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Distribution of Radioactivity in the Chondrichthyes *Squalus acanthias* and the Osteichthyes *Salmo gairdneri* Following Intragastric Administration of (9-^{14}C)Phenanthrene

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The increasing offshore oil exploitation and production of oil generally results in the risk of an increasing amount of oil in the marine environment. The components in oil creating the greatest concern are the polycyclic aromatic hydrocarbons (PAH), mainly because of their possible carcinogenic and/or mutagenic effects. Industrial processes, eg. aluminium smelters and ferro-silicon, iron- and coke-works are also an important sources of PAH pollution in the sea (PALMORK et al. 1973 and PALMORK 1974).

The fate of aromatic hydrocarbons in marine animals has recieved increasing attention in the last decade. Comprehensive reviews of this subject have been published by VARANASI & MALINS (1977) and NEFF (1979).

The present studies dealing with spiny dogfish (*Squalus acanthias*) and rainbow trout (*Salmo gaidneri*) are part of a series of experiments with different marine organisms (SOLBAKKEN et al. 1979, 1980 and PALMORK & SOLBAKKEN 1981). All the experiments were performed under the same laboratory conditions using intragastric administration of the PAH-component, phenanthrene. Thus it is possible to compare species differences of disposition of PAH in various marine organisms.

EXPERIMENTAL

The spiny dogfish (*Squalus acanthias*) were hooked on longlines in the coastal area near Bergen. The rainbow trout (*Salmo gairdneri*) were received from a commercial hatchery. Fish of both sexes were used in the experiments, and the weight (mean$\pm$S.D.) were 1498$\pm$323 and 113$\pm$30g for the spiny dogfish and the rainbow trout, respectively. The fish were not fed one week prior to and four days after dosing. In experiments of longer duration the spiny dogfish were fed thawed frozen sprat (*Sprattus sprattus*), and the rainbow trout were given commercially available dry pellets. (Tess salmon diet nr. 5, Skretting A/S, Norway). Dosing was performed as described by SOLBAKKEN et al. (1979). The spiny dogfish received a dose of 18.5 μg (2.0 μCi) of [9-^{14}C]phenan-

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threne (sp. act. 19.3 mCi/mmol), and the rainbow trout were given 15.8 μg (1.0 μCi) of [9-^{14}C]phenanthrene (sp. act. 11.3 mCi/mmol). The spiny dogfish were placed in 1100-L containers (flow rate 20 L/min, 9°C, 34°/oo) for experiments lasting less than one week, or 35000-L containers (flow rate 50 L/min, 8°C, 34°/oo) for experiments of longer duration. The rainbow trout, adapted to seawater, were kept in 260-L containers (flow rate 7 L/min, 10-11°C, 34°/oo), each containing five fish.

At appropriate times samples of fish were frozen and maintained at -20°C until required. After thawing,

TABLE 1. Distribution of radioactivity in some tissues of spiny dogfish (Squalus acanthias) at various times following intragastric administration of [9-^{14}C]phenanthrene (18.5 μg/fish).

	24h	96h	168h	672h
Liver	21.3* (4,16.1)**	64.06 (5,14.12)	74.2 (5,7.38)	34.3 (4, 12.9)
Bile	0.07 (4,0.04)	1.2 (5, 1.0)	0.5 (5, 0.3)	0.6 (4, 0.6)
Muscle	3.3 (4, 2.8)	20.34 (5, 8.23)	18.22 (5,4.05)	8.2 (4, 2.9)
Gonads	0.20 (4,0.06)	0.34 (5, 0.24)	0.30 (5,0.12)	0.22 (4, 0.10)
Kidney	0.21 (4,0.16)	0.46 (5, 0.25)	0.37 (5,0.11)	0.15 (4, 0.01)
Salt gland	0.02 (4,0.01)	0.03 (5,0.002)	0.02 (5,0.01)	0.01 (4,0.002)

* mean value, % of administered dose found in organ

** number of animals, standard deviation of mean

TABLE 2. Distribution of radioactivity in some tissues of rainbow trout at various times following intragastric administration of [9-^{14}C]phenanthrene (15.8 μg/fish).

	5 h	17 h	24 h	36 h	48 h
Liver	1.1* (4,0.9)**	1.2 (5,0.6)	1.8 (5,0.9)	1.5 (5,1.2)	0.9 (2,0.01)
Gall bladder	0.2 (4,0.2)	1.6 (4,1.1)	4.9 (4,2.8)	5.2 (5,4.4)	-
Muscle	1.8 (4,1.1)	5.6 (5,1.7)	6.4 (5,2.9)	4.9 (5,2.9)	5.3 (4,1.3)
Intestinal fat	0.6 (4,0.7)	5.0 (5,4.0)	6.4 (5,3.2)	6.3 (5,3.4)	5.7 (4,2.6)

	72 h	96 h	168 h	336 h	672 h
Liver	0.6 (5,0.4)	0.7 (5,0.5)	0.7 (5,0.2)	0.08 (5,0.01)	0.02 (5,0.004)
Gall bladder	14.0 (5,10.9)	4.5 (3,2.2)	2.6 (3,2.0)	0.1 (5,0.1)	0.002 (5,0.002)
Muscle	2.3 (5.1.3)	1.6 (4,0.5)	2.3 (5,0.7)	0.4 (5,0.1)	0.2 (5,0.03)
Intestinal fat	4.2 (5,2.7)	3.9 (4,0.3)	0.9 (5,0.9)	0.6 (5,0.3)	0.03 (5,0.02)

* mean value, % of administered dose found in organ

** number of animals, standard deviation of mean different tissues were removed and weighed. From spiny dogfish liver, muscle, bile, gonads, kidney and rectal gland were analysed for radioactivity and from rainbow trout liver, muscle, the entire gall bladder, and intestinal fat were examined. Two samples (approx. 100 mg) of each tissue, except for bile, were used to determine radioactivity. The method for measuring the radioactivity are discribed by SOLBAKKEN et al. (1979).

RESULTS AND DISCUSSION

Table 1 and 2 show the amounts of radioactivity (as % of dose) present at various times in different tissues of spiny dogfish and rainbow trout both given a single dose of [9-^{14}C]phenanthrene intragastrically.

The findings in table 1 show that the highest content of radioactivity in the spiny dogfish was found in the liver. The maximum value for radioactivity in liver occurred 168 h after dosing. In all other tissues the maximum accumulation was found 24 to 168 h after dosing. Only small amounts of radioactivity were found in bile, gonads, kidney and rectal gland. Surprisingly, the amounts of radioactivity in the tissues 672 h after dosing were 33 to 65% of the maximum values. These high amounts of radioactivity may be related to the high content of fat in sharks and/or the low mixed-function oxidase activities in the hepatic microsomes in spiny dogfish as noted by BEND et al. (1977). Quantitative studies of the metabolites of phenanthrene in spiny dogfish showed only a small amount of metabolites compared to the amount found in bony fish (SOLBAKKEN unpublished observations). Analyses of radioactivity in stomach and intestine (SOLBAKKEN unpublished observations) show that only small amounts (<0.5% of dose) remained 168 h after dosing. Qualitative and quantitative studies of the metabolites in spiny dogfish will be dealt with in a subsequent publication.

GUARINO et al. (1972) described the distribution of [^{14}C]naphthalene in plasma, hepatic and renal compartments in the spiny dogfish 24 h after administration of 0.05 mg/kg (route of administration unknown). Similarly, they found the greatest accumulation in the liver (22.65% of dose), and only a small fraction of the dose in bile and kidney (0.03 and 0.02%, respectively).

Table 2 shows a different distribution of radioactivity in the rainbow trout compared to that in the spiny dogfish. The greatest degree of accumulation of radioactivity in rainbow trout was observed in the gall bladder (14% of dose) and maximum accumulation occurred 48 to 96 h after dosing. In liver, muscle and intestinal fat the maximum accumulation occurred earlier, 17-36 h after dosing. The highest value of radioactivity in the liver was recorded earlier than in the gall bladder. This is in good agreement with the study described by SOLBAKKEN et al. (1979) where coalfish (*Pollachius virens*) were dosed intragastrically with [^{14}C]phenanthrene. In contrast with the radioactivity in spiny dogfish, the radioactivity in rainbow trout and coalfish decreased to background values 336 to 672 h after dosing.

A low content of radioactivity was observed in the liver of rainbow trout, and this is in contrast to the results in the experiment with coalfish reported by SOLBAKKEN et al. (1979). They found 72% of the dose in coalfish liver 17 h after dosing. Rainbow trout, however, have a small liver (approx. 10% of the weight of the liver in coalfish with equal total weight). Also, the liver in rainbow trout (a fat fish) is not as fatty as the liver in coalfish (a lean fish). The fat fish have the depot of lipid in the muscle, whereas the lean fish have the depot in the liver. Phenanthrene and PAH generally are lipophilic compounds and will accumulate accordingly in lipid-rich tissues. These factors might explain the lower content of radioactivity in the liver of rainbow trout (1% of dose) found 17 h after dosing. Analyses of stomach and intestine (SOLBAKKEN unpublished observations) show that only a small fraction of the dose remains 24 h after dosing (2 and 6% in stomach and intestine, respectively). Thus the finding of high concentrations in the gall bladder, which stores the metabolites from the liver before excreting them to the intestine, indicates that the elimination of radioactivity from the liver of rainbow trout is very efficient. A study of the metabolites in rainbow trout will be published later.

ROUBAL et al. (1977) found in studies using coho salmon (*Oncorhynchus kisutch*) dosed with [^{14}C]anthracene or [^{14}C]naphthalene, that the concentration of radioactivity in muscle (dpm/mg tissue) was about 10% of that present in the liver. This was discussed by SOLBAKKEN et al. (1979) who found the corresponding value to be 2% in the coalfish given phenanthrene. They concluded that a lower value in coalfish is due to the differences in the distribution of lipid in lean and fat fish. The corresponding value in rainbow trout (a fat fish) is 7%, and that is in accordance with the earlier assumption. WHITTLE et al. (1977) showed that most of the radioactivity of [^{14}C]benzo(a)pyrene fed to young herring was present in the lipid fraction of the tissues examined. MELANCON & LECH (1978) studied the distribution and elimination of [^{14}C]naphthalene in rainbow trout during short- and long-term exposures in seawater. They found the highest concentration in the visceral fat and the lowest in muscle. This is in accordance with the present results using phenanthrene given intragastrically. The amounts of radioactivity are lower for the intestinal fat than the muscle because of a low total weight of the intestinal fat. In experiments using coho salmon ROUBAL et al. (1977) and COLLIER et al. (1978) showed that ^{14}C-labelled naphthalene and anthracene given intragastrically accumulated to a greater extent in muscle compared with other tissues. In an experiment with juvenile starry flounder (Platicthys stellatus) and rock sole (Lepidopsetta bilinieata) force-fed with [^{3}H]naphthalene, the radioactivity in all tissues of rock sole was considerably greater than the radioactivity in the corresponding tissues of starry flounder (VARANASI et al. 1979). At 24 h after dosing the muscle contained the highest amount of radioactivity, but 168 h after dosing the greatest amount was recovered in bile. Our studies, using rainbow trout, show a similar pattern of distribution to that found in flatfish, which also is a fat fish. Fat fish exposed to aromatic hydrocarbons will accumulate the greatest amounts of the compounds in the gall bladder and the muscle. Lean fish (e.g. codfish), however, will accumulate most of the compounds in the liver.

The most pronounced differences in the disposition of phenanthrene between bony fish and cartilaginous fish in our studies are that the maximum value of radioactivity in the liver of cartilaginous fish occurred several days later than the corresponding value in bony fish. Furthermore, the radioactivity in cartilaginous fish was retained at a high level beyond 672 h (28 days), a time at which the radioactivity in bony fish is near the background values.

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