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# Effects of Benzo(a)pyrene on Food Assimilation and Growth Efficiency in *Porcellio scaber* (Isopoda)

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Benzo(a)pyrene is a well-known carcinogen, belonging to the polycyclic aromatic hydrocarbon group (PAH). These substances are emitted into the environment by several anthropogenic activities, including oil spills and coal burning. In the terrestrial environment, PAH accumulate in the upper horizons of the soil profile (Thomas *et al.* 1984). As a consequence of diffuse pollution concentrations of PAH in soils from rural areas have increased fivefold since the last century (Jones *et al.* 1988). At former gas works in the Netherlands, extremely high PAH concentrations in soil have been found (Visschers and Verschueren 1988). Little is known about the possible threat of this contamination to life in the soil.

Life in soil includes a variety of invertebrates, such as earthworms, isopods, springtails and mites. Many of these organisms live on decaying matter; by means of their saprotrophic activities, they contribute to the decomposition of dead organic matter and the maintenance of soil fertility. Contamination of soil may affect saprotrophic activities and may thus endanger soil processes. The animal component of soil life often reacts more sensitive to contamination than microbial activity (*cf.* Killham and Wainwright 1981).

PAH may affect biological activity in soil both positively and negatively. Some of the low-molecular weight PAH may be degraded by microbes, but high molecular weight PAH appear to be quite stable. Because of the strong sorption of PAH to dead organic matter, uptake by plants is minimal (Edwards 1983); however, soil organisms ingesting dead organic matter (saprotrophs) will be exposed to high concentrations.

Although the metabolic effects of benzo(a)pyrene have been investigated extensively in rodents, little is known about its effects in the terrestrial environment and nothing is known about its effects on soil invertebrates. In this study, we have choosen the woodlouse *Porcellio scaber* (Latr.) (Crustacea, Isopoda) as a representative from the soil invertebrate community. Experiments were designed to analyse the consequences of benzo(a)pyrene exposure for the energy metabolism. The partitioning of energy between respiration and 'scope for growth' is considered as an ecologically relevant criterion for studying effects of contaminants in the environment (Calow 1989). PAH might affect this partitioning by inducing biotransformation reactions that make demands on the assimilated energy.

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## MATERIALS AND METHODS

Isopods (*Porcellio scaber* Latr.) were collected from a wood-stack and were kept at 17°C, in terraria containing poplar leaf litter. For experiments, males and non-pregnant females were selected in a weight range of 50 to 80 mg fresh weight. During experiments, animals were housed individually in polystyrene pots (diameter 5 cm), equipped with a perforated lid. Each pot contained a moistened baked clay ball (diameter 1 cm) to provide an inert substrate for shelter and water supply. Food was prepared from poplar leaf litter, collected from the field in April (Expt. I) and October (Expt. II). Leaves were dried, ground to powder and re-moistened; food was offered in polythene dishes (diameter 9 mm, height 3 mm), each dish containing approximately 100 mg fresh weight of food. The pots were placed on a tray, in a fume cabinet in a climatized room at 17°C and were exposed to 12 hours of dimmed light per day. To secure a constant relative humidity, the bottom of the tray was covered with moistened filter paper, with on the top of the tray a glass plate with holes.

Benzo(a)pyrene (BaP) was dissolved in acetone (500 $\mu g\ ml^{-1}$) and dilutions were made such that, when mixing 250 µl with 1 g of dry food, the food contained 0 (acetone only), 1, 5, 25, and 125 $\mu g\ g^{-1}$ after evaporation of the acetone. Analysis of contaminated food using reversed phase HPLC and fluorescence detection showed that losses of BaP during evaporation of the solvent did not exceed 10% (T.C. van Brummelen, pers. comm.). 1.2 ml of deionized water was added to each g of food to obtain a suitable consistency.

Each week the food was prepared anew and the animals were transferred to fresh pots. Following drying of the pots, weekly food consumption was determined for each individual from the weight difference of the food dish and defecation was determined following collection of faecal pellets. For each individual, consumption (C) and defecation (F) were accumulated over the experimental period (5 weeks). The dry weight increase of isopods ($\Delta W$) was determined by weighing before (fresh weight), and after (fresh weight and dry weight), assuming the water content to be constant. Assimilation efficiency (AE) was calculated as $AE = (C-F)/C$ and growth efficiency (GE) as $GE = \Delta W/(C-F)$.

Respiration was measured by placing an individual isopod in a perspex chamber (volume 6.9 c.c.), equipped with an inlet and an outlet hole, through which a flow of carbon dioxide free air (80% $N_2$, 20% $O_2$) was led at 2 l $hr^{-1}$. The carbon dioxide concentration of the outflow was recorded using an infra red gas analysing system. Details of the equipment have been given by Van Wensem (1989). Following 20 minutes of equilibration, the $CO_2$ concentration was recorded for a further 20 minutes. Respiration (µl $CO_2\ g^{-1}\ hr^{-1}$) was calculated from the integrated concentration profile, the flow rate and the fresh weight of the isopod. Each individual was measured once a week for a period of 5 weeks. To reduce inter-individual variation, gut contents were depurated by starving the isopods for one day preceding respiration measurements.

Two main experiments were conducted, one to determine the effects of BaP on consumption, assimilation and growth, the other to determine the effects on respiration. In both experiments, the exposure period was 4 weeks, preceded by one week acclimation using uncontaminated food. Experiment I involved 110 individuals, assigned to 5 exposure levels (including controls), 2 sexes (male and female), and 10 replications, except for controls with 15 replications. Experiment II involved 50 individuals assigned to 5 exposure levels, 2 sexes and 5 replicates. The data were analysed by means of the TOXSTAT software package.

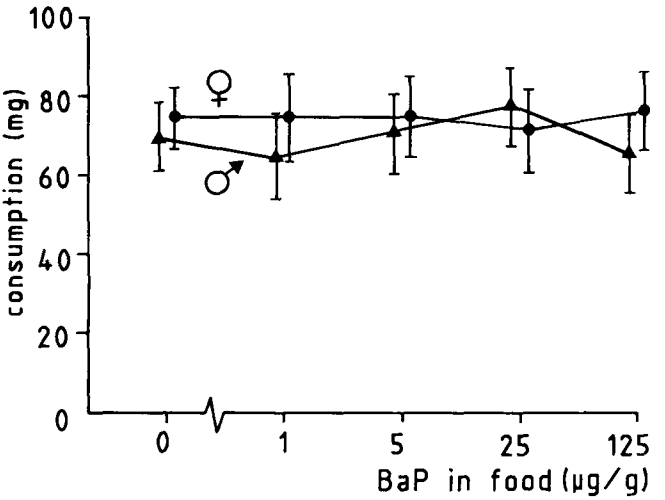


Figure 1. Food consumption by male and female *Porcellio scaber*, as a function of benzo(a)pyrene concentration. Each point gives a mean based on 9 to 15 replicates; bars indicate standard errors for the means, calculated from the pooled error mean square.

## RESULTS AND DISCUSSION

Mortality was minimal in both experiments (6 dead out of 160), and was not related to the benzo(a)pyrene exposure level. In accordance with the results of studies using other invertebrate species (Neff 1979), BaP does not seem to be toxic in terms of acute mortality.

Consumption was not affected by benzo(a)pyrene additions to the food up to 125 $\mu g\ g^{-1}$ (Fig. 1). Isopods did not avoid the food when contaminated with BaP. Thus, all animals had ingested a dose of BaP proportional to the concentration in the food; this facilitates the interpretation of the effects. Other contaminants often affect feeding activity in isopods. For example, when cadmium and zinc salts were added to food, isopods decreased their consumption (Van Capelleveen 1990). Fig. 1 also clearly demonstrates that males and females did not differ in their consumption.

Consumed food was assimilated with an efficiency of 25.6%, when averaged over all observations (Fig. 2). This is in good agreement with similar isopod studies using ground poplar leaves as food (M.H. Donker, unpublished). When other food types are used, assimilation efficiency may be higher, especially in the case of high quality food composed of potatoes and carrots (Van Capelleveen 1990). Assimilation efficiency of *Porcellio scaber* is in the range reported for other soil invertebrates (Petersen and Luxton 1982).

At the highest BaP exposure, male isopods showed a significantly higher assimilation (34%) compared to the control (26%), judged on William's isotonic regression model. Since consumption was not significantly affected (Fig. 1), the residence time of food in the gut will be constant. The increased assimilation must therefore be due to an active mechanism, induced by BaP exposure, that enhances digestion of food or absorption of digestive products. Such a mechanism could be interpreted as an increased need for energy. Increased assimilation was not observed in females (Fig. 2).

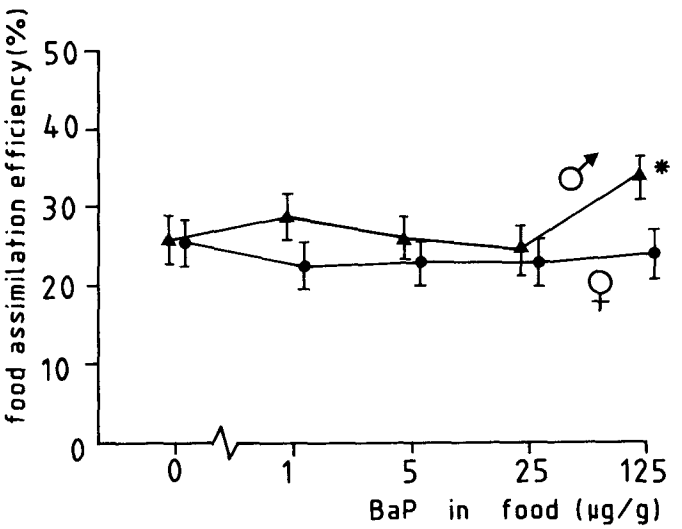


Figure 2. Food assimilation, expressed as a percentage of consumption, by male and female *Porcellio scaber*, as a function of benzo(a)pyrene concentration. Each point gives a mean based on 9 to 15 replicates; bars indicate standard errors for the means, calculated from the pooled error mean square. The asterisk indicates a mean differing significantly from the control (William's test, $p < 0.05$).

Assimilation efficiency may be influenced by the microbial population of the gut, although its role in food digestion is under discussion (Hassal and Jennings 1975; Griffiths and Wood 1985). Bacterial isolates from the gut of *Oniscus asellus* were able to oxidize aromatic acids occurring in humic compounds (Kaplan and Hartenstein 1978). When exposed to BaP, the gut flora could be induced to enhance the degradation of recalcitrant compounds and thereby to increase the availability of digestive products. However, this hypothesis cannot explain the different responses of males and females, unless the gut flora differs between the sexes.

Growth of isopods varied considerably between individuals, which is reflected in large standard errors for the means (Fig. 3). When averaged over all observations, growth efficiency was 11%; this is a rather low value which can be explained by the fact that only adult animals were used for the experiments. There were marked sex-dependent responses to BaP-exposure. In males, growth efficiency decreased from 11% in the controls to 1.7% at 125 µg $g^{-1}$, but in females it increased from 10% in the controls to 20.5% at 1 µg $g^{-1}$ (Fig. 3). Growth efficiency at the highest dose level was significantly affected in males (William's test), but not in females. The response of males is consistent with the interpretation that BaP induces an increased need for energy uptake at the 125 µg $g^{-1}$ exposure level (*cf.* Fig. 2). In females the response is similar to the 'hormetic' effects discussed by Stebbing (1982), which are usually interpreted as an overcompensative reaction of a cybernetic system to low levels of growth inhibitors. Whether these interpretations are correct cannot be decided at the moment.

Respiration (carbon dioxide production) decreased during the first week of the experiment, after which it stabilized between 40 and 60 µl $g^{-1}$ $h^{-1}$ (Fig. 4). This is somewhat on the low side as other authors have reported metabolic rates ranging

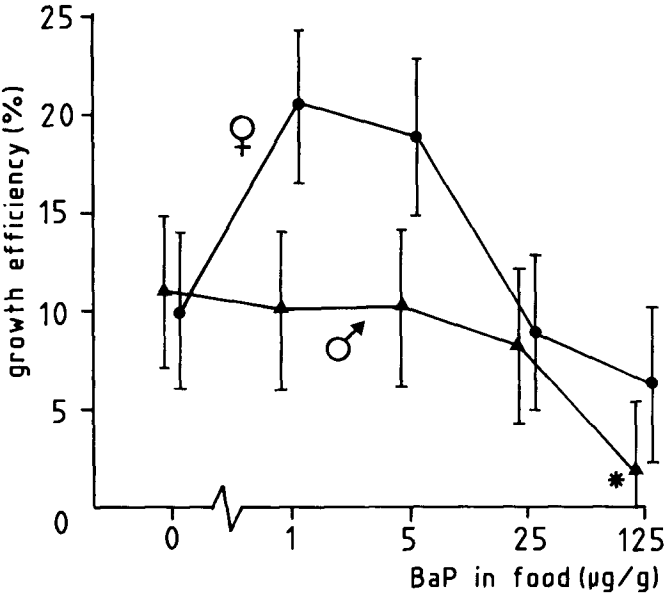


Figure 3. Body growth, expressed as a percentage of assimilation, by male and female *Porcellio scaber*, as a function of benzo(a)pyrene concentration. Each point gives a mean based on 9 to 15 replicates; bars indicate standard errors for the means, calculated from the pooled error mean square. The asterisk indicates a mean differing significantly from the control (William's test, $p < 0.05$).

between 120 to 210 µl $g^{-1}$ $h^{-1}$ for *Porcellio* (Wieser and Oberhauser 1984; Van Wensem 1989). Since the metabolic rate is highest in well-fed animals, this might indicate that food quality was not optimal in this experiment. Another explanation could be that isopods were starved for one day prior to respiration measurements. This not only decreased the average respiration, but it also considerably reduced inter-individual variation.

Males seemed to have a slightly higher metabolic rate than females (Fig. 4), but no effect of BaP could be noted in either sex. The increased assimilation and decreased growth, as observed in male isopods exposed to 125 µg $g^{-1}$ BaP, is not balanced by an increased respiration. It seems that an interpretation of the present results in terms of an energy partitioning model may be too simple.

Benzo(a)pyrene is a well-knewn inducer of the microsomal cytochrome P-450 mono-oxygenase system in the vertebrate liver (Timbrell 1982). Marked sex differences in biotransformation capacity have been reported for some species, such as the rat, which are due to the influence of sex hormones. It is not known whether similar biotransformation reactions would be effective in *Porcellio scaber*. On the basis of studies using larger crustaceans such as crayfish (James 1989) one would expect the biotransformation process to be situated in the hepatopancreas.

The concentration of BaP affecting *Porcellio* in this study (125 µg $g^{-1}$) is relatively high compared to concentrations in contaminated soils in the Netherlands (Visschers and Verschueren 1988). On the basis of the present results, BaP in soil

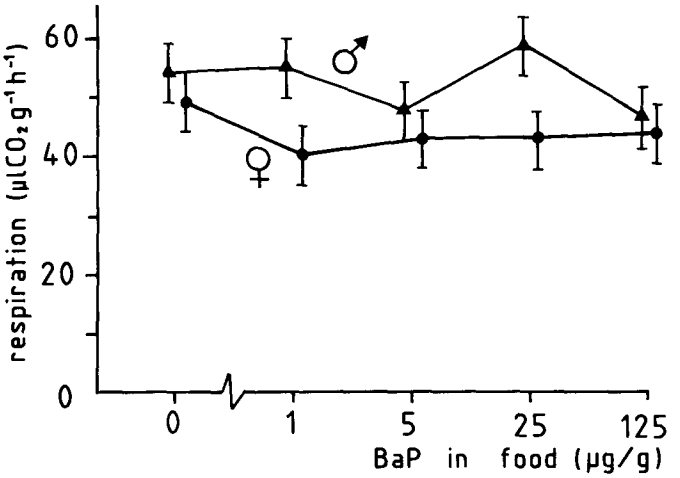


Figure 4. Metabolic rate, expressed as carbon dioxide production per hour per gram of fresh weight, by male and female *Porcellio scaber*. Each point gives a mean based on 5 replicates, where each replicate is a total of 4 measurements, at different times, on the same individual. Bars indicate standard errors for the means, calculated from the pooled error mean square.

is not expected to affect isopods seriously. However, we know of no other study in which soil invertebrates have been exposed to BaP; therefore, it cannot be decided whether the effects on *Porcellio* are representative for other soil fauna. Further research must clarify what the ecotoxicological consequences would be of chronic PAH exposure in a soil community. *Porcellio scaber* seems to be a suitable model for analysing metabolic adaptation following PAH exposure in soils.

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## REFERENCES

Calow P (1989) Physiological ecotoxicology; theory, practice and application. In: Løkke H, Tyle H, Bro-Rasmussen F (ed) Proc 1st European Conf Ecotoxicology. DIS Congress Service, Vanløse, pp 23-35

Edwards NT (1983) Polycyclic aromatic hydrocarbons (PAH) in the terrestrial environment - a review. *J Environ Qual* 12: 427-441

Griffiths BS, Wood S (1985) Microorganisms associated with the hindgut of *Oniscus asellus* (Crustacea, Isopoda). *Pedobiologia* 28: 377-381

Hassal M, Jennings JB (1975) Adaptive features of gut structure and digestive physiology in the terrestrial isopod *Philoscia muscorum* (Scopoli 1763). *Biol Bull* 149: 348-364

James MO (1989) Cytochrome P450 monooxygenases in crustaceans. *Xenobiotica* 19: 1063-1076

Jones KC, Stafford JA, Waterhouse KS, Johnston AE (1988) Long-term changes in the polynuclear aromatic hydrocarbon content of agricultural soils. In: Mathy P (ed) Air Pollution and Ecosystems. D Reidel, Dordrecht, pp 792-797

Kaplan DL, Hartenstein R (1978) Studies on monooxygenases and dioxygenases in soil macroinvertebrates and bacterial isolates from the gut of the terrestrial isopod, *Oniscus asellus* L. *Comp Biochem Physiol* 60B: 47-50

Killham K, Wainwright M (1981) Deciduous leaf litter and cellulose decomposition in soil exposed to heavy atmospheric pollution. *Environ Pollut Ser A* 26: 79-85

Neff JM (1979) Polycyclic aromatic hydrocarbons in the aquatic environment. Applied Science Publ, London

Peterson H, Luxton M (1982) A comparative analysis of soil fauna populations and their role in decomposition processes. *Oikos* 39: 287-388

Stebbing ARD (1982) Hormesis - the stimulation of growth by low levels of inhibitors. *Sci Total Environ* 22: 213-234

Thomas W, Rühling Å, Simon H (1984) Accumulation of airborne pollutants (PAH, chlorinated hydrocarbons, heavy metals) in various plant species and humus. *Environ Pollut Ser A* 42: 295-310

Timbrell JA (1982) Principles of Biochemical Toxicology. Taylor & Francis, London

Van Capelleveen HE (1990) The ecotoxicity of zinc and cadmium for the woodlouse *Porcellio scaber*. *Environ Pollut*, in press

Van Wensem J (1989) A terrestrial micro-ecosystem for measuring effects of pollutants on isopod-mediated litter decomposition. *Hydrobiologia* 188/189: 507-516

Visschers M, Verschueren K (1988) Teer- en PAK-problematiek bij onderzoek van bodemverontreinigingsgevallen met teerachtige stoffen. Soil Protection Series, Vol 72, SDU, The Hague

Wieser W., Oberhauser C (1984) Ammonia production and oxygen consumption during the life cycle of *Porcellio scaber* (Isopoda, Crustacea). *Pedobiologia* 26: 415-419

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