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Effects of seven organic pollutants on soil nematode *Caenorhabditis elegans*

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Abstract

Caenorhabditis elegans is a free-living soil nematode that is commonly used as a model for toxicity tests. The aim of this study was to investigate the toxicity of seven organic pollutants: four azaarenes (quinoline, acridine, phenazine, and 1,10-phenanthroline), short-chain chlorinated paraffins, and two organochlorinated pesticides (toxaphene and hexachlorobenzene). The exposure to all chemicals was carried out in three test media (soil, agar, and aquatic medium), and adult mortality was evaluated after 24 and 48 h. Toxaphene was the most toxic substance with LC_{50} (48 h) of 379 mg/kg in the soil and 0.2 mg/L in the aquatic medium. Quinoline was the most toxic chemical in agar test with LC_{50} (48 h) of 10 mg/L. HCB showed a very low toxicity in all tests, maybe due to its very low water solubility. Longer than 24-h test duration was found necessary for getting more correct data on toxicity. In comparison with other studies, *C. elegans* was less sensitive than other soil invertebrates. Different response might be attributed to different exposure routes and shorter test duration. Equilibrium partitioning theory was used to calculate K_{oc} from results of soil and aquatic tests but this approach was found not working. Our results suggest that the tests with nematode *C. elegans* should be included to the battery of tests for risk assessment of POPs in soil.

Keywords: *Caenorhabditis elegans*; Azaarenes; Toxaphene; Hexachlorobenzene; Chlorinated paraffins; Toxicity

1. Introduction

Nematodes are the most abundant metazoans on the Earth. They are also the most abundant invertebrates in the soil ecosystem where they perform many ecological functions and particularly bacterivorous nematodes play an important role in the nutrient cycling (Wood, 1988). Soil nematodes belong to microfauna living in the pore-water of soil top layer, where they can be exposed substantially to the soil contaminants that are usually accumulated here. The thin cuticle covering their body is water-permeable, which makes them very sensitive to the uptake of dissolved fraction of contaminants. After exposure and effects of toxicants, the ability of nematodes to play their ecological roles may be impaired with possible deleterious effects at the ecosystem level. In ecological studies, change in nematode community structure is used as a sensitive marker of environmental stress as well as of pollution (Yeates, 2003). For these reasons, it is wise to include nematode species in the test battery for ecological risk assessment of chemicals in soil.

Impact of soil pollution on nematodes has been studied intensively in recent years in the field studies as well as in laboratory experiments. Standardized toxicity tests using nematodes *Caenorhabditis elegans* or *Plectus acuminatus* have been introduced by ASTM (2001) and by Kammenga et al. (1996), respectively. Nematodes, particularly *C. elegans*, are suitable test organisms due to simple breeding in agar medium, easy test performance, little demands on place and material, and short duration of test (Williams and Dusenbery, 1990; Donkin and Dusenbery, 1993; Peredney and Williams, 2000; Boyd and Williams, 2003). The detailed review of the use of nematodes in soil ecotoxicology was published recently by Sochová et al. (2006). *C. elegans* was found the most frequently used test species in nematode studies, although *P. acuminatus* is recommended as more ecologically relevant (Kammenga et al., 1996).

C. elegans started initially to be used as a model organism in genetics (Brenner, 1974). Since 1990s, it has been used as test organism in soil, aquatic and agar toxicity tests (Williams and Dusenbery, 1990; Donkin and Dusenbery, 1993; ASTM, 2001; Boyd and Williams, 2003) with possibility to determine variety

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of endpoints for toxic effect (Dhawan et al., 2000; Anderson et al., 2001). As reviewed by Sochová et al. (2006) more than 40 studies about toxicity of pollutants to *C. elegans* have been published so far. Most of the studies were focused on metals and pesticides, whereas information is missing about toxicity of organic compounds to nematodes. Persistent organic pollutants (POPs) are very important and priority environmental contaminants, because they show high persistency in the environment, strong bioaccumulation tendency and many possible toxic effects (Holoubek, 1999; Jones and de Voogt, 1999). Effects of POPs have been very intensively investigated with regard to human and mammal health, effects in aquatic ecosystems have been also studied many times, but effects on soil biota have been only sporadically studied.

The aim of this study is to provide information on toxicity of selected persistent organic pollutants to soil nematode *C. elegans*. Chemicals were selected to represent different types of POPs: (i) azaarenes: quinoline, acridine, phenazine, and 1,10-phenanthroline are examples of industrial by-products, (ii) chlorinated paraffins are examples of industrial high-volume produced chemicals, and (iii) toxaphene and hexachlorobenzene (HCB) are examples of organochlorinated pesticides. Negative effects of all these chemicals were found for aquatic organisms but they have been tested for toxicity on soil organisms only sporadically and never on toxicity to *C. elegans*. In our study, toxicity of all seven chemicals for *C. elegans* was tested in soil, aquatic medium and agar. In this paper, the suitability of these tests is evaluated for routine testing of POPs and toxicity of seven chemicals for *C. elegans* and for other species is compared. Moreover, the possibility of extrapolation the toxicity results between soil and aquatic tests is studied and discussed in this paper.

2. Material and methods

2.1. Chemicals

Quinoline (98% purity), acridine (97% purity), phenazine (98% purity), 1,10-phenanthroline (99% purity), hexachlorobenzene (99% purity), toxaphene (Supelco technical mixture) were purchased from Sigma-Aldrich Ltd. (CR). Chlorinated paraffins (labeled as C_{12}, 64% chlorine content by weight) were provided by Novácké závody Inc. (Slovakia). This technical mixture included all short-chain paraffin fractions ($C_{10–13}$) with a composition of C_{10} 6%, C_{11} 37%, C_{12} 32% and C_{13} 25%. All chemicals were dissolved in acetone but HCB in ethanol (both solvents of HPLC purity; Merck, CR) to get stock solutions. Then appropriate dilution series were prepared to get desired concentrations after spiking as described bellow.

2.2. Nematode culture

C. elegans, wild-type strain N2 varieta Bristol, was used. The culture has been kept on NGM agar plates, with bacterial lawn of a uracil-deficient strain of *Escherichia coli* (OP50) as food source and maintained at 20 °C (Brenner, 1974). Tests were conducted with age-synchronized 3-4 day old adult organisms obtained by procedure described in Boyd and Williams (2003).

2.3. Experimental soil

A natural soil was used in the soil tests. This soil was collected from the top layer of a field near Brno city (CR). It was a loamy sand cambisol with 68.9% sand, 16.9% silt and 21.2% clay. It had pH_{KCl} 6.48, organic carbon 2.35%, and total nitrogen 0.27%. Contents of organic pollutants and heavy metals were comparable to the background levels according to the Czech Republic limits (www.env.cz). The soil was air-dried at room temperature and then sieved (<2 mm), defaunated by deep freezing and stored under dry conditions in the dark before use.

2.4. Soil toxicity test

Soil toxicity testing was performed according to ASTM guideline (ASTM, 2001) and Donkin and Dusenbery (1993). Ten grams of dry weight (dw) soil was weighed into glass jars, spiking solutions were dropped on the soil surface and jars were left in the fume hood overnight to allow evaporation of the solvent. Final concentrations of tested compounds were 100, 500, 1000, 1500, 2000, and 2500 mg/kg dw for quinoline, acridine, phenazine, and 1,10-phenanthroline, 500, 1000, 3000, 6000, and 10,000 mg/kg dw for chlorinated paraffins, 50, 100, 250, 500, and 1000 mg/kg dw for toxaphene, and 50, 100, 300, 600, and 1000 mg/kg dw for HCB. These final tested concentrations were derived on the base of preliminary range finding tests. Control soil without solvent or chemicals and control soil with only solvent added were prepared too. After evaporation of solvent, soil in each jar was thoroughly mixed and 2.33 g dw was weighted into Petri dish (diameter 40 mm) using 3 replicates each concentration. Soil in Petri dish was moistened with 1.5 mL water which makes about 80% of soil water holding capacity then. Then 10 organisms from a 3-4 day old egg plate were transferred into each Petri dish, covered by lid and incubated at 20 °C for 24 and 48 h. After exposure period, surviving organisms were extracted using Ludox procedure (ASTM, 2001) and counted under dissecting microscope.

2.5. Aquatic toxicity test

Aquatic toxicity test was performed in K-medium according to Williams and Dusenbery (1990). About 10 mL of K-medium were added into beaker, mixed with the spiking solutions, and left in the fume hood overnight to allow evaporation of the solvent. Final tested concentrations were 0.8, 1.6, 3.125, 6.26, 12.5, 25, 50, 100, 500, 1000 mg/L for quinoline, 0.8, 1.6, 3.125, 6.26, 12.5, 25, and 38 mg/L for acridine, 0.8, 1.6, 3.125, 6.26, 12.5, and 16 mg/L for phenazine, 0.4, 0.8, 1.6, 3.125, 6.26, 12.5, 25, and 50 mg/L for 1,10-phenanthroline, and 0.000375, 0.00075, 0.0015, 0.003, and 0.006 mg/L for HCB. The concentration ranges for these compounds were determined by their solubility in water and then using dilution factor of two. Concentration scale for toxaphene and chlorinated paraffins was 0.03125, 0.0625, 0.125, 0.25, and 0.5 mg/L in the first test. Although the water solubility of both chemicals is ca. 0.5 mg/L, the preparation of much more higher concentrations is allowed in this case, because these viscous liquids are miscible well with water. Therefore, also higher concentrations of 50, 100, 500, 1000, and 3000 mg/L were tested in the second experiment. The results of both tests were consistent and were put together. However, the results must be interpreted carefully. Clear K-medium only control and control for effects of solvent were prepared by the same way. After evaporation of solvent, *E. coli* as food source was added. Spiked K-medium and controls were divided into 2 mL portions into 5-mL beakers (5 replicates per concentration) and 5 organisms were introduced into each beaker. Beakers were covered by parafilm and incubated at 20 °C. Surviving organisms were counted after 24 and 48 h under dissecting microscope.

2.6. Agar toxicity test

Agar toxicity test was performed on NGM agar and the procedure was inspired by Anderson et al. (2001) and Dhawan et al. (2000). Spiking solutions were added to freshly prepared hot agar (ca. 60–70 °C) and solvent evaporated very fast then. The solubility of compounds was verified before testing and it was found markedly higher in hot agar than in K-medium of aquatic test. This allowed broader range of tested concentrations which were 1.6, 3.125, 6.25, 12.5, 25, 50, 100, and 500 mg/L for quinoline, 6.25, 12.5, 25, 50, 100, and 500 mg/L for acridine, 25, 50, 100, 500, 1000, 1500, and 2000 mg/L for 1,10-phenanthroline and phenazine, 100, 500, 1000, 3000, and 6000 mg/L for chlorinated paraffins, 10, 25, 50, 100, 500, and 1000 mg/mL for toxaphene, and 0.1, 0.5, 1, 3, and 6 mg/L for HCB. Spiked hot agar was poured into 40-mm Petri dishes (5 replicates per concentration). Clear agar only control and control for effects of