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Disposition of Low and High Environmental Concentrations of PCBs in Snapping Turtle Tissues

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Organisms living in water are sensitive to changing environmental conditions and may, due to the general equilibrium between the biotic community and its environment, be utilized to monitor toxics in aqueous ecosystems. Snapping turtles, as a result of their ability to store high concentrations of PCBs in their fat (Stone et al. 1980; Helwig and Hora 1983; Olafsson et al. 1983), provide an excellent screen for the detection of trace toxic substances in water.

Snapping turtles may also be of value in the monitoring of the disposition of environmental pollutants in the tissues of organisms living in a particular ecosystem. Many organochlorine compounds are only slowly metabolized by animals and consequently the parent compounds tend to persist in the tissues. For a number of years, it was assumed that these compounds were practically irreversibly retained in the adipose tissue due to their high lipid solubility, a property which was anticipated to result in a low blood-fat partition. However, it has now been amply documented that the blood of humans and animals exposed to PCB's contain readily detectable quantities of such highly lipophilic substances. The presence of exceedingly hydrophobic chemicals in such an aqueous medium as blood has been attributed to dissolution in blood components, such as lipids (Morgan and Roan 1970), plasma proteins (Moss and Hathway 1964), and particularly plasma lipoproteins (Winter et al. 1975; Skalsky and Guthrie 1978) which may be a key factor in establishing the distribution in the body of at least some chlorinated hydrocarbons (Morrison 1971; Skalsky and Guthrie 1977).

Differences in the degree of dissolution of various polychlorinated hydrocarbons in blood may be attributed to differences in the relative solubility of these compounds in one or more of the blood components. These binding characteristics provide an explanation for the fact that Kepone, which resembles other halogenated hydrocarbons in polarity and other physical properties, differs from them in terms of relative disposition between adipose tissue and blood. The fat to blood ratio of

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chemical workers exposed to large quantities of Kepone was only 7 to 1 (Cohen et al. 1978) whereas the comparable distribution of DDT was shown to be 280 to 1 (Morgan and Roan 1974). Although both these compounds are bound by plasma lipoproteins, Kepone appears to interact mainly with ß-lipoproteins (high-density fraction) in marked contrast to DDT which binds primarily to ß-lipoproteins (low-density fraction) (Winter et al. 1975; Skalsky and Guthrie 1977) a factor which may well account for the difference in fat to blood partitioning. Similarly it could account for the tendency of Kepone and tetrachlorodibenzodioxin (TCDD) to accumulate in the liver at higher concentrations than in adipose tissue (Egle, et al. 1978; Boylan et al. 1978).

Skalsky and Guthrie concluded that binding of individual organochlorine compounds by lipoproteins and albumin involved slowly reversible hydrophobic interactions with a quasi steady state between adipose tissue, blood and remaining tissues. If indeed such a dynamic equilibrium is involved, it might be anticipated that, under the impact of a multi-component pollutant such as an Aroclor, the biological system would respond in the same manner over a wide range of concentrations provided that the binding of each the congeners involved reversible interactions with the quasi steady state. The net result would be that the order of quantitative disposition of PCBs in the various tissues would be maintained as the concentration in each site increased. It is the objective of this study to determine if such a pharmacodynamic equilibrium is operative in snapping turtles (Chelydra serpentina, the species native to New York State) (New York State Conservation Dept., 1939; Petokas et al. 1980) subjected to widely differing degrees of PCB environmental contamination. Two specimens were chosen, one from a region of very low contamination (a 21 lb. male turtle caught at a farm pond near Columbus, Chenango County, New York) and the other from a highly polluted area (a 10.5 lb. turtle caught at the G. E. Moreau dump site near South Glen Falls, Saratoga County, New York).

MATERIALS AND METHODS

The two snapping turtles were killed by decapitation, the blood drained, plastrons removed and fat bodies and appropriate organs taken. Representative fat samples from these reptiles were examined for the presence of organochlorine residues.

Fat samples (5 gm) were subjected to Soxhlet extraction with 2:1 acetone-hexane for one hour, and then 1:1 acetone-hexane for six hours. The residual liquid was evaporated to low volume on a Kuderna Danish and then treated with conc. H_2SO_4. The hexane layer was removed, washed with a small amount of water and dried over anhydrous sodium sulfate. The resulting solution was made up to 10 ml in volumetric flask. A sample of this solution (1 ml) was submitted for gas chromatographic analysis. The sample (2µl for splitless glass capillary analysis) was injected into the port of a 5880 Hewlett-Packard chromatograph maintained at 225°C. An Apiezon L column was temperature programmed to achieve the best resolution of the PCBs in a standard

1:1:1:1 mixture of Aroclors 1016, 1221, 1254, 1260 and Mirex. The carrier gas consisted of helium (1-2 ml/min) with a makeup gas of argon/methane 95:5 (15-20 ml/min). The analytical data were obtained as outlined in the literature (Bush et al. 1982).

RESULTS AND DISCUSSION

Chromatograms of PCBs from adipose tissue samples of the comparative turtles indicate that they have been subjected to very different concentrations of the same types of PCBs (Table 1).

Table 1. PCB congener concentrations in adipose tissue of specimen turtles.

PCB Congener	Columbus Turtle ppm	Columbus Turtle R.T.(Min)	Moreau Turtle ppm	Moreau Turtle R.T.(Min)
3,2'4'	.012	27.24	.080	23.14
3,2'3' & 4,2'4'	.017	27.65	.228	23.57
4,2'3'	.038	28.18	.069	24.12
25,2'5'	.123	29.18	.282	25.16
24,2'5'	.075	29.81	.167	25.81
23,2'5'	.051	30.15	.140	26.18
24,2'4'	.021	30.51	.304	26.53
4C	.634	31.44	.036	27.48
5A	.046	33.31	.014	27.91
24,3'4'	.151	35.89	272.	32.18
4,2'3'4'	.033	36.66	29.4	32.95
236,2'3'6'	.117	36.97	297.	33.32
25,2'4'5'	.063	37.43	7.96	33.72
24,2'4'5'	.054	38.21	166.	34.52
25,2'3'4'	.302	38.78	13.5	35.13
23,2'3'4'	.093	39.80	99.6	36.02
236,2'3'4'6'	.332	44.45	152.	40.90
34,2'3'4'	.159	45.93	171.	42.45
245,2'4'5'	.269	46.23	80.0	42.69
234,2'4'5	.264	47.78	87.6	44.23
234,2'3'4'	.084	49.38	35.7	45.83
2356,2'3'5'6'	.075	49.83	16.8	46.26
234,2'3'4'6'	.017	50.64	10.2	47.06
236,2'3'4'5'6'	.009	52.58	10.1	49.02
34,2'3'4'5'	.123	54.10	43.4	50.48
245,2'3'4'5'	.390	55.22	41.6	51.60
234,2'3'4'5'	.138	56.82	23.7	53.16
2345,2'3'5'6'	.136	57.07	14.5	53.42
245,2'3'4'5'6'	.126	58.50	9.80	54.68
234,2'3'4'5'6'	.067	60.48	7.93	56.35
345,2'3'4'5'	.159	65.81	10.88	60.17

A function of blood protein is to reversibly bind sparingly soluble endogenous materials for transport from one site to another (Skalsky and Guthrie 1975, 1978). Exogenous materials upon entering an organism are often distributed from tissue to tissue by the same blood proteins. The tissue in which such a substance becomes preferencially located is dependent on the solubility characteristics of that compound in the lipid, protein, plasma protein or lipoprotein associated with that particular site. The comparative data for disposition of PCBs in the tissues of the two turtles are presented in Table 2.

Table 2. Relative disposition of PCBs in tissues of specimen turtles

Low Pollution Environment Columbus Chenango County, NY		High Pollution Environment Moreau Saratoga County, NY
Tissue	PCB Conc. (ppm)	PCB Conc. (ppm)
Fat	4.2	1600
Testes	1.6	100
Brain	1.0	82
Liver	1.0	72
Heart	0.64	49
Kidney	1.2	48
Pancreas	1.2	48
Lungs	0.41	13

Despite the environmental stress placed on the turtle from Moreau, the tissues showed similar order of disposition as for the turtle from Columbus. These data suggest that the binding of PCB congeners by lipoproteins and albumin involve slowly reversible hydrophobic interactions with a quasi steady state between adipose tissue, blood and remaining tissues over the extremes of PCB concentrations.

Toxicological implications, of lipoprotein binding to exogenous materials, have been attributed to selective absorption of low density lipoproteins by cells, followed by the metabolism of the lipoprotein with a specialized group of enzymes. The residual procarcinogen is thus provided with a site of activation in close proximity to the nucleus (Skalsky and Guthrerie; 1975, 1977).

An alternate cause of toxicity involves the inhibition of an enzyme (Westlake et al. 1983). In this case, the resulting toxic response is dependent not only on the degree of inhibition but also on the initial level of enzyme present. Consequently changes in enzymes may provide indirect evidence of cellular damage. Species with low levels of detoxifying enzymes may be vulnerable to chronic effects from exposure to chemicals as a result of slow metabolism and consequently bioaccumulation. Accordingly the disposition of a toxic substance such as a PCB in a target tissue may be utilized to establish the cause of death in a particular organism.

In the case of birds, the mortality level is generally reached with an accumulation of 300 ppm PCBs in the brain (Sileo et al. 1977). The corresponding lethal dose for snapping turtles has not yet been established. Recent research suggests that turtles will survive much higher levels of PCBs than can birds. Turtles (Mauremys caspia rivulata, Emydidae chelonia) withstand the lethal action of parathion due to a low K_i value for brain esterase inhibition. In the turtle this K_i value is about 250-fold lower than that for the barn owl (Yawetz et al. 1978). This lack of sensitivity of the enzyme may afford the turtle with an efficient biochemical defense mechanism against parathion poisoning. It has been suggested (Anderson et al. 1977) that the brain cholinesterase of the turtle differs from that found in mammals and birds but resembles more the enzyme found in amphibians.

Wildlife monitoring focusses on the pollution of the wildlife itself, but the use of selected specimens may also be employed to monitor the pollution of the habitat. The data obtained in this study markedly reflect the difference in pollution levels of the sites from which the turtles were obtained. Despite the wide difference in degree of exposure to PCBs, their accumulation within the reptile is not random but rather shows an ordered preference for specific locations, eg. fat, testes and brain. The higher concentrations found in the latter regions reflect the lipoprotein solubility of PCBs. In the remaining tissues the relative order is probably due to the corresponding concentrations of the appropriate enzymes required for metabolism of these substances.

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