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Disposition of Toxic PCB Congeners in Snapping Turtle Eggs: Expressed as Toxic Equivalents of TCDD

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Studies of snapping turtles, taken from the region of the Upper Hudson River, in New York State, revealed exceedingly high levels of PCBs in the adipose tissue (Olafsson, 1983). There is evidence to suggest that large reserves of fat provide protection against chlorinated hydrocarbon toxicity (Matthews and Anderson 1975). Wyss et. al. (1982) concluded that highly lipophilic PCB congeners, such as 2,2',4,4',5,5'-hexachlorobiphenyl, appear to be irreversibly stored in the fat of rats having large adipose tissue depots. Such irreversible storage may protect snapping turtle eggs from disposition of toxic PCB congeners and account for the apparent absence of reports regarding detrimental effects on the hatchability of eggs from turtles living in the vicinity of the upper Hudson River. The present study was undertaken to determine if indeed these eggs are protected against disposition of toxic PCB congeners by the presence of large reserves of fat.

Absorption of a toxic PCB congener into the blood is the first step which must occur if that congener is to reach its site of action (Matthews, 1975). Lipoproteins are involved in transporting such hydrophobic substances. As the degree of halogenation increases, not only does the lipid solubility increase but also the possibility of binding to lipoproteins. In vivo rat studies have shown that 60% of 2,2',4,5,5'- pentachloro and 80% of 2,2',4,4',5,5'-hexachlorobiphenyl partition into the lipoprotein fraction. Thus, the transportation of halogenated aromatics in the blood appears to occur via partition into various lipid rich phases rather than by specific binding.

Although tissue volumes, such as those of the liver and muscle, play an important role in determining the initial site of disposition, the major factor controlling the elimination of these compounds involves metabolism. For simple halogenated benzenes as well as for more complex halogenated biphenyls, oxidative metabolism, catalyzed by P-448, occurs primarily at the site of two adjacent unsubstituted carbon atoms via arene oxide formation (Weisburger 1980) leading to the formation of water soluble metabolites. Alternatively, the resulting

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intermediate epoxide may act through its strong electrophilic character to bind covalently to cellular macromolecules, the most probable target being DNA (Miller, 1978; Heidelberger, 1975). Considerable evidence suggests that covalent adduct formation represents both the initial and critical step in carcinogenesis.

Toxicological studies have also demonstrated that the most toxic PCB congeners, isosteriomers of tetrachlorodibenzo-p-dioxin (TCDD), require no metabolic activation. These compounds have chlorine atoms in the meta and para positions of both rings (McKinney, 1982). These findings in conjunction with x-ray data obtained for TCDD suggest that the four lateral substituents must be capable of lying in the same plane, ie. the rings must be able to assume coplanarity if they are to lie within a rectangle 3x10Å so as to bind, as does TCDD, to a cytosolylic protein, the Ah receptor and evoke a toxic response at the target site. The avidity with which a given congener binds to the Ah receptor, correlates with the chemical's potency as a cytochrome P-448 inducer (Parkinson, 1980). The latter enzyme manifests its presence through aryl hydrocarbon hydroxylase (AHH) activity in PCB metabolism via arene oxide formation.

It may be concluded that the structures of PCB congeners and isomers which favor induction of cytochrome P-448 are also those which are toxic and resist metabolism. These compounds possess a minimum of four chlorine atoms and therefore highly lipophilic. It is the objective of the present study to determine if the heavy fat bodies of the female turtle provide a sufficiently large sink to retain the toxic congeners and prevent their incorporation into the eggs.

MATERIALS AND METHODS

After carefully washing the shell, the yolk was separated from the white, weighed and then homogenized in the presence of an equal volume of anhydrous sodium sulfate. The resulting mixture was placed in a soxhlet, which had previously been extracted with hexane to remove any PCB impurities. The yolk was extracted with hexane over a period of eight hours. The resulting extract was worked up in the usual manner for gas chromatographic analysis.

The PCB mixtures were subjected to high resolution capillary gas chromatographic analysis. The sample (2µl for splitless gas chromatographic analysis) was injected into the port of a 5890 Hewlett Packard gas chromatograph maintained at 225°C. A DB-5 fused silica column was temperature programmed to achieve the best resolution of the PCBs in the sample. The carrier gas consisted of helium (1-2 ml/min with a make-up gas of argon/methane 95:5 (15-20 ml/min). Injections were made with an automatic injector. The white and shell were analyzed in the same manner.

The DB-5 column was calibrated in terms of a hexane solution containing known amounts of the pure synthetic toxic PCB congners and isomers (Table 1) in order to permit accurate quantitation of these compounds.

Table 1. Toxic polychlorobiphenyls utilzed in the calibration of the DB-5 column.

3,3'4,4',5-pentachloro-	2,3,4,4',5-pentachloro-
3,3',4,4',5,5'-hexachloro-	2,3,3',4,4',5-hexachloro-
3,3',4,4'-tetrachloro-	3,4,4',5-tetrachloro-
2,3,3',4,4'-pentachloro-	2,3,3',4,4',5,5'-heptachloro-
2',3,4,4',5-pentachloro-	2,3',4,4',5-pentachloro-
2,3,3',4,4',5-hexachloro-	2,3',4,4',5,5'-hexachloro-

RESULTS AND DISCUSSION

The gas chromatograms (Fig. 1) obtained for the egg extracts indicate the presence of toxic PCB congeners and isomers.

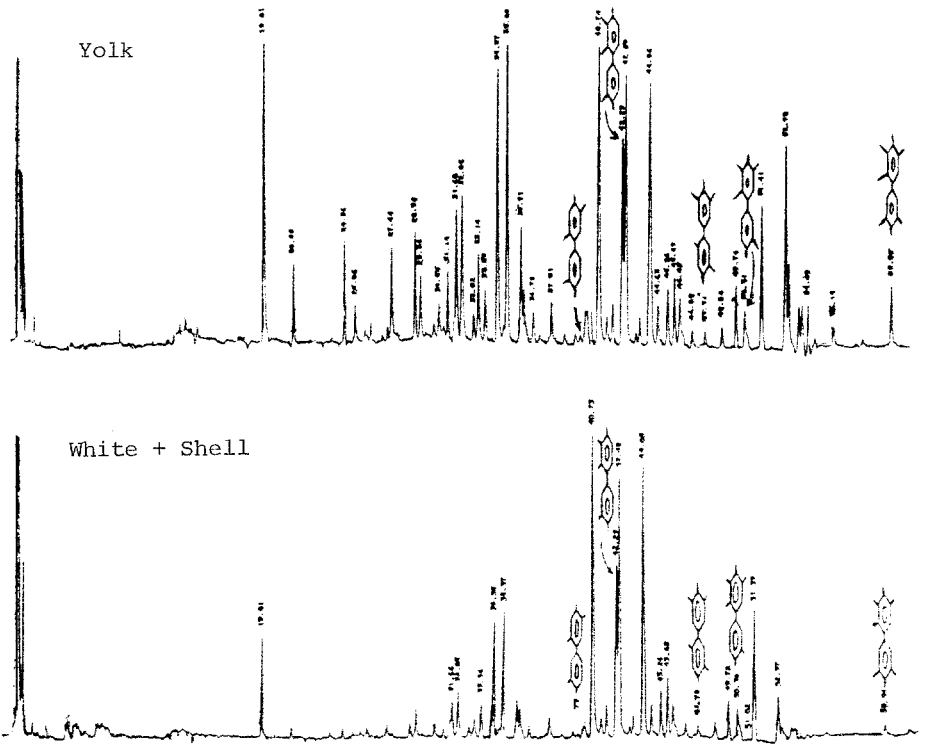

Figure 1. High resolution gas chromatographic analysis showing disposition of PCB congeners in the yoke vs shell and white of a snapping turtle egg.

Differences in partitioning of the organochlorine compounds between the yolk vs the white and shell is evident from differences in the earlier peaks. The strong preference for disposition in the yoke is very apparent, particularly in terms of the comparative heights of the first peak, hexachlorobenzene (RT 19.01 minus) which is present in both components.

A comparison of the relative PCB toxicity associated with environmental samples, on the basis of total concentration of PCBs, is difficult. The environments from which the sample were taken may have differed markedly in the types of Aroclor to which they were subjected. Arochor 1016 contains a much lower concentration of toxic congeners than Arclor 1260. Since toxic congeners have from four to seven chlorine substituents, the toxicity of an Aroclor increases with increasing chlorine content. A further complication in the utilization of total PCB content, as a monitor of toxicity in environmental samples, lies in the fact that the lower molecular weight congeners may have been removed to different extents due to metabolism via the food chain, a factor related to the time lapse since the polutant was introduced into that area.

Toxic assessments based on the total concentration of toxic PCB congeners, while an improvement, do not take into account the wide differences in toxicity between the individual toxic congeners. This difficulty has been circumvented in the present study by converting the amount of each toxic congener to the equivalent amount of TCDD required to produce the same degree of toxicity. The total toxic impact of the PCB mixture can then be computed by summation of the toxic equivalents of the individual toxic PCB congeners and isomers.

The total equivalence factors (TEFs) required to convert the amounts of toxic PCB congeners to equivalent toxic amounts of TCDD were obtained through the relationship between AHH activity and toxicity. Several studies have shown that the avidity with which a compound binds to the Ah receptor correlates to a degree with the chemical's toxicity (Poland and Knutson 1982). Binding in itself is not the sole determining factor. It appears that other events subsequent to ligand - receptor complex formation are responsible for toxic responses to halogenated aromatics such as TCDD. Since the Ah locus controls not only the expression of AHH activity but also several other genes, it is possible that TCDD and its isostereomers exert their toxic effects through the expression or repression of one or more genes controlled by the receptor.

Bradlaw and Casterline (1979) established, through bioassay studies, a relationship between AHH activity and toxicity involving TCDD and its isostereomers. In a continuation of this approach Sawyer and Safe (1982) studied the in vivo quantitative structure activity relationships (QSARs) of several PCB congeners with their in vitro activities as AHH inducers of rat hepatoma H-4-II E cells. An excellent linear correlation was established between the in vivo -log ED_{50} values for several mediated toxic responses such as body weight loss and thymic atrophy and the in vitro - log EC_{50} values for AHH induction. Quantitative estimates of AHH induction potencies for toxic PCB congeners and isomers, obtained as EC_{50} concentrations (Safe 1987) were utilized in the present study to compute the necessary TEFs (Olafsson, 1987) and calculate the toxic equivalents of TCDD (Table 2).

The toxic equivalents obtained in Table 2 were utilized to calculate the total toxic equivalents of TCDD and to determine the relative disposition of toxicity in the eggs (Table 2).

Table 2. Total equivalents of TCDD disposed in yolk and in the white and shell of a snapping turtle egg.

Polychlorinated Biphenyls	Conc. in PPM	T.E.Fs	Toxic Equiv. of TCDD (PPM)
Yolk A			
2,3',4,4',5-	0.0317	8.35×10^{-6}	0.265×10^{-6}
2,3,3',4,4'-	0.695	1.10×10^{-3}	0.765×10^{-3}
2,3',4,5',5'	0.115	7.22×10^{6}	0.830×10^{-6}
2,3,5',4,4',5-	0.202	1.35×10^{-4}	0.273×10^{-4}
2,3,3',4,4',5,5'	0.0625	8.50×10^{-6}	0.530×10^{-6}
White & Shell A			
2,3'4,4',5-	0.00268	8.35×10^{-6}	0.00224×10^{-6}
2,3,3',4,4'-	0.0605	1.10×10^{-3}	0.0665×10^{-3}
2,3',4,4',5,5'-	0.0122	7.22×10^{-6}	0.0875×10^{-6}
2,3,3',4,4',5-	0.0455	1.35×10^{-4}	0.0615×10^{-4}
2,3,3',4,4',5,5'	0.00375	8.50×10^{-6}	0.0319×10^{-6}
Yolk B			
2,3',4,4',5-	0.016	8.35×10^{-6}	0.134×10^{-6}
2,3,3',4,4'-	1.89	1.10×10^{-3}	2.08×10^{-3}
2,3',4,4',5,5'-	0.281	7.22×10^{-6}	2.03×10^{-6}
2,3,3',4,4',5-	0.556	1.35×10^{-4}	0.75×10^{-4}
2,3,3',4,4',5,5'-	0.119	8.50×10^{-6}	1.01×10^{-6}
White & Shell B			
2,3',4,4',5-	0.0083	8.35×10^{-6}	0.069×10^{-6}
2,3,3',4,4'-	0.272	1.10×10^{-3}	0.299×10^{-3}
2,3',4,4'5,5'-	0.0379	7.22×10^{-6}	0.273×10^{-6}
2,3,3',4,4',5	0.140	1.35×10^{-4}	0.189×10^{-4}
2,3,3',4,4',5,5'	0.0173	8.50×10^{-6}	0.147×10^{-6}

Table 3. Relative distribution of equivalent TCDD toxicity in yolk vs shell & white.

Sample	Toxic Equiv. TCDD (PPB)	Weight of Sample (g)	Wt. of TCDD Equiv. (ng)	% Toxicity in Sample
Yolk(A)	0.794	4.142	3.29	95.9
White & Shell	0.0728	1.917	0.140	4.08
Yolk(B)	2.18	4.579	9.98	95.0
White & Shell	0.318	1.542	0.490	4.67

The data obtained in Tables 2 and 3 indicate that the fat depots of the female turtle did not prevent toxic congeners from being dispersed in the egg. Of the five toxic congeners present in the yolk

as well as in the white and shell, two, 2,3,3',4,4'-pentachlorobiphenyl and 2,3,3',4,4',5-hexachlorobiphenyl made up more than 99% of the total toxicity. Furthermore over 95% of the total toxicity resided in the yolk.

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