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Comparative chronic toxicity of homo- and heterocyclic aromatic compounds to benthic and terrestrial invertebrates: Generalizations and exceptions

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ABSTRACT

The aim of the present study was to elucidate consistent patterns in chronic polycyclic aromatic compound (PAC) toxicity to soil and sediment inhabiting invertebrates. Therefore we examined our experimental dataset, consisting of twenty-one chronic effect concentrations for two soil invertebrates (*Folsomia candida* and *Enchytraeus cripticus*) and two sediment invertebrates (*Lumbriculus variegatus* and *Chironomus riparius*) exposed to six PACs (two homocyclic isomers, anthracene and phenanthrene; two azaarene isomers: acridine and phenanthridine; and two azaarene transformation products, acridone and phenanthridone). In order to determine if effect concentrations were accurately predicted by existing toxicity- K_{ow} relationships describing narcosis, chronic pore water effect concentrations were plotted jointly against $\log K_{ow}$. Fifteen of the twenty-one effect concentrations (71%) were above the lower limit for narcosis, showing that narcosis was the main mode of action for the majority of the tested homo- and heterocyclic PACs during chronic exposure. Toxicity of all tested compounds to soil organisms was accurately described by the toxicity- K_{ow} relationship. However, for the sediment invertebrates exposed to some of the tested heterocyclic PACs deviations from narcosis were identified, related to specific physicochemical properties of the test compounds and/or species specific sensitivities. It is concluded that existing toxicity- K_{ow} relationships describing narcosis in some cases underestimate chronic PAC toxicity to sediment inhabiting invertebrates.

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1. Introduction

Invertebrates inhabiting PAC contaminated soils and sediments are chronically exposed to a variety of homocyclic and heterocyclic compounds (Lahr et al., 2003; Liu et al., 2004; Uhler et al., 2005). Until now, risk assessment for PACs is based on only a limited set of homocyclic compounds, ignoring the vast number of substituted heterocyclic compounds and transformation products. This omission has gradually been recognized, and consequently heterocycles are receiving a growing scientific attention (Bleeker et al., 1999a; Wiegman et al., 2001; Sverdrup et al., 2002a; Feldmann et al., 2006). For some groups of heterocyclic compounds, enough data have now become available to answer the urgent question if standards for the limited set of homocyclic PACs also sufficiently protect against heterocyclic PACs.

All organic toxicants, including PACs, induce narcosis to some extent. Many studies have shown that narcosis is strongly related to the lipophilicity of the compound, often expressed as the n-octanol-water partition coefficient (K_{ow}) (Könemann, 1981; De Voogt et al., 1988; Swartz et al., 1995; Chen et al., 1997; Schultz and Bearden, 1998). However, specific modes of action (i.e. other than narcosis) cause deviations from such relationships (Bearden and Schultz, 1998; Escher and Hermens, 2002). Hence, the relationship between toxicity and $\log K_{ow}$ can be used in search for generalizations as well as for identifying exceptions. Bleeker et al. (2002a) applied this approach to azaarenes, heterocyclic compounds in which a single carbon atom has been substituted by a nitrogen atom. Previously, azaarene concentrations were thought to be lower (1–10%) than those of the homocyclic analogues, but recently De Voogt and Laane (in press) reported that the sum of the concentrations of azaarenes and azaarene transformation products in marine sediments ranged from almost equal to up to one order of magnitude higher than that of their homocyclic analogues. Bleeker et al. (2002a) compared the acute toxicity of azaarenes to the midge *Chironomus riparius* to that of homocyclic compounds by plotting the 96 h LC50 values of both groups of

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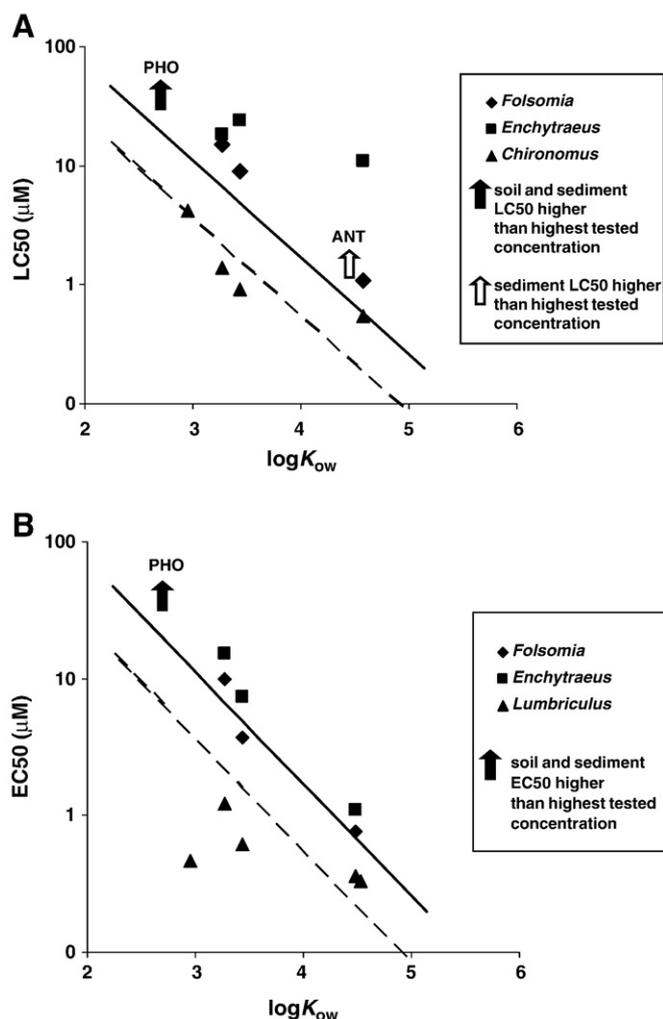


Fig. 1. Effect concentrations for the effect of six polycyclic aromatic compounds (PACs) on survival (LC50, A) and reproduction (EC50, B) of terrestrial and benthic invertebrates (◆ *Folsomia candida*, ■ *Enchytraeus crypticus*, ▲ *Chironomus riparius*/*Lumbriculus variegatus*), as a function of $\log K_{ow}$. Solid line: linear $\log LC50\text{-}\log K_{ow}$ relationship for *Chironomus riparius* exposed for 96 h to similar PACs ($y = -0.8162x + 3.4936$, $r^2 = 0.986$, (Bleeker et al. 2002a)). Dashed line: ratio LC50 from Bleeker et al. (2002a)/3, lower limit for narcosis.

and $EC50_{reproduction}$ values were plotted against the $\log K_{ow}$ values of the tested compounds.

3. Results and discussion

Our experimental matrix consisted of six compounds tested on four invertebrates, with one (sub)lethal endpoint for the benthic

organisms and two endpoints, survival and reproduction, for the terrestrial organisms, resulting in thirty-six potential effect concentrations. In twenty-one of the thirty-six cases a reliable effect concentration was obtained (Table 2), in all other cases no toxic effects were observed. We plotted these chronic LC50 (Fig. 1A) and EC50 (Fig. 1B) values on the relationship between acute aquatic LC50 data and $\log K_{ow}$ by Bleeker et al. (2002a), which describes narcosis. Fig. 1 shows that all possible outcomes were actually observed: some compounds were less toxic (e.g. anthracene and phenanthridine) while others were more toxic than expected (e.g. phenanthrene). To aid the discussion on generalizations and exceptions, the results of the comparisons made in Fig. 1 are summarized in Table 3. Generalizations will be discussed first, followed by the five different types of exceptions that were found.

3.1. Generalizations

The main purpose of the present study was to answer the question if chronic effect concentrations for homo- and heterocyclic compounds and transformation products were accurately predicted by existing toxicity- K_{ow} relationships. To this purpose the lower limit for narcosis was estimated by dividing the acute LC50 values from the LC50- $\log K_{ow}$ relationship by a factor of 3, because in previous studies on soil invertebrates a LC50/EC50 ratio larger than 3 suggested sub-lethal effects of the tested compounds on reproduction, deviating from narcosis (Droge et al., 2006). Fifteen of the twenty-one effect concentrations (71%) were above this lower limit for narcosis (Fig. 1 and '=' marks in Table 3), meaning that narcosis was the main effect for the majority of the tested homo- and heterocyclic compounds during chronic exposure. Nevertheless, phenanthrene was the only compound for which nearly all effect concentrations did not deviate from the acute LC50- $\log K_{ow}$ relationship, except for the LC50 for *E. crypticus*, which was higher than expected (Table 3). The homocyclic compound phenanthrene is a well-known narcotic compound, and the results from this study are in agreement with the available literature (Landrum et al., 1992; Neilson, 1998; Sverdrup et al., 2002b). For the two heterocyclic compounds, the eight effect concentrations for the two terrestrial invertebrates did not deviate from the acute LC50- $\log K_{ow}$ relationship, except for the phenanthridine LC50 for *E. crypticus*, which was higher than expected (Table 3). Hence, in agreement with Sverdrup et al. (2002b), it can be concluded that chronic soil toxicity for homo- and heterocyclic PACs could be accurately explained by an acute effect- $\log K_{ow}$ relationship describing narcosis.

For the transformation products only two effect concentrations were obtained, one agreeing with the relationship (acridone LC50 for *C. riparius*) and one being lower than expected (acridone EC50 for *L. variegatus*) (Table 3). Due to the limited number of effect concentrations (only 2 out of 12 potential effect concentrations) a reliable comparison with toxicity of their heterocyclic parent compounds and homocyclic analogues cannot be made. On the other hand, this result shows that PAC metabolism generally results in transformation

Table 3

Summary of the comparison between chronic effect concentrations for soil and sediment invertebrates and acute LC50s (narcosis) from (Bleeker et al., 2002a).

		$\log K_{ow}$	Acute LC50 (μM , Bleeker et al. 2002a)	Soil				Sediment				
				<i>F. candida</i>		LC50/EC50	<i>E. crypticus</i>		LC50/EC50	<i>C. riparius</i>		<i>L. variegatus</i>
				LC50	EC50		LC50	EC50		LC50	EC50	
Homoc.	Anthracene	4.53	0.62	w	w			w	w	3.8	=	=
	Phenanthrene	4.48	1.16	=	=	1.4		=	=	3.8	=	=
Azaar.	Acridine	3.27	6.67	=	=	1.6		=	=	1.5	=	+
	Phenanthridine	3.44	4.85	=	=	3.6		-	=	3.0	+	+
Tr. pr.	Acridone	2.95	12.18	n	n			n	n		=	+
	Phenanthridone	2.7	19.5	c	c			c	c		c	c

w: not toxic at highest tested concentration, which was above water solubility; c not toxic at highest tested concentration, which was below the acute LC50; n not toxic at the highest tested concentration, which was above the acute LC50; - chronic effect concentration higher than acute LC50, = chronic effect concentration similar to acute LC50 (narcosis), + chronic effect concentration lower than acute LC50 (specific effects besides narcosis).

products that are harmless for most organisms, although unexpected high metabolite toxicity may be observed occasionally (see below).

3.2. Exceptions

3.2.1. Compounds for which the predicted effect concentration was above maximal water solubility

For the homocyclic compound anthracene the predicted effect concentration (0.62 μM , Table 3) is above maximal water solubility (0.37 μM , Table 1). Hence, no adverse effects of anthracene at maximal water solubility were expected, which was indeed the case for the two terrestrial invertebrates (Table 3). In the experiments with the midge *C. riparius*, anthracene was dissolved in acetone and added to wet sediment, but due to its low water solubility crystallization of anthracene occurred at higher concentrations in the sediment (León Paumen et al., 2008a). Therefore, the highest reliable test concentration (0.15 μM) was too low to observe any adverse effects. In contrast, in the experiments with the benthic oligochaete *L. variegatus* a different spiking method using dry sediment was applied, and reproduction was affected at a concentration (0.33 μM , (León Paumen et al. 2008b)) similar to the maximal water solubility (0.37 μM). The similarity between expected effect concentrations and water solubility makes the toxicity of anthracene rather unpredictable: due to species specific sensitivities, for some species effect concentrations may be above maximal water solubility and no effects will be observed. In contrast, for other species (*L. variegatus* in the present study) effect concentrations are below maximal water solubility, and because of high hydrophobicity of anthracene this results in the lowest effect concentration of the six tested compounds. Likewise, low reproduction EC10 values for anthracene compared to EC10s for other PACs were observed for the springtail *F. fimetaria* (Sverdrup et al., 2001), while the model used in that study predicted that no chronic toxicity of anthracene would be observed due its low water solubility.

3.2.2. Compounds for which the highest tested concentration was below the predicted effect concentration

Phenanthridone has the lowest lipophilicity (K_{ow}) and the highest water solubility (S_w) of all tested compounds (Table 1). Therefore, expected effect concentrations were highest (Table 3). Because of the low solubility of phenanthridone in acetone, the high expected pore water effect concentration was not achieved with the spiking method used, and phenanthridone did not exert adverse effects on survival, emergence or reproduction of any of the test organisms (Table 3).

3.2.3. Compounds which were not toxic at the highest tested concentration, although this concentration was above the predicted effect concentration

The transformation product acridone did not affect survival and reproduction of the terrestrial invertebrates, although the highest tested pore water concentration was far above the acute LC50. Hence, effects of acridone were expected, but not observed. In contrast, acridone affected survival or reproduction of the two benthic invertebrates at or even below expected concentrations (Table 3). Since narcosis is the minimal toxicity that a compound exerts, explanations have to be found for the lack of narcotic effects of acridone to the two terrestrial invertebrates. One reason could be the experimental K_{oc} value used for the calculation of pore water concentrations ((Jonassen et al., 2003) (Table 1)), which was much lower than the value for its isomer phenanthridone. This, together with the low organic carbon content of the soil, could lead to an overestimation of the pore water concentration in the soil. Another explanation could be the faster depletion in the soil compared to water-saturated sediment, which could limit the availability of the compounds to the test organisms (Jager et al., 2000). These considerations underline the fundamental problems in research on scarcely studied heterocyclic PAC and PAC transformation products: although there is an urgent need for insight in their fate, effects and

risk, it is still hard to find reliable values for their physicochemical properties and accurately estimate their availability in soil and sediment.

3.2.4. Compounds for which the observed effect concentration was above the predicted effect concentration

Two of the 21 observed effect concentrations were above the predicted effect concentration, i.e. effect concentrations for the homocyclic compound phenanthrene and its azaarene analogue phenanthridine on survival of the terrestrial oligochaete *E. crypticus*. *E. crypticus* is known for its low sensitivity to organic compounds compared to the springtail *F. candida* (Sverdrup et al., 2002a; Droge et al., 2006; Krogh et al., 2007; Kolar et al., 2008;), while toxic effects of heavy metals occur at the same range of concentrations for these two soil invertebrates (Lock and Janssen, 2003; Kuperman et al., 2004, 2006).

3.2.5. Compounds which were more toxic than predicted

Four of the twenty-one observed effect concentrations (19%) were lower than expected (Table 3). Since these were all sediment effect concentrations for heterocyclic compounds, this means that for benthic organisms, toxicity of heterocyclic PACs is underestimated using effect- K_{ow} relationships describing narcosis. Three out of the four exceptions were observed for reproduction of the oligochaete *L. variegatus* (EC50s for acridine, phenanthridine and acridone), and two out of the four exceptions were observed for the two benthic invertebrates exposed to phenanthridine.

The high sensitivity of *L. variegatus* reproduction to three of the four tested heterocyclic PACs could be related to its asexual mode of reproduction. The soil organisms used in this study and the midge *C. riparius* are oviparous, but the oligochaete *L. variegatus* reproduces via asexual fragmentation (architomy) under laboratory conditions. In the architomic fission process two fragments are formed and the missing segments are generated during a regeneration period that lasts more than a week (Leppanen and Kukkonen, 1998; Martinez et al., 2005). During this period the newly generated worms do not feed, and high sensitivity to toxicants in the sediment might be related to the high energy demands for segment regeneration, which could influence energy allocation to detoxification mechanisms (Penttinen and Kukkonen, 1998).

The high toxicity observed for the benthic organisms exposed to phenanthridine compared to its isomer acridine illustrates the drawback of using K_{ow} as a descriptor in studies dealing with isomers. K_{ow} is a macroscopic property, and therefore differences between K_{ow} values of isomers will be minimal. Hence, other more specific physicochemical properties are needed to explain toxicity differences between closely related compounds. Therefore, several physicochemical properties of the six compounds analyzed in this study were calculated using ChemProgPro™ and ClogP™ (i.e. charge of the N atom, charge of the O atom, dipole, HOMO-LUMO gap). For the tested azaarene isomers the dipole of the molecule, which defines its electronic conformation, differed by a factor of 2.5. This difference may have implications for toxicant-membrane interaction and receptor binding. In agreement, higher toxicity to mussels of phenanthridine compared to its isomers has been related to a combination of physicochemical properties describing the attractive and repulsive forces governing toxicant-membrane interaction (Kraak et al., 1997). However, at the moment too little information is available to link observed effects of PACs to physicochemical properties other than K_{ow} in a quantitative way.

4. Conclusions

Narcosis was the main mode of action of the tested homo- and heterocyclic PACs during chronic exposure of soil and sediment invertebrates. However, exceptions related to specific physicochemical properties of the compounds and/or species specific sensitivities were

also identified. Particularly benthic invertebrates were sometimes more sensitive to the tested heterocyclic PACs than expected, meaning that PAC sediment risk assessment based solely on a small set of homocyclic structures could be underprotective.

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