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Effects of Phthalate Esters on the Locomotor Activity of the Freshwater Amphipod *Gammarus pulex*

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In waters contaminated with persistent, lipophilic pollutants, a major part of the pollutants becomes adsorbed to organic particles (Al-Omran and Preston 1987). These particles eventually settle and become incorporated into sediments in oceans, lakes and streams. Benthic animals, like the freshwater amphipods, are living, foraging and burrowing at the sediment surface and will therefore be exposed to these pollutants to a greater degree than pelagic organisms.

The freshwater amphipod *Gammarus pulex*, mainly found in streams, is an important component of freshwater food webs while also contributing substantially to the decomposition of leaves (Iversen 1975). *G. pulex* has proved to be susceptible to a wide variety of toxicants and is well known as a test organism in aquatic toxicology (Stephensson 1983). Movements in *G. pulex* include passive drift (Iversen and Jessen 1977), active upstream movement (Hultin 1971), and minor changes of location in search for food and mates. The rheotactic behavior shows a diel periodicity pattern with increased activity at night. This pattern is mainly controlled by light and temperature fluctuations (Hultin 1971). Chemical stress has been shown to affect locomotor activity in fish (Kleerekoper 1976) and drift in benthic invertebrates (Crowther and Hynes 1977; Muirhead-Thomson 1978a).

Phthalates are of environmental concern owing to their large-scale annual production (about 2 million tons/year; US EPA 1980) and to their ubiquitous use as additives in the manufacture of plastics. Among the phthalates, di-2-ethylhexyl phthalate (DEHP) and dibutylphthalate (DBP) are the most commonly used compounds. Phthalates are lipophilic (log *n*-octanol/water partition coefficients between 3 and 9) with a relatively low water solubility (0.5 $mg \cdot L^{-1}$ for DEHP and 11.2 $mg \cdot L^{-1}$ for DBP; Howard et al. 1985), and show low acute toxicity to fish (Mayer and Sanders 1973) and selectively toxic to cladocerans (Streufert et al 1980). Little is known, however, about their effects on the behaviour, reproductive success or the growth of organisms (McCarthy and Whitmore 1985). In this investigation the locomotor activity of *G. pulex* was studied under phthalate stress. The aim of the study was to determine the effects of phthalates on overall locomotor activity of *G. pulex* and the impact of long term exposure on diel activity.

MATERIAL AND METHODS

The amphipods were kept in circular aquaria with continuously flowing tap water.

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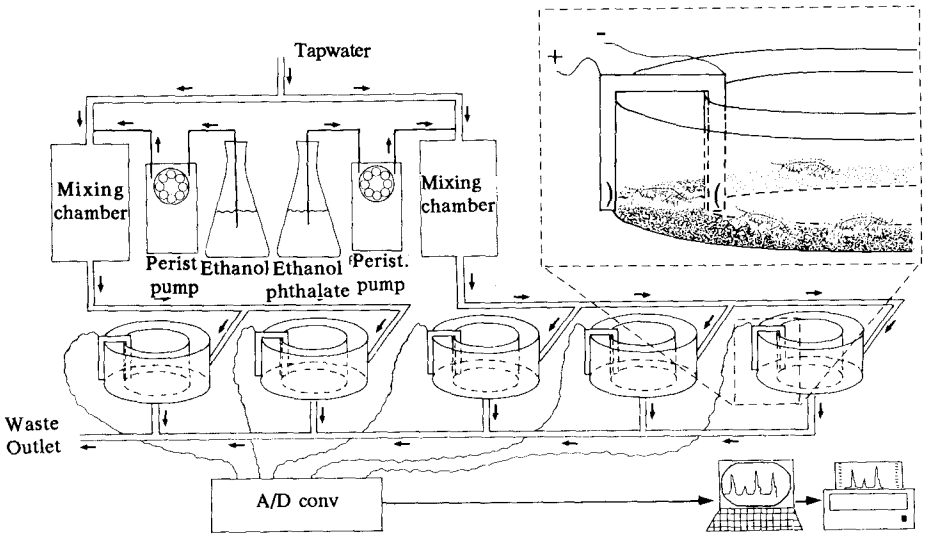

Figure 1. Schematic overview of the test system used to study the effects of phthalates on the locomotor activity of *G. pulex*. Each aquarium contained approx. 3 L of water (inner diam. 20 cm and outer diam. 30 cm) and 25 organisms. A/D conv. = analog to digital converter.

The aquaria were made of plexiglass, and polyethene was used as tubing. The experimental arrangement consisted of five aquaria, three of which were used for phthalate exposure while two were used as controls (Fig 1). The water temperature was kept at 10-12°C, which is close to natural temperature conditions (May-September). The water current was checked by adding small dust particles to the water surface and adjusting the velocity to 7.5 $cm \cdot s^{-1}$. This corresponded to a water flow of approx. 3.0 $L \cdot min^{-1}$, and is the optimum velocity for upstream movement. In a slower current the organisms will migrate with the current and in a faster current drift increases (Hughes 1970). The bottom substrate consisted of sand and decaying alder leaves and patches of pebbles. A natural light regime (light/dark, LD16:8-20:4) was simulated with a light intensity of 1500 lux at the water surface. To each aquarium 25 *G. pulex* (700 individuals$\cdot m^{-2}$) were added. They were obtained from a stream in the southern part of Sweden, which has been described previously by Hultin (1971). The animals were acclimated for five days before the activity was measured. Owing to limitations in the photocell detection system we had to use organisms > 12 mm in length. Animals in three of the aquaria were exposed to water contaminated with DBP or DEHP. Two aquaria were kept as controls. The phthalates were diluted in ethanol to make stock solutions. The solutions were continously added to the water via a capillary tube so that a concentration of 100 or 500 $\mu g \cdot L^{-1}$ was maintained (The concentrations do not exceed the water solubility of the compounds). Ethanol, which also was added to the reference aquaria, never exceeded a concentration of 0.0025% (v/v).

The activity of the animals was recorded by an IR light tray- photocell-passage (Müller and Schreiber 1967), where the numbers of lightbeam interruptions per hour were registered (Fig 1). The photocell had a column width of 5 mm and was positioned just above the bottom. The photocells were connected to an A/D-

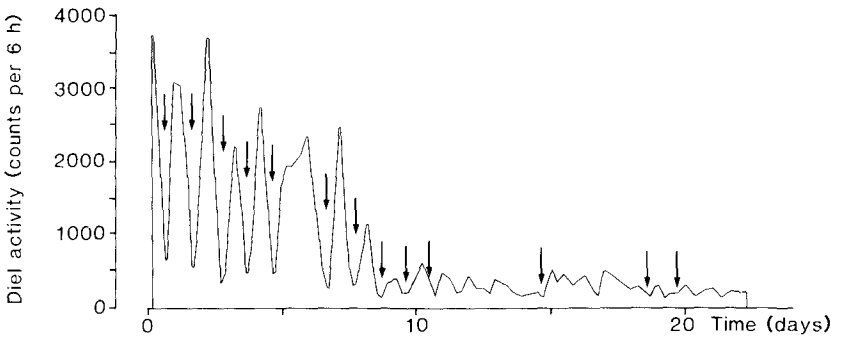

Figure 2. Diel activity patterns of non-exposed *G. pulex* (Days 1-20). The day has been divided into four periods (00-06, 06-12, 12-18 and 18-24), and activity has been calculated as the mean for each 6 hr period (Arrows indicates the period 12-18).

converter and to an ordinary 8-bit computer with a registration program written in BASIC. The computer registered the counts from each of the five photocells and the program reported the sum of counts·cell^{-1}·hour^{-1}. The effects of the phthalates on locomotory activity were studied making comparisons between exposed and non-exposed organisms. Activity was measured both on a 6-h basis, to study diel variation, and on a 24-h basis to study changes in overall locomotor activity. The activity measurements were made over a 25-day period which was divided into pre-exposure (5 days), exposure (10 d) and post-exposure (10 d) periods. The measurements were corrected for the number of organisms surviving the treatment.

After termination of the experiment, the number of organisms was determined and the amount of adsorbed and bioaccumulated phthalate estimated. The organisms (ca 0.5 g) were washed with 2 x 5 mL of ethanol in order to remove phthalates adsorbed onto the integument. To the ethanol fraction 5 mL petroleum ether and 40 mL water (with 5% NaCl to avoid emulsions) were added. The extract was cleaned up with sulphuric acid and evaporated to about 1 mL according to Thurén and Södergren (1987). After washing, the organisms were homogenized and the phthalates extracted with acetonitrile/petroleum ether (50:50 v/v). The extract was treated as above. The DBP and DEHP were quantified on a capillary gas chromatograph (GC) equipped with a flame ionization detector (FID) according to Thurén (1986, quality assurance and quality control described in article).

A Kruskal-Wallis nonparametric analysis of variance was used to determine the differences in activity between non-exposed and exposed animals (by comparing total activity sums for the 25-d period).

RESULTS AND DISCUSSION

There was no difference in mortality between phthalate-exposed and non-exposed organisms.

The activity of *G. pulex* was initially high, reaching a maximum level of around 600 passages·hr^{-1} (Fig 2). It took between 5 and 10 d for the animals to become acclimatized, after which their activity stabilized between 50 to 100 passages·hr^{-1}.

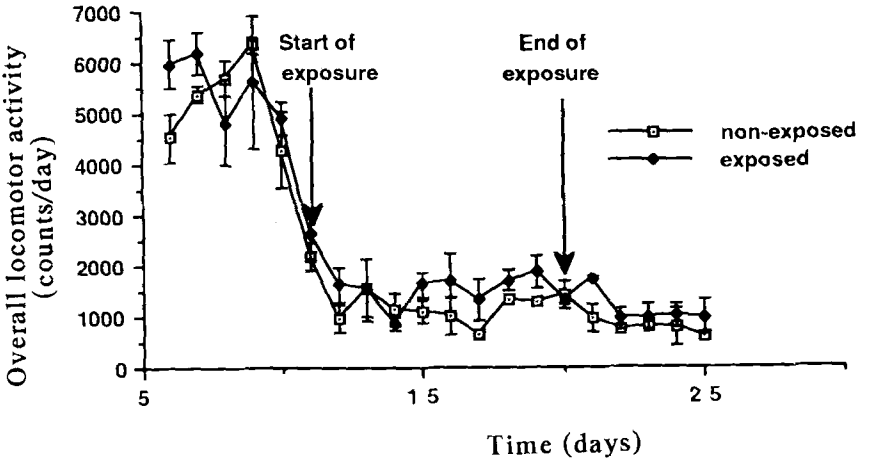

Figure 3a. Mean overall locomotor activity per day for non-exposed and exposed (100 µg DEHP·L^{-1}) *G. pulex*(n=3 for exposed animals and n=2 for non-exposed).

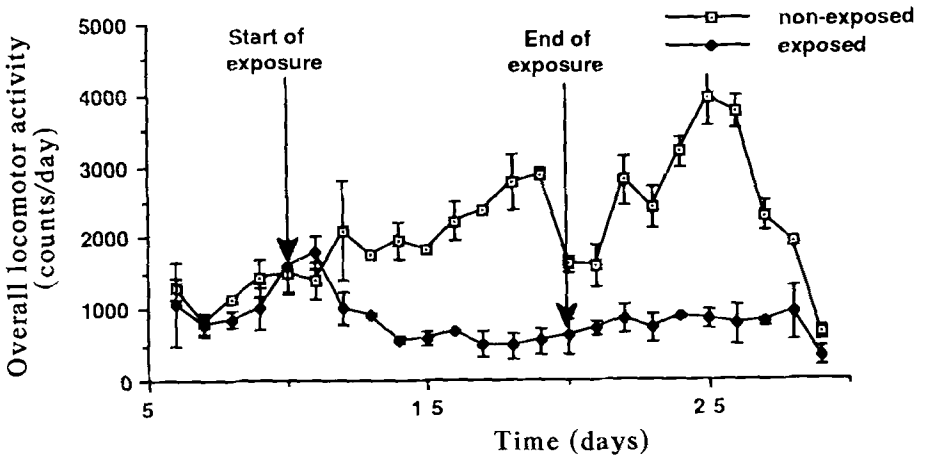

Figure 3b. Mean overall locomotor activity per day for non-exposed and exposed (500 µg DEHP·L^{-1}) *G. pulex*(n=3 for exposed animals and n=2 for non-exposed).

G. pulex was expected to show an activity peak after dawn, since the activity center (=the midpoint between onset and termination of activity) lies before dawn when darkness exceeds 8 hr/24 hr and after dawn when the dark period is of shorter duration. A number of studies have confirmed this in the field (Hultin 1971). During the pre-exposure period, there was a tendency towards higher activity

during the day. However, after 5 d, the diel activity pattern become irregular for exposed as well as non-exposed animals, and remained so for the duration of the study (Fig 2). Effects on diel activity were therefore excluded from the remainder of the study.

Exposure of *G. pulex* to DBP and DEHP at the 500 $\mu g \cdot L^{-1}$-level resulted in an increasing overall activity during the first day. This increase was probably attributable to drifting since we observed a higher degree of detachment in the initial phase of exposure. Although the system was primarily designed to measure upstream movements, a minor part of the total drift was also registered by the photocells. The *G. pulex* activity peak initially observed for each of the phthalates after exposure to 500 $\mu g \cdot L^{-1}$ may have resulted from catastrophic drift. This type of drift is considered to be an escape reaction in response to a rapid change in water quality. It has previously been used as an indicator to measure water conditions (Crowther and Hynes 1977 (de-icing salt): Muirhead-Thomson 1978b (permethrin): Wallace et al 1975 (methoxychlor)).

There was no significant difference in overall locomotor activity between non-exposed animals and animals exposed to 100 μg DEHP$\cdot L^{-1}$ ($p < 0.564$ for DEHP, Fig 3a). There was a tendency toward increased activity (approx. 35-40% greater than the controls) which even persisted throughout the post-exposure period. Exposure of *G. pulex* to DEHP (500 $\mu g \cdot L^{-1}$) decreased overall locomotor activity compared with non-exposed animals ($p<0.083$, , Fig. 3b). This effect continued throughout the post-exposure period ($p<0.083$).

The overall locomotor activity of the animals was unaffected by exposure to 100 μg DBP$\cdot L^{-1}$ ($p < 0.248$ for DBP, Fig 4a), with a tendency towards a decreased activity (by approx. 25-30%) compared with the non-exposed animals. After termination of the exposure activity returned to pre-exposure levels. When exposed to DBP (500 $\mu g \cdot L^{-1}$) the overall locomotor activity of the organism decreased compared with the non-exposed animals ($P<0.083$, Fig. 4b). This effect also persisted during the post-exposure period ($P<0.083$) .

The overall locomotor activity of the animals decreased when exposed to DEHP at 500 $\mu g \cdot L^{-1}$ but not at 100 $\mu g \cdot L^{-1}$. The effect persisted throughout the post-exposure period. Decapods and *G. pulex* have been found to have organs sensitive to water velocity (Lockwood 1968), and "olfactory" organs whose functioning might be disturbed by various pollutants (Muirhead-Thomson 1978a: Muirhead-Thomson 1978b: Wallace et al. 1975). Thus the large amount of DEHP adsorbed to and accumulated by the organisms could have caused these organs to malfunction thereby reducing mobility and affecting upstream movement. The decreasing activity following DEHP exposure could therefore have been the result of mechanical and/or physiological effects.
Exposure to 500 μg DBP$\cdot L^{-1}$ led to a decrease in total activity ($p < 0.083$) which persisted during the post-exposure period ($p<0.083$).Exposure to 100 $\mu g \cdot L^{-1}$ only resulted in a non-significant decrease in activity ($p<0.248$) with a return to pre-exposure levels after withdrawal of the phthalate.

G. pulex accumulated between 87 and 1998 $\mu g g^{-1}$ (fresh weight,f.w.) of DEHP depending on the degree of exposure . The amounts adsorbed to the animal's integument ranged from 62 to 2293 μg DEHP$\cdot g^{-1}$ (f.w.) (Table 1). After exposure to DBP the animals contained 14 to 32 $\mu g \cdot g^{-1}$ (f.w.) whereas the adsorbed amount ranged from 4.2 to 4.5 $\mu g \cdot g^{-1}$ (f.w.) (Table 1). The amount of DBP adsorbed to the animals was similar for both levels of exposure. However, compared with DEHP

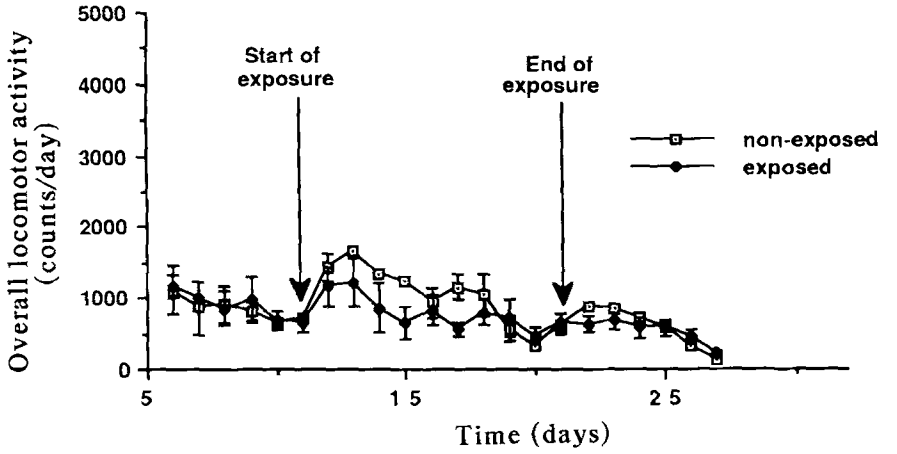

Figure 4a. Mean overall locomotor activity per day for non-exposed and exposed (100 µg DBP·L^{-1}) *G. pulex*(n=3 for exposed animals and n=2 for non-exposed).

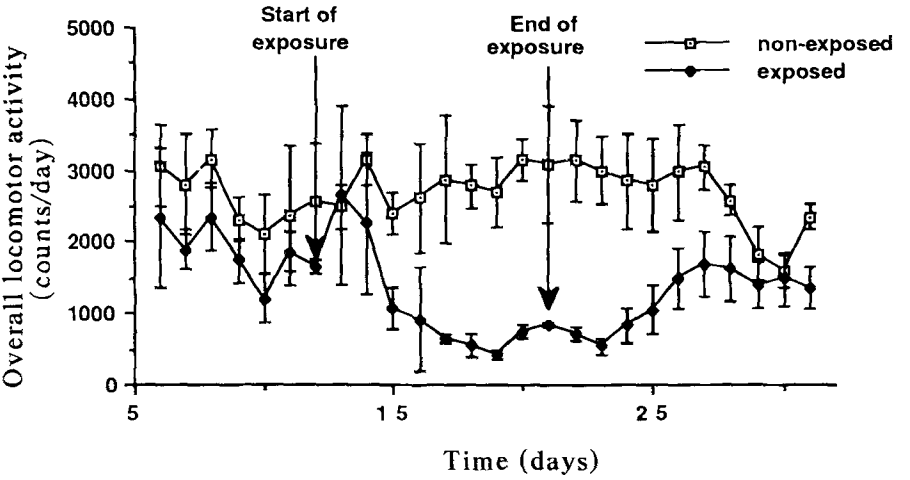

Figure 4b. Mean overall locomotor activity per day for non-exposed and exposed (500 µg DBP·L^{-1}) *G. pulex*(n=3 for exposed animals and n=2 for non-exposed).

the amount was approximately 500 times lower, indicating that decreased activity following DBP exposure is not due to an adsorptive effect on the animals "organs". The amount of DBP accumulated by the animals increased in relation to the exposure level. This suggests that DBP may cause a physiological disturbance resulting in a decreased activity (Fig. 4b) manifested at conc. >100 µg DBP·L^{-1}. The amount of phthalates adsorbed to and accumulated by the animals was higher for DEHP than DBP. This difference is probably attributable to the greater lipophilicity of DEHP (Howard et al. 1985), its higher adsorptive capacity to surfaces and particles (Al-Omraan and Preston 1987), and its greater persistency

Table 1. Distribution of phthalates in *G. pulex* exposed to DEHP or DBP for 10 days (n=3, numbers within brackets show the standard deviation).

	DEHP		DBP	
Concentration in water ($\mu g \cdot L^{-1}$)	100	500	100	500
Accumulated ($\mu g \cdot g^{-1}$)	87 (46)	1998 (747)	14 (1)	32 (9)
Adsorbed ($\mu g\ g^{-1}$)	62 (10)	2293 (801)	4.5 (2.7)	4.2 (1.4)
Adsorbed ($\mu g\ org^{-1}$)	1.8 (0.4)	48 (12)	0.10 (0.05)	0.10 (0.03)

compared with DBP.
Locomotor activity disturbances may have a negative impact on populations of freshwater amphipods. Amphipods living in streams have evolved morphological as well as behavioral adaptations to avoid the "washing-out" effect. One such adaptation is active upstream movement, which compensates for downstream drift. A pollutant-induced decrease in upstream movement could lead to a wash-out of certain organisms in contaminated streams, which might in turn alter the structure of the macrobenthic community. In natural aquatic environments DEHP and DBP would probably not affect the locomotor activity or upstream movements of *G. pulex*, since such effects were only observed at concentrations of 500 $\mu g \cdot L^{-1}$. In polluted areas the levels in water are about 0-5 $\mu g \cdot L^{-1}$ for DEHP and 0-3 $\mu g \cdot L^{-1}$ for DBP (Thurén 1986: Morita et al. 1974: Murray et al. 1981).
However, it has been shown that in many polluted areas most of the phthalate material becomes incorporated into the sediment (Thurén 1986: Malisch et al. 1981: Schwartz et al. 1979). Since phthalates are partitioned onto sediment from water (Sullivan et al. 1982), benthic invertebrates living at the sediment-water interface will be more heavily exposed than pelagic animals. Thus, the level of exposure to phthalates from contaminated sediments might be high enough to induce sublethal effects on macrobenthos, such as overall disturbances in locomotor activity.

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