BIOAVAILABILITY, BIOTRANSFORMATION AND ELIMINATION OF BENZO(A)PYRENE AND BENZO(A)PYRENE-7,8-DIHYDRODIOL IN THE LOBSTER, HOMARUS AMERICANUS.

Margaret O. James, John D. Schell and Vallery Magee
Department of Medicinal Chemistry and The Whitney Laboratory,
University of Florida, Gainesville, FL 32610

Benzo(a)pyrene (BaP), is a ubiquitous environmental procarcinogen. One pathway of bioactivation of BaP is by cytochrome P-450 and epoxide hydrolase dependent biotransformation to BaP-7,8-dihydrodiol (BaP-7,8D) followed by a second cytochrome P-450-dependent monooxygenation to BaP-7,8D-9,10-oxide, which can bind covalently to DNA and other macromolecules. Studies showed that lobsters held in pounds constructed of creosoted piling attained high concentrations of BaP in hepatopancreas and muscle (Dunn and Fee, 1979, J. Fish. Res. Bd. Can, 36: 1469), although the route of exposure was not clear. In laboratory studies, a single intrapericardial dose of 1 mg/kg [14-C]-BaP distributed mainly into hepatopancreas, the fatty digestive organ, and muscle, which accounts for about 40% of body weight (Foureman et al., 1978, Bull. MDIBL, 18: 93). At the 1 mg/kg dose, BaP was very slowly metabolized and eliminated from lobsters. In crustaceans, biotransformation of lipophilic xenobiotics to polar metabolites does not always increase the rate of elimination (e.g. James et al., 1987, Bull. MDIBL, 27: 9), but this has not been studied for BaP metabolites in lobster. The purpose of the present study was to determine the oral bioavailability and routes of biotransformation and elimination of low doses of BaP and a more polar and potentially toxic metabolite, BaP-7,8D in lobsters. Lobsters may be exposed to BaP-7,8D by consuming BaP-exposed prey. Both BaP and BaP-7,8D are potential human carcinogens.

Lobster, Homarus americanus, body weight 450-650 g were dosed with [14-C]-BaP or [14-C]-BaP-7,8D either intrapericardially from a DMSO solution or orally by gavage from a suspension of the worm Nereis virens. The dose was 0.26 $\pm$ 0.03 μmole/kg body weight (approximately 70 μg/kg). Serial hemolymph samples were taken at 1,2,4,15,30 min, 1,2,4,8 hr and 1,2,4,8 and 16 days after the intrapericardial doses. After oral administration, hemolymph samples were taken at 4,8,12 hr and 1,2,3,4,5,6,8 and 16 days after the dose. Total radioactivity in hemolymph was quantitated by scintillation counting of aliquots of each sample. The remaining hemolymph was analyzed for parent compound by deproteination with methanol followed by solid phase extraction with octadecylsilane cartridges (SEP-PAK C18). Groups of 3-4 lobsters were sacrificed and dissected at 8 and 16 days after BaP or BaP-7,8D and all tissues were removed and analyzed for [14-C].

Figures 1 and 2 show the concentration of [14-C] in hemolymph with time are presented in Figures 1 and 2. The early elimination from hemolymph after intrapericardial injection is shown in Figure 1 and the intrapericardial/oral comparisons are shown in Figure 2. Following intrapericardial injection, both BaP and BaP-7,8D were rapidly cleared from hemolymph (Figure 1) and the more lipophilic BaP cleared faster. Hemolymph concentrations of [14-C] following oral BaP did not reach a maximum until 3 days after the dose (Figure 2A), whereas maximum hemolymph concentrations of [14-C] following BaP-7,8D were attained by 8 hr after the dose (Figure 2B). Concentrations of radioactivity and percent of dose recovered in selected tissues with the highest values are presented in Tables 1 (BaP) and 2 (BaP-7,8-D). Muscle contained a relatively large percentage of the remaining dose for both compounds, even though the tissue levels were much lower than in hepatopancreas. Antennal gland contained high concentrations of radioactivity after both BaP and BaP-7,8D, but surprisingly urine contained 100-fold lower

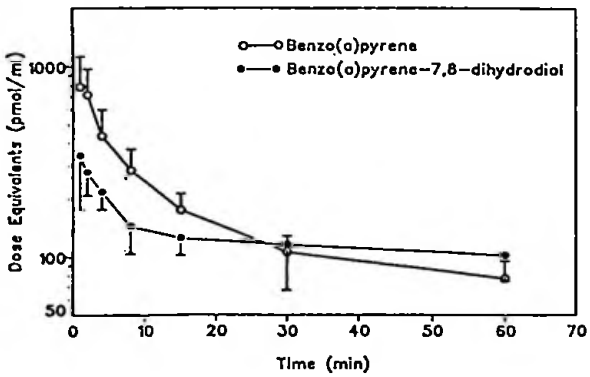

Figure 1: Hemolymph concentrations of radioactivity following intrapericardial doses of BaP or BaP-7,8D.

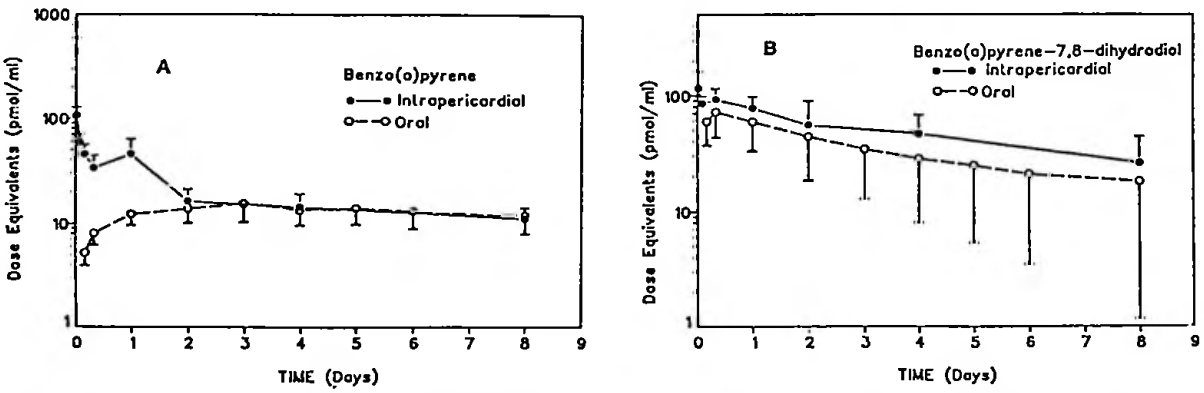

Figure 2: Hemolymph concentrations of radioactivity following oral or intrapericardial doses of BaP or BaP-7,8D.

Table 1. Distribution of radioactivity 16 days after administration of BaP.

	Percent dose in each tissue at sacrifice	
	Oral	Intrapericardial
Hemolymph	1.0 ± 0.2	0.7 ± 0.2
Hepatopancreas	62.8 ± 11.2	33.6 ± 21.0
Intestinal content	0.6 ± 0.3	0.8 ± 0.3
Muscle	16.8 ± 1.3	14.5 ± 6.8
Antennal gland	0.1 ± <0.1	0.1 ± <0.1
Urine	<0.1 ± 0.1	<0.1 ± <0.1
Total recovery, % dose	85.9 ± 11.4	54.2 ± 31.0
	Tissue concentration, pmol/g BaP equivalents	
	Oral	Intrapericardial
Hemolymph	13 ± 3	9 ± 3
Hepatopancreas	3895 ± 833	2592 ± 1566
Intestinal content	1167 ± 856	1818 ± 741.
Muscle	116 ± 9	100 ± 47
Antennal gland	584 ± 28	387 ± 58
Urine	8 ± 14	2 ± 2

All values are mean ± S.D., n = 4 (oral) or 3 (intrapericardial) lobsters.

Table 2. Distribution of radioactivity following doses of BaP-7,8D.

	Percent dose in each tissue at sacrifice			
	Eight days		Sixteen days	
	Oral	Intra-pericardial	Oral	Intra-pericardial
Hemolymph	2.7 ± 1.8	3.3 ± 2.1	0.2 ± 2.1	0.6, 3
Hepatopancreas	3.3 ± 1.2	5.5 ± 2.7	1.3 ± 1.0	1.7, 1.5
Intestinal contents	0.7 ± 0.2	1.9 ± 1.6	0.1 ± <0.1	0.2, 0.1
Muscle	2.4 ± 1.5	5.6 ± 2.3	0.6 ± 0.5	1.1, 1.0
Antennal gland	0.4 ± 0.3	0.6 ± 0.2	0.1 ± <0.1	0.1, 0.1
Urine	<0.1 ± <0.1	<0.1 ± <0.1	<0.1 ± <0.1	<0.1,<0.1
Total recovery, % dose	11.0 ± 4.5	19.9 ± 9.2	2.7 ± 2.0	4.6, 3.3

	Tissue concentration, pmol/g BaP-7,8D equivalents			
	Eight days after dose		Sixteen days after dose	
	Oral	Intra-pericardial	Oral	Intra-pericardial
Hemolymph	30 ± 20	36 ± 23	2 ± 1	7, 6
Hepatopancreas	191 ± 68	343 ± 185	73 ± 56	206, 39
Intestinal contents	1536 ± 358	4580 ± 3755	174 ± 87	539, 157
Muscle	15 ± 9	38 ± 9	4 ± 4	11, 3
Antennal gland	948 ± 776	1760 ± 1004	143 ± 107	323, 267
Urine	9 ± 8	17 ± 11	1 ± <0.1	2, 1

Values are mean ± S.D., n = 3 to 4 lobsters, or individual values.

concentrations of [14-C]. Radioactivity from BaP was retained in the hepatopancreas for longer than radioactivity from BaP-7,8D but for both compounds gastrointestinal elimination was important, as seen from the high concentrations in intestinal contents. Radioactivity was eliminated from BaP-7,8D-dosed lobsters more rapidly than from BaP-dosed lobsters. The tissue concentration data suggested that BaP was completely absorbed from the oral dose, even though the area under the hemolymph concentration-time curve was less than for the intrapericardial dose. On the other hand both tissue concentration and area under the hemolymph concentration-time curve suggested less than 100% absorption of orally administered BaP-7,8D.

Analysis of hemolymph showed that up to 1 hr after the dose greater than 90% of the radioactivity was parent BaP. Thereafter concentrations of polar metabolites increased with time. Analysis of hepatopancreas showed that less than 30% of the radioactivity present at 2 weeks after the dose was unchanged BaP. In contrast, Foureman et al. (1978, Bull. MDIBL 18: 93) found that after a 1 mg/kg dose greater than 90% of the [14-C] present in hepatopancreas at 6 weeks after the dose was parent BaP. Analysis of hemolymph and tissues from BaP-7,8D-dosed lobsters is in progress.

These studies showed that the more polar and toxic metabolite of BaP, Bap-7,8D, was cleared from lobsters more rapidly than parent BaP, but both compounds were cleared much more slowly from lobsters than flounder (McElroy and Kleinow, 1988, this volume). These studies also suggest that for very lipophilic compounds which are rapidly cleared from hemolymph, such as BaP, hemolymph concentrations of compound following oral administration may not accurately predict total body burdens. For both BaP and BaP-7,8D, two edible tissues, the muscle and hepatopancreas, accounted for most of the dose retained in the lobsters.

Supported by an L.P. Markey Fellowship, the NIH SCOR grant to MDIBL and an NCI/NIEHS/US Army grant CA 44297 to M.O.James.