

## RESEARCH REPORTS

1973 #1

### DISTRIBUTION OF $^{14}\text{C}$ -2,4,5,2',5'-PENTACHLOROBIPHENYL IN THE LOBSTER *Homarus americanus* AT VARIOUS TIMES AFTER A SINGLE INJECTION INTO THE PERICARDIAL SINUS

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The polychlorinated biphenyls (PCB) occur as widespread contaminants in the environment and are present as tissue residues in several marine species (Adamson and Guarino, Bull. MDIBL 12: 6, 1972). In an earlier investigation we found no significant decrease in the radioactive content of lobster hepatopancreas one week after a single parenteral dose of  $^{14}\text{C}$ -2,4,5,2',5'-pentachlorobiphenyl ( $^{14}\text{C}$ -PCB) as compared to hepatopancreas of lobsters sacrificed 24 hrs after treatment (Bend, Guarino, Briley, Rall and Fouts, Bull. MDIBL 12: 9, 1972). We now describe the distribution of radioactivity in lobster tissues and plasma two, four, and eight weeks after a single intravascular injection of  $^{14}\text{C}$ -PCB. In addition the chemical nature of the radioactivity remaining in hepatopancreas (at all three time points), green gland and feces was determined by extraction and thin-layer chromatography (TLC) of the extracts.

Adult lobsters of mixed sex weighing 320-560 g were purchased locally (Lunt's Lobster Pound, Trenton, Maine and Thurston's, Bass Harbor, Maine).  $^{14}\text{C}$ -PCB (10.08 mCi/mM) was purchased from Mallinckrodt.  $^{14}\text{C}$ -PCB (0.2 mg/kg,  $1.4 \times 10^7$  dpm/kg) was injected into the pericardial sinus of the lobster as a solution in Emulphor-water (38% v/v). Following treatment, lobsters were placed in a lobster crate (maximum of 15 per 50 pound crate) which was immersed under two feet of water in the MDIBL seal car in Laboratory Cove. The crates were raised three times per week and the lobsters fed small pieces of flounder or mackerel. Any dead lobsters were removed. Only animals that were alive and appeared to be healthy were dissected at the time points studied. Plasma was removed from the pericardial sinus just prior to sacrifice and the various tissues were dissected from the carcass and weighed. Duplicate aliquots of plasma (100  $\mu\text{l}$ ) or tissue (70-200 mg) were solubilized by digestion in 2 ml NCS (Amersham/Searle) by incubation at 50° C overnight. Liquid scintillation solvent (18 ml; 5.0 g PPO and 250 mg POPOP/liter toluene) was added and the radioactivity determined in a Nuclear Chicago Mark I liquid scintillation spectrometer. Counting efficiency was determined using the channels ratio procedure and varied from 60-82 percent for the solubilized biological samples.

TLC was performed on precoated silica gel GF plates (5 x 20 cm, 250  $\mu$  thick; Analtech, Inc.). The solvent systems used were hexane (solvent A) and benzene:ethyl acetate (12:1, v/v; solvent B). Plates were activated by heating at 110° C for 60 minutes prior to use. Nonradioactive 2,4,5,2',5'-pentachlorobiphenyl (Analabs) was used as a chromatographic standard. It was located on the fluorescent plates under short wavelength UV light. After solvent development (10 cm) the plates were sequentially scraped (in 1 cm segments) and the radioactivity determined by counting the silica

gel in vials containing 10 ml fluoralloy (Beckman) - dioxane liquid scintillation cocktail (214.4g fluoralloy scintillation cocktail mix/2 liters scintillation grade dioxane). Radioactive recovery from the plates using this procedure was normally greater than 95 percent.

TABLE 1  
SPECIFIC ACTIVITY OF VARIOUS LOBSTER TISSUES FOLLOWING PERICARDIAL INJECTION  
OF  $^{14}\text{C}$ -2,4,5,2',5'-PENTACHLOROBIPHENYL (0.2 mg/kg)

Tissue	dpm/mg tissue or / 1 plasma Time after administration				
	24 Hours*	1 Week*	2 Weeks	4 Weeks	8 Weeks
Hepatopancreas	183.4 ± 24.7 (6)**	192.9 ± 33.4 (7)	170.9 ± 40.0 (5)	131.2 ± 34.7 (6)	77.4 ± 28.4 (9)
Green gland	20.1 ± 13.0 (6)	8.4 ± 2.4 (7)	6.1 ± 1.9 (5)	4.8 ± 1.2 (6)	3.4 ± 1.4 (9)
Intestine	14.0 ± 7.0 (6)	14.3 ± 5.9 (7)	17.6 ± 18.7 (5)	4.3 ± 0.9 (6)	2.2 ± 1.5 (9)
Heart	8.8 ± 5.4 (6)	2.7 ± 0.4 (7)	2.2 ± 0.7 (5)	1.6 ± 0.3 (6)	1.1 ± 0.3 (9)
Tail Muscle	5.1 ± 1.8 (6)	1.6 ± 0.1 (7)	1.3 ± 0.3 (5)	0.8 ± 0.2 (6)	0.4 ± 0.2 (9)
Claw muscle	5.0 ± 3.1 (6)	1.5 ± 0.2 (7)	1.3 ± 0.1 (5)	0.8 ± 0.1 (6)	0.6 ± 0.2 (9)
Stomach	4.5 ± 1.1 (6)	4.8 ± 2.1 (7)	4.3 ± 2.0 (5)	2.2 ± 1.0 (6)	1.3 ± 0.4 (9)
Gill	3.4 ± 1.8 (6)	3.2 ± 1.5 (7)	3.1 ± 2.3 (5)	1.4 ± 0.9 (6)	0.8 ± 0.3 (9)
Male gonad	8.3 (1)	1.6 (1)	2.9 (2)	2.1 (2)	1.1 + 0.2 (5)
Egg masses	16.6 ± 9.7 (5)	7.7 ± 6.6 (6)	10.2 ± 6.0 (3)	8.6 ± 4.0 (6)	7.7 ± 4.0 (4)
Plasma	1.33 ± 0.41 (6)	0.49 ± 0.17 (7)	0.62 ± 0.07 (5)	0.36 ± 0.13 (5)	0.20 ± 0.05 (9)
Feces	---	---	142.5 ± 70.7 (5)	21.5 ± 6.6 (6)	20.1 ± 10.5 (9)

\* From MDIBL Bulletin 12: 10, 1972.

\*\* Mean ± S.D. (N)

The specific activity of various lobster tissues sacrificed at different time points after  $^{14}\text{C}$ -PCB administration is shown in Table 1. The data reported last year for the early time points (24 hours and one week) are included for comparison. The hepatopancreas contained much higher concentrations of radioactivity (per mg tissue) than any other organ (Table 1) and by far the greatest percentage of administered radioactivity (Table 2). In fact the radioactivity remaining in hepatopancreas at 24 hours, one week, two weeks, four weeks, and eight weeks accounted for 91, 93, 95, 96, and 95 percent respectively of the total recovered radioactivity in these lobsters. The half-life ( $t_{1/2}$ ) of radioactivity in the various tissues was estimated from semi-logarithmic plots of the specific activity (dpm/mg) vs. time (days). In general the tissues could be separated into two groups; those that retained radioactivity for several weeks ( $t_{1/2} > 3$  weeks) and those that lost most of their radioactivity relatively quickly ( $t_{1/2} < 5$  days). The hepatopancreas ( $t_{1/2} = 45$  days), egg masses (39 days), stomach (28 days), gill (24 days), and intestine (23 days) fell into the former

TABLE 2

% ADMINISTERED RADIOACTIVITY REMAINING IN LOBSTER AFTER PERICARDIAL INJECTION OF  $^{14}\text{C}$ -2,4,5,2',5'-PENTACHLOROBIPHENYL (0.2 mg/kg)

Tissue	Time after injection				
	24 Hours*	1 Week*	2 Weeks	4 Weeks	8 Weeks
Hepatopancreas	61.4 ± 8.3 (6)**	60.5 ± 10.8 (7)	61.4 ± 14.6 (5)	55.2 ± 13.4 (6)	31.2 ± 12.5 (9)
Green gland	0.19 ± 0.12 (6)	0.07 ± 0.01 (7)	0.07 ± 0.03 (5)	0.05 ± 0.01 (6)	0.04 ± 0.02 (9)
Intestine	0.10 ± 0.09 (6)	0.11 ± 0.05 (7)	0.21 ± 0.27 (5)	0.04 ± 0.01 (6)	0.03 ± 0.02 (9)
Heart	0.09 ± 0.04 (6)	0.03 ± 0.01 (7)	0.04 ± 0.02 (5)	0.02 ± 0.01 (6)	0.01 ± 0.01 (9)
Tail muscle	4.36 ± 0.84 (6)	1.57 ± 0.26 (7)	1.54 ± 0.41 (5)	0.96 ± 0.26 (6)	0.45 ± 0.02 (9)
Stomach	0.36 ± 0.14 (6)	0.53 ± 0.39 (7)	0.45 ± 0.22 (5)	0.20 ± 0.10 (6)	0.12 ± 0.04 (9)
Gill	0.61 ± 0.31 (6)	0.59 ± 0.31 (7)	0.60 ± 0.41 (5)	0.25 ± 0.15 (6)	0.16 ± 0.07 (9)
Male gonad	0.06 (1)	0.02 (1)	0.03 (2)	0.04 (2)	0.01 ± 0.01 (5)
Egg masses	0.48 ± 0.34 (5)	0.37 ± 0.56 (6)	0.46 ± 0.44 (3)	0.64 ± 0.38 (4)	0.67 ± 0.53 (4)
Mean total recovery	67.59%	64.77%	64.80%	57.40%	32.69%

\* From MDIBL Bulletin 12: 11, 1972.

\*\* Mean ± S.D. (N)

category. Those in the second group included plasma (5 days), green gland (4.5 days), heart (3.5 days), male gonad (3.5 days), tail muscle (3.5 days), and claw muscle (3.5 days).

Virtually all of the radioactivity in hexane extracts of hepatopancreas from lobsters sacrificed two weeks, four weeks or eight weeks after treatment was present as unchanged 2,4,5,2',5'-pentachlorobiphenyl. More than 90 percent of the hepatopancreas radioactivity was extracted into hexane ( $93.2 \pm 3.4\%$ ; mean ± S.D., N = 7) after 4 extractions. Over 95 percent of this hexane soluble radioactivity co-chromatographed with authentic standard in solvent systems A ( $R_f = 0.7-0.8$ ) and B ( $R_f = 0.9-1.0$ ). The radioactivity in hexane extracts of the green gland at both two and four weeks after treatment also consisted of more than 90 percent  $^{14}\text{C}$ -PCB. However, less than 50 percent of the total radioactivity was extracted with hexane and acidified toluene suggesting the presence of polar metabolites in the aqueous residue. With feces which were obtained from the intestine at the time of sacrifice from lobsters treated two weeks and eight weeks earlier only about 10 percent of the activity was extractable into hexane and acidified toluene. About 70 percent of the extracted material was chromatographically identical to 2,4,5,2',5'-pentachlorobiphenyl in both TLC systems. The nature of the fecal water-soluble metabolite(s) has not yet been studied but presumably it is a conjugate of phenolic metabolite(s) of  $^{14}\text{C}$ -PCB similar to those which have been demonstrated in our laboratory in the rat (Chen and Matthews, unpublished observations).

It appears that the feces (Table 1) are the major site of elimination of  $^{14}\text{C}$ -PCB and its metabolite(s) in the lobster. The extended time period required for the removal of this compound is consistent with the negligible levels of oxidative xenobiotic metabolism found in subcellular fractions

prepared from lobster hepatopancreas or green gland (Pohl, Bend, Devereux and Fouts, Bull. MDIBL 13: 1973).

This study has shown that the lobster hepatopancreas is the principal site of storage for a single, purified polychlorinated biphenyl isomer after parenteral administration. The prolonged period required for excretion of this compound is consistent with the observed presence of lipophilic environmental contaminant residues in hepatopancreas of lobsters in nature. It further suggests that lobster hepatopancreas might be avoided as a foodstuff if one is interested in minimizing his dietary source of organochlorine chemical residues.

1973 #2

**GLUTATHIONE S-ARYLTRANSFERASE: DISTRIBUTION IN SEVERAL MARINE SPECIES AND PARTIAL CHARACTERIZATION IN HEPATIC SOLUBLE FRACTIONS FROM LITTLE SKATE *Raja erinacea* LIVER**

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The biliary excretion of several xenobiotics (or their conjugated metabolites) appears to be an important step in their elimination by some marine animals (Adamson, Fed. Proc. 26: 1047, 1967; Adamson and Guarino, Comp. Biochem. Physiol. 42A: 171, 1972). Sulfobromophthalein (BSP) is excreted in the bile of the dogfish (Boyer, Bull. MDIBL 11: 2, 1971; Guarino *et al.*, Bull. MDIBL 12: 41, 1972) and of the rainbow trout (Schmidt, Weber, Proc. West. Pharmacol. Soc. 15: 40, 1972). In mammals biliary excretion of BSP is normally associated with its conjugation to glutathione (GSH), catalyzed by the enzyme GSH S-aryltransferase. Therefore, soluble fractions isolated from liver or hepatopancreas of several marine species were assayed for GSH S-aryltransferase activity (towards 1,2-dichloro-4-nitrobenzene). Several properties of this enzyme and its distribution in extrahepatic organs of this species were also examined.

Little skates (650-1,300 g), large skates (1,400-2,400 g), thorny skates (1,400-1,500 g), dogfish (1,280-1,600 g), killifish (approximately 1 g), eels (110-180 g), hagfish (220-250 g), King of Norway (850-1,300 g), winter flounder (205-280 g), mackerel (510-770 g), rock crabs (120-190 g), and lobsters (435-620 g) of mixed sex were captured in local Maine waters or purchased from commercial fishermen. All animals were maintained in live-cars or tanks equipped with flowing seawater at MDIBL for at least 24 hours before use with the exception of the mackerel whose livers were frozen just after death prior to assay the following day. Tissues were homogenized as described previously (Bend, Pohl and Fouts, Bull. MDIBL 12: 12, 1972). Cell debris, nuclei, and mitochondria were sedimented from the homogenate by centrifugation at 10,000 g for 20 minutes. The microsomal supernatant ("soluble fraction") resulting after centrifugation of the 10,000 g supernatant at 177,700 g for 40 minutes (Beckman 60Ti rotor, 50,000 rpm) was used as the enzyme source. Protein concentration of soluble fractions was determined by the procedure of Lowry *et al.* (J. Biol. Chem. 193: 265, 1951).