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## Life cycle responses of the midge *Chironomus riparius* to polycyclic aromatic compound exposure

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*Emergence of Chironomus riparius is a sensitive endpoint to detect life cycle effects of PACs.*

### Abstract

During acute exposure, polycyclic aromatic compounds (PACs) act mainly by narcosis, but during chronic exposure the same compounds may exert sublethal life cycle effects. The aim of this study was therefore to evaluate the chronic effects of sediment spiked PACs on the emergence of the midge *Chironomus riparius*. Three isomer pairs were selected, and 28-day LC<sub>50</sub> values and 50% emergence times (EMt<sub>50</sub>) were determined. Concentration–response relationships were observed for phenanthrene, acridine, phenanthridine and acridone. Anthracene and phenanthridone had no effect on total emergence, but did cause a delay in emergence. Calculated porewater LC<sub>50</sub> values correlated well with logK<sub>ow</sub> values, suggesting narcosis as mode of action. In contrast, effect concentrations for delay in emergence (EMt<sub>50</sub>) deviated from narcosis, suggesting a specific mode of action during chronic exposure. It is concluded that emergence is a powerful endpoint to detect life cycle effects of PACs on a key sediment inhabiting invertebrate.

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**Keywords:** *Chironomus riparius*; Polycyclic aromatic compounds; Emergence

### 1. Introduction

Polycyclic aromatic compounds (PACs) are ubiquitous contaminants, present in the environment mainly as a result of anthropogenic activities (industry, oil drilling and vehicle emissions). Smaller amounts of PACs are generated in natural processes, like volcanism and forest fires (Neilson, 1998). Because of their hydrophobicity, PACs tend to accumulate in sediments and soils, which act not only as a sink, but also as

a source of PACs for benthic organisms (Wilcke, 2000; Van Metre and Mahler, 2005). In sediment cores from 38 urban and reference lakes across the United States, total sediment ΣPAH concentrations (ΣPAH = sum of 13 homocyclic PACs used for the consensus-based sediment quality guidelines in the USA) ranged from 0.5 to 10 mg/kg (Van Metre and Mahler, 2005).

Populations of benthic invertebrates are exposed to a variety of PACs in the sediment, but little is known about their chronic effects, since research has mainly focused on acute exposure (Bleeker et al., 1998; Neilson, 1998; Wiegman et al., 2001). During such acute high-dose exposures PACs act mainly by narcosis (Neilson, 1998), but during chronic exposure the same compounds may exert mutagenic, teratogenic and carcinogenic effects and cause developmental disturbances. This

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has been documented for homocyclic PACs and azaarenes (Cronin and Bickham, 1998; Bleeker et al., 1999a,b; Mitchelmore and Hyatt, 2004).

Azaarenes are a group of heterocyclic PACs with an in-ring nitrogen substitution, comprising up to 10% of the total PAC concentration in contaminated sediments (Neilson, 1998). However, azaarene concentrations are not monitored in sediments and few ecotoxicological data are available for this group of heterocyclic compounds. Like homocyclic PACs, azaarenes are actively biotransformed by invertebrates (Guerrero et al., 2002; Stroomberg et al., 2004) and biodegraded by bacteria (Johnsen et al., 2005; Sartoros et al., 2005), resulting in a vast number of heterocyclic transformation products. Azaarenes and their transformation products, more polar than their homocyclic analogues, can interact specifically with cell membranes and DNA, and this interaction may influence their toxicity (De Voogt et al., 1999). Since isomer specific toxicity of PACs has been established in several studies (Kraak et al., 1997; Bleeker et al., 1999a; De Voogt et al., 1999; Wiegman et al., 2001; Droge et al., 2006), three isomer pairs were selected: two three-ringed homocyclic compounds, two three-ringed azaarenes and the two main transformation products of the two azaarenes. The aim of this study was to determine if the closely related homo- and heterocyclic compounds affected emergence of the midge *Chironomus riparius* during chronic exposure to PAC spiked sediments. Emergence is a crucial life cycle parameter, which was proven to be affected by sediment-associated toxicants (Ristola et al., 1999b; De Haas et al., 2002).

## 2. Materials and methods

### 2.1. Test organism

The non-biting midge *Chironomus riparius* (Diptera, Chironomidae) is widely used in ecotoxicity tests (Ristola et al., 1999a; De Haas et al., 2004). Larvae of this insect burrow tubes in the sediment and feed on detritus particles surrounding the tube. Its life cycle consists of four larval stages, a pupa stage and a non-feeding adult stage. Under favourable conditions the life cycle is completed in 3–4 weeks (Gower and Buckland, 1978). Three of the four larval stages are in close contact with the sediment, which makes *C. riparius* a suitable organism for chronic sediment toxicity testing. In addition, a standard OECD guideline for sediment toxicity testing with this organism has recently become available (OECD guideline 218) (OECD, 2004).

### 2.2. Test compounds

Six test compounds were chosen: two three-ringed homocyclic compounds, anthracene (99% purity) and phenanthrene (98% purity); two of their azaarene analogues, acridine (97% purity) and phenanthridine (99% purity), and two Phase I azaarene transformation products, acridone (99% purity) and phenanthridone (99% purity). Several properties of the selected compounds are listed in Table 1. All compounds were provided by Sigma-Aldrich Chemie BV (Zwijndrecht, the Netherlands).

### 2.3. Sediment spiking

Drontermeer sediment, a Dutch reference sediment (12–14% Organic Matter determined by loss on ignition, 15.5% clay and all individual PAH concentrations below 0.01 mg/kg dry weight; d.w.), was used for the toxicity tests. The sediment was provided by AquaSense/Grontmij (Amsterdam, the

Netherlands). After sampling, it was homogenised and frozen to eliminate indigenous fauna, and kept at  $-20^{\circ}\text{C}$  until use. Three days before the spiking procedure was started the sediment was thawed at  $4^{\circ}\text{C}$ .

The sediment was spiked to obtain the following nominal PAC concentrations: 28, 84, 168 and 337  $\mu\text{mol/kg}$  d.w. for anthracene (5–60 mg/kg d.w.); 112, 449, 898, 1795 and 3591  $\mu\text{mol/kg}$  d.w. for phenanthrene (20–640 mg/kg d.w.); 139, 279, 556, 1116 and 2232  $\mu\text{mol/kg}$  d.w. for acridine and phenanthridine (25–400 mg/kg d.w.); 154, 307, 640, 1281 and 2561  $\mu\text{mol/kg}$  d.w. for acridone (30–500 mg/kg d.w.); and 154, 307, 640 and 1281  $\mu\text{mol/kg}$  d.w. for phenanthridone (30–250 mg/kg d.w.). The spiking was done in the dark to prevent photodegradation of the compounds. Acetone was used as carrier solvent, and for each PAC concentration series equal volumes of acetone were added to all treatments. Controls and solvent controls were included as well. Solvent control emergence values were used for statistical analysis of the results, because they reflected better the experimental conditions of the spiked sediment. There were seven replicates per concentration. Two replicates were sacrificed to perform PAC measurements at the beginning and half-way through the experiment, leaving five replicates for the toxicity test.

Two different methods were used to spike the sediment with the compounds, according to their solubility in acetone. The homocyclic compounds and the azaarenes were added in small volumes of acetone (20 ml for anthracene and phenanthrene; 50 ml for acridine and phenanthridine) to a mixture of 2100 ml of Dutch Standard Water (deionised water with 200 mg/L  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 180 mg/L  $\text{MgSO}_4 \cdot \text{H}_2\text{O}$ , 100 mg/L  $\text{NaHCO}_3$  and 20 mg/L  $\text{KHCO}_3$ ; pH  $\approx 8.2$ ) and 630 g of wet sediment in 3-L glass bottles. A 20:1 w/w mixture of Trouvit<sup>®</sup> (Trouw, Fontaine-les-Vervins, France) and TetraPhyll<sup>®</sup> (Tetra Werke, Melle, Germany), diluted in 50 ml of deionised water, was used as additional food source in order to minimise food shortage related effects on the development of the midge larvae (De Haas et al., 2002). Food quantities (0.15 mg/larvae/day) were added to the water–sediment mixture according to the OECD 218 guideline (OECD, 2004). The glass bottles filled with the water–sediment–food mixture were placed on a roller bank (30 rpm) for 24 h to homogenise. Next, the homogenised water–sediment–food mixture was divided over seven replicate 400-ml glass beakers and allowed to settle for 7 days, in order to obtain a stable sediment layer and to equilibrate the compound with the sediment, while the acetone evaporated.

For the two transformation products, less soluble in acetone, a pre-spiking procedure was followed. The spiking solution (260 ml acetone for acridone and 300 ml for phenanthridone) was added to one-quarter of the total amount of sediment, and the acetone was allowed to evaporate overnight in a fume cupboard. The day after, the remaining sediment was added and the same mixing and equilibration procedure as for the other compounds was followed.

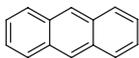
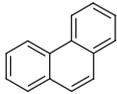
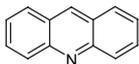
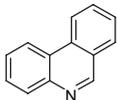
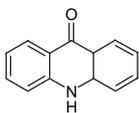
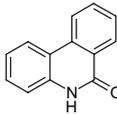
### 2.4. Toxicity tests

Twenty-eight-day toxicity tests were performed according to the OECD 218 guideline (OECD, 2004), with slight modifications. The tests were conducted in an incubator at a constant temperature of  $20 \pm 1^{\circ}\text{C}$ , and mercury lamps (Powertone HPI-T Plus, Philips, the Netherlands) were used to provide a light regime of 16 h of light (light intensity of approximately 50  $\mu\text{mol}$  quanta/ $\text{m}^2/\text{s}$ ) and 8 h of darkness. UV filters were used to suppress UV-B radiation and minimise photodegradation of the tested compounds. The glass beakers were covered with plastic foil and constantly aerated to keep the oxygen concentration stable during the test. Deionised water was added when necessary to compensate for evaporation losses in the beakers.

Ten freshly laid *C. riparius* egg ropes obtained from our laboratory culture were kept in a Petri dish filled with Dutch Standard Water for 72 h, and the hatched first instar larvae, less than 24 h old, were used for the toxicity tests. At the start of the experiment, ten first-instar *C. riparius* larvae were carefully introduced in each of the test beakers using a blunt glass Pasteur pipette. The larvae were allowed to settle on the sediment for 4 h before aeration of the vessels was restarted. Half-way through the experiment, the larvae were fed a second time to provide them with fresh food. Emergence was recorded daily starting at day 14, when the first adult midges started to emerge, until day

Table 1

Polycyclic aromatic compounds (PACs) selected for this study and some of their properties: CAS-number, molecular weight (MW),  $\log K_{ow}$ ,  $\log K_{oc}$ , water solubility (Sw)

Compound	Structure	CAS number	MW	$\log K_{ow}$	$\log K_{oc}$	Sw ( $\mu\text{M}$ )
<i>Homocyclic PACs</i>						
Anthracene		120-12-7	178.23	4.53 <sup>a</sup>	4.3 <sup>f</sup>	0.37 <sup>b</sup>
Phenanthrene		85-01-8	178.23	4.48 <sup>a</sup>	4.2 <sup>f</sup>	7.20 <sup>b</sup>
<i>Heterocyclic PACs (azaarenes)</i>						
Acridine		260-94-6	179.22	3.27 <sup>a</sup>	4.1 <sup>f</sup>	260 <sup>b</sup>
Phenanthridine		229-87-8	179.22	3.44 <sup>a</sup>	3.8 <sup>f</sup>	25.7 <sup>d</sup>
<i>Azaarene transformation products</i>						
Acridone		578-95-0	195.22	2.95 <sup>c</sup>	3.9 <sup>f</sup>	670 <sup>d</sup>
Phenanthridone		1015-89-0	195.22	2.70 <sup>c</sup>	4.3 <sup>f</sup>	1428 <sup>d</sup>

<sup>a</sup> Experimental values from Helweg et al., 1997.

<sup>b</sup> Values from Pearlman et al., 1984.

<sup>c</sup> Experimental values from Thomsen, 2002.

<sup>d</sup> Calculated with WSKOW, version 1.40.

<sup>e</sup> Values from Bleeker et al., 1999a.

<sup>f</sup> Experimental values from Jonassen, 2003; Jonassen et al., 2003.

28, when the test was terminated. Emerged midges were removed during the daily inspections.

pH and oxygen concentration in the overlying water were measured at the beginning and the end of the experiment. pH ranged from 6.0 to 8.5 and oxygen concentration was always above 60% air saturation, fulfilling the requirement of the OECD 218 test guideline (OECD, 2004).

### 2.5. PAC measurements

Sediment was sampled at the beginning, at the half-way point and at the end of the experiment and stored frozen at  $-20\text{ }^{\circ}\text{C}$  until analysis. Ten grams of centrifuged sediment (water content approximately 10%) were mixed with equal amounts of anhydrous  $\text{NaSO}_4$  (Merck, Darmstadt, Germany), and Soxhlet extracted for 5 h in cellulose extraction thimbles. Hexane (Sigma-Aldrich Chemie) was used as extraction solvent for the homocyclic compounds and the azaarenes, and acetonitrile (Biosolve BV, Valkenswaard, the Netherlands) was used for the transformation products. The hexane-extracted samples were transferred into acetonitrile by blowing off the hexane using a gentle stream of nitrogen, and measured using high-performance liquid chromatography

(HPLC). The HPLC system consisted of a  $5\text{ }\mu\text{m}$  Valco LiChrosorb<sup>®</sup>  $250 \times 4.6\text{ mm}$  RP-18 analytical column (Varian, the Netherlands) with a  $5\text{ }\mu\text{m}$  LiChrospher<sup>®</sup> 100 RP-18 guard column (Merck, Darmstadt, Germany), connected to a Spectroflow 980 fluorescence detector and an Applied Biosystems 785A UV-Diode Array detector.

Recovery checks were performed for each of the compounds to validate the Soxhlet extraction procedure. Known amounts of spiking solution in the same concentration range as tested in the experiments were added to clean sediment, and recovery after the extraction procedure was determined. Actual concentrations were corrected for recovery.

### 2.6. Statistical analysis

The average exposure concentration of the test compound in the sediment at which 50% reduction in number of emerged midges occurred compared to the solvent control ( $\text{LC}_{50}$ ) was calculated according to Haanstra et al. (1985). The following logistic curve was fitted through the concentration–response data using the average of the measured concentrations at the beginning,

Table 2  
 LC<sub>50</sub> values (μmol PAC/kg d.w.) for the effect of the tested PACs on the survival (total emergence) of the midge *Chironomus riparius*, and PAC concentrations in the sediment significantly delaying emergence compared to the control (μmol PAC/kg d.w.)

	LC <sub>50</sub> (μmol/kg d.w.) with 95% C.I.	Emergence sign. delayed compared to control ( $p < 0.05$ ) (μmol/kg d.w.)	Porewater LC <sub>50</sub> (μM)	Porewater EMt <sub>50</sub> value diff. from control (μM)
<i>Homocyclic compounds</i>				
Anthracene	>162	81	–	0.07
Phenanthrene	597–841	597–841	0.71	–
<i>Azaarenes</i>				
Acridine	926 (680–1173)	753	2.04	1.66
Phenanthridine	705 (595–815)	367	0.78	0.40
<i>Transformation products</i>				
Acridone	2708 (1909–2773)	635	5.20	0.74
Phenanthridone	>2145	506	–	0.64

half-way through and at the end of the experiment as the average exposure concentration:

$$Y = c / (1 + e^{b(\log(X) - \log(a))}) \quad (1)$$

where  $Y$  is the effect parameter (emergence),  $X$  is the average exposure concentration (μmol/kg sediment dry weight),  $a$  is the LC<sub>50</sub>,  $b$  is the slope of the logistic curve and  $c$  is the average emergence in the solvent control.

For each test concentration, the day at which 50% emergence occurred (EMt<sub>50</sub>) was calculated by fitting a logistic curve through the cumulative emergence plot using the data of the five replicates, also according to Haanstra et al. (1985). This was done for males and females separately because of the differences in emergence time between both sexes. The sex ratio was close to one. In eq. (1),  $X$  were the days were emergence was recorded,  $a$  was the EMt<sub>50</sub> and  $c$  was the average emergence per jar at the end of the experiment. For the acridone data the logistic fit was not possible, and a Weibull curve was used instead. To determine at which PAC concentrations emergence was significantly delayed, EMt<sub>50</sub> values for the different test concentrations were compared to the solvent control value using generalised likelihood ratio tests (Van Gestel and Hensbergen, 1997). For phenanthridone, the comparison was done using the lowest concentration. Statistical analysis was performed using SPSS® 11.0 for Windows and SYSTAT® 5.2.

### 3. Results

#### 3.1. PAC measurements

Actual concentrations of the test compounds in the sediment were corrected for extraction losses using the results of the recovery check experiment, in which recovery ranged from 61% for acridine to 88% for acridone. For all test compounds, actual concentrations at the beginning of the experiment after correcting for recovery ranged from 70% to 91% of the nominal value. The concentrations of anthracene, phenanthrene, acridine and phenanthridine in the test sediment decreased by 25%, 42%, 18% and 55% respectively during the experiment, probably due to microbial degradation (Van Herwijnen et al., 2003; Johnsen et al., 2005). Actual concentrations of acridone and phenanthridone in the sediment remained nearly constant during the experiment, indicating that these transformation products were not further degraded. The averages of the measured concentrations at the three sampling points during the experiment were used as actual exposure concentrations for the data analysis.

#### 3.2. Survival (total emergence)

Emergence of *C. riparius* in the acetone controls was at least 80%, in accordance with the OECD 218 guideline (OECD, 2004). Clear concentration–response relationships were observed for phenanthrene, acridine, phenanthridine and acridone. Anthracene, due to its low solubility, had only a slight effect on total emergence at the highest possible test concentration, and phenanthridone had no effect on emergence. At the end of the test, the sediment was sieved to check for remaining larvae in the sediment, and no larvae were found. Since midge larvae either emerged from the sediment or died, survival equalled total emergence, and emergence data ( $t = 28$  days) were used to calculate actual LC<sub>50</sub> values with 95% confidence intervals (C.I.) (Table 2). The average values of the PAC concentrations measured in the sediment during the experiment were used to perform the LC<sub>50</sub> calculations. Phenanthrene inhibited emergence completely at the highest tested concentration (841 μmol/kg sediment d.w.), while the next lower concentration in the range, 597 μmol/kg sediment d.w., allowed emergence of all larvae. For this reason, no logistic model fit could be used, and these two concentrations are given to encompass the LC<sub>50</sub>. The LC<sub>50</sub> values of the two azaarene isomers were similar, namely 926 μmol/kg sediment d.w. (95% C.I. 680–1173 μmol/kg sediment d.w.) for acridine and 705 μmol/kg sediment d.w. (95% C.I. 595–815 μmol/kg sediment d.w.) for phenanthridine. From the two tested transformation products, only acridone hampered emergence of the midge larvae, and the LC<sub>50</sub> value for this compound was 2708 μmol/kg sediment d.w. (95% C.I. 1909–2773 μmol/kg sediment d.w.).

#### 3.3. Emergence time

In this study, an increasing delay in emergence with increasing PAC concentrations in the sediment was apparent for male midges, but not for females. Male EMt<sub>50</sub> values increased gradually with increasing PAC concentrations in the sediment for all tested compounds except phenanthrene (Fig. 1), while female EMt<sub>50</sub> values did not. In order to

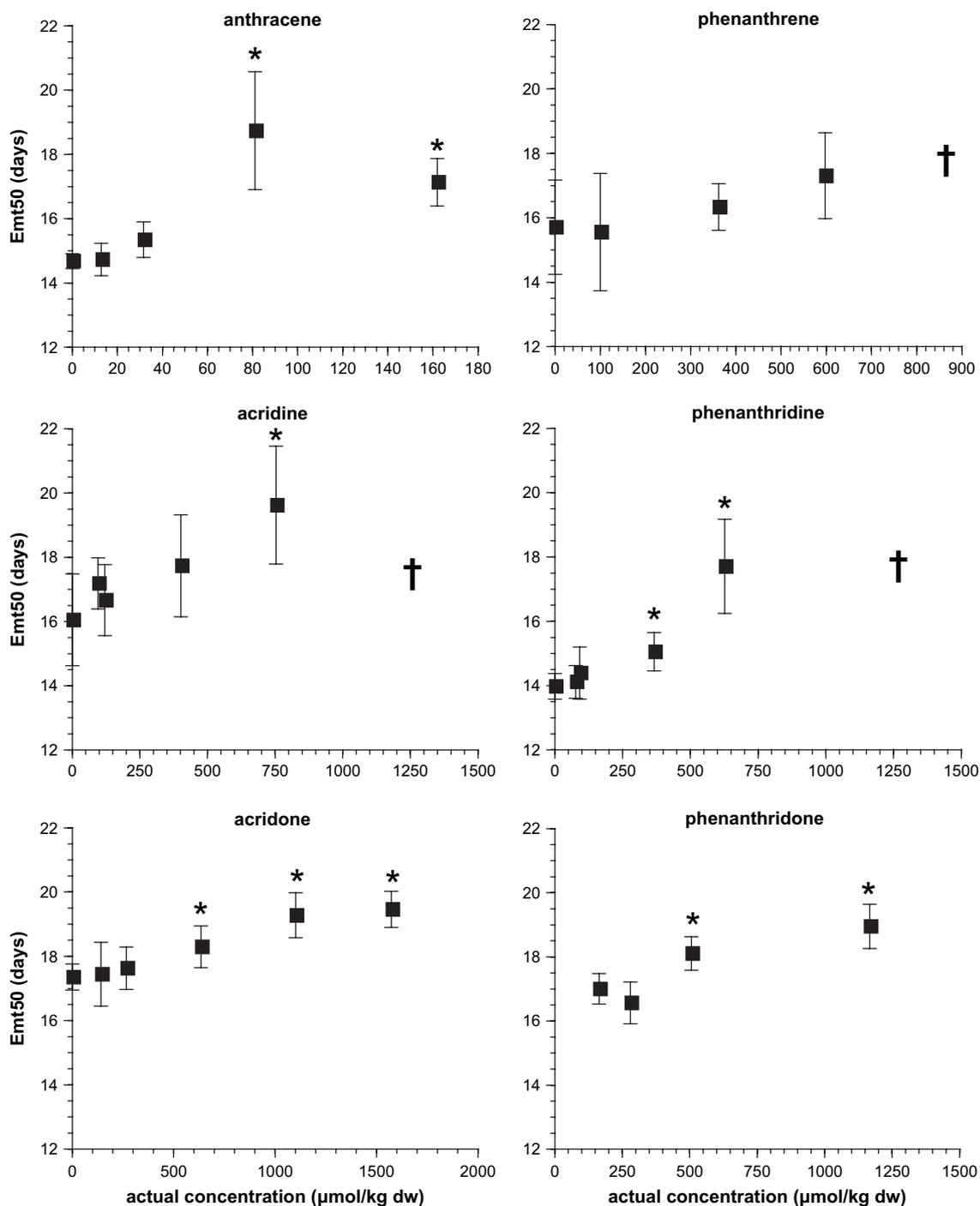


Fig. 1. Effect of six single polycyclic aromatic compounds (PACs) on the emergence time of *C. riparius* males from spiked sediments. X-axis: actual concentrations of the compounds measured in the sediment. Y-axis: 50% male emergence time (EMT<sub>50</sub>, days, average plus standard deviations). †Concentrations with no emerging midges. \*EMT<sub>50</sub> value significantly different from control value ( $p < 0.05$ ).

determine the lowest concentration at which emergence was significantly delayed, EMT<sub>50</sub> values of exposed male midges were compared to the corresponding acetone control EMT<sub>50</sub> values using likelihood ratio tests. For phenanthridone, likelihood ratio tests were performed using the lowest test concentration (164 µmol/kg sediment d.w.) instead of the acetone control, because the EMT<sub>50</sub> value for the control was significantly higher than the control values of the other experiments,

and also significantly higher than the three other phenanthridone concentrations tested.

Anthracene had the most pronounced effect, causing a significant delay in emergence at 81 µmol/kg sediment d.w. Both azaarenes delayed emergence at the highest tested concentrations, but the difference from the control was significant at a lower concentration for phenanthridine (367 µmol/kg sediment d.w.) than for acridine (753 µmol/kg sediment d.w.).

Also the two transformation products, acridone and phenanthridone, caused clear sublethal effects, with  $EMt_{50}$  values significantly different from the control at 635 and 506  $\mu\text{mol/kg}$  sediment d.w., respectively. The data for anthracene and phenanthridone showed the high sensitivity of the  $EMt_{50}$  parameter. Due to its low solubility and subsequent low concentrations in the sediment, no  $LC_{50}$  value could be obtained for anthracene, while it delayed emergence at the lowest concentration of all tested compounds. Comparing the two transformation products, it was observed that phenanthridone delayed emergence at a comparable concentration as its isomer acridone, while acridone was the only transformation product that caused substantial mortality of the larvae.

## 4. Discussion

### 4.1. Emergence of males and females

Studies using emergence of chironomids as an endpoint have been performed before (Taylor et al., 1993; Sibley et al., 2001; De Haas et al., 2002; Pery et al., 2002; Leslie et al., 2004), and emergence was usually more sensitive than growth (Ristola et al., 1999b; Smith and Kokkinn, 2004). This is probably due to the fact that effects on emergence involve three moultings and the very complex pupation process. Not only delay has been reported, also accelerated emergence has been found for 2,4,5-trichlorophenol and several endocrine-disrupting chemicals (Ristola et al., 1999b; Watts et al., 2001), probably due to disturbance of the moulting and pupation processes.

In our study, clear differences between effects on emergence of males and females were found. For the six tested compounds, male emergence was significantly delayed, while no significant difference from the control was found for female emergence. Differences in sensitivity of males and females have been reported in studies on different types of chemicals, including metals, pesticides, PAHs, surfactants and endocrine-disrupting chemicals (Kahl et al., 1997; Ristola et al., 1999b; Watts et al., 2001; Pery et al., 2003; Sanchez et al., 2005). So far, however, no convincing explanation is available for the observed sex related differences in toxic effects. Generally, female emergence was found to be more sensitive than male emergence (Liber et al., 1996; Ristola et al., 1999b; Sanchez et al., 2005), and it has been hypothesised that this is caused by the longer residence time in the sediment of female larvae compared to male larvae. The higher lipid content of females, needed for adult egg production, could also lead to a higher accumulation of lipophilic compounds (Sibley et al., 1997; Pery et al., 2002). However, higher lipid content could also result in a higher toxicant accumulation in the females' body before a toxic effect would appear. The significantly delayed male emergence in our study supports this conjecture. In addition, larger delay in emergence for males was previously observed for *C. riparius* exposed to phenanthridine, one of the PACs tested in our study (Bleeker et al., 1999b).

### 4.2. Narcosis versus specific effects

Polycyclic aromatic compounds, like all organic compounds, produce narcosis to some extent. Several studies have shown that narcosis is strongly related to the lipophilicity of the compound, commonly expressed as the n-octanol-water partition coefficient ( $K_{ow}$ ) (De Voogt et al., 1988). Consequently, deviations from the relationship between effect concentrations and  $K_{ow}$  values may indicate a different mode of action (Bearden and Schultz, 1998; Escher et al., 2005). This was clearly demonstrated previously for soil invertebrates chronically exposed to several PACs (Sverdrup et al., 2001; Droge et al., 2006), and therefore in the present study we followed the same approach. Since for the tested compounds, having a  $\log K_{ow}$  value  $<5$ , porewater exposure of the midge larvae was expected (Belfroid et al., 1996), chronic  $LC_{50}$  values ( $\mu\text{M}$ ) were calculated from the chronic sediment  $LC_{50}$  values ( $\mu\text{mol/kg}$  sediment d.w., Table 2), using a set of empirically determined organic carbon-water partition coefficients ( $K_{oc}$ ) from (Jonassen, 2003; Jonassen et al., 2003) and correcting for the organic carbon content of the sediment. A similar calculation was made using the exposure concentrations at which the  $EMt_{50}$  value differed significantly from the control. For phenanthrene, the porewater effect concentration was calculated using the geometric mean of the concentration interval in which effects on emergence appeared (Table 2).

The calculated porewater  $LC_{50}$  and  $EMt_{50}$  values were plotted against the  $\log K_{ow}$  values of the tested compounds, and

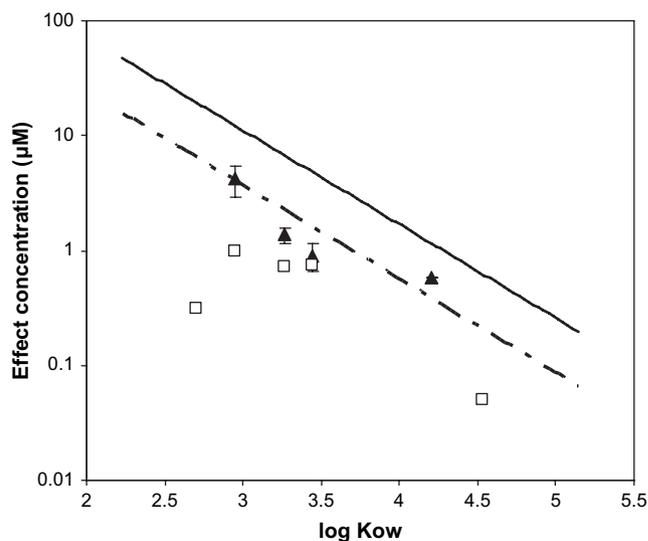


Fig. 2. Calculated 50% lethality porewater concentrations ( $LC_{50}$ , with 95% C.I. bars) after 28 days of exposure and male  $EMt_{50}$  porewater concentrations (lowest concentrations at which male  $EMt_{50}$  significantly differed from control  $EMt_{50}$ ,  $p < 0.05$ ) for the effect of the tested polycyclic aromatic compounds (PACs) on the emergence of *Chironomus riparius* from spiked sediments. X-axis:  $\log K_{ow}$  values of the tested compounds. Y-axis: pore-water  $LC_{50}$  values ( $\blacktriangle$ ,  $\mu\text{M}$ ) and  $EMt_{50}$  values ( $\square$ ,  $\mu\text{M}$ ). Solid line: linear  $\log LC_{50}$ - $\log K_{ow}$  relationship for *C. riparius* exposed for 96 h to similar PACs ( $y = -0.8162x + 3.4936$ ,  $r^2 = 0.986$  (Bleeker et al., 2002)). Dashed line: ratio  $LC_{50} = (EMt_{50})/3$ , lower limit for narcosis.

compared to the  $LC_{50}$ – $\log K_{ow}$  relationship obtained by Bleeker et al. (2002) for exposure of *C. riparius* to PACs (Fig. 2). In those acute high-dose experiments, narcosis was the main mode of action. The lower limit for narcosis was estimated by dividing the acute  $LC_{50}$  values from the  $LC_{50}$ – $\log K_{ow}$  relationship by a factor of 3, because in previous studies on soil invertebrates a  $LC_{50}/EC_{50}$  ratio larger than 3 suggested sublethal effects of the tested compounds on reproduction, deviating from narcosis (Droge et al., 2006). Hence, we assumed that specific effects on emergence occurred if the effect concentrations obtained in the present study were below the calculated lower limit for narcosis. The porewater  $LC_{50}$  values for the homo- and heterocyclic compounds tested in our study correlated well with the  $LC_{50}$ – $\log K_{ow}$  relationship (Fig. 2). It can therefore be concluded that the tested homo- and heterocyclic compounds did not differ in their effect on total emergence, and narcosis was probably the main mode of action. The  $EMt_{50}$  values for the two tested azaarenes, acridine and phenanthridine, were close to the  $LC_{50}$  values (Fig. 2). In contrast,  $EMt_{50}$  effect concentrations for anthracene and the two transformation products were lower than the  $LC_{50}$  and below the estimated limit for narcosis, suggesting possible specific effects on emergence time ( $EMt_{50}$ ) of these compounds during chronic exposure.

For all compounds except anthracene, concentrations at which effects on emergence time of the tested compounds appeared were more than one order of magnitude higher than total  $\Sigma$ PAH concentrations measured in lake sediments from the USA (Van Metre and Mahler, 2005). Concentrations of azaarenes and other heterocyclic PACs are nearly never monitored in the field, but estimated to be up to 10% of the total PAC concentration in the sediment (Neilson, 1998). Thus, the obtained single compound effect concentrations from this study were about two orders of magnitude higher than estimated azaarene concentrations in the field. However, midge populations in the field are never exposed to a single PAC and other stress factors may play a role during chronic exposure. Therefore, chronic toxicity of the tested heterocyclic compounds should not be neglected.

## 5. Conclusion

When compared to acute and chronic  $LC_{50}$  values, it can be concluded that delay in emergence is an effective endpoint to detect life cycle effects of PACs on the midge *Chironomus riparius*.

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