanganate solution was added. Eight mL of 5 % potassium persulfate solution was added to each sample, and allowed to sit at room temperature overnight. Ten mL of 10% hydroxylamine hydrochloride solution was added, causing the purple color to disappear. Five mL of 10% stannous chloride solution was added, and the bottle was immediately attached to the bubbler assembly on an LDC Analytical mercury Monitor 3200. Nitrogen gas at a flow rate of 1000mL/min was used to aerate the system, and total mercury concentrations were determined by total area under the curve (AUC) as compared

to AUC for a series of mercuric chloride standards. The limit of quantitation was 60 ppb.

RESULTS AND DISCUSSION

Total mercury (Hg) concentrations in brain tissue ranged from 100-600 ppb, with a mean concentration of 240 ppb (see Table 1). The average concentration in male fishers was 180 ppb (N = 3), while that in females was 290 ppb (N = 11). Fishers occupy a variety of habitats and consume a variety of food (Arthur et al 1989). Therefore, it is not surprising that the concentrations of Hg varied between individuals.

Cholinesterase activity values are also reported in Table 1. The mean value for cholinesterase activity in all fishers was 5.68 umoles/g/min with a range of 3.66 to 7.48 umoles/g/min. The male mean brain cholinesterase activity was 6.42 umoles/g/min (N = 3), while the female mean was 5.75 umoles/g/min (N = 11). Organophosphate (OP) insecticide concentrations were not determined on brain tissue and more suitable tissue such as liver or stomach contents were not available for analysis. Since all animals captured appeared normal and healthy at the time of harvest, the cholinesterase activity may be representative of normal values. The cholinesterase data reported may aid in the diagnosis of potential OP exposure in future studies, as no information regarding normal brain cholinesterase activity in the fisher could be located.

Only heptachlor and lindane were detected in fisher brain tissue (Table 1). No distinct bioaccumulative trends could be determined regarding organochlorine compounds in fisher brains. Sample size limits the strength of these observations, and increased sample sizes are needed to further assess the bioaccumulation of OC compounds in the fisher.

Three main PCB congeners were found in fisher brain tissue including 28, 49, and 153 (see Table 1). All three congeners contained substitutions in at least one ortho and para position. The degree of chlorination and position of the chlorine about the biphenyl structure have profound effects on subsequent metabolism (Parkinson and Safe 1987; Sipes and Schnellman 1987). In mammals, the para and meta positions are preferred sites of biphenyl hydroxylation, and adjacent unsubstituted carbon atoms in these positions facilitate biotransformation (Billings and McMahon 1978; Burke and Bridges 1975; Matthews and Tuey 1980). Therefore, PCBs with substitutions in the para and meta position, such as PCB 153, would be expected to accumulate in mammalian tissues.

Conversely, PCBs 28 and 49 contain adjacent unsubstituted carbon atoms making them more susceptible to biotransformation and less likely to accumulate. One explanation for environmental occurrence in brain tissue is that lower chlorinated PCBs more readily cross the blood-brain barrier and accumulate. Laboratory studies involving rodents and non-human primates determined that these lightly

chlorinated ortho substituted PCBs can cross the blood brain barrier and affect the dopaminergic system (Seegal et al 1991; Shain et al 1991).

Table 1. Cholinesterase Activity and Contaminant Concentrations (wet weight) in Fisher Brains Harvested in Northern Wisconsin.

Number	Unit **	Sex	Total Hg ppb	PCB Congener-ppb	Brain OC's ppb	Cholinest-erase (umoles/ g/min)
1	U-3	M	260	28-1.47 49-10.15	LOQ	5.79
2	U-3	F	430	LOQ	LOQ	7.48
3	U-5	M	180	LOQ	LOQ	6.37
4	U-5	F	390	LOQ	Lindane 6.07	6.61
5	U-5	F	580	LOQ	Heptachlor 9.49	6.69
6	U-3	F	120	LOQ	Lindane 0.73	6.11
7	U-5	F	260	LOQ	LOQ	6.69
8	U-3	F	400	LOQ	LOQ	4.34
9	U-5	F	280	49-1.64	LOQ	4.51
10	U-3	F	100	49-3.91 153-1.30	LOQ	3.66
11*	U-4	F	LOQ	LOQ	LOQ	6.84
12	U-5	M	100	49-7.87 153-1.54	LOQ	7.11
13	U-2	F	200	153-0.47	LOQ	6.57
14	U-2	F	LOQ	153-0.49	LOQ	4.79

*Died During Immobilization (Ketamine HCl).
**Wisconsin Deer Management Unit
LOQ- Below Limit Of Quantitation.

The fisher appears to accumulate specific PCB congeners in brain tissue in a manner similar to laboratory animals (Ness et al 1994; Shain et al 1991; Gerstenberger et al, unpublished data). In addition, low levels of Hg and selected OCs have the potential to accumulate in fisher brain tissue. Further studies are needed to define the absorption, metabolism, distribution, and excretion of PCB

congeners and other environmental contaminants in the fisher and similar species. The contaminant levels and cholinesterase activity reported may be indicative of background or normal levels respectively, since all fishers appeared to be healthy at the time of harvest.

Acknowledgments. We thank Michelle Hannah, Gary Bordson and Jerry Bargren of the Veterinary Diagnostic Laboratory at the University of Illinois for analytical assistance. Also, the assistance of Cathy Stolls at the University of Wisconsin-Superior was appreciated.

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