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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

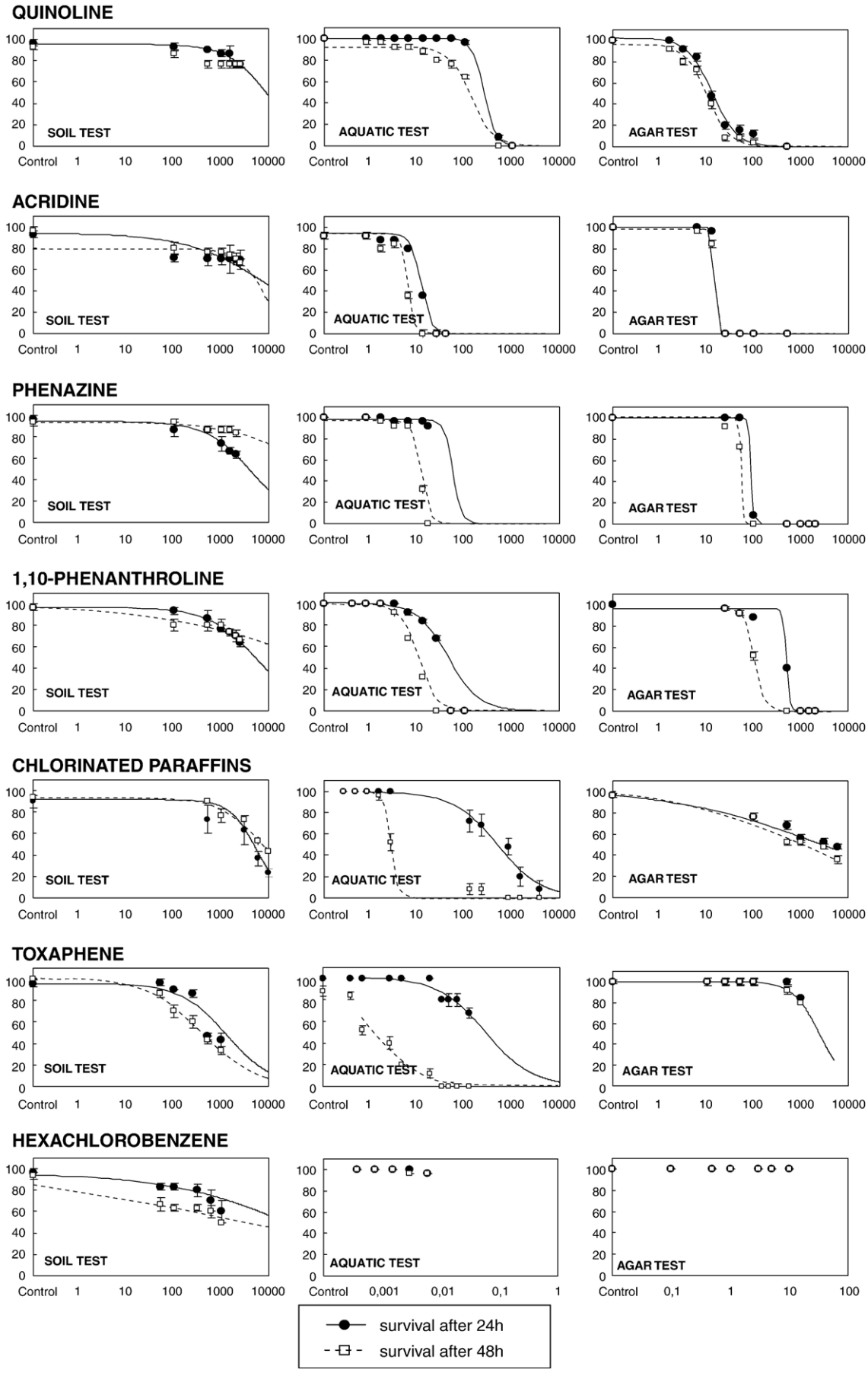
Soil toxicity testing was performed according to ASTM guideline (ASTM, 2001) and Donkin and Dusenbery (1993). Ten grams of dry weight (dw) soil was weighed into glass jars, spiking solutions were dropped on the soil surface and jars were left in the fume hood overnight to allow evaporation of the solvent. 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


QUINOLINE
ACRIDINE
PHENAZINE
1,10-PHENANTHROLINE
CHLORINATED PARAFFINS
TOXAPHENE
HEXACHLOROBENZENE
SOIL TEST
AQUATIC TEST
AGAR TEST
Control
survival after 24h
survival after 48h

solvent were prepared by the same way. After agar solidification, a drop of *E. coli* culture was added and spreaded on the surface of each plate and plates were incubated at 37 °C overnight. Next day 10 age-synchronized adult organisms were transferred to each dish. Dishes were covered by lids and incubated at 20 °C. Mortality was evaluated after 24 and 48 h under dissecting microscope.

2.7. Statistical analysis and toxicity calculation

Differences between solvent controls and water controls were tested by Student's *T*-test. Estimation of concentrations causing 50% and 10% reduction of survival (LC_{50} and LC_{10} values, respectively) and their 95% confidence intervals were calculated from logistic regression model. Model described in Haanstra et al. (1985) was used for LC_{50} and model described in Sverdrup et al. (2001) was used for LC_{10}. All statistical analyses were done using STATISTICA 6.0 software (StatSoft, 2001).

2.8. Equilibrium partitioning method

The equilibrium partitioning method (EqP-method) can be used to calculate soil toxicity (expressed in mg/kg) from aquatic toxicity (expressed in mg/L) using a partitioning coefficient (van Beelen et al., 2003; Ditoro et al., 1991). Reversibly in our study, organic carbon–water partitioning coefficient K_{oc} was calculated for all tested compound from the measured toxicity results (LC_{10} and LC_{50} after 24 and 48 h) in soil and aquatic medium. These calculated values were compared with values found in literature. K_{oc} values were calculated from the following equations: $K_d = LC_{soil}/LC_{aquatic}$ and $K_{oc} = K_d/f_{oc}$, where K_d is soil–water partitioning coefficients, f_{oc} is the fraction of organic carbon in particular soil (i.e. 0.0235 in our soil), LC_{soil} is toxic concentration in soil test (mg/kg dw) and $LC_{aquatic}$ is toxic concentration in aquatic test (mg/L). For LC values, both 10% and 50% mortality was used after 24 and 48 h of exposure, respectively, giving four K_{oc} values for each chemical.

3. Results and discussion

3.1. C. elegans tests for POPs toxicity testing

Adult mortality in all controls and solvent controls was never above 10% and fulfilled required validity criteria (ASTM, 2001). Water and solvent controls were not significantly different (*T*-test, $p > 0.05$) in all tests and therefore the influence of solvent residues on tests results may be neglected. Coefficients of variance were usually far bellow 30% which is, in our opinion, variability acceptable and common in toxicity testing. Variability was higher in the soil tests and it was sometimes increasing with increasing effect. Hence, more than 3 replicates are strongly recommended for soil test to decrease the variability and enable exclusion of outlying values.

For all chemicals with exception of low toxic HCB, logistic models of dose–response curves were allowed to be constructed (Fig. 1) and curve parameters to be computed (Table 1). LC_{10} and LC_{50} values are directly parameters of the logistic curve in these regression models (Haanstra et al., 1985; Sverdrup et al., 2001). It means that in that case when regression model was not statistically significant, LC value is only a rough estimate and has no confidence interval (Table 1). It was most frequent in soil tests, because at the highest concentration tested was still not reached 50% mortality.

The results of selected POPs toxicity (Table 1; Fig. 1) showed that they were strongly dependent on the test medium and test duration. In aquatic tests, the dose–response curves (Fig. 1) show that substances were more toxic after 48 h than 24 h. It was most apparent in the case of chlorinated paraffins and toxaphene which were not toxic after 24 h but the most toxic chemicals after 48 h. The increase of toxicity in time can be expected because as the exposure duration is longer more toxicant is probably taken up. Hence, longer exposure time is probably necessary to get the correct information about toxicity of this kind of chemicals. In soil and agar tests, the toxicity after 24 h and 48 h was not changing substantially. It seems that decreasing bioavailability in time due to sorption in soil and agar may compensate longer exposure duration. Also in these tests, longer duration is probably more appropriate.

3.2. Toxicity of selected POPs to C. elegans

It is hard to conclude any ranks of toxicity of tested compounds because their LC values were changing substantially between exposure times and the order of values was different for different media. Results of azaarenes toxicity on *C. elegans* survival (Table 1; Fig. 1) show that the most toxic azaarene in aquatic medium was acridine and the least toxic was quinoline. Surprisingly, quinoline was the most toxic in agar test and the least toxic was 1,10-phenanthroline. In the soil tests, there were almost no differences in toxicity to *C. elegans* between the tested azareenes. Toxic effects of chlorinated paraffins and toxaphene on survival of *C. elegans* in soil seem stronger than effects of azaarenes or HCB (Fig. 1). This is surprising because paraffins and toxaphene are more strongly bound in soil and their bioavailability is lower (see K_{oc} values in Table 2). Higher toxicity of chlorinated paraffins and toxaphene was found also in aquatic test after 48 h of exposure.

3.3. Sensitivity of C. elegans test compared to other soil and aquatic tests

Results of toxicity tests on *C. elegans* may be compared to other studies of azaarenes effects on invertebrates that have been published. *C. elegans* has LC_{50} value by one order of magnitude higher than *Enchytraeus crypticus* and *Eisenia veneta* and by 1–2 orders of magnitude higher than *Folsomia candida* and *Folsomia fimetaria* (Sverdrup et al., 2002; Bleeker et al., 2003). In the aquatic test the sensitivity of *C. elegans* is in the same range as sensitivity of *Chironomus tentans*, *Chironomus riparius*, *Daphnia pulex*, and *Daphnia magna* (Southworth et al., 1978; Parkhurst et al., 1981). In the test with phenazine, nematode has one order of magnitude higher LC_{50} value and in test with 1,10-phenanthroline it has a one order of magnitude lower LC_{50} value than *Daphnia magna* (Southworth et al., 1978; Parkhurst et al., 1981; Feldmannová et al., 2006).

C. elegans LC_{50} for chlorinated paraffins in soil was by one order of magnitude lower than LC_{50} *Eisenia fetida* and *Enchytraeus albidus* and was similarly sensitive as *F. candida* (Bezchlebová et al., in press). In aquatic medium, *C. elegans* has 1 and 3 orders of magnitude higher LC_{50} value for chlorinated paraffins than *Daphnia magna* and *C. tentans*, respectively (European Commission, 1999).

In the soil test, *C. elegans* has about 1–2 orders of magnitude lower LC_{50} for toxaphene than *Enchytraeus crypticus* and was similarly sensitive as *E. fetida*. *F. candida* has 2 orders of magnitude lower LC_{50} value than *C. elegans* (Bezchlebová et al., 2004). LC_{50} value for toxaphene is 1 and 2 orders in magnitude lower for *D. pulex* than for *C. elegans*.

We found no toxicity of HCB in tests in our study at tested concentrations. Only in the aquatic test, a lower mobility of organisms was observed. The reason is the low solubility of HCB which determines the highest tested concentration. Similarly to our results, no significant effect on toxicity for *Leptocheirus plumulosus*, *Hyalella azteca* and *C. tentans* was observed in known studies (Fuchsman et al., 1998).

Fig. 1. Dose–response curves showing toxicity of seven persistent organic pollutants to *C. elegans* in soil, aquatic and agar test. Survival was measured after 24 h (solid line, full circles) and 48 h (dashed line, open squares). Error bars show standard errors of a mean ($n = 3$ for soil test and $n = 5$ for aquatic and agar test).

Table 1
The results of *C. elegans* toxicity tests in soil, aquatic medium and agar for seven chemicals expressed as 50% and 10% lethal concentrations (mg/kg dw or mg/L) after two exposure times

Medium	Exposure (h)		Quinoline	Acridine	Phenazine	1,10-Phenanthroline	Chlorinated paraffin	Toxaphene	HCB
Soil	24	LC_{50}	>2500	>2500	>2000	>2500	5450 (2887–8012)	1285 (587–1983)	>1000
		LC_{10}	1122 (114–2130)	~100–1000*	374.2 (ND)	421.5 (ND)	1291.3 (ND)	108.2 (ND)	>1000
	48	LC_{50}	>2500	>2500	>2000	>2500	8833 (6002–11664)	376 (247–504)	>1000
		LC_{10}	~100–1000*	1788 (231–3346)	1760.4 (ND)	16.9 (ND)	895.9 (ND)	21.7 (1.1–42.4)	800 (ND)
Aquatic	24	LC_{50}	248.3 (211.9–284.7)	11.1 (10.1–12.1)	54.7 (51–58.3)	43.1 (29.4–56.9)	>0.5	>0.5	>0.006
		LC_{10}	132.8 (101.7–164)	6.3 (4.8–7.8)	34.1 (28.3–39.9)	8.1 (4.5–11.6)	>0.5	>0.5	>0.006
	48	LC_{50}	131.3 (103.5–159.1)	5.9 (5.1–6.7)	10.8 (9.8–11.9)	10.1 (8.5–11.7)	0.5 (0.4–0.5)	0.2 (0.1–0.2)	>0.006
		LC_{10}	40.3 (25.5–55)	3.7 (2–5.4)	7.1 (5.1–9.1)	3.9 (2.3–5.4)	0.3 (0.2–0.4)	0.012 (0.002–0.021)	>0.006
Agar	24	LC_{50}	12.6 (9.6–15.6)	14.1 (ND)	86.9 (ND)	488.8 (100.4–877.1)	2372.3 (ND)	>1000	>1000
		LC_{10}	3.3 (1.4–5.2)	13 (ND)	81.3 (ND)	403.7 (ND)	~10–100*	108.2 (ND)	>1000
	48	LC_{50}	10 (7.8–12.2)	14.2 (ND)	53.3 (ND)	103.9 (95.3–112.4)	869.9 (ND)	376 (247.4–504.5)	>1000
		LC_{10}	3.1 (1.5–4.6)	12.1 (7.7–16.5)	47.3 (ND)	61.6 (32.2–91.1)	~10–100*	21.7 (1.1–42.4)	>1000

The values were calculated from logistic regression and 90% confidence intervals are shown in parentheses. ND means that confidence interval was not possible to determine. Asterisks mean rough non-calculated estimate from dose–response curve.

To summarize these comparisons, *C. elegans* was frequently less sensitive in soil test than tests using other soil invertebrates. First apparent reason for these observations might be different duration of the tests – 24/48 h for *C. elegans* vs. 28 days for *F. candida* and *E. crypticus* and 2 or 8 weeks for *E. fetida*. On the other hand, the duration of the *C. elegans* test is related to its life-cycle duration similarly like duration of other tests. This issue needs to be answered experimentally in the future. Other factors, which might be the reason of different sensitivity of the *C. elegans* soil test, are inter-species variability of sensitivity to different chemicals and different exposure routes for different types of organisms. Because the sensitivity of *C. elegans* is comparable to other species in aquatic medium, lower sensitivity in the soil test might be also caused by different exposure route. It seems that exposure of nematodes is limited strictly to pore-water dissolved toxicants while ingestion may contribute to the exposure of larger species.

3.4. Equilibrium partitioning calculations

Using the toxicity results from soil and aquatic tests with *C. elegans* the K_{oc} values were experimentally determined for all tested compounds on the base of toxicity tests results and equilibrium partitioning method (van Beelen et al., 2003). This possibility is quite rare advantage of some test species that can be exposed in both soil and aquatic medium in the same conditions and time duration. Both LC_{10} and LC_{50} values after 24 and 48 h of exposure were used to derive four calculated K_{oc} values for each compound (Table 2).

Table 2
K_d and K_{oc} values calculated according to EqP-method from LC in soil and aquatic tests

f_{oc}=0.0235	$K_{d\ (LC_{10}\ 24\ h)}$	$K_{oc\ (LC_{10}\ 24\ h)}$	$K_{d\ (LC_{10}\ 48\ h)}$	$K_{oc\ (LC_{10}\ 48\ h)}$	$K_{d\ (LC_{50}\ 24\ h)}$	$K_{oc\ (LC_{50}\ 24\ h)}$	$K_{d\ (LC_{50}\ 48\ h)}$	$K_{oc\ (LC_{50}\ 48\ h)}$	K_{oc}
Quinoline	8.4	359.5	2.5–24.8	106.4–1055	>10.1	>429.8	>19.0	>808.5	66 [a] 692 [b]
Acridine	15.9–158.7	676.6–6753	483.2	20,564	>225.2	>9583	>423.7	>18,030	1549 [a] 18,836 [b]
Phenazine	11.0	467.0	247.9	10,551	>36.6	>1557	>185.2	>7881	427 [a] 1549 [c]
1,10-Phenanthroline	52.0	2214.3	4.3	184.4	>58.0	>2468	>247.5	>10532	37 [a] 8318 [b, d]
Chlorinated paraffin	>2583	>109,915	2986	127,078	<10,900	>463,830	<17,666	>7,517,454	199,500 [e] 13,110 [f]
Toxaphene	>226.4	>9634	1808	76,950	<2570	>109,362	1880	80,000	210,000 [g] 6000 [h]

Organic carbon content f_{oc} for tests and calculations was 0.0235. Last column shows for comparison K_{oc} values from literature.
[a] Karickhoff et al. (2003).
[b] Jonassen (2003).
[c] Physical Properties Database (PHYSPROP) www.piskac.cz/ETD/Default.htm.
[d] K_{oc} for 1,10-phenanthroline was not available; hence K_{oc} for phenanthridine was used.
[e] HSDB (2001).
[f] European Commission (1999).
[g] Thompson et al. (1998).
[h] Ecotoxicological database at www.piskac.cz/ETD/Default.htm.

Calculated K_{oc} values were very different when based at different exposure times or from LC_{10} and LC_{50} values. Experimentally obtained values were substantially different from the values found in literature or databases. From these constraints it can be concluded that in the case of *C. elegans* this approach is not working. This can have numerous reasons. It is possible that toxicity, particularly mortality, is very robust parameter to assess sorption in soil. Further, organic matter in soil can be different and its sorption characteristics can also vary a lot. Moreover, compounds can be sorbed also by other soil constituents like clays (Seki and Yurdakoç, 2005). It is hard to compare results from different studies performed with different soils. In short, it is hard to compare our experimental values with values from other studies where different soil was used and also different methods from standardized procedures to model calculations from K_{oc}.

4. Conclusions

C. elegans is model species representing very important group of soil invertebrates – nematodes, which has key functions in the soil environment. This is the first reason why they should be part of the test batteries for risk assessment of soil contamination by POPs. Further, it was shown by our study that *C. elegans* aquatic and soil tests are suitable for POPs testing although some constraints exist. Agar test is hard to interpret, because exposure and bioavailability are not clear. The sensitivity of *C. elegans* was found lower than of other invertebrates in soil but comparable in aquatic medium. Duration of the test is recommended to be kept 48 h minimally and prolongation might increase the sensitivity of the tests. The tests with nematodes should be included to the test battery for risk assessment of POPs in soil. The question is if other, maybe more ecologically relevant and more sensitive nematode species should be used instead of *C. elegans*.

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References

Anderson GL, Boyd WA, Williams PL. Assessment of sublethal endpoints for toxicity testing with the nematode *Caenorhabditis elegans*. Environ Toxicol Chem 2001;20:833–8.

ASTM. ASTM Standard E 2172-01: standard guide for conducting laboratory soil toxicity with the nematode *Caenorhabditis elegans*. Annual Book of ASTM Standards, vol. 11. American Society for Testing Materials; 2001. p. 1606–16.

Bezchlebová J, Černohlávková J, Lána J, Sochová I, Hofman J. Ecological risk assessment of toxaphene residues in soils. Poster presentation, NICOLE Network Meeting, Sofia, Bulgaria, November 15–16, 2004; 2004.

Bezchlebová J, Černohlávková J, Lána J, Kobetičová K, Sochová I, Přibylová P, et al. Effects of short chain chlorinated paraffins on soil organisms. Ecotox Environ Safe; in press. doi:10.1016/j.ecoenv.2006.12.015.

Bleeker EAJ, Wiegman S, Droge STJ, Kraak MHS, van Gestel CAM. Towards an improvement of the risk assessment of polycyclic (hetero)aromatic hydrocarbons. Report 2003-01, department of aquatic Ecology and Ecotoxicology. Amsterdam, The Netherlands: University of Amsterdam; 2003. p. 27–32.

Boyd WA, Williams PL. Comparison of the sensitivity of three nematode species to copper and their utility in aquatic and soil toxicity tests. Environ Toxicol Chem 2003;22:2768–74.

Brenner S. The genetics of *Caenorhabditis elegans*. Genetics 1974;77:71–94.

Ditoro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, et al. Technical basis for establishing sediment quality criteria for nonionic organic–chemicals using equilibrium partitioning. Environ Toxicol Chem 1991;10:1541–83.

Dhawan R, Dusenbery DB, Williams PL. A comparison of metal-induced lethality and behavioral responses in the nematode *Caenorhabditis elegans*. Environ Toxicol Chem 2000;19:3061–7.

Donkin SG, Dusenbery DB. A soil toxicity test using the nematode *Caenorhabditis elegans* and an effective method of recovery. Arch Environ Contam Toxicol 1993;25:145–51.

European Commission. European union risk assessment report. Alkanes, C10-13, chloro-, CAS-No.: 85535-84-8, EINECS-No:287-476-5, Luxembourg, European Chemical Bureau; 1999.

Feldmannová M, Hilscherová K, Maršálek B, Bláha L. Effect of *N*-heterocyclic polyaromatic hydrocarbons (*N*-PAHs) on survival, reproduction and biochemicals parameters in *Daphnia magna*. Environ Toxicol 2006;21:425–31.

Fuchsman PC, Barber TR, Sheehan PJ. Sediment toxicity evaluation for hexachlorobenzene: spiked sediment tests with *Leptocheirus plumulosus*, *Hyalella azteca*, and *Chironomus tentans*. Arch Environ Contam Toxicol 1998;35:573–9.

Haanstra L, Doelman P, Voshaar JHO. The use of sigmoidal dose–response curves in soil ecotoxicological research. Plant Soil 1985;84:293–7.

Holoubek I. Persistent bioaccumulative and toxic chemicals in Central and Eastern Europe: levels and risks. Crit Rev Anal Chem 1999;29:179–85.

HSDB Toxaphene toxicology data file number 1616. U.S. Department of Health and Human Services. Hazardous Substances Data Bank (HSDB, online database – Toxicology data network – TOXNET). National Toxicology Information Program, National Library of Medicine. Bethesda, MD; 2001.

Jonassen KEN. Determination of physico-chemical constants in sorption of polycyclic aromatic compounds to soil organic matter – investigated by use of soil organic matter HPLC columns. Ph. D. Thesis in Environmental Chemistry, Riso National Laboratory, Plant research department, Roskilde, Denmark and Roskilde University, Institute of life science and chemistry, Roskilde, Denmark.

Jones KC, de Voogt P. Persistent organic pollutants (POPs): state of the science. Environ Pollut 1999;100:209–11.

Kammenga JE, van Koert PHG, Riksen JAG, Korthals GW, Bakker J. A toxicity test in artificial soil based on the life-history strategy of the nematode *Plectus acuminatus*. Environ Toxicol Chem 1996;15:722–7.

Karickhoff SW, Brown DS, Scott TA. Sorption of hydrophobic pollutants on natural se diments. Water Res 13: 241–248. In: Doucette WJ. 2003. Quantitative structure–activity relationships for predicting soil–sediment sorption coefficients for organic chemicals. Environ Sci Technol 1979;22:1771–88.

Parkhurst BR, Bradshaw AS, Forte JL, Wright GP. The chronic toxicity to *Daphnia magna* of acridine, a representative azaarene present in synthetic fossil fuel products and wastewaters. Environ Pollut 1981;24.

Peredney CL, Williams PL. Utility of *Caenorhabditis elegans* for assessing heavy metal contamination in artificial soil. Arch Environ Contam Toxicol 2000;39:113–8.

Seki Y, Yurdakoç K. Paraquat adsorption onto clays and organoclays from aqueous solution. J Colloid Interface Sci 2005;287:1–5.

Sochová I, Hofman J, Holoubek I. Using nematodes in soil ecotoxicology. Environ Int 2006;32:374–83.

Southworth GR, Beauchamp JJ, Schmieder PK. Bioaccumulation potential and acute toxicity of synthetic fuel effluents in freshwater biota: azaarenes. Environ Sci Technol 1978;12:1062–6.

StatSoft, Inc. STATISTICA for Windows [Computer program manual]. Tulsa, OK: StatSoft, Inc., 2300 East 14th Street, Tulsa, OK 74104, phone: (918) 749–1119, fax: (918) 749–2217, email: info@statsoft.com, WEB: http://www.statsoft.com; 2001.

Sverdrup LE, Kelley AE, Krogh PH, Nielsen T, Jensen J, Scott-Fordsmand JJ, et al. Effects of eight polycyclic aromatic compounds on the survival and reproduction of the springtail *Folsomia fimetaria* (Collembola, Isotomidae). Environ Toxicol Chem 2001;20:1332–8.

Sverdrup LE, Krogh PH, Nielsen T, Stenersen J. Relative sensitivity of three terrestrial invertebrate tests to polycyclic aromatic compounds. Environ Toxicol Chem 2002;21:1927–33.

Thompson RS, Gillings E, Cumming RI. Short-chain chlorinated paraffin (55% chlorinated): determination of organic carbon partition coefficient. Zeneca (Zeneca Confidential Report BL6426/B). UNECE Dossier; 1998.

van Beelen P, Verbruggen EMJ, Peijnenburg WJGM. The evaluation of the equilibrium partitioning method using sensitivity distributions of species in water and soil. Chemosphere 2003;52:1153–62.

Williams PL, Dusenbery DB. Aquatic toxicity testing using the nematode, *Caenorhabditis elegans*. Environ Toxicol Chem 1990;9:1285–90.

Wood WB, editor. The nematode *Caenorhabditis elegans*. New York: Cold Spring Harbor Laboratory; 1988.

Yeates GW. Nematodes as soil indicators: functional and biodiversity aspects. Biol Fertil Soils 2003;37:199–210.