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Uptake and Metabolism of Benzo(a)pyrene Absorbed to Sediment by the Freshwater Invertebrate Species *Chironomus riparius* and *Sphaerium corneum*

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The polyaromatic hydrocarbon (PAH) Benzo(a)pyrene (BP) is a widespread contaminant, which is known to be carcinogenic in mammals after ic activation. BP is released into the environment and the water as a by-product of combustion of fossil and recent material (fuel, wood) in industry, traffic and households and is also released by natural sources. Most of the PAHs are highly lipophilic and therefore bound to humic substances, dissolved macromolecules and particulate matter which are at least deposited in the aquatic sediments (WERSHAW, 1986). The BP concentrations in sediments of pristine waters do normally not exceed 1 µg/g dry weight (dw). In polluted waters of industrial areas, the BP concentration may increase up to 100 µg/g dw (HANNAH et al., 1983). The risk for environmental health caused by such sediment bound PAHs can be assessed by using BP as a model substance.

One aim of this study was to investigate if the sediment bound BP is bioavailable to sediment dwelling organisms. For this purpose we examined the uptake of sediment bound BP.

The metabolism of PAHs in insects has been investigated, however, only little is known about the Phase I and Phase II metabolism in clams, especially in freshwater species (HARITOS *et al*., 1994; Lu *et al.,* 1977; MICHEL *et al.,* 1995). The organisms we choose were two sediment inhabiting invertebrates, the larvae of the midge *Chironomus riparius* and the European fingernail clam *Sphaerium corneum.* We, therefore, investigated whether the BP taken up by the test organisms undergoes metabolic activation, since the toxicity of BP is modulated by metabolism.

MATERIALS AND METHODS

We performed two experiments to determine if BP was available to sediment dwelling invertebrates. An uptake and elimination experiment was carried out in spiked sediment with *C. riparius* and *S. corneum* using

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radiolabelled BP. A metabolism study was carried out using BP spiked water. BP and its metabolizes in tissue homogenates or released into the water were identified using HPLC.

The sediment used for the uptake and elimination study was desalinated Poole Harbour sediment which was chosen as a clean reference sediment in several studies (EC REPORT 3738, 1994). It was obtained from the top layer and oxidized during the desalination procedure. The water used for all experiments was reconstituted water according to a protocol for Dutch Standard Water (NEN 6503, 1980). The characteristics of the sediment and the water are shown in Table 1 and 2.

Table 1. Characteristics of desalinated Poole Harbour Sediment

Particle Size Distribution	percent (by weight)
<2000 µm	95.79
<1000 µm	70.36
<500 µm	40.75
<250 µm	20.25
<125 µm	8.01
<90 µm	5.57
<73 µm	4.17
<63 µm	3.13
<45 µm	2.09

Table 2. Characteristics of Dutch Standard Water (NEN 6503, 1980)

pH	8.2
Hardness	210 mg/l CaCO3
T	20°C

The sediment was spiked using the following procedure:
16 µL of BP (100 mM) dissolved in dimethylsulfoxide (DMSO) were added to 2.6 µmol of dry radiolabelled BP (26 Ci/mmol) and DMSO was added up to a final volume of 1 mL. This solution was added dropwise into 400 mL of wet sediment with a dry weight of 8%. The spiked sediment was shaken overnight at 4°C. The final concentration expressed in dry weight of sediment was 10 µg/g with a radioactivity of 100 µCi (2.210^8 dpm / 400 mL wet sediment, approximately $5.5.10^5$ dpm / mL wet sediment). Experiments performed earlier have shown that the uptake of BP by Chironomus in the range of 10 to 100 µg/g dw follows first order kinetics.

For the uptake experiment the following procedure was chosen:
Second instar larvae of *Chironomus riparius* (10 days old) from a laboratory culture and *Sphaerium corneum* collected 2 weeks before testing in a small stream in northern Germany (Hagener Au, near Kiel) were each exposed to the spiked sediment. After five days of exposure animals were sifted out and transferred into clean sediment for the elimination phase. The frequency of sampling is shown in Table 3.

Table 3. Frequency of sampling for uptake and elimination study

Time [h]	Uptake	Time [h]	Elimination
0	S.c./C.r.	120	S.c./C.r.
1	S.c./C.r.	121	S.c./C.r.
2	S.c./C.r.	122	S.c./C.r.
4	S.c./C.r.	124	S.c./C.r.
8	S.c./C.r.	128	S.c./C.r.
21	S.c./C.r.	142	S.c./C.r.
45	S.c./C.r.	168	S.c./C.r.
72	S.c./C.r.	192	S.c./C.r.
120	S.c./C.r.	240	S.c.

Chironomids were exposed in 50 mL crystallization dishes containing 40 mL of the wet sediment (8% dry weight) without aeration. Oxygen was not below critical concentrations during the experiment. For each sample one crystallizing dish was used. Each sample consists of 3 to 5 pools of larvae, 10 to 20 larvae per pool. For sampling, a pool of larvae were taken out of the sediment, washed, blotted dry and weighed.
The clams were exposed in aerated 2 L glass aquaria, containing 400 mL of sediment/water mixture (dry weight 8%) .In this case aeration was essential to avoid anoxic stress. For sampling clams were taken out of the sediment, the soft tissue was dissected and then treated in the same way as the larvae. Three to 5 replicates were taken of each sample.
All samples were kept frozen until radioactivity was measured, which was achieved by combustion in a Zinsser Biological Oxidiser. The radioactivity was measured with a Beckman Liquid Scintillation Counter (LSC).

The metabolism study was performed in water spiked with radiolabelled BP (2.5 nMol / L = 1 µCi / mL). As exposure system two 200 mL glass vessels were used for *C. riparius* or *S. corneum.* The water was not aerated during the exposure time. After 20 hours of exposure clams or lar-

vae were homogenized in 0.2 M Na-Acetate buffer, pH 5.0. Homogenates were divided in two aliquots, one of each was incubated with Glucuronidase/Sulfatase at 37°C for 1 hour. After this time, water was added to the homogenates up to a volume of 20 mL and nonpolar metabolites were extracted with hexane. Polar metabolites were extracted by using an Extrelut column (Merck, Darmstadt) and ethylacetate as organic solvent. The combined extracts were evaporated and the residue was dissolved in 500 µL DMSO. Fifty µL of each extract was injected into an HPLC (Merck/Hitachi, RP 18/10 column.; initial solvent acetonitrile/ water:10/90, up to 100% acetonitrile) and the eluate was fractionated in 40 steps of 1 minute each. The radioactivity was again measured with an LSC. For the determination of metabolites in the water 100 µL of water (clam or larvae experiment) were incubated with 10 µL HCl (10 N) for 1 h at 95°C, centrifuged and the supernatant was injected into the HPLC.

RESULTS AND DISCUSSION

The data of BP accumulation in *C. riparius,* measured as total radioactivity, show a fast uptake during the first 8 hours followed by a slower uptake until 120 hours (Fig. 1 a). A similar biphasic accumulation was observed for BP in *S. corneum.* In contrast to the kinetics obtained in *C. riparius,* the phase of fast uptake lasted about 24 hours in *S. corneum* (Fig 1b).

A biphasic kinetic could also be detected for the elimination phase of radioactivity for C. *riparius* as well as for S. *corneum* (Fig 1a+1b). In both cases an initial rapid phase during the first 8 hours was followed by a slower phase. In contrast to the results of Leversee *et al*. (1982) who

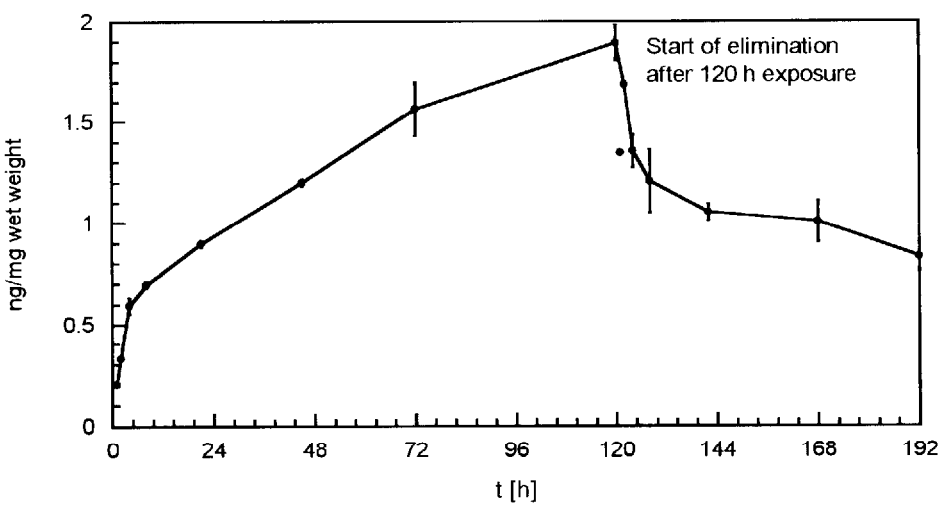

Figure 1a. Accumulation of sediment sorbed BP by larvae of *C. riparius*. Each point is the mean ± stddev. of 3 replicates.

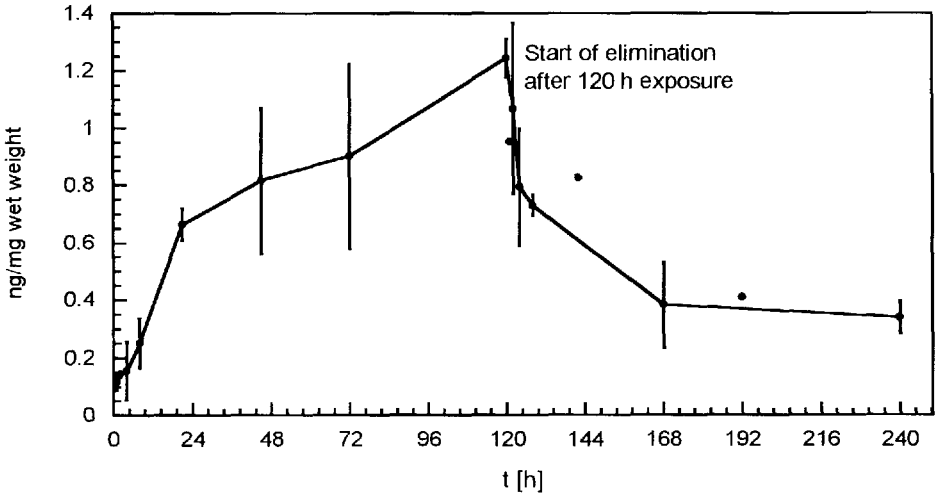

Figure 1 b. Accumulation of sediment sorbed BP by *S. corneum.* Each point is the mean ± stddev. of 5 replicates.

found steady state conditions after 8 hours for the uptake of BP by *Chironomus* from the water, we observed no steady state for the uptake of BP from the sediment until 120 hours. The results of our uptake and elimination study show that sediment bound BP is bioavailable to aquatic organisms, such as *C. riparius* and *S. corneum.* Both species are predated by higher animals, such as fish and birds. This may lead to a food chain transfer of these highly lipophilic and potentially carcinogenic compounds.

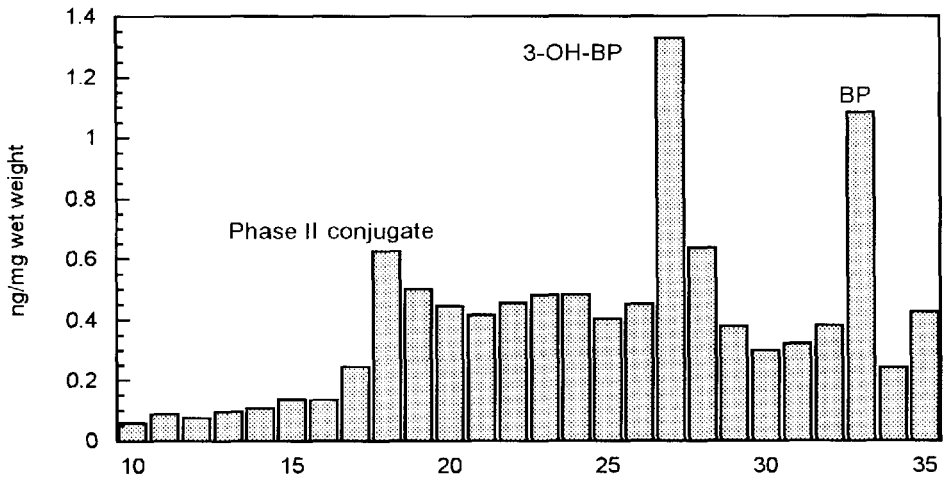

Figure 2a. HPLC chromatogram of BP metabolites in the larvae of *C. riparius* after 20 hour exposure.

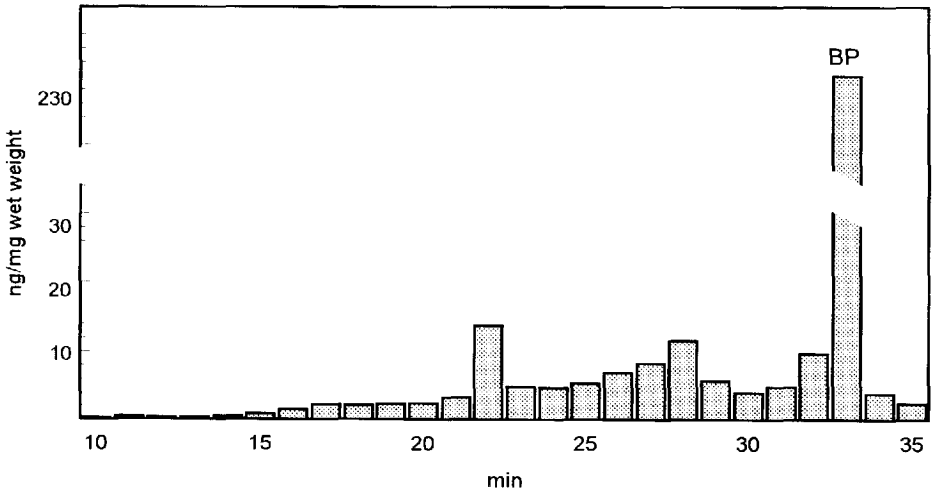

Figure 2b. HPLC chromatogram of BP metabolites in the mussel S. *corneum* after 20 hour exposure.

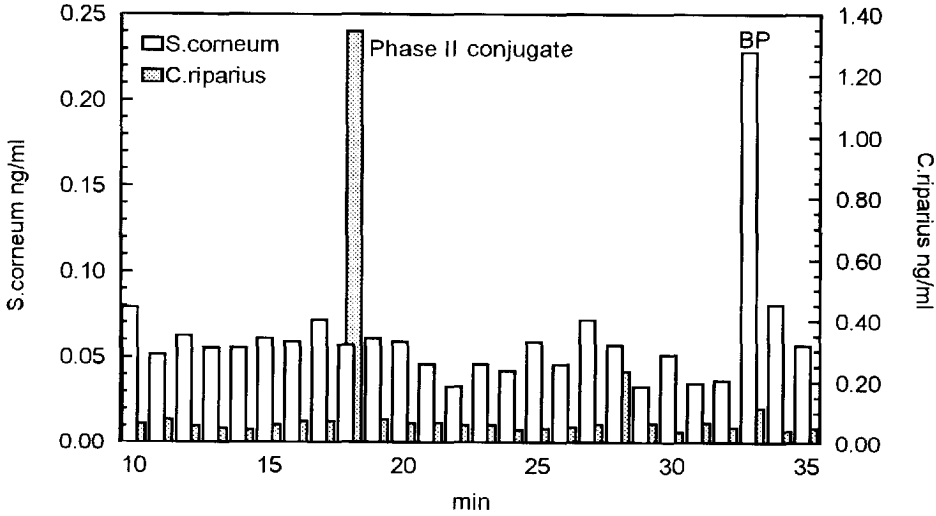

Figure 3. HPLC chromatogram of BP metabolites in water after 20 hour exposure. The phase II conjugates were hydrolized with glucuronidase/sulfatase before fractionating.

The HPLC chromatogram of BP metabolites (Fig. 2a) shows the capability of *C. riparius* to produce a wide range of BP metabolites. We observed Phase 1 and Phase II metabolites in the larvae homogenates. The major Phase I metabolite was identified as 3-hydroxy-BP while the Phase II metabolites were conjugates of 3-hydroxy-BP.

The HPLC chromatogram of the BP metabolites (Fig. 2b) produced by *S. corneum* shows only a small amount of a Phase I metabolite which could

be identified as 3-Hydroxy-BP. Associated conjugates also occurred. We observed that the major metabolites of BP released by the *Chironomus* larvae were Phase II metabolites while almost all of the radioactivity identified in the water containing the clams belonged to the parent compound (Fig. 3). The estimated percent parent compound and metabolites for both organisms are shown in table 4.

Table 4. Estimation of percent parent compound of BP and metabolites in tissue extracts after 20 h of exposure

Compound	*Sphaerium corneum*	*Chironomus riparius*
unidentified metabolites	18%	46 %
phase II conjugate	-	14%
3OH-BP	8 %	23 %
BP	74 %	17%

These findings are in agree with the results of Lu *et al* (1977) which reported that after 3 days of exposure, the major amount of BP in the snail *Physa spec.* was 88% parent compound while in the mosquito *Culex pipiens quinque fasciatius* only 22% of the parent compound was detected.

It can be stated that BP is taken up by the organisms and metabolized. A genotoxicity caused by these metabolites can be considered as previously shown by BURGEOT *et al*. (1995). During our experiments we could not detect any dead larvae or clams. These findings agree with previous studies, where BP concentrations up to 1000 µg/g dw in sediment did not cause any lethality (HOFFMANN, 1993).

Since the occurrence of Cyt P_{450} and MFO activities in marine clams is well known (LIVINGSTONE, 1991) and the capability of the species *Mytilus galloprovincialis* to detoxify BP in a phase I and phase II metabolism was demonstrated by MICHEL *et al.* (1995) it is still questionable to what amount the freshwater species *S. corneum* is able to metabolize PAHs like BP and which pathway of detoxification is used by this species.

The present study demonstrated that sediment bound BP is bioavailable to sediment inhabiting organisms such as clams and midge larvae and that it is metabolized to potentially genotoxic substances. Whether this is of health concern for these organisms or their predators warrants further investigations.

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