Bull. Environm. Contam. Toxicol. 26, 196-201 (1981)

Distribution and Elimination of [9-^{14}C]Phenanthrene in the Horse Mussel (*Modiola modiolus*)

Karsten H. Palmork and Jan Erik Solbakken

Institute of Marine Research, Directorate of Fisheries, Nordnesparken 2, 5011 Bergen-Nordnes, Norway

Few studies have been reported describing the distribution and elimination of polycyclic aromatic hydrocarbons (PAH) in mussels after a single exposure, e.g. LEE et al. (1972), NEFF & ANDERSON (1975) and NEFF et al. (1976).

The horse mussel (Modiola modiolus) is a bivalved mollusc which lives on hard bottoms below the littoral region. This species is closely related to the blue mussel (Mytilus edulis) and is common in northern waters (WIBORG, 1946). PALMORK (1974) reported findings of horse mussels containing PAH in fjords polluted by PAH from industries using Søderbergs electrodes. The PAH compound of highest concentration in the area was phenanthrene, which was therefore used in the study of PAHs in the horse mussel.

This study is part of a series conducted under identical experimental conditions using various marine animals (PALMORK & SOLBAKKEN 1981, SOLBAKKEN et al. 1979, 1980 and SOLBAKKEN & PALMORK 1980).

EXPERIMENTAL

Organisms. The horse mussels (Modiola modiolus) were collected at Sotra near Bergen, which is a non-polluted area. Mussels of both sexes were used and the mean weight (mean ± S.D.) of the meat and blood was 42 ± 12g. They were not fed during the exposure experiment in order to reduce gastrointestinal absorption. In the elimination study the mussels were given mixed thawed krill (Meganyctiphanes norvegica).

Treatment and maintenance. [9-^{14}C]Phenanthrene (600 µg containing 60 µCi, sp. act. 19.3 mCi/mmol) was dissolved in 150 µL ethanol and mixed in a small volume of seawater which subsequently was mixed with 12 L of seawater. Ten mussels were placed in each of three tanks (20 L) containing 4 L of the labeled seawater. The seawater was aerated using small air pumps. Samples of seawater were analysed for radioactivity at the beginning of the experiment.

0007-4861/81/0026-0196 $01.20

The mussels were exposed 48 h to the labeled water and then transferred to a tank containing clean seawater (260 L, 34°/oo S, flowrate 8 L/min.). The temperature was 11.5 to 15.0° during the exposure experiment and 9.0° in the elimination experiment.

Sample preparation. At the appropriate times the mussels were frozen and maintained at -20° until required. The mussels were then thawed and hepatopancreas, gills, kidney, genital tissue (including mesosoma) and mantle were carefully removed and weighed. The mussels were washed with methanol to remove radioactivity from the total surface. Two samples (approx. 100 mg) of each tissue were analysed for radioactivity using standard methods (Soluene-350 and Dimilume-30, Packard Instrument Co.) and [^{14}C]toluene as internal standard. The remaining parts of the mussels analyzed at the end of the exposure and elimination experiment were saponified (FARRINGTON & MEDEIROS 1975). The radioactivity of the solvent fraction was then measured.

RESULTS AND DISCUSSION

Nearly 40% of the radioactivity was "dissolved" in the seawater. The radioactivity measured in the three tanks was $17.2 \times 10^6 \pm 1.6 \times 10^6$ (S.D.) disintegrations per minute (dpm) which corresponds to a concentration of 20 ppb. The total amount of radioactivity found in the horse mussels at the end of the exposure experiment was $6.2 \times 10^5 \pm 2.6 \times 10^5$ dpm which correspond to 3.6% of the radioactivity in the seawater. This gives a concentration of 62 µg/kg wet weight.

The amounts of radioactivity present in different tissues in horse mussel exposed to [9-^{14}C]phenanthrene mixed in seawater are given in table 1. The highest amounts of radioactivity were found in samples taken immediately after transferring to clean seawater. The hepatopancreas contained the highest amounts of radioactivity and this is in accordance with a study performed by PALMORK & SOLBAKKEN (1981) where Norway lobsters (Nephrops norvegicus) were dosed with [9-^{14}C]-phenanthrene intragastrically. After 2-4 days in clean seawater about half of the radioactivity in the tissues was lost. Thereafter the elimination decreased and in contrast to the study with Norway lobsters, the horse mussels contained a high level of radioactivity even 28 days after exposure. This is also reported by SOLBAKKEN & PALMORK (1980) in an experiment using dogfish (Squalus acanthias) dosed with [9-^{14}C]phenanthrene. They found that between 33 to 65% of the maximum value found in liver, gall bladder, muscle, gonads, kidney and salt gland were left 28 days after dosing and concluded that

this may be related to the high content of fat in sharks and/or a low enzymatic capacity in the liver. Experiments in which phenanthrene (9.8 µg/mussel) was injected into horse mussels, showed no detectable levels (<0.1µg/mussel) of metabolic products (SOLBAKKEN unpublished observations).

TABLE 1

Distribution of radioactivity in some tissues of horse mussel at various times following [9-^{14}C]-phenanthrene exposure in seawater (20 ppb).

	0 day	1 day	2 days
Hepato pancreas	217.1* (4; 82.3)**	143.0 (5; 60.2)	82.2 (5; 39.1)
Gills	149.0 (5; 77.5)	71.0 (5; 23.8)	63.4 (5; 34.8)
Genital tissue	77.2 (4; 26.0)	64.8 (5; 29.6)	50.9 (5; 28.0)
Mantel	40.5 (5; 16.9)	21.4 (5; 6.1)	19.3 (5; 11.4)
	4 days	**7 days**	**28 days**
Hepato-pancreas	70.6 (5; 23.7)	57.3 (5; 23.2)	36.5 (5; 18.0)
Gills	49.1 (5; 27.2)	49.0 (5; 20.1)	32.2 (5; 10.8)
Genital tissue	29.4 (5; 28.0)	19.9 (4; 7.3)	6.4 (5; 2.0)
Mantel	16.3 (5; 8.5)	17.8 (5; 13.4)	7.2 (5; 4.8)

* mean value, total activity (dpmx10^{-3})

** number of animals; standard deviation of mean

The values for radioactivity in the kidney are shown in table 2, and they are given as dpm per unit of wet weight of the tissue because of the difficulty involved in measuring the total weight.

The corresponding values of hepatopancreas are also given for comparison. Table 2 shows that the kidney has a much higher concentration of radioactivity than the hepatopancreas. The level of radioactivity in the kidney reached its maximum after one day in clean seawater probably because of the excretory function of the kidney and only a small decrease in the concentration was found between 1 and 28 days after exposure.

TABLE 2

Distribution of radioactivity in some tissues of horse mussel at various times following [9-^{14}C]-phenanthrene exposure in seawater (20 ppb).

	0 day	1 day	2 days
Kidney	6.1* (5; 3.2)**	17.1 (4; 13.2)	12.9 (5; 9.4)
Hepato-pancreas	15.6 (4; 3.9)	9.3 (5; 3.2)	8.0 (5; 3.6)

	4 days	7 days	28 days
Kidney	13.0 (5; 5.5)	10.7 (5; 6.0)	10.3 (5; 6.0)
Hepato-pancreas	6.7 (5; 2.6)	5.1 (5; 2.6)	2.4 (5; 0.9)

* mean value, concentration of radioactivity ((dpm/g)x10^{-4})

** number of animals; standard deviation of mean

LEE et al. (1972) described the accumulation of [^{14}C]-naphthalene and [^{3}H]benzo(a)pyrene dissolved in seawater by the mussel Mytilus edulis. The gills were the site of the highest concentration of radioactivity, a finding in contrast to the present results. If the values in table 1 are calculated as dpm per unit of weight the gills are found to have the lowest concentrations. LEE et al. (1972) found the highest amount of radioactivity, however, in the gut which in their study refers to all tissues including hepatopancreas, except the mantle, gills and adductor muscle. This corresponds with our results where most of the radioactivity was

found in the hepatopancreas. NEFF & ANDERSON (1975) studied the accumulation, release and distribution of [^{14}C]benzo(a)pyrene in the clam Rangia cuneata after 1 day exposure (30.5 ppb). After 30 days only 1.2% of the radioactivity found after 1 day remained in the tissues. The highest amount of radioactivity was also in these experiments found in the viscera.

In a study performed by NEFF et al. (1976) the relative rates of accumulation and release of naphthalene, phenanthrene, chrysene and benzo(a)pyrene from seawater by the clam Rangia cuneata were observed. The concentrations of the compounds were nearly the same in each experiment. Phenanthrene accumulated most rapidly and was eliminated most slowly. Naphthalene showed the most rapid release and this is expected since naphthalene has the highest water-solubility of these compounds. The fact that phenanthrene showed a lower degree of elimination than chrysene and benzo(a)pyrene, which have a lower water-solubility, is unexpected.

The release of benzo(a)pyrene from environmentally contaminated mussels, Mytilus edulis, was described by DUNN & STICH (1976). They found about 30% of the benzo(a)pyrene concentration remaining in the mussels after 28 days in clean seawater. The initial concentration of benzo(a)pyrene was 45 µg/kg. In the present study the corresponding value for the whole mussel is 38% (unpublished results). Comparison of these two experiments should be done with caution since the experimental conditions were different and the mussels are of different species.

Acknowledgements. This investigation was supported in part by the Norwegian Marine Pollution Research and Monitoring Programme. We would like to thank The Aquarium in Bergen for supplying the mussels, A.M. Tveit, Bergen for her assistance and R.R. Scheline, Department of Pharmacology, University of Bergen, for reviewing the manuscript.

REFERENCES

DUNN, B.P., and H.F. STICH: Bull. Environm. Contam. Toxicol. 15: 398 (1976).

FARRINGTON, J.M., and G.C. MEDEIROS: In: Proc. 1975 Conference on Prevention and Control of Oil Pollution. American Petroleum Institute. Washington, D.C. p. 115 (1975).

LEE, R.F., R. SAUERHEBER, and A.A. BENSON: Sci. 177, 344 (1972).

NEFF, J.M., and J.W. ANDERSON: In: Proc. 1975 Conference on Prevention and Control of Oil Pollution. American Petroleum Institute. Washington, D.C. p. 469 (1975).

NEFF, J.M., J.W. ANDERSON, B.A. COX, R.B. LAUGHLIN, Jr., S.S. ROSSI, and H.E. TATEM: In: Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment. American Institute. Washington, D.C. p. 515 (1976).

PALMORK, K.H.: Niónde Nordiska Symposiet om Vattenforskning. Nordforsk, 99 (1974).

PALMORK, K.H., and J.E. SOLBAKKEN: Bull. Environm. Contam. Toxicol. 26, in press (1981).

SOLBAKKEN, J.E., and K.H. PALMORK: Distribution of Radioactivity in the Chondrichthyes Squalus acanthias and the Osteichthyes Salmo gairdneri Following Intragastric Administration of [9-^{14}C]phenanthrene. In prep. (1980).

SOLBAKKEN, J.E., K.H. PALMORK, T. NEPPELBERG, and R.R. SCHELINE: Bull. Environm. Contam. Toxicol. 23, 100 (1979).

SOLBAKKEN, J.E., K.H. PALMORK, T. NEPPELBERG, and R.R. SCHELINE: Acta Pharmacol. et Toxicol. 46, 127 (1980).

WIBORG, K.F.: Rep. Norw. Fishery mar. Invest. 8, 1 (1946).