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Different Pathways for the Uptake of Benzo(a)pyrene Adsorbed to Sediment by the Mussel *Mytilus galloprovincialis*

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Polynuclear aromatic hydrocarbons (PAHs) are a major class of organic contaminants in the marine environment and may not only affect productivity of marine organisms but may ultimately affect the human health. In the aquatic habitat, many organisms readily accumulate PAHs from the environment and store them at a relatively high level in their tissues (Farrington *et al.* 1982; Bender *et al.* 1988). Consequently, it is of interest to determine the bioavailability of PAHs for marine species such as mussels consumed by humans.

Most of the studies on experimental accumulation and depuration of PAHs in marine organisms were carried out by addition of either water solubilized PAHs (Broman and Ganning 1986; Suteau *et al.* 1987) or sediment adsorbed compounds (Neff and Anderson 1977; Roberts *et al.* 1989) to a clean environment. To test the bioavailability of PAHs adsorbed in sediment, the present study describes the release of labelled B(a)P from contaminated sediment and its transfer to water and mussels (*Mytilus galloprovincialis*). The effect of sediment suspension was also investigated.

MATERIALS AND METHODS

The experiment was carried out in January 1989. Mussels of uniform shell length (20-30 mm) were collected from a low polluted site at the entry of the Arcachon Bay (South-West of France). The mussels were externally cleaned, and placed in a fiberglass tank (stock tank, size 50 L) during 1 wk. All studies were conducted at 18°C.

Contaminated sediment was prepared as follows : muddy sediment (particle size $< 100\mu$, containing 8.5 ug PAH $.g^{-1}$ as previously reported by Ribera *et al.* (1989)) was collected in the Toulon Bay (South of France) and then dried under vacuum at 80°C. A sample of 500 g was mixed 1 hr with 1.5 mg 7-10^{14}C B(a)P (5 mCi/mmol, purchased from Amersham, France) in 500 mL acetone. The solvant was then evaporated by means of a Rotavapor.

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Samples were taken to control the adsorbed radioactivity (corresponding to 3.0 $\pm$ 0.2ug B(a)P.g^{-1}). Contaminated sediment (200g) was introduced into 2 glass tanks (size 20 L) and 15 L of sea water (from the Arcachon Bay) were then carefully added in each tank. A tray containing 40 mussels was placed in each tank (A and B) as shown in Fig 1. In tank B the sediment was resuspended in water by 5 min stirring 48 and 96 hr after starting the experiment.

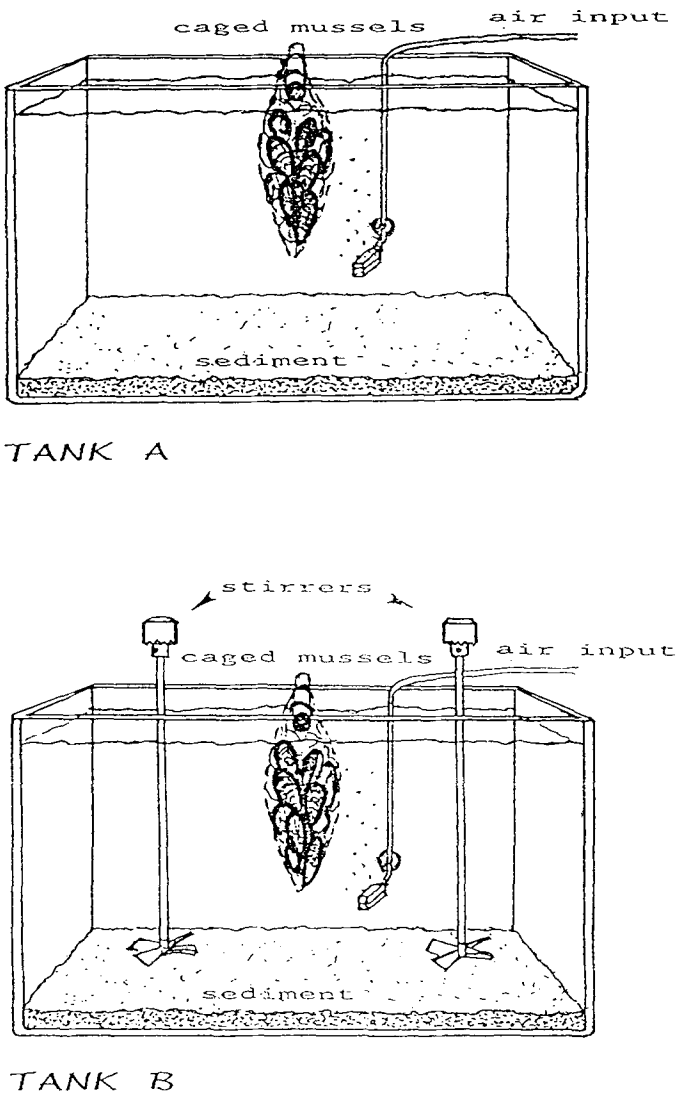

Figure 1. Experimental conditions : tank A was without stirrers whereas tank B was fitted with stirrers to resuspend sediment.

At intervals samples of mussels (2 animals each) and water were collected from each tank. Mussels collected in tanks A and B were substituted by mussels from the stock tank in order to maintain a constant number of animals in each experimental tank. Mussels were shucked, the tissues were drained in acetone to remove any surface adsorbed B(a)P. After weighing (650 $\pm$ 112 mg as on an average per animal) the tissues were solubilized in soluene 350 (purchased from Packard, France) one night at 50°C under gentle agitation. After complete solubilization, scintillation mixture was added and samples were dark-equilibrated one night before liquid scintillation counting (on a Searle Delta 300). Water samples (consisting of 3 replicates of 1 mL each) were collected near the trays and added to 15 mL scintillation mixture before counting.

Groups of values were compared by the methods of *F*- and *t*- tests as described by Schwartz 1963.

RESULTS AND DISCUSSION

Figure 2 shows the uptake of radiolabelled B(a)P from contaminated sediment with or without stirring steps (tanks B and A respectively). At zero time the water was introduced into the tanks and part of sediment fraction was resuspended. The B(a)P concentration in water consequently increased to about 6 ug.L^{-1} (Figure 2b). Then the sediment particles were decanted and the water concentration rapidly decreased to a minimum value of about 2 ug.L^{-1} within 12 hr The kinetic parameters of these changes in tanks A and B were reported in Table 1 (A1 and B1). In tank A the B(a)P concentration in water increased during the remaining experimental time and could be judged to follow a straight line. This increase corresponded to the dissolution of B(a)P in water. The rate of this kinetic was 510 ug.hr^{-1} or 34 ng.L^{-1}.hr^{-1} (Table 1, A).

As shown by B(a)P-derived radioactivity, the amount of suspended particles in water compartment was strongly increased after stirring (B2, B3) and then decreased, following an exponential curve to finally reach the same values as measured in tank A. This result indicated that sediment agitation did not increase the dissolution of B(a)P in water.

The accumulation of radiolabelled B(a)P by mussels is shown in Figure 2a. After 48 hr the rate of B(a)P accumulation from water dissolved fraction (tank A) follows a straight line (23 ng.hr^{-1} for transfer of B(a)P between water and mussel compartment in tank A, corresponding to nearly 1 ng.g^{-1}.hr^{-1} for mussel intake). In tank B, the kinetics for B(a)P accumulation after stirring steps (B2 and B3) shows a curve similar to that of the initial agitation (A1 and B1). The initial rapid increase (5 to 15 ng.g^{-1}.hr^{-1} within the first 2 hr) is related to a strong increase in suspended particles in water compartment (B(a)P-derived radioactivity twice higher within the first hour after stirring). Thus in our experimental conditions, the uptake of B(a)P in mussels was more than 5 times

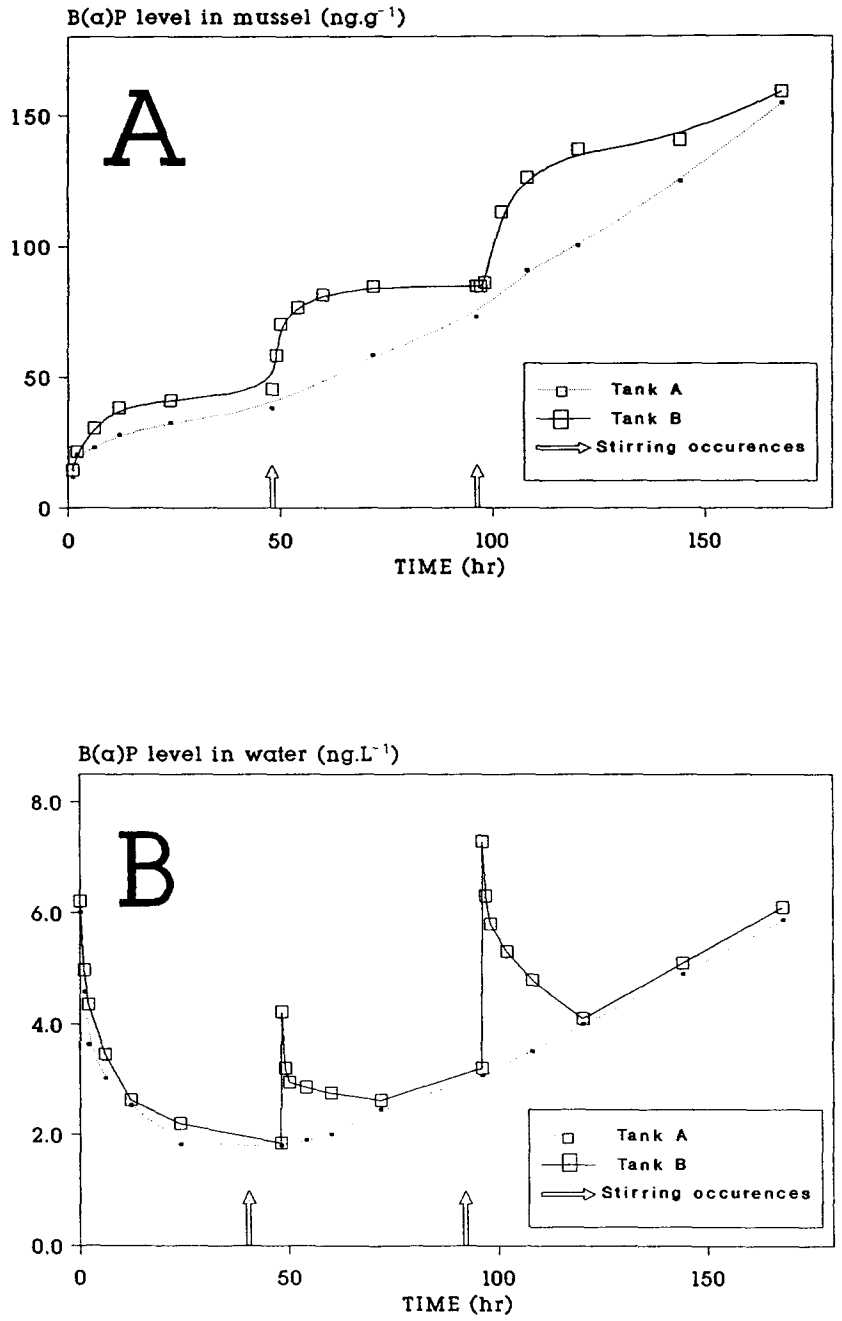

Figure 2. B(a)P concentration in (A) the mussels Mytilus galloprovincialis (ng.g^{-1} wet weight) and (B) the water (ng.L^{-1}).

Table 1 . Rates of B(a)P interchanges between sediment, water and animal compartments

Experiment[1]	Compartment	Relationship (ug)	Ordinant Zero intercept (ug/hr)	Slope	Correlation coefficient	n	Significance P <
A	Water	linear	1.290	0.509	0.998	9	0.01
	Mussel	linear	-0.136	0.023	0.988	7	0.01
	Water+Mussel	linear	1.460	0.528	0.997	7	0.01
B	Water	linear	-0.426	0.537	0.996	6	0.01
	Mussel	linear	-0.098	0.024	0.996	4	0.01
	Water+Mussel	linear	-0.866	0.566	0.996	4	0.01
A+B	Water	linear	0.498	0.522	0.996	15	0.01
	Mussel	linear	-0.151	0.024	0.988	14	0.01
	Water+Mussel	linear	0.316	0.546	0.996	15	0.01
A1	Water	log	61	-0.021	0.833	5	0.05
	Mussel	log	0.495	-0.028	0.996	4	0.01
B1	Water	log	68	-0.22	0.880	5	0.05
	Mussel	log	0.608	0.026	0.863	4	0.05
B2	Water	log	50	-0.013	0.683	6	ns
	Mussel	log	1.716	0.011	0.916	4	0.01
B3	Water	log	97	-0.019	0.930	6	0.01
	Mussel	log	2.369	0.018	0.835	4	0.05

[1] Values used for calculation corresponding to times :
A : 48, 72, 96, 108, 120, 144, 168 hr for mussels and water+mussel.
B : 48, 72, 96, 120, 144, 168 hr for water and 48, 96, 144, 168 hr for mussels and water+mussel.
The kinetics after stirring were calculated from times 0, 1, 2, 6 and 12 hr for water and 2, 6, 12 and 24 hr for mussels.
ns : not significant

higher when sediment particles were suspended in water. It is possible that mussel filtration rate was increased in presence of sediment as suggested by McLeese and Burridge (1983). In the case of oyster, Fortner and Sick (1985) reported that B(a)P was accumulated in greater amounts from dissolved form rather than from adsorbed on particulate organic matter. As for mussels, the curve of B(a)P accumulation, 12 hr after stirring, tended to flatten into a segment which is nearly horizontal. This phase corresponded to a decrease in particle concentration in water and the rate of B(a)P accumulation was therefore nearly zero. However we did not measure any release of B(a)P from mussels during this period. Dunn and Stich (1976) reported half life of 16 d for B(a)P from contaminated mussels (45 ug.kg^{-1} B(a)P) transferred to clean water, and a decrease of over 20% was measured in the first 3 d. The plateau in B(a)P accumulation corresponded therfore to a decrease in particulate concentration with simultaneous increase in dissolved B(a)P form. There was no statistically significant difference ($p>5\%$, t-test of student) in the B(a)P content in mussels from the two experimental tanks 48 hr after stirring occasions. Thus the rate of B(a)P dissolution from sediment can be calculated in tank B from the values measured at 48, 96, 144 and 168 hr. The results shown in Table 1 (B) are not significantly different from those measured in tank A. Thus a calculation of the transfer rates in our experimental system (200 g sediment, 15 L water, 26 g mussel tissues) shows that the rate of B(a)P dissolution was 546 ng.hr^{-1} , the rate of B(a)P accumulation in mussels was 24 ng.hr^{-1}, and the rate of B(a)P released from mussels was nearly zero (Figure 3). All along the experiment, these rates were constant. At the end of this study, only 15% of the B(a)P initially present in the sediment pool was dissolved and 0.7% was transferred into the mussel compartment.

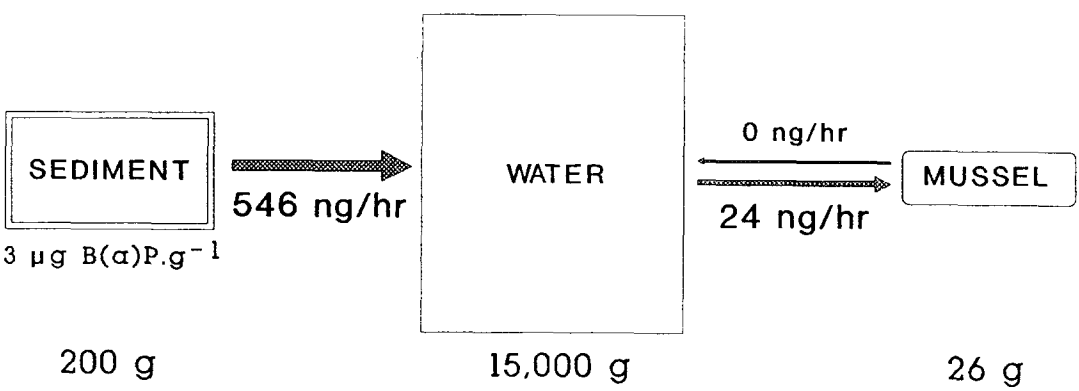

Figure 3. An illustration of the compartment analysis model.
Pool sizes were 200 g for sediment, 15, 000 g for water and 26 g for mussels.
At zero time 600μg of 14C B(a)P were adsorbed in sediment (giving a B(a)P concentration in sediment of 3 μg/g)

The ability of mussels to accumulate PAHs either released to the water column or remaining in the sediments makes these molluscs attractive for pollution monitoring and for the assessment of PAH biological effects. However the low rate of excretion may provide discrepancy between water concentration and tissue content of B(a)P over a period of some days (about 1 wk). Moreover, in polluted areas where mussels are generally cultivated, these animals must not be collected either for human consumption or for pollution monitoring within 1 wk after a storm which may be responsible for sediment suspension increase.

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