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Organochlorine Residue Levels in Mississippi River Water Snakes in Southern Louisiana

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The tremendous wetland areas and subtropical weather conditions in southern Louisiana provide an ideal habitat for many species of reptiles. These animals generally occupy the top of their food chains, and should have significant value as indicators of persistence and biological magnification of various pollutants (Bauerle et al. 1975; Stone et al. 1980). In addition, since snakes frequently spend much of their lives within a few kilometers of their birthplace (Hirth et al. 1969), they provide an opportunity for the researcher to consider the distribution of pollutants on a local and/or regional basis.

Our study was designed to determine the usefulness of water snakes in pollution monitoring. This was accomplished by assessing the organochlorine load in tissues of snakes inhabiting three sites along the Mississippi River near Baton Rouge, Louisiana. The significance of this load becomes evident upon consideration that the Mississippi pours an estimated 400 billion gallons of water into the Gulf of Mexico per day (Helfman 1965). Two species of water snakes, Nerodia rhombifera and Nerodia cyclopion, were chosen for analysis of chlorinated hydrocarbons. Mushinsky and Hebrard (1977) determined that fishes account for 95.2 and 98.4%, respectively, of the total volume of food consumed by N. rhombifera and N. cyclopion. Thus, the organochlorine load of both species should reflect considerable biomagnification relative to water column levels. Dense populations of both species of snakes generally occur in borrow pits between the levee and the Mississippi River.

MATERIALS AND METHODS

Snakes were captured live from borrow pits at one of three sites between 1977 and 1979: one north and two south of the city of Baton Rouge. After collection at either Thomas Point, Brusly, or Belle Helene the snakes were transported to Louisiana State University, Baton Rouge, USA. In the laboratory a group of N. rhombifera was dissected to obtain fat bodies, liver and muscle tissue. The skin and heads were removed from a second group, including members of both species (whole snakes). In four cases, females snakes contained embryos. These embryos were removed for

separate analysis. All samples were weighed and stored frozen at -20°C in acid-washed, hexane rinsed glass containers for processing at a later date.

For residue analysis 20 whole N. rhombifera (weight range 58-479 g) and 9 N. cyclopion (weight range 59-894 g) were thawed and homogenized in a Hobart Meat Grinder. Following this procedure, 25 g or less of the whole animal homogenate was used for analysis of chlorinated hydrocarbons.

The individual tissues examined from N. rhombifera comprised 21 fat samples, 28 liver samples, and 28 muscle samples. The frozen tissues were thawed and hand homogenized with a mortar and pestle in petroleum ether and anhydrous Na_2SO_4.

Sample extraction in petroleum ether, acetonitrile partitioning, saponification, and Florisil column cleanup procedures were employed as described in the Pesticides Analytical Manual (U.S. FDA). The successful elutions of the Florisil column were 6% alcoholic ethyl ether/94% petroleum ether and 20% alcoholic ether/80% petroleum ether. These eluates were analyzed with a Perkin Elmer Model 3920 gas-liquid chromatograph equipped with a ^{63}Ni electron capture detector. Two columns were used for analyses. The first was glass (183 x 0.64 cm), packed with 10% DC-200 on Chromosorb Q 80/100 mesh; the second was glass (92 x 0.64 cm), packed with 6% SE-30/4% OV-210 on Chromosorb Q 80/100 mesh. Injector, column, and detector temperatures were 210° C, 200° C, and 275° C, respectively. The carrier gas was 5% methane in argon flowing at 100 ml/min (DC-200) or 60 ml/min (SE-30/OV-210). Peaks which indicated the presence of α- and β-chlordane, phorate and trifluralin were occasionally detected. These were qualitatively confirmed (mixed column: 1.5% OV-17, 1.95% QF1) and did not represent large residues. Average standard recoveries from Florisol column procedures ranged from 73 to 101%. Method recoveries from spiked samples ranged from 81 to 97%. Data were not adjusted for recoveries.

All data are reported as arithmetic means. Geometric means determined for these data did not differ appreciably from arithmetic means. Analysis of variance (ANOVA) was used to determine the significance of variation among means of organochlorine level and content over sites. When significant variation occurred, groupings were determined by Duncan's Multiple Range Test. Student t-tests were used to assess whether differences occurred in tissue and whole body organochlorine residue levels in relation to sex, length or weight.

RESULTS AND DISCUSSION

The levels (ppm) of PCB's, DDT and its metabolites (DDE and DDD) and other organochlorine residues in the individual tissues of Nerodia rhombifera are summarized in Table 1. Highest concentrations of organochlorine compounds detected were in the fat bodies, intermediate in liver samples and lowest concentrations in

TABLE 1. Mean values for organochlorine residue levels in fat bodies, (F), liver (L), and muscle (M) tissue of Nerodia rhombifera collected in South Louisiana. Data in g g wet tissue weight-1 (ppm); N.D. = none detected; mean expressed based on full sample size.

SITE	Thomas Point			Brusly			Belle Helene		
TISSUE	F	L	M	F	L	M	F	L	M
SAMPLE SIZE	7	9	12	7	13	9	7	6	7
LINDANE	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	1.47(7)[1]	N.D.	N.D.
ENDRIN	0.01(2)	0.02(5)	N.D.	0.01(1)	<0.01(4)	N.D.	N.D.	N.D.	N.D.
ALDRIN	0.02(1)	0.01(1)	N.D.	0.01(1)	N.D.	N.D.	N.D.	<0.01(1)	<0.01(1)
DIELDRIN	0.58(1)	0.13(6)	<0.01(6)	0.27(5)	0.05(11)	<0.01(1)	0.24(7)	0.01(4)	<0.01(3)
HEPTACHLOR EPOXIDE	0.70(4)	0.05(5)	<0.01(6)	0.35(2)	0.02(3)	<0.01(1)	0.21(2)	0.09(6)	0.03(5)
HCB	0.22(7)	N.D.	N.D.	0.20(7)	0.01(4)	N.D.	1.72(7)	0.03(6)	0.01(5)
DDT	0.77(4)	N.D.	N.D.	0.14(2)	N.D.	N.D.	1.77(6)	N.D.	N.D.
DDE	4.24(7)	0.21(8)	0.02(11)	1.92(7)	0.12(2)	0.01(7)	4.66(7)	0.08(6)	0.02(7)
DDD	N.D.	0.02(1)	N.D.	N.D.	N.D.	N.D.	N.D.	<0.01(1)	N.D.
DDTs	5.02(7)	0.23(8)	0.02(11)	2.06(7)	0.12(12)	0.01(7)	6.43(7)	0.08(6)	0.02(7)
PCBs	8.16(7)	0.66(5)	0.05(4)	5.15(7)	0.30(6)	N.D.	13.65(6)	0.17(4)	0.02(1)

[1]Number within parenthesis is number of samples in which the residue was detected.

muscle tissue. No correlation existed between organochlorine load and sex, length, or weight ($P > 0.05$ in all cases). However, a limited sample size may have precluded suitable tests for these relations.

DDT was only found in fat tissues (12 of 21 samples) while almost all tissue samples, 72 of 77, had detectable levels of DDE. Recovery of DDE in snake tissue nearly one decade after DDT was banned was consistent with known persistence of this metabolite. Fleet and Plapp (1978) found that DDT concentrations in fat bodies of the related Natrix (=Nerodia) erythrogaster and Natrix (=Nerodia) fasciata decreased by 52 and 62%, respectively, between the years 1971 and 1974-5. The levels of DDE determined in the present study are considerably lower than those reported for snakes by Fleet and Plapp, but comparable to those found in Hudson River snapping turtles by Stone et al. (1980). The study site chosen by Fleet and Plapp (1978) had been sprayed repeatedly with DDT for more than 20 years.

PCB's were detected in 52% of all the tissue samples, and 20 or 21 fat samples. Highest concentrations of PBC's in fat tissue were found in samples from Belle Helene. One individual sample contained 38.2 ppm Aroclor 1260. ANOVA, however, showed no significant differences among the three sites ($0.10<P<0.13$) because of the high variability in PCB concentrations.

Dieldrin, heptachlor epoxide, and hexachlorobenzene (HCB) also occurred in the majority of examined tissues. Significant levels of all three compounds were found in fat tissues at all sites, while only trace amounts could be detected in muscle. Site differences were not observed for dieldrin in either fat or liver samples although mean concentrations in Thomas Point samples were higher (ANOVA, $P > 0.10$ for fat; $P > 0.10$ for liver). The parent compound, aldrin, and the dieldrin isomer, endrin, were occasionally found. These were primarily in snake tissues from Thomas Point and Brusly. Their average levels were always less than 20 ppb of wet tissue weight.

Heptachlor was not detected in any tissue examined. Levels of heptachlor epoxide indicate that the heptachlor is rapidly converted to its metabolite. Heptachlor epoxide was present in 44% of all tissues and 50% of the fat samples. Again, there were not signficant differences in residues in fat samples from snakes in the three study sites.

Hexachlorobenzene, present in 100% of the fat samples, reached an average of nearly 2 ppm in fat tissue from Belle Helene. One-way ANOVA for sites was highly significant ($P < 0.001$). Duncan's multiple range test revealed that the mean for the Belle Helene site was significantly greater than means for the other two sites studied. Low levels of HCB were determined for liver and muscle tissue; the trend was again for Belle Helene samples to have higher HCB concentrations than the other sites. HCB occurred in

18 of the 20 snake tissue samples taken from the Belle Helene location.

Other organochlorine residues identified were not present in all tissues or at all sites. Lindane ($\sqrt{}$-BHC) was detected in all seven fat samples taken from Belle Helene.

Whole animal burdens of organochlorine residues were determined for 20 N. rhombifera taken from the three sampling sites. Nine N. cyclopion were collected from the Thomas Point and Belle Helene locations. The occurrence and concentrations of residues were similar for both species (Table 2), reflecting the tissue burdens recorded for N. rhombifera. The two species have remarkably similar feeding habits (Mushinsky and Hebrard, 1977); hence, this result is not surprising. Both species of snakes heavily prey upon the numerous small fish that occupy the shallow waters along the shoreline. Fish such as Gambusia affinis, Heterandria formosa and Poecilia latipinna account for the vast majority of the diet of water snakes. These prey species, with their very high turnover rate, would quickly assimilate any water-borne chemicals and in a very short time period pass them up the food chain.

Residues of DDT's and PCB's were found in virtually every snake examined. The average levels of these residues ranged from 0.1 to 0.6 ppm of wet weight, which is similar to reports for fishes from fresh water (Veith et al. 1979) and marine environments (Butler and Schutzmann 1979). Earlier reports for aquatic reptiles have been concerned with residue levels in individual tissues and eggs (Fleet and Plapp 1978; Hall et al. 1979; Punzo et al. 1979; Stone et al. 1980).

Aroclor 1260 and DDE accounted for the majority of the PCB's and DDT's, respectively, simiar to the results of individual analysis. ANOVA indicated no site differences for the levels of these compounds.

Concentrations of heptachlor epoxide in intact N. rhombifera ranged from below detection to 0.69 ppm (present in 18 of 20 samples) and in intact N. cyclopion from below detection to 0.15 ppm (present in 7 of 9 samples). Heptachlor was present only in trace amounts in N. rhombifera collected from Thomas Point.

The concentration of HCB was significantly greater ($P < 0.05$) in snakes of both species taken from Belle Helene, by a ten-fold increase over snakes taken from the other sites. This confirms the pattern established for individual tissues of snakes from Belle Helene. The Belle Helene sample location lies within an area which received considerable attention due to HCB contamination of cattle in 1973 (Chemical Week, 112:21, April 25, 1973). Soil and grasses sampled during 1973 contained HCB ranging from 0.014 to 4.560 ppm (Louisiana Air Control Commission 1973). This 1973 collection site was within three miles of our site for 1978

TABLE 2. Mean values for organochlorine residue levels in whole Nerodia rhombifera and Nerodia cyclopion collected in South Louisiana. Data based on whole animal wet weight. Detail as in TABLE 1.

SITE	Thomas Point		Brusly	Belle Helene	
SPECIES	rhombifera	cyclopion	rhombifera	rhombifera	cyclopion
SAMPLE SIZE	6	5	6	8	4
LINDANE	N.D.	N.D.	N.D.	N.D.	0.10(3)
ALDRIN	N.D.	N.D.	<0.01(2)	0.01(6)	N.D.
DIELDRIN	0.06(6)	0.06(4)	0.08(6)	0.02(7)	0.03(4)
HEPTACHLOR	<0.01(3)	N.D.	N.D.	N.D.	N.D.
HEPTACHLOR EPOXIDE	0.06(5)	0.05(5)	0.03(5)	0.22(8)	0.05(2)
HCB	0.02(4)	<0.01(5)	0.02(5)	0.20(8)	0.07(4)
DDT	N.D.	N.D.	0.11(6)	N.D.	0.04(3)
DDE	0.22(6)	0.13(5)	0.20(6)	0.10(8)	0.10(4)
DDD	N.D.	N.D.	0.07(4)	N.D.	N.D.
DDTs	0.22(6)	0.13(5)	0.38(6)	0.10(8)	0.14(4)
PCBs	0.58(5)	0.28(5)	0.39(6)	0.25(8)	0.27(4)

TABLE 3. Mean values for organochlorine residue levels in embryos from aquatic snakes collected in South Louisiana. Detail as in TABLE 1.

SPECIES	N. rhombifera		N. cyclopion
SITE	Thomas Point	Belle Helene	Belle Helene
SAMPLE SIZE	1	2[1]	1
EMBRYO MEAN WEIGHT (g)	110.0	136.8	354.8
LINDANE	N.D.	N.D.	0.64
ENDRIN	0.01	N.D.	N.D.
ALDRIN	N.D.	0.13	N.D.
DIELDRIN	0.12	0.09	0.09
HEPTACHLOR EPOXIDE	0.13	1.76	N.D.
HCB	0.04	0.27	0.14
DDT	N.D.	N.D.	0.07
DDE	0.31	0.53	0.28
DDTs	0.31	0.53	0.35
AROCLOR 1260	0.80	1.28	1.33

[1]In the case where residues were detected they were found in embryos from both females examined.

and 1979. Residents of the area in 1973 contained significantly greater plasma levels of HCB than a control group (Burns and Miller 1974). Elevated plasma HCB concentrations have been correlated with the condition of cutaneous porphyria in humans (Cam and Nigogosyan 1963). HCB is used as a seed dressing fungicide in wheat and other grains and is also a common starting material and by-product in the chemical industry (Gilbertson and Reynolds 1972). The presence of elevated levels of HCB in the snake tissues indicates that either the HCB contamination problem of 1973 still exists or noteworthy persistence of HCB has occurred in this locale. The persistence of HCB and its resistence to photolysis has been demonstrated (Plimmer and Klingebeil 1976). Since the size and/or weight of a snake was not correlated with the presence of HCB contamination, it is possible that newborn snakes are still being exposed to high levels of HCB in the area of Belle Helene.

Much attention has been directed towards the presence of organochlorines in eggs and developing embryos of aquatic vertebrates (Livingston and deLaCruz 1977; Guiney et al. 1979). Table 3 lists organochlorine residue levels in pooled embryos taken from four female snakes at two sites. The occurrence of residues in the embryos reflected patterns observed for adult whole snakes (Table 2). All four embryo samples had considerable levels of PCB's, ranging from 0.8 to 1.33 ppm. The embryos were analyzed with the yolk sac included, therefore it is likely that the lipid portion of the yolk contributed most of the measured hydrocarbons. Dieldrin, DDE, and HCB were also detected in every embryo sample. Levels of 0.81 and 2.70 ppm heptachlor epoxide were observed in the two embryo samples of N. rhombifera taken from Belle Helene. However, no heptachlor epoxide was detected in the N. cyclopion embryo sample taken in the following year from the same site. Heptachlor was not detected in any embryo sample.

Butler and Schutzmann (1979) have demonstrated that organochlorines are passed from females into developing embryos of the spiny dogfish. Similarly, Guiney et al. (1979) determined that elimination of a PCB isomer, 2,5,2',5'-tetrachlorobiphenyl, from fishes was enhanced during the spawning season. Our results for the few embryos examined are in agreement with this phenomenon. Indeed, organochlorine residues may adversely effect vertebrate development.

The chlorinated hydrocarbon burdens measured in tissues and intact snakes indicate that, despite considerable individual variability, these reptiles are useful as pollution indicators. Detection of local "hot spots" is possible, as demonstrated in the case of hexachlorobenzene at Belle Helene. This feature is attributable in part to the limited migratory behavior of these snake species. In regions where snakes are abundant, a periodic survey of certain deleterious substances contained in either fat bodies or whole snakes would be informative and integrate well into any local or regional monitoring program.

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