DISPOSITION OF DIETARY BENZO(a)PYRENE IN AQUATIC SPECIES: WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS) AND GREEN CRAB (CARCINUS MAENAS)

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Polycyclic aromatic hydrocarbons (PAH) comprise a class of persistent pollutant compounds that have been accumulating in soils and sediments worldwide (National Research Council, Oil in the Sea, 1985). Although not particularly toxic in themselves, PAH can be biologically transformed into metabolic products with mutagenic, teratogenic, and carcinogenic potential. Most aquatic organisms have the ability to metabolize PAH, but very little is known about what actually happens to metabolic products in these organisms or in the environment (Richards and Jackson, EPA Symposium: Carcinogenic Polynuclear Aromatic Hydrocarbons in the Marine Environment, EPA-600/9-82-013, 1982). Investigation of in vivo PAH metabolism has shown that PAH metabolic products have longer residence times than the parent compound in aquatic organisms (Little et al., Toxicol. Appl. Pharm. 77:325-333, 1985; Malins and Roubal, Environ. Res. 27:290-297, 1982; McElroy, Mar. Environ. Res. 17:133-136, 1985). The present study was undertaken to better determine production rates, identities, and residence times of PAH metabolites in two common marine organisms, the winter flounder (Pseudopleuronectes americanus) and the green crab (Carcinus maenas).

Winter flounder (146-390 g) and green crab (65-106 g) were collected locally from the Gulf of Maine and maintained in running seawater at 11-14 ^{o}C. In captivity animals were fed a prepared diet containing cod liver oil, gelatin, and Tetramin fish food daily. Food was withheld from experimental organisms for 48 hours prior to administration of a single dose of [7-^{14}C-] benzo(a)pyrene (BaP) (Pathfinder Laboratories, purity >98% on HPLC). Organisms were sacrificed at periods of up to 12 days, and all major organs and tissues collected for determination of total radioactivity and metabolic class determination where appropriate.

BaP was administered to fish by gavage in a carrier of corn or cod liver oil homogenized with the polychaete Nereis virens. Fish were maintained in individual tanks of running seawater and sacrificed 8, 24, 48, and 96 hours after dosing. Prior to removal for sacrifice by severing the spinal cord, fish were anesthetized by adding MS222 to their tank water (final concentration 200 mg/L). Radioactivity was followed in blood, bile, urine, liver, kidney, stomach, intestine, heart, gill, gonad, muscle, skin, spleen, urinary bladder, gall bladder, and feces. Individual crabs were placed in tanks of flowing seawater and fed squares of BaP labeled diet ad lib. Prior to collection, 24, 48, 96, and 288 hours after dosing, crabs were immobilized by exposure to dry ice. Crabs exposed for longer than 48 hours were fed unlabeled food daily. Hepatopancreas, hemolymph, stomach, green gland, shell, gill, heart, testes, muscle, intestine, and feces were collected from crabs. Small tissues (<0.3 g) were counted whole after digestion with Protosol (New England Nuclear). The detection limit was set at 0.01% of the dose given, which was always $\geq$2 times background radioactivity. Larger tissues were subsampled for digestion and the remainder frozen for subsequent metabolite analysis.

Preliminary determination of metabolite formation was made in flounder liver and crab hepatopancreas. Tissues were ground in anhydrous sodium sulfate and then extracted with a mixture of chloroform/methanol/water to

separate organic soluble compounds (parent BaP and primary metabolites), water soluble metabolites (conjugates) and unextractable material (bound residues). To minimize photooxidation all exposures and sample analyses were conducted under red light.

Table 1. Percent of Dose Remaining in Pseudopleuronectes americanus

	Time (hours) 8	24	48	96
Blood[a]	3.87 ± 2.22	1.30 ± 0.89	<0.01	<0.01
Bile	0.60 ± 0.36	2.11 ± 1.49	3.81 ± 1.58	5.08 ± 0.49
Liver	1.55 ± 0.68	1.47 ± 0.31	1.27 ± 0.56	1.10 ± 0.29
Intestine	8.12 ± 3.89	12.93 ± 3.83	7.41 ± 2.78	1.18 ± 0.35
Urine[b]	<0.01	0.09 ± 0.04	0.06 ± 0.02	0.03 ± 0.01

Values expressed as mean ± 1 SE. a: Calculated assuming a 3% wet weight blood volume (Hoar and Randall, Fish Physiology, Vol. 4, 1970). b: Terminal urine sample. N=4 for 8 and 96 hrs. N=5 for 24 and 48 hrs.

Total radioactivity remaining in selected compartments of the flounder is shown in Table 1. Values are expressed as the percent of dose given on a whole tissue basis. The highest percentage of the dose was found initially in intestinal tissue (intestinal contents were removed and the tissue rinsed prior to collection). Total radioactivity in this tissue remained high for 48 hours. Radioactivity in the liver remained fairly constant over 96 hours, while that in the blood decreased to near background by 48 hours, and levels in the bile increased throughout the exposure period. At all time points terminal urine contained little radioactivity. Table 2 presents similar data for the tissues retaining the most radioactivity in green crab. Hepatopancreas retained the highest percentage of the dose with levels dropping only 50% from 1 to 12 days. Approximately 1 to 3% of the dose was found in the stomach, muscle tissue, and shell, while levels in the muscle and shell remained fairly constant for 12 days.

Table 2. Percent of Dose Remaining in Carcinus maenas

	Time (days) 1	2	4	12
Hepato-pancreas	10.86 ± 3.31	5.97 ± 2.23	6.25 ± 3.58	5.07 ± 1.25
Green Gland	0.45 ± 0.23	0.59 ± 0.05	0.08 ± 0.05	0.07 ± 0.02
Stomach	3.04 ± 1.06	1.10 ± 0.48	1.18 ± 0.61	1.13 ± 0.07
Muscle	1.45 ± 0.72	0.93 ± 0.49	0.91 ± 0.19	1.57 ± 0.24
Shell	3.70 ± 0.84	2.18 ± 0.47	1.66 ± 0.14	3.63 ± 0.46

Values expressed as mean ± 1 SE. N=4.

Preliminary analysis of fish liver and crab hepatopancreas indicates that both these organisms metabolize BaP very rapidly. In the flounder at all time points 53 to 70% of recovered radioactivity was found as conjugated metabolites, 11 to 31% as bound residues with only 4 to 15% found as organic soluble material (parent and primary metabolites). BaP appeared to be less extensively metabolized in the green crab hepatopancreas with 30 to 46% found as conjugated metabolites, 18 to 33% as bound residues, and 26 to 42% found in the organic extract.

In vivo BaP disposition and metabolism following gastric administration in the Winter flounder can be compared with a similar study conducted on English sole by Varanasi et al. (Xenobiotica 12:417-425, 1982). In ripe English sole, the concentration of BaP-derived radioactivity remained constant in liver of females but appeared to decrease in males from 24 to 168 hours after a single oral dose. The percent of dose retained was somewhat less (0.1 to 0.9) than that reported here for Winter flounder. Regardless of sex or length of exposure >90% of radioactivity in the liver was found as conjugated metabolites. Total bound metabolites were not reported, but measurable amounts of radioactivity were determined to be covalently bound to liver DNA and protein.

Lee et al. (Mar. Biol. 37:363-370, 1976) fed BaP to the blue crab (Callinectes sapidus). As in the green crab, most of recovered radioactivity was found in the hepatopancreas. Assimilated radioactivity was highly variable, but after 2 days an average of 3% of the dose was found in hepatopancreas of which approximately 58% was present as unmetabolized BaP. Although the extraction procedures used in the two studies were somewhat different, hydroxylated metabolites predominated in extracts of blue crab hepatopancreas and the presence of unextractable radioactivity was observed. In contrast to the work reported here, Lee found relatively little radioactivity in other tissues of blue crab, less than 1% of the dose was found in stomach, blood, gill, muscle or gonad. However, neither the shell nor the green gland were analyzed.

In summary, two species of locally abundant marine organisms metabolize dietary BaP in vivo extensively to conjugated and bound metabolites showing patterns of BaP accumulation similar to that reported for other closely related marine organisms. Although a relatively small proportion of the dose given was assimilated, significant levels of BaP-derived compounds remained in selected tissues of winter founder and green crabs for at least 4 to 12 days respectively. The importance of gastrointestinal tissues as a repository for BaP derived radioactivity in both these species is also noteworthy. This work was supported by the Lucille P. Markey Charitable Trust and the Center for Membrane Toxicity Studies at the MDIBL, and the National Cancer Institute (NCI) and the US Army Medical Research and Development Command under contract #CA44289 from the NCI to AMc. The technical assistance of Mr. J. Sisson on portions of this work is greatfully acknowledged.