1973 #23

DISTRIBUTION AND BLOOD DISAPPEARANCE OF ^{14}C-2,4,5,2^{1},5^{1}-PENTACHLOROBIPHENYL IN THE DOGFISH SHARK *Squalus acanthias* AFTER INTRAVASCULAR ADMINISTRATION

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The polychlorinated biphenyls (PCB) are widespread in the waters of the North Atlantic (Harvey *et al.*, Science, 180, 643, 1973). Due to their high chemical stability and lipid solubility there is a persistence of PCB residues in vertebrate organisms which may in part be attributed to storage in adipose tissue. A recent report indicated that pure mono-, di- and tetrachlorobiphenyl isomers were

TABLE 1

SPECIFIC ACTIVITY (dpm/mg) OF VARIOUS TISSUES AND FLUIDS OF THE DOGFISH SHARK WITH TIME AFTER INTRAVENOUS ADMINISTRATION OF ^{14}C-2,4,5,2^{1},5^{1}-PENTACHLOROBIPHENYL (0.03 mg/kg)

Tissue or fluid	dpm/mg tissue or /ul fluid Time after administration			
	6 hours	24 hours	3 days	7 days
Liver	14.004 ± 2.147 (3)+	21.633 ± 4.796 (3)*	18.984 ± 5.772 (3)	14.052 ± 2.391 (4)
Salt gland	3.117 ± 0.908 (3)	0.715 ± 0.073 (3)***	0.600 ± 0.071 (3)***	0.355 ± 0.054 (4)***
Spleen	1.864 ± 1.044 (3)	0.591 ± 0.047 (3)***	0.611 ± 0.081 (3)***	0.455 ± 0.061 (4)***
Pancreas	2.310 ± 0.769 (3)	0.488 ± 0.075 (3)***	0.532 ± 0.159 (3)***	0.327 ± 0.045 (4)***
Kidney	5.394 ± 0.722 (3)	1.036 ± 0.221 (3)***	0.647 ± 0.053 (3)***	0.494 ± 0.054 (4)***
Gill	1.343 ± 0.375 (3)	0.779 ± 0.046 (3)*	0.554 ± 0.126 (3)***	0.695 ± 0.211 (4)**
Heart	0.820 ± 0.096 (3)	0.361 ± 0.022 (3)***	0.413 ± 0.112 (3)***	0.152 ± 0.038 (4)***
Brain	0.915 ± 0.304 (3)	1.068 ± 0.235 (3)	0.396 ± 0.163 (2)**	0.158 ± 0.029 (4)***
Muscle	-	-	0.872 ± 0.416 (2)	0.664 ± 0.083 (4)
CSF	0.017 ± 0.029 (3)	0.029 ± 0.051 (3)	0.055 ± 0.048 (3)	0.044 ± 0.044 (4)
Blood	0.804 ± 0.023 (3)	0.322 ± 0.083 (3)**	0.498 ± 0.228 (3)	0.135 ± 0.132 (4)***
Bile	0.408 ± 0.209 (3)	1.349 ± 0.510 (3)**	2.708 ± 0.578 (2)***	12.034 ± 4.834 (4)***

+Mean ± S.D. (N).
*p < 0.10
**p <0.05
***p < 0.01
} compared with 6 hrs.

hydroxylated by rat and pigeon but not by a fresh water fish, brook trout (Hutzinger *et al.*, Science, 178, 312, 1972). A hexachloro isomer was not hydroxylated by any of these species. In our laboratory we have shown recently that a pentachloro PCB isomer injected intravenously was taken up rapidly by the liver in rats and excreted in the bile both as unchanged compound and as more polar hydroxylated and conjugated — hydroxylated metabolites. In view of the apparent lack of metabolism of PCB in a fresh water fish we thought it of interest to study the distribution, excretion, and metabolism of the pentachloro PCB isomer in a marine species, the dogfish shark *Squalus acanthias*.

Female dogfish, 3.0-5.1 kg, were caught locally and stored in tanks equipped with circulating sea water at MDIBL during the experimental period. 2,4,5,2^1,5^1-Pentachlorobiphenyl-(2^1,5^1-dichlorophenyl-^{14}C) (^{14}C-PCB), 10.08 mCi/mM, was obtained from Mallinckrodt Nuclear. ^{14}C-PCB (0.03 mg/kg; 2.2×10^6 dpm/kg) was injected into the caudal vessels as a solution in Emulphor (R)-water (36%, v/v). Blood samples (serially in disappearance studies or terminally in distribution studies) were drawn from the caudal vessels and put in heparinized vials. Fish were killed by spinal cord transection at six and 24 hours, at three, seven and 12 days after injection and tissues were removed and weighed. At the same time, bile samples were aspirated from the gall bladder and cerebrospinal fluid (CSF) was taken from the ventricular system. Solubilization and measurement of radioactivity were done as described elsewhere in this Bulletin (Bend *et al.*, Bull. MDIBL, 13, 1973). Statistical analysis of the data was performed using Dunnett's multiple comparisons test. Log transformation was used to equalize the variances.

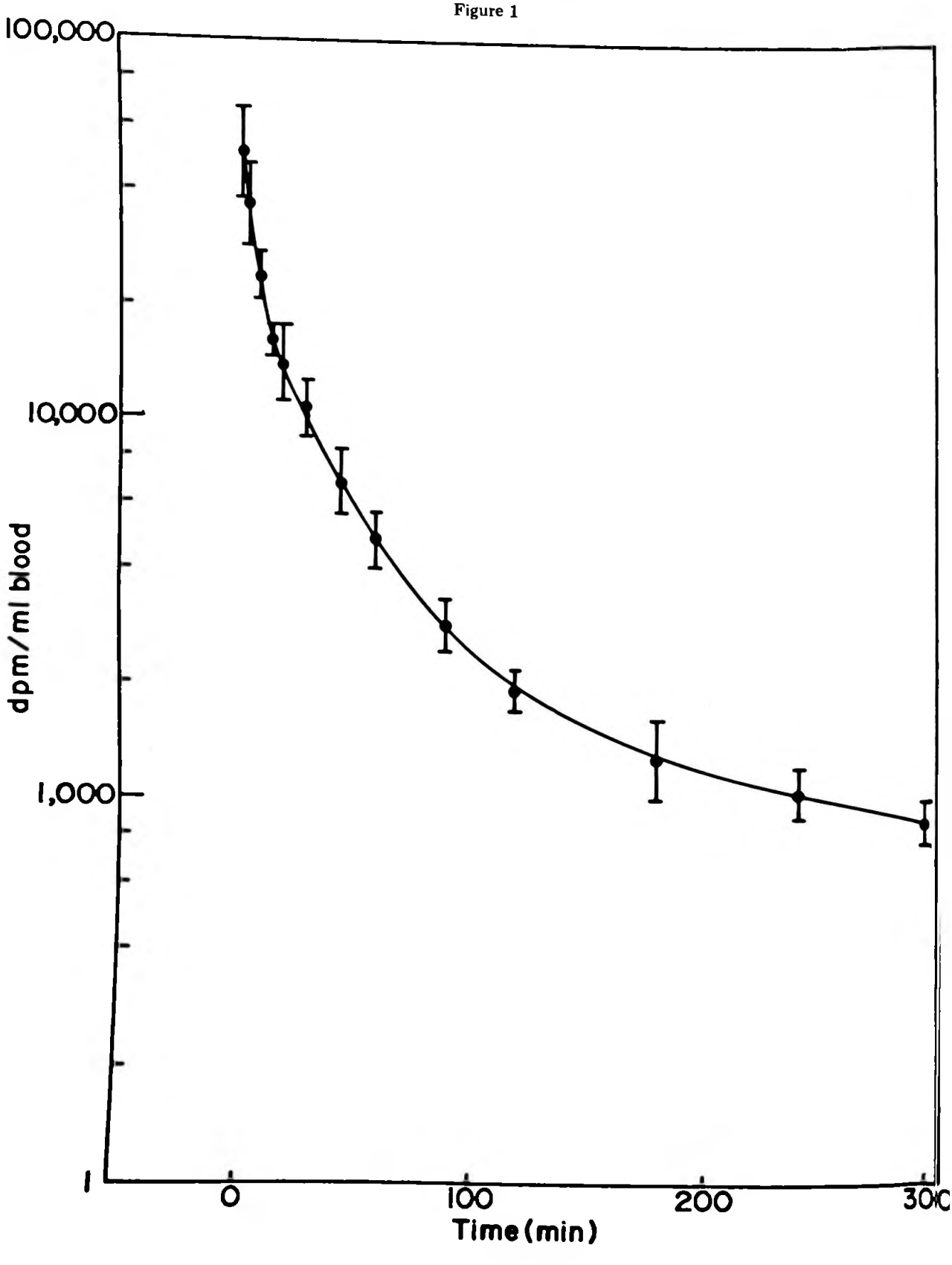
TABLE 2

% ADMINISTERED RADIOACTIVITY REMAINING IN TISSUES OF DOGFISH SHARK WITH TIME AFTER INTRAVENOUS INJECTION OF ^{14}C-2,4,5,2^1,5^1-PENTACHLOROBIPHENYL (0.03 mg/kg)

	Time after injection			
Tissue	6 hours	24 hours	3 days	7 days
Liver	75.216 ± 6.106 (3)*	94.020 ± 24.654 (3)	79.552 ± 12.770 (3)	89.424 ± 11.170 (4)
Salt gland	0.077 ± 0.019 (3)	0.018 ± 0.002 (3)***	0.010 ± 0.001 (3)***	0.008 ± 0.001 (4)***
Spleen	0.211 ± 0.038 (3)	0.081 ± 0.020 (3)**	0.060 ± 0.018 (3)***	0.073 ± 0.031 (4)***
Pancreas	0.249 ± 0.033 (3)	0.054 ± 0.018 (3)***	0.045 ± 0.020 (3)***	0.032 ± 0.004 (4)***
Kidney	0.430 ± 0.155 (3)	0.064 ± 0.006 (3)***	0.059 ± 0.011 (3)***	0.060 ± 0.016 (4)***
Heart	0.052 ± 0.005 (3)	0.014 ± 0.002 (3)***	0.016 ± 0.004 (3)***	0.008 ± 0.003 (4)***
Brain	0.033 ± 0.011 (3)	0.028 ± 0.006 (3)	0.010 ± 0.001 (2)**	0.005 ± 0.002 (4)***
Mean total recovery	76.268%	94.279%	79.752%	89.610%

*Mean ± S.D. (N).
**p <0.05, ***p <0.01 compared with 6 hrs.

Figure 1



As shown in Table 1 the specific activity (dpm/mg) in the liver was higher than for any other tissue at all time periods. Furthermore specific activity actually increased in the liver from six to 24 hours and remained at or above the activity at six hours throughout the course of the study (20.275 dpm/mg at 12 days). In most of the other tissues there was a significant decrease in activity between six and 24 hours. There were only trace levels of radioactivity in CSF at all times. Urine was collected continuously in a few fish after cannulation of the urinary papillae. Only small amounts of radioactivity were present in the urine (0.103 dpm/µl at six hours to 0.069 dpm/µl at 96 hours). However in bile there was a consistent increase in activity with time, peak levels being attained at seven days. By 12 days biliary radioactivity had declined to 2.854 dpm/µl. The delayed pattern of biliary excretion of radioactivity in dogfish is in contrast to that in a mammalian species, the rat, where ^{14}C-PCB and its metabolites were rapidly excreted in the bile after an IV injection. At all times (Table 2) most of the recovered radioactivity was found in the liver; there were only trace amounts (<1%) in the other tissues at six hours and the percentages found were significantly lower at all times after six hours (except in the brain at 24 hours). Serial blood samples were drawn in three fish at times ranging from 2.5 to 300 minutes and the decline in radioactivity (dpm/mg) was plotted semilogarithmically versus time. As shown in Figure 1, blood disappearance of radioactivity appears to follow a multiple exponential decay with time; the initial phase of which could correlate with the rapid uptake by the liver over this early time period (Table 2).

In an attempt to characterize the radioactivity present, aliquots of liver sampled at various time points were homogenized and extracted four times with benzene. Thin-layer chromatographic (TLC) analysis of the extracted radioactivity on precoated 250 µ silica gel GF plates (activated at 110°C for one hour and developed in hexane) demonstrated that most of this radioactivity co-chromatographed with authentic 2,4,5,2^{1},5^{1}-pentachlorobiphenyl (R_f 0.7-0.8) at all time periods studied. However the percent of total sample radioactivity extracted into the benzene decreased with increasing time after injection of ^{14}C-PCB (from 100 percent at six hours to 56 percent at seven days).

In conclusion ^{14}C-PCB is rapidly cleared from the blood after intravascular injection in dogfish and taken up almost exclusively by the liver. Due to the high lipid content of dogfish liver (~50 percent by weight), this organ appears to function both as the major site of rapid uptake and the major site of storage for PCB (and perhaps other highly lipid soluble compounds). The delayed excretion into bile of PCB-derived products may be due to a poor partitioning of the compound from hepatic lipid into the parenchymal cell where metabolism and excretion can occur. Further the low hydroxylating activity of dogfish liver (Bend *et al.*, Bull. MDIBL, 12, 12, 1973) may also contribute to the delayed excretion of PCB.

1973 #24

STUDIES OF THE FATE OF PHENYLACETIC ACID IN SOME FISH

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A large number of nonnutrient carboxylic acids are detoxified in mammalian species by com-