Fol. 41 E

This paper not to be cited without prior reference to the authors

International Council for
the Exploration of the Sea

C.M. 1979/E: 51
Marine Environment
Quality Committee

ACCUMULATION AND METABOLISM OF PHENANTHRENE IN RAINBOW TROUT (SALMO GAIDNERI)

by

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ABSTRACT

Phenanthrene labelled with carbon-14 was administered to rainbow trout, Salmo gaidneri, in dry food and the radioactivity in the different tissues were measured at intervals using a Packard Tri-Carb scintillation-counter. Carbon-14 was rapidly accumulated in the liver and thereafter high concentrations was found in the gall bladder. Two weeks later most of the carbon-14 had disappeared.

The metabolites of phenanthrene were isolated from the gall bladder and intestine 48 hours after the administration of 25 mg. The analyses of the TMS-derivatives of the metabolites were performed using a computerized gas chromatograph mass spectrometer. Quantitative studies were carried out using selected ion monitoring. A total of eight metabolites were isolated and identified. The main metabolite in gall bladder was 1.2-dihydro-1.2-dihydroxy-phenanthrene, which constituted 93% of the total metabolic products. The fraction of free metabolites was 75 and 8% in gall bladder and intestine, respectively.

RESUMÉ

(Accumulation et métabolisme de phenanthréne chez la truite arc-en-ciel (Salmo gaidneri). Phenanthréne étiquete avec carbone-14 a été administré a la truite arc-en-ciel, dans la nourriture seche et la radioactivité dans les tissues différent ont été mesurés par intervalls en employant un compteur á scintillation - Packard Tri-Carb. Carbone-14 a été rapidement accumulé dans le foie et ensuite hautes concentrations ont été trouvées dans la vésicule biliaire. Après deux semaines la plupart du carbone-14 est disparue.

Les métabolites de phenanthréne ont été isolées de la vésicule biliaire et l'intestine 48 heures après l'administration de 25 mg. Utilisant la chromatographie en phase gazeuses sur capillaire de verre et des analyses en spectrometre de masse, nous avons analysé les TMS-dérivatives des métabolites. Des études quantitative ont été accomplies par fragmentographie de masse. Une somme totale de huit métabolites a été isolée et identifiée. Le métabolite principal trouvé, a été 1.2-dihydro-1.2-dihydroxyphenanthréne, qui constituaient 93% de tous les produits métaboliques. Le fraction des métabolites libre etait 75 et 8% dans la vésicule biliaire et intestine, respectivement.

INTRODUCTION

The increasing oil exploitation in the North Sea and offshore production of oil generally results in the risk of an increasing amount of oil finding its way to the sea. The components in oil creating the most concern, are the polycyclic aromatic hydrocarbons (PAH) mainly because of their possible carcinogenic and/or mutagenic effects (Anon, 1977). Industrial processes, e.g. aluminium smelters, ferro-silicon-, iron- and coke-works are also a source of PAH pollution in the marine environment (Palmork et al., 1973, Palmork,1974).

The biotransformation of polycyclic aromatic hydrocarbons in marine organisms has become a topic of research only in recent years. The subject has been reviewed by Varanasi and Malins (1977).

This paper deals with uptake, depuration and metabolism of phenanthrene, as a representative of the polycyclic aromatic hydrocarbons. The first experiments in our laboratory using saithe, a lean fish (Palmork et. al., 1978, Solbakken et al., 1979, 1980) showed that the main metabolite of phenanthrene in saithe was different from the main metabolite of phenanthrene in mammals (Sims, 1962 and Chaturapit and Holder, 1978). For the experiment described in this paper we have chosen rainbow trout (Salmo gaidneri), a fat fish to establish the fate of phenanthrene (PAH) in fish as a start of comparative studies of various species based on experiments performed under the same laboratory conditions utilizing basicly the same experimental techniques.

MATERIAL AND METHODS

The rainbow trout (both sexes) used in this study were acclimated to seawater (9,5°C, 35°/oo S) one week before dosing. The main weights were 113±30 g (S.D.) and 152±39 g (S.D.) for the ^{14}C-experiments and for metabolic studies, respectively.

All fish in the ^{14}C-experiments were given the same amount (15.8µg containing 1.0µCi) of [9-^{14}C] phenanthrene intragastricly

(The Radiochemical Centre, Amersham, England). The technique used is described in detail by Palmork et al., (1978) and Solbakken et al. (1980) and the experiments were performed under the same laboratory conditions.

The radioactivity was measured in liver, intestinal fat, muscle and gall bladder at intervals after dosing, using standard methods. Soluene-350, Dimilume-30 (Packard Instruments Co) and an internal standard ([^{14}C] toluene) were employed in the scintillation counting. Intestine and stomach were extracted with 50 ml toluene and one ml of the extract was analysed for radioactivity (one fish only each time). In the metabolic studies each of 10 fish was given 25 mg of phenanthrene and 48 h after dosing the gall bladder and intestine were removed. The samples from 10 fish were combined. Qualitative and quantitative studies of TMS-derivated hydroxylated metabolites of phenanthrene were analysed according to Palmork et al., (1978) and Solbakken et al., (1979).

RESULTS AND DISCUSSION

Accumulation and depuration

Table 1 shows the amounts of radioactivity (as % of given dose) present after various times in liver, intestinal fat, muscle and gall bladder. The table shows that the greatest degree of accumulation of radioactivity was in the gall bladder, and maximum accumulation occurred 48-96 h after dosing. A much lower content of radioactivity was recovered in the liver of rainbow trout and this is different to the results obtained for saithe (Palmork et al., 1978 and Solbakken et al., 1980). They found 72% of the dose in saithe liver 17 h after dosing. Rainbow trout however, have a small liver (approx. 10% of the liver in saithe) and the liver in rainbow trout (fat fish) is not as fatty as the liver in saithe (lean fish). Phenanthrene or PAH generally are lipophilic compounds and will accordingly accumulate in lipid rich tissues and these factors might explain the lower content of radioactivity in the liver of rainbow trout.

Table 1. Distribution of radioactivity in some organs 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	72 h	96 h	168 h	336 h	672 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)	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	0.2 (4, 0,2)	1.6 (4, 1.1)	4.9 (4, 2.8)	5.2 (5, 4.4)	-	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	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)	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	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)	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.

The analyses of stomach and intestine shows that only a small fraction of the dose is left 24 h after dosing (2 and 6% in stomach and intestine respectively). This indicates that the depuration of radioactivity in the liver of rainbow trout is very efficient, and this might explain the high concentration found i gall bladder, which store the metabolites from the liver before excreting them to the intestine.

Most of the radioactivity was depurated 14 days after dosing and there was no indication that the radioactivity was stored for more than 28 days (672 h) in any of the analysed organs, not even in intestinal fat. These results are in good agreement with the results reported by Lee et al., (1972), Corner et al., (1976), Roubal et al., (1977), Palmork et al., (1978) and Solbakken et al., (1980).

Metabolism

Table 2 gives the values in µg/g of the metabolites of phenanthrene in gall bladder and intestine. The main metabolite in gall bladder was found to be 1,2-dihydro-1,2-dihydroxyphenanthrene (93% of the total metabolites) and not 9,10-dihydro-9,10-dihydroxyphenanthrene, which is reported to be the main metabolite in mammals (Sims, 1962 and Chaturapit and Holder, 1978) and Norway lobster (Palmork and Solbakken, 1979). This is in agreement with earlier results in studies using saithe dosed with phenanthrene (Palmork et al., 1978 and Solbakken et al., 1979). However, the main metabolite in the rainbow trout intestine was 9,10-dihydro-9,10-dihydroxyphenanthrene which constitutes 61% of the intestine metabolites, and only 15% of the 1,2-dihydro-1.2-dihydroxyphenanthrene. This might be a result of intestinal microbial biotransformation of phenanthrene. In saithe intestine 47% of given dose phenanthrene (25 mg) was found unchanged 48 h after dosing, but no attempt was made to identify the metabolites.

The fraction of free metabolites was 75 and 8% in gall bladder and intestine, respectively. In Saithe gall bladder only 10% of free metabolites was found.

Table 2. Concentrations of metabolites in gall bladder and intestine of rainbow trout 48 h after intragastric administration of phenanthrene (25 mg/fish).

Compound	Gall bladder			Intestine		
	Unconjugated µg/g	Total µg/g	% unconjugated	Unconjugated µg/g	Total µg/g	% unconjugated
1-Hydroxyphenanthrene	1.2	5.9	20	0.1	0.3	33
2-Hydroxyphenanthrene	0.1	0.7	14	*	2.0	0
3-Hydroxyphenanthrene	0.1	0.5	20	*	0.1	0
4-Hydroxyphenanthrene	0.3	0.6	50	*	*	-
9-Hydroxyphenanthrene	0.1	0.2	50	0.1	0.1	50
1,2-Dihydro-1,2-dihydroxyphenanthrene	117.0	146.3	80	0.5	1.6	31
3,4-Dihydro-3,4-dihydroxyphenanthrene	0.3	0.5	64	*	*	-
9,10-Dihydro-9,10-dihydroxyphenanthrene	*	3.2	0	*	6.4	0

* trace (<0,05 µg/g)

\- not detected

The experiments with [^{14}C] phenanthrene in saithe and rainbow trout shows that the maximum concentration of radioactivity in gall bladder occurs at 24-48 h and 48-96 h, respectively. This might explain the difference in the concentration of free metabolites in the gall bladder. The sampling of the gall bladder (48 h after dosing) gives us the metabolites before the maximum in rainbow trout and after the maximum in saithe. A higher concentration of the free metabolites might therefore be expected in the rainbow trout gall bladder.

ACKNOWLEDGEMENTS

This investigation was supported in part by the Norwegian Marine Pollution Research and Monitoring Programme which is gratefully acknowledged.

The authors want to thank dr. Emmy Egidius and Kari Andersen, Institute of Marine Research, Bergen, for supplying the Rainbow trout and The Aquarium in Bergen for all help in keeping the animals.

We also want to thank Anders Goksøyr, Bergen, for his assistance in this work.

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