



UvA-DARE (Digital Academic Repository)

Invertebrate life cycle responses to PAC exposure

León Paumen, M.

Publication date

2009

Document Version

Final published version

[Link to publication](#)

Citation for published version (APA):

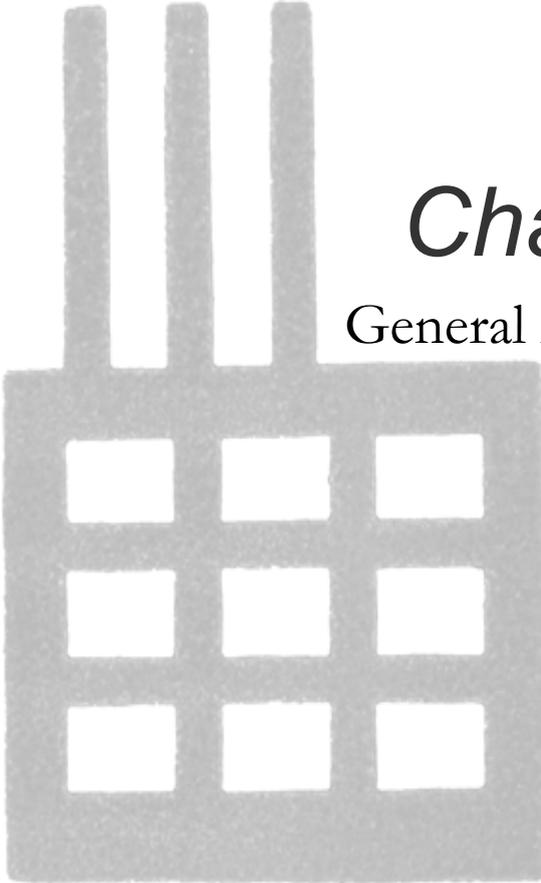
León Paumen, M. (2009). *Invertebrate life cycle responses to PAC exposure*. Universiteit van Amsterdam.

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.



Chapter 1

General introduction

Do you realise how many of the things we say are just echoes?

Doris Lessing, the Golden Notebook

Polycyclic Aromatic Compounds (PACs) have been classified as priority toxic substances by the European Commission (European Commission, 2006), and frequently determine the need for soil and sediment remediation (Lahr et al., 2003). These organic toxicants, generated mainly by incomplete combustion of organic materials, have always been present at background concentrations in soils and sediments around the world (Nam et al., 2008; Neilson, 1998). Recently, PACs have



Fig 1. NASA Spitzer Space Telescope image of the spiral galaxy M81, located some 12 million light years from Earth. The infrared radiation emitted by nitrogen-containing PAC molecules is shown in red. Taken from the NASA Ames research Center website.

also been detected throughout space, and emission of infrared radiation by large heterocyclic PACs containing nitrogen is now being used as tracer of star formation (Figure 1, Hudgins, 2005). Some astronomers even consider complex PACs possible multi-tasking molecules in the origin of life (Ehrenfreund and Foing, 1995; Ehrenfreund et al., 2007), since their stable and radiation-resistant structure could have been the primitive building material of bacterial-like membranes and metabolic pathways (<http://proto-cells.lanl.gov/>). Due to their omnipresence in the environment, degradation and biotransformation mechanisms for organic substances have evolved, allowing bacteria and higher organisms to cope with background PAC concentrations (Van Herwijnen et al., 2003; Van Straalen and Roelofs, 2006).

Natural emission sources of PACs include volcanism and forest fires, but the dramatic concentration increase that started at the end of the 19th century is mainly of anthropogenic origin (Prevedouros et al., 2004; Van Metre and Mahler, 2005). Due to coal burning in manufactured gas plants, industrial activities, oil drilling and refining (Lima et al., 2003; Lu et al., 2005; Nam et al., 2008; Srogi, 2007), concentrations of

PACs in soils and sediments have greatly increased (Osborne et al., 1997; Wild and

Jones, 1995). Current background concentrations of individual PACs in soil are around 1-10 $\mu\text{g}/\text{kg}$ dry soil, while average concentrations of the $\Sigma 16\text{EPA-PACs}$ (the 16 homocyclic PACs for which risk assessment is performed by the US EPA) can reach 4 mg/kg dry soil in urban soils (Wilcke, 2000), and even 1000 mg/kg dry soil have been measured at highly contaminated sites (data from Bioclear, a Dutch remediation company).

In the second half of the 20th century, ecosystem degradation due to increased concentrations of PACs and other toxicants (e.g. pesticides, dioxins, PCBs, metals) was acknowledged, and measures were adopted in western countries to reduce or ban PAC emissions (Hellmann, 2003). Thanks to these measures, industrial emissions have decreased and for some PACs even ceased, but contrary to other contaminants, total PAC concentrations in the environment remain far above background levels and in some cases are still increasing (Figure 2; Lima, 2003, Van Metre, 2005). Thus, although the release of PACs from point sources in western countries is diminishing, diffuse emission sources

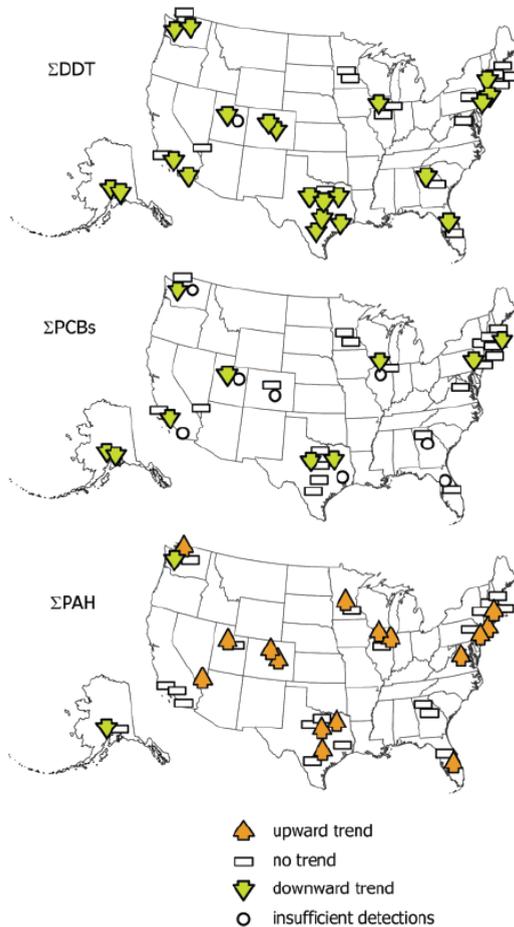


Figure 2. Trends in three groups of contaminant concentrations in USA lake sediment cores from 1970 to 2001 (from Van Metre and Mahler 2005). ΣDDT : sum of DDT, DDD and DDD; ΣPCBs : sum of Aroclors 1016/1242, 1254, and 1260; ΣPAH : sum of the main Polycyclic Aromatic Hydrocarbon fractions.

persist and hamper reduction of PAC concentrations in the environment. Meanwhile, the economic globalization process started in the end of last century is resulting in incredibly fast growth and uncontrolled industrialization in eastern countries like

China and India, with a tremendous increase in the use of fossil fuels (e.g. coal, oil, Figure 3). Due to this increase, a coupled increase in PAC concentrations in air, soil and sediment is taking place (Liu et al., 2005; Xu et al., 2006). Hence, it can be stated that industrial PAC emissions are not decreasing, only moving eastwards with unknown implications for global environmental quality.

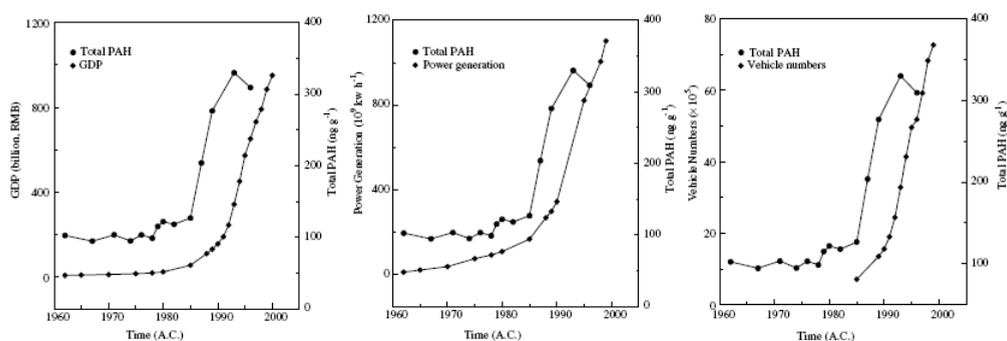


Figure 3. Relationship between the development in time of total PAH concentrations in a sediment core and socioeconomic indicator data (gross domestic product (GDP), power generation and vehicle numbers) of the Pearl River Delta region (from Liu et al. 2005).

Heterocyclic PACs

Because of their origin, diffuse emission sources and long-range transport, PACs accumulated in soils and sediments usually occur as a complex mixture. The main components of this mixture are homocyclic hydrocarbons, fused aromatic rings of carbon and hydrogen atoms only. Due to their high hydrophobicity, homocyclic PACs bind to organic matter and their availability in soils and sediments is usually lowered (Cornelissen et al., 2005; Jonker et al., 2007; Landrum et al., 1992; Moermond et al., 2007; Xiao et al., 2004). In smaller fractions than homocyclic PACs, a myriad of heterocyclic structures with in ring substitutions of e.g. nitrogen, oxygen, sulfur and chlorine atoms occur in the mixture (Neilson, 1998). Due to these substitutions, heterocyclic PACs are more water soluble than their homocyclic analogues, and therefore more available in pore water (Chen and Preston, 2004). Although they are present in fairly high concentrations and their availability is higher compared to their homocyclic analogues, heterocycles are not included in PAC risk assessment, and are rarely included in ecotoxicological test schemes. Hence, little information is available about their occurrence, fate and effects (Chen and Preston, 1998; Chen and Preston, 2004; Kozin et al., 1997; Hogenboom et al., 2008; Osborne, 1997; Wilhelm et al.,

2000). Heterocycles deserve therefore much more scientific attention than they have received so far.

Azaarenes (heterocyclic PACs with an in ring nitrogen substitution of a carbon atom) are the most ubiquitous group of heterocycles, comprising up to 10% of the total PAC concentration (Chen and Preston, 1998; Chen and Preston, 2004; Neilson, 1998; Osborne et al., 1997; Preston et al., 1997). Like their homocyclic analogues, they are mainly generated by partial combustion of N-containing organic materials (Wilhelm et al., 2000), but they are also released in the environment from additional sources, like wood preservation activities using tar oils and, in smaller amounts, pharmaceutical moieties (Pereira, 1983; Reineke et al., 2007; Westerhoff et al., 2005). Because of their fairly high environmental concentrations and widespread occurrence, azaarenes have been chosen as ‘model’ heterocyclic toxicants by our research group (PhD thesis E.A.J. Bleeker, 1999, PhD thesis S. Wiegman, 2002). Results from these projects showed that short term toxic effects of azaarenes often occur in the same range as toxicity of their homocyclic analogues, underlining that their toxicity should certainly not be neglected (Bartos et al., 2006; Bleeker et al., 2002; Feldmannova et al., 2006; Jung et al., 2001; Kraak et al., 1997b; Wiegman et al., 2001).

Transformation products

When not sorbed to solid surfaces, PACs are degradable (Guerrero et al., 2002; Johnsen et al., 2005; Lei et al., 2005; Stroomberg et al., 2004; Van Herwijnen et al., 2004), and stable degradation products are often found at PAC contaminated sites (Blotvogel et al., 2007; Pereira, 1983; Pereira, 1988). In the biological transformation process, PACs are made less hydrophobic by incorporating an oxygen atom in the molecule. In insects and mammals, hydroxylated PACs are subsequently conjugated to glutathione or sulfate, ‘carrier’ molecules that facilitate excretion (Barreira et al., 2007; Paskova et al., 2006). This mechanism allows organisms to cope with the presence of a wide range of organic toxicants, ubiquitous in terrestrial and benthic environments. However, biotransformation can also result in activation of the molecules, which become reactive and form adducts with proteins and DNA before they can be excreted (Xue and Warshawsky, 2005). DNA adducts are eliminated by repair systems in the cell, but during continuous exposure mutagenic and teratogenic effects of transformation products have been observed, mainly in mammalian systems, but also in other organisms (Aouadene et al., 2008; Cronin and Bickham, 1998; Lemieux et al., 2008; Neilson, 1998; Papa et al., 2008; Warshawsky, 1992; Wood et al., 1983). For example, the azaarene metabolite acridone has shown high mutagenicity in the Mutatox[®] test compared to its parent compound acridine and its isomer

phenanthridone (Bleeker et al., 1999b), demonstrating the unpredictable consequences of the transformation process even for closely related compounds. This unpredictability creates great uncertainties in PAC effect prediction.

PAC exposure of soil and sediment inhabiting organisms

Invertebrates are exposed to PACs accumulated in soil and sediment mainly through the dissolved fraction of the toxicant in pore water (Belfroid et al., 1996). When taken up by the organism, non-polar organic contaminants commonly exert direct toxicity via narcosis, a reversible state caused by non-specific interaction of lipophilic molecules with biological membranes (Escher and Hermens, 2002). Because of its non-specificity, all compounds that act via narcosis exert toxic effects (in equilibrium conditions) at a narrow lipid based concentration range in the organism's membranes (McCarty and Mackay, 1993). Consequently, effects observed for one test organism might be extrapolated to other organisms correcting for lipid content (Hendriks, 1995; Landrum et al., 2003). As a result, research on organic contaminants, PACs included, has mainly focused on narcotic effects determined in short-time water-only experiments. Toxicity data for homocyclic PACs obtained from these short-term high-dose experiments were successfully related to compound lipophilicity, expressed as the octanol-water partition coefficient (K_{ow}) (Di Toro et al., 2000; Hermens et al., 1984). In this way, successful effect predictions for PACs and other organic compounds could be made using quantitative structure-activity relationships (QSARs) (Bundy et al., 2001; De Voogt et al., 1988; McCarty et al., 1992; McGrath et al., 2004; Schultz et al., 2003; Van Leeuwen et al., 1992). However, these short-term experiments also showed that toxicity of closely related compounds, like isomers, can differ greatly (Bleeker et al., 2002; Kraak et al., 1997a; Wiegman et al., 2001), and specific effects other than narcosis were found for some PACs, complicating effect prediction (Bartos et al., 2006; Bleeker et al., 1999a; Bleeker et al., 2002; Bleeker et al., 1999b; Jung et al., 2001; Machala et al., 2001). Some of these effects, like phototoxicity, are explained by specific properties of the molecules (in this case the HOMO-LUMO gap, (Bleeker et al., 2002; Wiegman et al., 2001)), but physicochemical properties causing teratogenic or mutagenic effects are less well characterized in invertebrates, because research has focused mainly on carcinogenic effects of epoxide PAC metabolites in mammals (Neilson, 1998).

During the last years, ecotoxicological research on PACs has slowly moved from experiments with mortality as the only endpoint to chronic exposure experiments testing effects of PAC contaminated soils and sediments on sub-lethal parameters (e.g. growth, reproduction), more relevant for population effects (Bell et al., 2004; Erickson

et al., 1999; Kreitinger et al., 2007; Landrum et al., 2003; Lotufo, 1998; Shor et al., 2004; Sverdrup et al., 2002a; Sverdrup et al., 2002d; Van Brummelen et al., 1996; Verrhiest et al., 2001). One of the main questions is, therefore, if effect- K_{ow} relationships established for acute water exposure also hold for chronic exposure to PACs in soils and sediments. In a study by Sverdrup et al. (2001), chronic soil toxicity data for the springtail *Folsomia fimetaria* were compared to a QSAR for acute effects, showing that narcosis was also the main mode of action of homo- and heterocyclic PACs during chronic exposure, although exceptions were observed as well. This promising approach will be further explored in this thesis.

Multi-generation exposure

It has frequently been stated that specific effects of PACs besides narcosis, leading to DNA damage, will only become evident after prolonged exposure, which due to the persistent nature of these contaminants is also frequently occurring in the field (Musch, 1996; Neilson, 1998; Street et al., 1998; Van Brummelen et al., 1996). Cumulative effects of PACs at the population level can only be determined if exposure of the test organisms lasts longer than one generation (Brennan et al., 2006; Campiche et al., 2007). Still, very few studies dealing with the effects of multi-generation exposure of soil and sediment inhabiting invertebrates have been reported. The few published studies on organic compounds indicate that damage accumulation due to successive generations of exposure to toxicant concentrations below the EC50 affects the fitness of invertebrate populations; and contrary to metals, no adaptation has been observed (Brennan et al., 2006; Campiche et al., 2007; Postma and Davids, 1995; Tominaga et al., 2003; Vogt et al., 2007). The lack of adaptation to organic compounds is striking, because they are as widespread as metals and organisms inhabiting soils and sediments have always been exposed to both. Therefore, studies comparing single and multi-generation effects of PACs are definitely needed to determine the real threat they pose to exposed populations.

Environmental risk assessment of PACs

Due to the impossibility of assessing the occurrence and toxicity of the vast amount of aromatic compounds present in the environment, PAC environmental risk assessment is performed using a limited set of merely homocyclic structures. This set consists, for example, of 16 PAHs in the U.S. and 10 PAHs in the Netherlands. For these compounds, total effect concentrations in soil or sediment are used to set quality standards. Risk assessment neglects the occurrence of heterocyclic structures, while recent projects aiming to quantify and rank the occurrence of PAC heterocycles have shown that they are ubiquitous in e.g. groundwater systems (Blotevogel et al., 2007).

Moreover, the available ecotoxicity data on heterocycles show that they are usually as toxic as their homocyclic analogues (Bartos et al., 2006; Bleeker et al., 2002; Feldmannova et al., 2006; Jung et al., 2001; Kraak et al., 1997b; Sverdrup et al., 2001), and cancer risk due to mutagenic activity of polar (heterocyclic) fractions in complex PAC mixtures could be significant (Lemieux et al., 2008). This underlines the importance of generating accurate data on scarcely studied compounds and endpoints, in order to judge if PACs can be considered to some extent a homogeneous group of toxicants. The uncertainties that complicate effect prediction (e.g. isomer-specific toxicity, mutagenicity of heterocycles and transformation products, changes in availability, specific effects due to prolonged exposure) also hinder the reliability of PAC risk assessment.

Aim and objectives

The aim of this thesis is to diminish the uncertainties that complicate PAC effect prediction and risk assessment.

To this purpose, the following objectives have been set:

- To determine life cycle effects of homo- and heterocyclic PACs on soil and sediment inhabiting invertebrates.
- To validate the application of K_{ow} -based effect relationships to describe general patterns and exceptions in the outcome of life cycle PAC toxicity experiments.
- To establish multi-generation effects of a homocyclic PAC on a soil inhabiting invertebrate, and compare them to effects of single generation exposure.
- To review the validity of the present PAC risk assessment by examining the implications of the long-term toxicity data generated in this thesis.

Test compounds

For the experiments described in *chapter 2*, eleven PACs belonging to three important structural groups were chosen as test compounds: six homocyclic PACs, three azaarenes and two Phase I azaarene transformation products (Figure 4). The homocyclic structures belong to the 16 EPA PAHs, and the azaarenes are analogues of three of the chosen homocyclic PACs. The two transformation products have been detected in laboratory experiments with aquatic invertebrates (de Voogt et al., 1999) and in the field (Blotevogel et al., 2007). For the experiments performed in *chapters 3*

and 4, a subset of three three-ring PAC isomer pairs (two homocycles, their two azaarene analogues and the two main azaarene transformation products; grey panel in Figure 5) was used. In the multi-generation experiment (*chapter 6*), the homocyclic PAC phenanthrene was used as test compound.

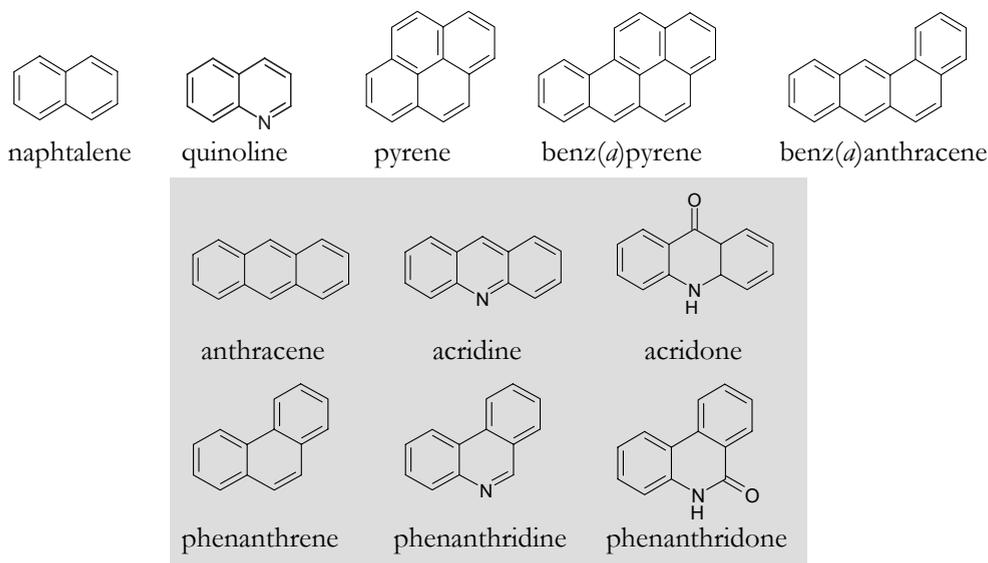


Figure 4. Test compounds used for the life cycle and multi-generation experiments. All compounds were tested in the experiments performed in *chapter 2*. Grey square: compounds tested in *chapters 3 and 4*. Phenanthrene was used for the multi-generation experiment performed in *chapter 6*.

Test organisms

Four test organisms were chosen to perform the life cycle experiments: two soil inhabiting invertebrates, the springtail *Folsomia candida* and the oligochaete *Enchytraeus crypticus*, and two sediment inhabiting invertebrates, the oligochaete *Lumbriculus variegatus* and the non-biting midge *Chironomus riparius*.

The cosmopolitan springtail *Folsomia candida* (Collembolla, Isotomidae, Figure 5) is a unpigmented invertebrate that inhabits soil pores and feeds on fungal hyphae. It is considered a tramp species, and has been found mainly in caves and mines, but recently also in forests and rural areas all around the world. It is in contact with its environment mainly via the ventral tube, which is used for fluid exchange and is probably the main route of exposure to chemicals dissolved in pore water (Fountain and Hopkin, 2005). Populations of *F. candida* consist exclusively of parthenogenetic females. Their reproductive organs are infected with bacteria of the genus *Wolbachia*, which probably cause the parthenogenesis. *F. candida* has a short generation time, is

easy to culture in the laboratory and its reproduction is a sensitive endpoint, and therefore it has often been used to determine effects of chemicals in soil. Studies have shown that *F. candida* is more sensitive to organic toxicants than to metals (Fountain and Hopkin, 2005; Herbert et al., 2004; Kolar et al., 2008; Kuperman et al., 2006; Sverdrup et al., 2002d; Van Gestel and Hensbergen, 1997). An ISO guideline to determine effects of chemicals on survival and reproduction of *F. candida* is available (ISO, 1999).

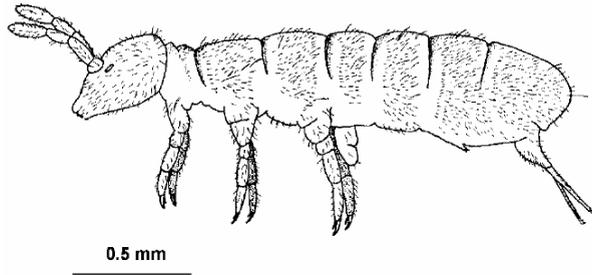


Figure 5. Adult *Folsomia candida* (Fountain and Hopkin, 2005).

The potworm *Enchytraeus crypticus* (Figure 6) belongs to the Enchytraeidae family, which are highly abundant in acid organic soils with relatively high moisture content (Kristufek et al., 1999). They contribute to the breakdown of detritus in the soil by direct consumption of dead organic material and to soil structure by their excrements and mucus (Bauer et al., 2001). *E. crypticus* is hermaphroditic and easy to culture in the laboratory, and therefore often used in toxicity testing (Jensen et al., 2003; Kolar et al., 2008; Krogh et al., 2007; Kuperman et al., 2006; Kuperman et al., 2004; Sverdrup et al., 2002a; Sverdrup et al., 2002d). An OECD guideline to determine effects of chemicals on survival and reproduction of *E. crypticus* is available (OECD, 2004b).

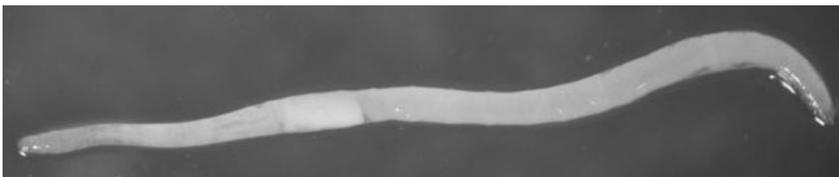


Figure 6. Adult of *Enchytraeus* sp. (www.tropicarium.ru).

The non-biting midge *Chironomus riparius* (Figure 7) is a member of the Chironomidae family, which are the most widely distributed and often the most abundant insects in freshwater (Armitage et al., 1995). Consequently, several biological indices concerning evaluation and monitoring of water quality rely heavily on them

(Armitage et al., 1995). Changes in species composition, dominance of pollution tolerant species, and frequency of deformities of larval head capsules are some of the commonly used parameters in these evaluations. In sediments, increasing densities of *Chironomus riparius* indicate a heavy load of organic matter. Larvae of *C. riparius* 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. Three of the four larval stages are in close contact with the sediment, making *C. riparius* a suitable organism for chronic sediment toxicity testing (Bleeker et al., 1999a; de Haas et al., 2004; Forbes and Cold, 2005; Hatch and Burton, 1999; Kim and Lee, 2004; Meregalli et al., 2001; Pery et al., 2003; Taylor et al., 1993). An OECD guideline for sediment testing using *C. riparius* is available (OECD, 2004a).

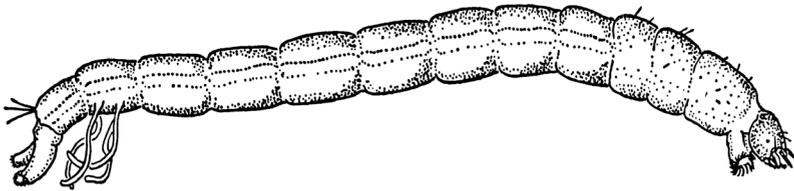


Figure 7. Late instar of *C. riparius* larva (De Pauw and Vannevel, 1990).

The endobenthic oligochaete *Lumbriculus variegatus* (Figure 8) is found in shallow marshes and ponds in Europe and North America, where it feeds on decaying plant material and microorganisms. It burrows in the sediment and also ingests sediment particles, and is therefore frequently used in bioaccumulation experiments (Kukkonen et al., 2004; Leppanen and Kukkonen, 2004; Lyttikainen et al., 2007; Schuler et al., 2004; Wiegand et al., 2007). In the laboratory, *L. variegatus* reproduces via fragmentation, which makes it easy to culture. Recently, a standardized protocol to determine effects of toxicants on reproduction has been developed by the OECD (OECD, 2006).

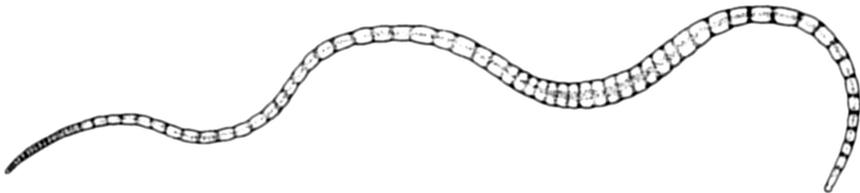


Figure 8. *Lumbriculus* sp. (De Pauw and Vannevel, 1990).

Outline of the thesis

During acute exposure PACs act mainly by narcosis, but during chronic exposure the same compounds may exert sub-lethal life cycle effects. At present, however, chronic PAC toxicity data are scarce. Therefore, in *chapter two* the effects of PACs on survival and reproduction of the two soil invertebrates are assessed, and in *chapter three* effects of PACs on midge emergence are determined. To evaluate if specific effects emerge besides narcosis during long-term exposure, the obtained chronic sub-lethal effect data are compared to an acute effect- K_{ow} relationship.

Chronic toxicity experiments using sediment or soil deal with the uncertainty of uncontrolled exposure, because in the spiked substrate various processes (e.g. degradation, sorption) modify the availability of the test substance to the test organisms and hamper an accurate determination of exposure concentrations. In order to calculate accurate effect concentrations for reproduction of the oligochaete *Lumbriculus variegatus*, Solid Phase Micro-Extraction (SPME, (Hawthorne et al., 2005; Jonker et al., 2007)) is used in *chapter four* to estimate the freely dissolved PAC concentrations in sediment pore water.

In *chapter five*, results of chapters two to four are combined in order to elucidate patterns in chronic PAC toxicity to sediment and soil invertebrates. Therefore, pore water effect concentrations are calculated from the sediment/soil effect concentrations and compared to an acute effect- K_{ow} relationship, assuming that soil and sediment inhabiting organisms are exposed primarily via pore water to the type of compounds tested.

Consequences of prolonged exposure to PACs can only be quantified if test organisms are exposed for several successive generations. In order to determine changes in toxicity occurring during multi-generation exposure, *chapter six* presents a multi-generation experiment in which the springtail *Folsomia candida* is exposed for ten consecutive generations to soil contaminated with the homocyclic PAC phenanthrene.

Due to the lack of chronic toxicity data, risk assessment for homocyclic PACs can not be performed according to the best available method. Besides, PAC heterocycles are neglected in actual PAC risk assessment. Therefore, in *chapter seven* a risk limit derivation performed with the obtained toxicity data is used to discuss the implications of our data for the current and future PAC risk assessment.