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A COMPARATIVE STUDY ON THE DISPOSITION OF THREE AROMATIC HYDROCARBONS IN FLOUNDER (*PLATICHTHYS FLESUS*)

BY

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ABSTRACT

SOLBAKKEN, J.E., SOLBERG, M. and PALMORK, K.H. 1983. A comparative study on the disposition of three aromatic hydrocarbons in flounder (*Platichthys flesus*). *FiskDir. Skr. Ser. HavUnders., 17: 473–481.*

The disposition of three aromatic hydrocarbons was studied in flounder (Platichthys flesus). The components, ^{14}C-naphthalene, ^{14}C-phenanthrene and ^{14}C-benzo(a)pyrene, were all given intragastrically. The radioactivity was analysed in several tissues and body fluids at various times after dosing. This study, performed under identical laboratory conditions, made it possible to compare the fate of the three components in flounder. The accumulation was greatest in the case of phenanthrene, whereas the elimination was most efficient with naphthalene. There was a close relationship in the disposition of radioactivity between the liver and bile as well as between blood plasma and muscle. The results indicate that the biliary and urinary excretion is less important with napthalene-derived components than with phenanthrene- and benzo(a)pyrene-derived components. Different factors that might affect the disposition of the aromatic hydrocarbons are discussed.

INTRODUCTION

Several studies describing the disposition of aromatic hydrocarbons in fish have been published during the last decade (e.g. COLLIER, THOMAS and MALINS 1978; DIXIT and ANDERSON 1977; LEE, SAUERHEBER and DOBBS 1972; MELANCON and LECH 1978, 1979; ROUBAL, COLLIER and MALINS 1977; SOLBAKKEN andPALMORK 1980, 1982; SOLBAKKEN *et al.* 1979, SOLBAKKEN, KNAP and PALMORK 1982; THOMAS and RICE 1981; VARANASI and GMUR 1980, 1981; VARANASI, GMUR and TRESELER 1979; WHITTLE *et al.* 1977). It is apparent that the large variations in experimental parameters (e.g. route of administration, components, species) make general conclusions difficult. In our laboratory the biological disposition of phenanthrene in several marine organisms has been studied under similar laboratory conditions (PALMORK and SOLBAKKEN 1980, 1981; Solbakken and Palmork 1980, 1981; Solbakken, Palmork, Neppelberg and Scheline 1979, 1980).

The present study describes the disposition of three aromatic hydrocarbons, naphthalene (Nph), phenanthrene (Phe), and benzo(a)pyrene (BaP), in the flounder *Platichthys flesus*. This is the first comparative study on the uptake and elimination of napthalene, phenanthrene and benzo(a)pyrene in a fish species. Our main interest was to compare the disposition of aromatic hydrocarbons of lower and higher molecular weight (Mw) than Phe with our previous results obtained with the latter compound. Nph (Mw: 128) is more hydrophilic and BaP (Mw: 252) is more lipophilic than Phe (Mw: 178). These characteristics are expected to influence the biological disposition of the components, depending primarily on the lipid content of the tissues.

MATERIALS AND METHODS

Flounder (*P. flesus*) of both sexes were collected from a shellow coastal area near Bergen and kept in an aquarium in the dark for 4 weeks prior to the experiments. They were before and during the experiment fed twice a week with thawed frozen krill (*Meganyctiphanes norvegica*). Dosing was performed as described by Solbakken *et al.* (1979), but the fishes were not anesthetized. The experimental conditions are given in Table 1.

At various times (1, 2, 4, 6 and 14 d and also after 12 h in the Nph and BaP experiments) after the start of the experiments, 5 fish were killed, quickly frozen, and stored at –20°C for up to one week. After thawing, samples of liver, white muscle (taken near the head), bile, urine and blood plasma were taken and analysed for radioactivity.

Bile and urine were sampled by puncturing the bladder with a Pasteur pipette. Blood (approx. 1 mL) was collected from the caudal vein and blood plasma obtained after treatment with heparin (5000 I.U./mL) and centrifugation. Aliquots (approx. 100 mg) of each tissue were digested (Soluene-350) and mixed in 10 mL of Dimilume-30 (Packard Instruments Co.). The radioactivity was determined using a Packrd 300 CD scintillation counter.

RESULTS AND DISCUSSION

The consentrations of radioactivity in different tissues and body fluids are given in Fig. 1. Radioactivity derived from three aromatic hydrocarbons was found in all tissues and body fluids within 12–24 h after dosing. The highest concentrations of radioactivity were found with Phe. There were great variations in the elimination of radioactivity in the different tissues and body fluids. The most distinct decreases were found in liver, blood plasma and

Table 1. The experimental conditions during the studies of the disposition of labelled naphthalene, phenanthrene and benzo(a)pyrene in flounder.

Experimental conditions	Experiment [1(4,5,8)-^{14}C]Naphthalene (Amersham)	[9-^{14}C]Phenanthrene (Amersham)	[7,10-^{14}C]Benzo(a)pyrene (Amersham)
The organisms:			
Mean wet weight ± SD (g)	74 ± 25	79 ± 21	75 ± 20
Total number of fishes	35	25	35
The experimental system:			
Volume of containers (L)	260	260	260
Number of containers	3	2	3
Number of organisms per container	10, 12, 13	12, 13	10, 12, 13
Flowrate of seawater (L/min)	5	5	5
Temperature (°C)	8.5	8.5	8.5
Salinity (‰)	34	34	34
Dosing:			
Spesific activity (mCi/mmol)	5	19.3	21.7
Dose per fish			
μg	9.25	9.25	9.25
μCi	0.36	1.00	0.80
nmole	72	52	37

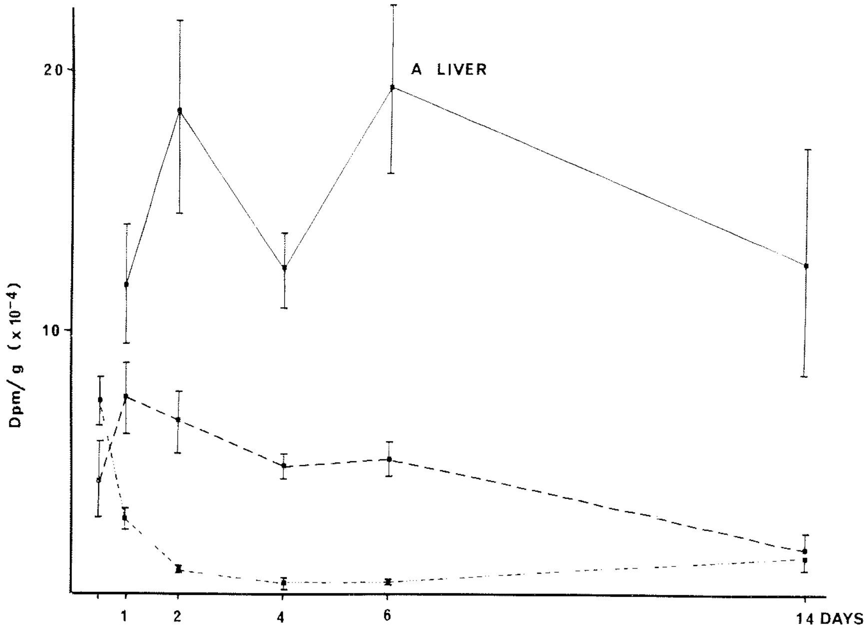
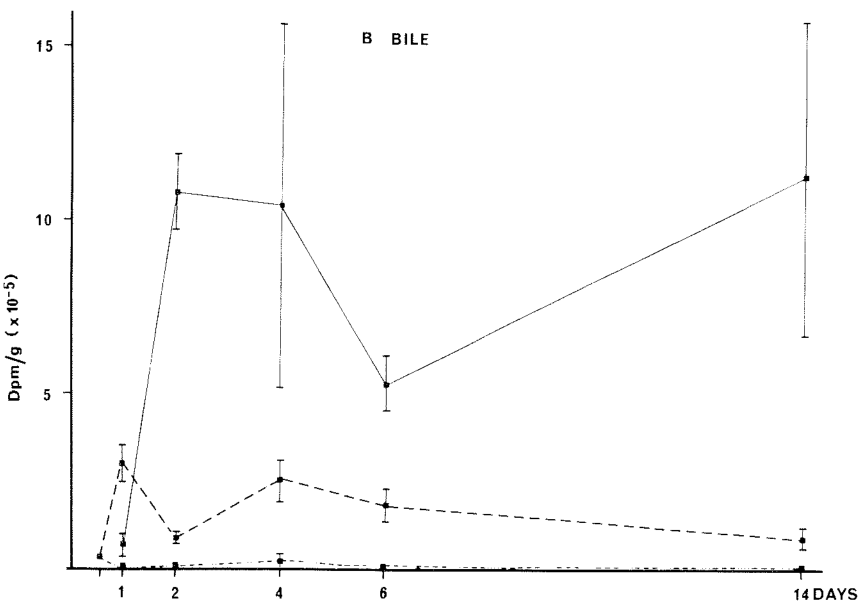
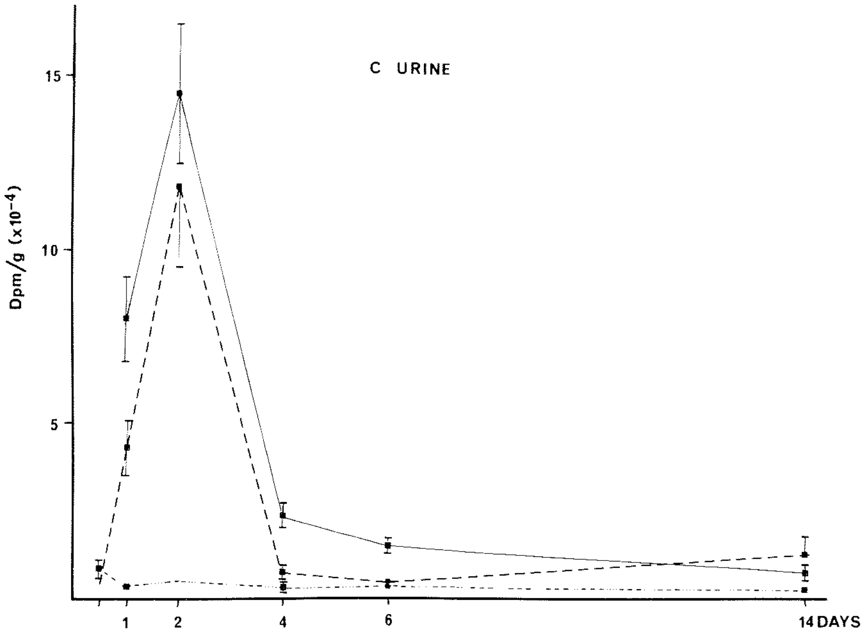
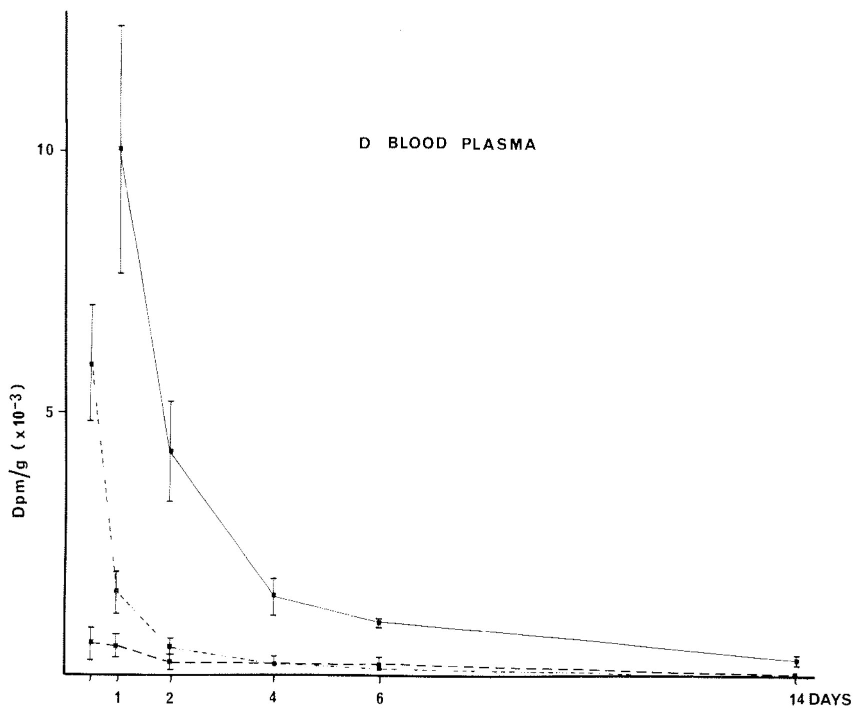
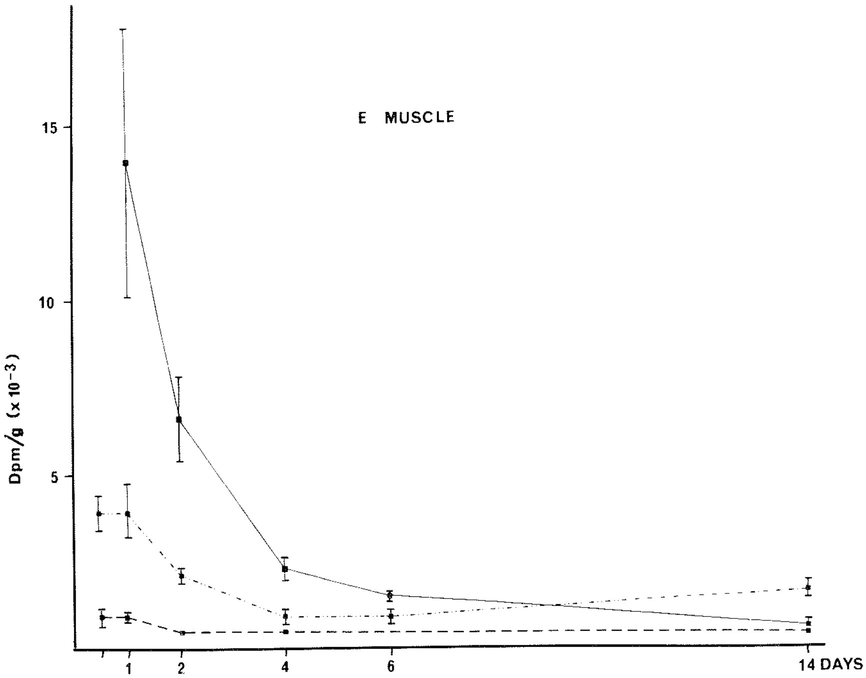


Fig. 1. Time-course of radioactivity in (A) liver, (B) bile, (C) urine, (D) blood plasma and (E) muscle of flounder after oral administration of 9.25 μg ^{14}C-labelled-naphthalene (dot-and-dash line), -phenanthrene (unbroken line), and -benzo(a)pyrene (broken line). Each point represent the mean (±SE) of 5 fish. The values of radioactivity of Nph and BaP have been corrected to correspond to the same specific activity as for Phe (i.e., measured radioactivity of Nph or BaP x $\frac{\text{spes.act. Phe}}{\text{spes.act. Nph or BaP}}$).




C URINE
Dpm/g (x10⁻⁴)
15
10
5
1
2
4
6
14 DAYS


D BLOOD PLASMA
Dpm/g (x10⁻³)
10
5
1
2
4
6
14 DAYS



muscle with Nph, in urine with BaP and in blood plasma, urine and muscle in the case of Phe. The concentrations of Phe-derived radioactivity in the liver and bile did not change markedly over the experimental period (14 d).

The consentrations of the Phe-derived compounds in the liver (Fig. 1A) were nearly twice the concentration of the BaP compounds. The time course of the radioactivity in the Nph-study decreased markedly during the first two days. Thereafter low concentrations of radioactivity were found. In the Phe and BaP study there was only a slight decrease of radioactivity in the liver during the 14 d experimental period. A more efficient elimination of Nph-derived radioactivity was expected since Nph is less lipophilic than Phe and BaP. These results are in accordance with findings from an experiment in which starry flounder (*P. stellatus*) was given labelled BaP and Nph (Varanasi and Gmur 1980). They found that the concentration of BaP-derived radioactivity in the liver 7 d after dosing was 5-fold greater than the corresponding value of Nph. Roubal *et al.* (1977) reported much higher concentrations of radioactivity in the liver of coho salmon (*Oncorhynchus kisutch*) when the fish was given labelled anthracene as compared to naphthalene.

Many authors have previously stressed the importance of biliary excretion of aromatic hydrocarbons (e.g. Lee *et al.* 1972; Solbakken *et al.* 1979, 1980). In the present investigation the time courses for the analyses of the bile and liver samples in the Phe and BaP studies (Fig. 1A and B) were roughly similar, indicating a close relationship between the liver and bile in the excretion of aromatic hydrocarbons.

In urine and bile (Fig. 1B and C) only low concentrations of Nph-derived radioactivity were found in spite of high concentrations in liver and blood plasma. It therefore seems likely that the biliary and urinary excretion are less important in the case of Nph than with Phe and BaP. This is in accordance with results published by THOMAS and RICE (1981). They found that the gills were the most important site of excretion of Nph-derived radioactivity from fish (*Salvelinus malma*) after oral administration. The excretion from the gills was more than 10 times greater than via the urine and intestine.

There was also a great similarity in the results from the blood plasma and the muscle analyses (Fig. 1D,E) which might reflect a close connection between blood and muscle tissue. However, the concentrations were only approximately 5% of the concentrations found in the liver. The fat content of the muscle used in this study was 6.5% of the fat content in the liver which may explain the different concentrations of the lipophilic xenobiotics in these tissues. Low concentrations of radioactivity in muscle of cod (*Gadus morhua*) given a single oral dose of BaP was also reported by CORNER, HARRIS, WHITTLE and MACKIE (1976).

SOLBAKKEN and PALMORK (in prep.) carried out an experiment where Phe was given to flounder in the winter season. The present experiment was performed in the summer, using corresponding laboratory conditions. The temperature was the same in the two experiments. The results, however, were diverging. Solbakken and Palmork found much lower concentrations of radioactivity in liver, muscle, urine, and bile compared to the present study. The elimination was less efficient in the present investigation. Thus, the time course of radioactivity in the liver (Fig. 1A) was closer to a corresponding curve in an experiment performed at 3°C (SOLBAKKEN and PALMORK, in prep.). These differences were probably due to seasonal variations in the biochemistry and physiology of the species, and this stresses the need of more knowledge about such variations to be able to give a meaningful evaluation of the results.

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