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Toxicity of four nitrogen-heterocyclic polyaromatic hydrocarbons (NPAHs) to soil organisms

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Abstract

The aims of this study were: (i) to investigate the toxicity of N-heterocyclic polyaromatic hydrocarbons (NPAHs) quinoline, acridine, phenazine, and 1,10-phenanthroline to the soil invertebrates *Eisenia fetida*, *Enchytraeus crypticus*, *Folsomia candida*, and *Caenorhabditis elegans*, (ii) to compare the toxicity of four NPAHs and the species sensitivity, and (iii) to discuss possible risks of these compounds in soils. Different toxicities were found for the tested NPAHs which might be partially explained by their structure and properties. Effect concentrations expressed as soil pore-water concentrations were related to log K_{ow}, which indicated narcosis as the most probable mode of toxic action. The species sensitivity decreased in the rank: springtails > enchytraeids = earthworms > nematodes. Predicted no-effect concentration (PNEC) values were calculated for all tested species giving values from 0.5 to 6.8 mg/kg. It is unlikely that there is a risk for soil organisms in natural soils where lower NPAHs concentrations are expected.

Keywords: NPAHs ecotoxicity; *Eisenia fetida*; *Enchytraeus crypticus*; *Folsomia candida*; Risk assessment

1. Introduction

N-heterocyclic polyaromatic hydrocarbons (NPAHs) are N-heterocycles of polycyclic aromatic hydrocarbons (PAHs), i.e. one or more carbon atoms in the fused ring structure are replaced by nitrogen atom(s). NPAHs tend to occur in a strong association with PAHs in the environment because they come from the same sources. They are formed during the incomplete combustion of organic matter, including wood, waste, and fossil fuels (such as gasoline, oil, and coal) in the presence of nitrogen containing substances (Chuang et al., 1991; Furlong and Carpenter, 1982; Osborne et al., 1997). NPAHs are also released into the environment from spills, wastes and effluents of several industrial activities such as oil drilling, refining and storage, coal tar processing, chemical manufacturing, and wood preservation (Pereira et al., 1983; Lopes and Furlong, 2001).

NPAHs are ubiquitous environmental pollutants that have been detected in all environmental matrices. Generally, they occur in the environment in amounts up to 1–10% of their homocyclic analogues (Nielsen et al., 1999; Wild and Jones, 1995; Lopes and Furlong, 2001). Concentrations of NPAHs have been predominantly measured in air (Wild and Jones, 1995, Chuang et al., 1991) or in waters (van Genderen et al., 1994; Pereira et al., 1983). However, NPAHs strongly accumulate in sediments showing significant concentrations (Furlong and Carpenter, 1982; Wakeham, 1979; Osborne et al., 1997). The levels of NPAHs in contaminated lake or river sediments have been reported from several tens to several hundreds of μg/kg (Osborne et al., 1997; Lopes and Furlong, 2001; Wakeham, 1979). Whilst soils and sediments strongly accumulate NPAHs, soil levels of individual NPAHs are rarely measured (Švábenský et al., 2007). Some authors have reported the concentrations of PAHs and NPAHs mixtures that can reach several tens or hundreds of mg/kg at industrial sites (WHO, 2004; Walsh et al., 1997; Erstfeld and Snow-Ashbrook, 1999; Hofman et al., 2004).

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The substitution of a nitrogen atom in the fused-ring structure has a large effect on the physical/chemical/toxicological properties of NPAHs. It was found that the NPAHs are more reactive, more soluble, and consequently more mobile in water than their parental PAHs compounds (Pearlman et al., 1984). Their higher solubility and lower K_{ow} can explain why NPAHs were detected less frequently in sediment than PAHs (Lopes and Furlong, 2001). Similarly as in the aquatic environment, the presence of nitrogen(s) may alter behaviour of NPAHs in soils and consequently the rate of their uptake and toxicity for soil organisms. Higher reactivity of NPAHs in comparison with the homocyclic PAHs may cause substantial volatilization, lower sorption, and higher degradation in soils (WHO, 2004). When compared to parental PAHs, NPAHs are weak bases and can be protonated in the soil solution which is dependant on their pK_b and pH of the soils. Weak bases are in molecular form at the pH of most natural soils. As the soil pH decreases, protonated (cationic) forms may be produced (Bintein and Devillers, 1994). These forms can be bound to soil cation exchange sites which may increase their retention in the soil (Bintein and Devillers, 1994; Doucette, 2003).

It has been shown that N-heterocycles can be strongly bioaccumulated (Southworth et al., 1979) or metabolized (de Voogt et al., 1999; Bleeker et al., 2002). The same effects as with homocyclic compounds were described for mammals including humans: mutagenity, carcinogenity, genotoxicity, teratogenity (Bleeker, 1999; Bleeker et al., 2002). NPAHs similarly to PAHs show a number of ecotoxic effects—acute toxicity, effects on growth and reproduction have been frequently reported (e.g. Dijkman et al., 1997; Kraak et al., 1997; Bleeker, 1999; Bleeker et al., 2003; Wiegman, 2002). The potency of NPAHs to modulate the biomarkers for detoxification and oxidative stress has been intensively studied (Feldmannová et al., 2006; Burýšková et al., 2006; Pašková et al., 2006). Few studies investigate the toxic effects of NPAHs on terrestrial organisms. Effects of few selected NPAHs (quinoline, acridine, phenanthridine, carbazole) were investigated for soil microorganisms (Sverdrup et al., 2002a), terrestrial plants (Sverdrup et al., 2003), springtails (Sverdrup et al., 2001; Bleeker et al., 2003), earthworms (Sverdrup et al., 2002c), enchytraeids (Sverdrup et al., 2002b; Bleeker et al., 2003), and snails (Sverdrup et al., 2006).

The main goal of our study was to investigate the toxicity of four NPAHs to soil organisms. Four low molecular weight NPAHs with different structures and properties containing one (quinoline, acridine) or two (phenazine, 1,10-phenanthroline) nitrogen atoms were selected to research the impact of different structures and properties on the measured toxicity. A test battery was used including the earthworm *Eisenia fetida*, the enchytraeid *Enchytraeus crypticus*, the springtail *Folsomia candida*, and the nematode *Caenorhabditis elegans.* They represent different soil invertebrates with different size, physiology, ecology, and exposure pathways. The sensitivity of the invertebrates to the tested compounds was compared. Toxicity measured in our study was compared to available data from the literature and the possible risk of NPAHs in the soil environment was discussed.

2. Materials and methods

2.1. Test organisms

All invertebrates (*E. fetida*, *E. crypticus*, *F. candida*, and *C. elegans*) have been permanently cultured at RECETOX laboratories (Czech Republic). *E. fetida* was cultured in the mixture of sphagnum peat and horse manure in the ratio of 1:1 (w:w), which was adjusted to pH of 6–7 with $CaCO_3$. The water content of the substrate was approximately 80% of its maximum water holding capacity (WHC_{max}). Feeding was not necessary (horse manure served as food was sufficient). The culture was maintained at $20\pm2\,^{\circ}C$ in the dark. *E. crypticus* was cultured in OECD artificial soil moisturized to 50% of WHC_{max}. Enchytraeids were maintained at $18\pm2\,^{\circ}C$ in the dark and were fed with oat flakes weekly. *F. candida* was kept on plaster of Paris and pulverized activated charcoal in ratio of 9:1 (w:w) at $18\pm2\,^{\circ}C$ in the dark. Dry baker's yeasts were added as a food weekly. *C. elegans*, wild type strain N2, var. Bristol, culture was kept on NGM agar with bacterial lawn of uracil-deficient strain of *Escherichia coli* (OP50) as a food source and was maintained at $20\pm2\,^{\circ}C$ in the dark.

2.2. Preparation of soil

OECD artificial soil was prepared for *E. fetida*, *E. crypticus*, and *F. candida* tests. The soil composition was 70% sand, 20% kaolin clay and 10% finely ground sphagnum peat (OECD, 2000a). The organic matter content was 4.68%. The pH_{KCl} was set to 6.0 ± 0.5 with $CaCO_3$ at the beginning of the tests and was found to increase to 6.5 ± 0.5 at the end of tests. The soil moisture was adjusted to 50% WHC_{max} by distilled water (3 ml per 10 g dry weight soil) after addition of the tested chemicals and before the addition of the organisms. The artificial soil was found to be an unsuitable substrate in the *C. elegans* test because the firm peat floated on the surface during the extraction procedure and disabled counting of surviving worms. Therefore, the natural uncontaminated soil from the top layer of a field was used for the *C. elegans* test. It was loamy sand cambisol, with pH_{KCl} of 6.5, and organic carbon of 2.35%. This soil was air-dried at room temperature, sieved (<2 mm), defaunated (deep freezing), and stored dry.

2.3. Preparation of tested chemicals and spiking

Quinoline (CAS 92-22-5), acridine (CAS 260-94-6), phenazine (CAS 92-82-0), 1,10-phenanthroline (CAS 66-71-7) were purchased from Sigma-Aldrich Ltd. (CR) Purity of all compounds was minimally 97%. Selected properties of these chemicals are in Table 1. The tested compounds were dissolved in acetone (99.8% purity, Chromservis Ltd., CR) in stock solutions corresponding to the highest tested concentrations. The stock solutions were diluted with acetone to get desired concentration ranges after spiking each 10 g dry weight soil with 1 ml of the appropriate spiking solution. After dropping the spiking solutions on the top of the dry soil in the test vessels, samples were thoroughly mixed. Pure acetone (1 ml per each 10 g dry weight soil) was used for the control samples. After spiking, the solvent was evaporated in a fume hood overnight. Soil moisture was adjusted the next day before start of the tests.

2.4. E. fetida test

The toxicity test was performed according to OECD guideline 222 (OECD, 2000a). The exposure concentrations 100, 500, 1000, 1500, 2000, 2500 mg/kg + control were used for all compounds. Three containers (1 l volume) per each concentration with 500 g dry weight soil were