Bull. Environm. Contam. Toxicol. 23,100-103 (1979)

Distribution of Radioactivity in Coalfish (*Pollachius virens*) Following Intragastric Administration of [9-^{14}C] Phenanthrene

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Biological disposition of hydrocarbons in marine animals has received increasing attention. This subject was reviewed by VARANASI and MALINS (1977) who included data on marine fish. Their summary makes it clear that relatively little is known on the latter subject; however both immersion and feeding studies have been reported on the uptake and excretion of some aliphatic and aromatic hydrocarbons in a few species of marine fish. It is also apparent that the large variations in experimental parameters (route of administration, exposure time, time of sample collection, type or species of fish used, etc.) make general conclusions difficult. Our interest in this area has been directed towards obtaining a better understanding of the biological disposition of polycyclic aromatic hydrocarbons (PAH's). Phenanthrene, a relatively simple PAH, was chosen as a representative compound because of its high abundance in crude oil and its presence as a byproduct formed in industry using Söderberg electrodes or coal and coke (PALMORK et al. 1973, PALMORK 1974). The coalfish (Pollachius virens) was chosen because of its abundance in Norwegian coastal waters, its commercial importance and also bause it is a lean rather than fat fish, the latter type having usually been selected in similar studies.

EXPERIMENTAL

Fish The coalfish (P. virens) used in this study were obtained from the coastal area near Bergen. Two groups containing fish of both sexes were used which weighed (mean ± S.D.) 54 ± 15 and 188 ± 37 g. They were not fed three days prior to dosing and they refused food the first four days afterwards. In experiments of longer duration they were regularly fed thawed frozen krill (Magnyctiphanes norvegica).

0007-4861/79/0023-0100 $01.00

Treatment All fish were given the same amount (15.8 µg containing 1.0 µCi) of [9-^{14}C] phenanthrene. This material had a specific activity of 11.3 mCi/mmol and a purity of 99 %. It was dissolved in CS_2 to give a solution containing 1.0 µCi/20 µL and this amount was added to a small amount of Tess salmon diet nr. 3 (Skretting A/S, Stavanger, Norway) placed in a gelatin capsule (size nr. 2, Parke, Davis & Co.). Prior to use about a fourth of each capsule was cut away. This allowed easy application of the radioactive solution with a micro syringe, ready evaporation of the CS_2 and improved bioavailability of the material. The capsule was introduced into the stomach of the fish anesthetized with benzocaine with the help of a modified 5-mL plastic syringe affixed to a plastic tube into which the capsule was inserted. By attaching a wire to the plunger of the syringe the capsule could be ejected from the tube.

Maintenance of fish Following dosing the fish were placed in 260-L containers for experiments lasting less than one week or 2000-L containers for experiments of longer duration. The seawater has a salinity of 34 °/oo, a temperature of 7-9°C, and a flow rate of 7 L/min in the smaller containers which held up to five fish and 30 L/min in the larger containers.

Sample preparation At the appropriate times the fish were frozen and maintained at -20°C until required. They were then thawed and the liver and gall bladder carefully removed and weighed. Two samples (approx. 100 mg) were taken of the liver (at end of lobes) and of muscle (white muscle behind head and over ventral line) whereas the entire gall bladder was used to determine radioactivity. Standard methods, using Soluene-100 or Soluene-350 and Dimilume-30 (Packard Instrument Co.) and an internal standard ([^{14}C] toluene), were employed in the scintillation counting.

RESULTS AND DISCUSSION

Table 1 shows the amount of radioactivity (as % of dose) present after various times in the liver, gall bladder and muscle of coalfish given [9-^{14}C] phenanthrene intragastrically. The dose employed was only 15.8 µg which corresponds roughly to 0.08 or 0.3 mg/kg in the two groups used. Of the organs studied, the liver showed by far the greatest degree of accumulation of radioactivity. Maximum accumulation occurred from 10 to 24 h after dosing and nearly 72 % of the dose was present in this organ after 17 h. Large amounts of radioactivity were also found in the gall bladder and, not surprisingly, the highest values were recorded at somewhat later times (24-48 h). The gall bladder values

TABLE 1

Distribution of radioactivity in some organs of coalfish (Pollachius virens) at various times following intragastric administration of [9-^{14}C] phenanthrene

	0.5 h	1 h	2 h	5 h	10 h	17 h	24 h
Liver	0.01*	0.2	0.5	1.5	1.5	71.9	47.1
	(3; 0.008)**	(4; 0.13)	(4; 0.39)	(4; 1.2)	(3; 0.2)	(5; 9.3)	(4; 32.1)
Gall bladder	0.002	0.003	0.007	0.007	0.03	3.0	3.3
	(6; 0.001)	(5; 0.002)	(3; 0.002)	(4; 0.002)	(3; 0.02)	(5; 1.7)	(4; 3.4)
Muscle	0.1	0.1	0.3	0.6	5.1	6.4	3.9
	(3; 0.06)	(3; 0.04)	(3; 0.1)	(3; 0.3)	(3; 2.3)	(5; 2.9)	(4; 1.7)

	36 h	48 h	72 h	96 h	168 h	336 h	672 h
Liver	28.5	20.7	21.2	15.5	10.9	1.0	0.5
	(5; 13.5)	(5; 10.0)	(5; 8.2)	(4; 8.9)	(5; 8.4)	(5; 0.6)	(3; 0.7)
Gall bladder	12.7	7.9	6.8	6.9	2.9	0.1	0.007
	(5; 2.8)	(5; 15.1)	(5; 6.0)	(4; 7.4)	(5; 1.8)	(5; 0.09)	(4; 0.006)
Muscle	3.5	3.3	3.6	2.4	0.9	0.9	2.8
	(5; 1.9)	(5; 1.6)	(5; 2.5)	(4; 0.6)	(5; 0.2)	(5; 0.6)	(4; 2.1)

* mean value, % of administered dose found in organ

**number of fish; standard deviation of mean

remained fairly high until 96 h, a finding which is no doubt related to the fact that the fish were fasted until this time. LEE et al. (1972) noted the delayed accumulation of radioactivity in the gall bladder of several species of marine fish exposed to ^{14}C-labelled naphthalene or benzo(a)pyrene. The importance of the biliary route in the excretion of aromatic hydrocarbons (largely as metabolites) was stressed by ROUBAL et al. (1977) and CORNER et. al. (1976) who studied coho salmon and codlings, respectively. The nature of the biliary and urinary metabolites in the present study will be dealt with in a subsequent publication.

CORNER et al. (1976) described the tissue distribution of radioactivity in codlings given a single oral dose of [^{14}C]benzo(a)pyrene. Little radioactivity was detected in the muscle and, after 96 h, none of this was due to unchanged compound. Our findings indicate a more substantial accumulation of radioactivity in the muscle of the coalfish.

ROUBAL et al. (1977) found that the concentration of radioactivity in muscle (expressed as radioactivity/mg tissue) in coho salmon given [^{14}C]anthracene or [^{14}C] naphathalene was about 10 % of that present in the liver. The corresponding value in the present study is about 2 %. A lower value in the coalfish is to be expected in view of the differences in the distribution of lipid in lean and fat fish and the resultant influence on the concentration of lipophilic substances in the tissues.

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