Arch. Environ. Contam. Toxicol. 2[illegible]-94 (1994)

Ref

59258

ARCHIVES OF
Environmental
Contamination
and Toxicology
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Patterns of Sensitivity to Cadmium and Pentachlorophenol among Nematode Species from Different Taxonomic and Ecological Groups

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Received: 19 July 1993/Revised: 6 December 1993

Abstract. The variation of acute toxicity data among nematode species belonging to different taxonomic and ecological groups was investigated. Twelve different nematode species were extracted from the soil and directly exposed to cadmium and pentachlorophenol. LC_{50}-values were estimated after 24, 48, 72, and 96 h of exposure in aqueous solutions. The species exhibited large differences in sensitivity. LC_{50}-values (72 h) for pentachlorophenol ranged from 0.5 to more than 34.5 μmol/L and for cadmium from 29 to more than 800 μmol/L. These toxicity data could be described by a log-logistic distribution function.

LC_{50}-values for cadmium were not correlated with those for pentachlorophenol. Species of the subphylum Secernentia were less sensitive to pentachlorophenol than species of the subphylum Penetrantia, while no differences were observed for cadmium. In addition, no relationship was found between toxicity data and life-history strategies. Slow colonizers (K-strategists, *sensu lato*) were not more sensitive to cadmium and pentachlorophenol than opportunistic species (r-strategists, *sensu lato*). Nematodes appeared to be as sensitive to pentachlorophenol as other soil invertebrates. Nematodes were generally tolerant to cadmium.

Nematodes, or threadworms, are ubiquitous in aquatic and terrestrial habitats and constitute one of the largest animal phyla. Population densities depend on various biotic and abiotic factors and may range to $1.10^7/m^2$ in soil (Sohlenius 1980).

Soil inhabiting nematodes can be divided into different feeding groups: plant, bacterial and fungal feeders, predators, and omnivores (Yeates *et al.* 1993). Plant feeding nematodes are of agricultural importance because of their detrimental effects to crops (Wallace 1977). Bacterivorous and fungivorous nematodes are key intermediaries in decomposition processes of organic matter in soil (Freckman 1988). These nematodes stimulate N-mineralization and play an important role in the nutrient cycle (Anderson *et al.* 1981; Yeates and Coleman 1982; Woods *et al.* 1982). Predators and omnivores regulate the density of prey populations by feeding on nematodes, algae, and other soil organisms.

Nematodes offer perspectives for ecotoxicological research because of their abundance, species diversity, and differences in sensitivity to chemicals. They can be extracted from the soil efficiently, and identification has become within reach of non-specialists (Bongers 1988). A large number of species can be reared in the laboratory and are relatively easy to handle.

Nematodes live in the interstitial water between soil particles; therefore, toxicity studies can be conducted in water. Since the bioavailability of toxicants for soil organisms depends largely on the concentration in the interstitial water (Van Gestel and Ma 1988; Aben *et al.* 1992), nematodes are directly exposed to environmental contaminants.

Various papers have investigated the toxicity of chemicals, *e.g.*, pesticides (Bunt 1980; Kämpfe and Wischgoll 1984) and heavy metals (Vranken and Heip 1986; Williams and Dusenbery 1990; Vranken *et al.* 1991) to nematodes. However, only a limited number of species have been tested, and routes of uptake and test conditions differed too much to allow for a proper comparison. Therefore, these studies provide no insight in the patterns of sensitivity among species in the phylum Nematoda.

This paper proposes to (i) obtain insight in the sensitivity of nematode species for two distinct toxicants and (ii) determine to what extent acute toxicity data are correlated with taxonomic and ecological similarities. Therefore, 12 species, representing distinct ecological groups and belonging to different subphyla, families and genera, were exposed to cadmium and pentachlorophenol in aqueous solutions. Both compounds are ubiquitous in the environment and have different modes of action, and their properties are well documented (Ros and Slooff 1988; Anonymous 1987). Cadmium occurs in zinc and phosphate ores and is mainly emitted into the environment by ore mining industries. Cadmium accumulates in the top layer of the soil

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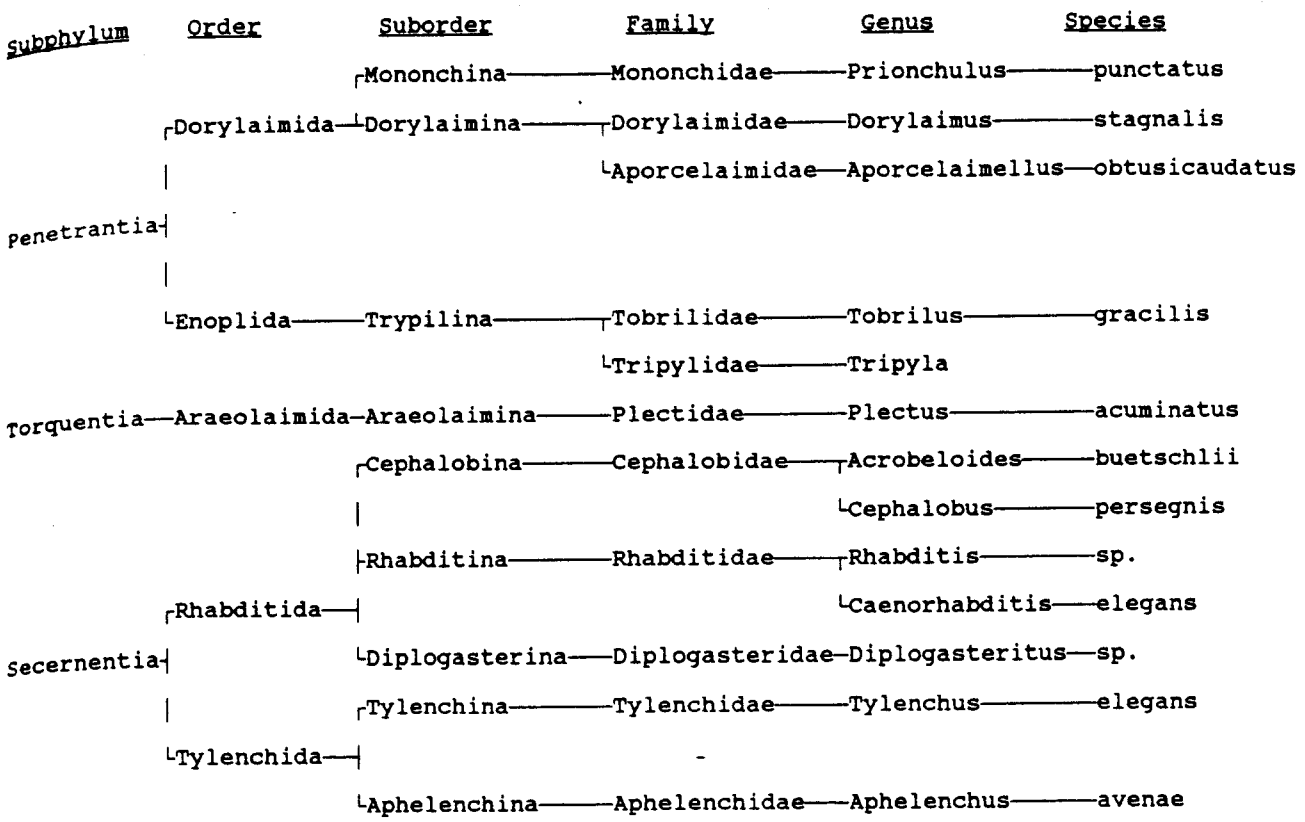

Fig. 1. Taxonomical classification of the nematode species tested

where it may affect terrestrial organisms. Pentachlorophenol is widely used as a wood preservative or fungicide. Once emitted into the air, pentachlorophenol is readily deposited on the soil.

Materials and Methods

Chemicals

Cadmium chloride ($CdCl_2$) was purchased from Merck, Darmstadt, Germany, and pentachlorophenol from Fluka Chemie A.G., Switzerland. The compounds were >99% pure. All other chemicals (Merck) were of the highest analytical grade.

Chemical Analysis

Analysis of cadmium and pentachlorophenol in water was performed with Atomic Absorption Spectrophotometry (Perkin Elmer 3030 AAS, flame furnace, detection-limit: 0.1 μg/L) and HPLC (Spectro Flow 757, Lichrosorb RP18 column (7 μm × 20 cm), UV-detector, 254 nm, detection limit: 50 μg/L, flow rate 0.7 ml/min), respectively.

Nematodes

Nematodes were extracted from the top mineral layer of arable soil, forest floor and stream sediment. Sites were selected where different species predominate. Samples were taken near Wageningen, The Netherlands, and recovered from the soil by means of the modified Oostenbrink-method (Oostenbrink 1954; 1960) during late spring. In this way multi-species suspensions were obtained, free from debris and soil particles. Before toxicity testing, adult nematodes were identified to species level. Representatives of different trophic levels were selected: bacterial feeders (*Rhabditis* species, *Cephalobus persegnis*, *Plectus acuminatus*, *Acrobeloides buetschlii*, and *Diplogasteritus* species), a fungal feeder (*Aphelenchus avenae*), plant feeders (*Tylenchus elegans*), carnivores (*Prionchulus punctatus*, *Tobrilus gracilis*) and omnivores (*Dorylaimus stagnalis*, *Aporcelaimellus obtusicaudatus*). The bacterial feeder *Caenorhabditis elegans* (Bristol, N2 strain), was obtained from a laboratory culture. Representatives of the genera *Rhabditis* and *Diplogasteritis* could not be identified to species level due to inconsistent taxonomical status. Figure 1 shows the taxonomical classification of the phylum Nematoda and the species which were used for this study.

Toxicity Tests

LC_{50}-tests were performed in water containing a defined mixture of minerals with concentrations resembling those found in interstitial water of sandy forest soils (K^+: 0.1 mmol/L, Na^+: 0.2 mmol/L, Ca^{2+}: 0.35 mmol/L, Mg^{2+}: 0.3 mmol/L, NH^{4+}: 0.3 mmol/L, NO^{3-}: 1.7 mmol/L, Cl^-: 0.3 mmol/L) (Schouten and Van der Brugge 1989). The pH was adjusted to 6.0 ± 0.1 with NaOH and checked at the end of each experiment. In the control, all tested nematode species survived in this water for at least 3 days. Pentachlorophenol was initially dissolved in ethanol (96%), after which stock solutions in water were made. The stock solutions were sonificated for 60 min. The amount of ethanol did not exceed 0.1 ml/L of the total test volume. The control contained 0.1 ml/L ethanol.

Two replicates of the tests were carried out in multi-dishes (Greiner, 24 compartment plate, no. 662160) with lid and sealed with parafilm to minimize volatilization. Dishes were stored at 20 ± 0.1°C in the dark.

Table 1. Acute toxicity of cadmium to nematodes in aqueous solutions. LC_{50}-values (μmol/L) of cadmium at subsequent time intervals (95% confidence intervals in brackets) (—: not determined)

Species	Exposure time (h) 24	48	72
Prionchulus punctatus	216.0 (124.1–375.9)	—	—
Dorylaimus stagnalis	336.0 (170.2–663.2)	154.1 (130.8–181.6)	91.8 (65.9–127.9)
Aporcelaimellus obtusicaudatus	—	214.9 (171.4–269.3)	131.1 (101.0–170.2)
Tobrilus gracilis	204.0 (163.9–253.9)	177.1 (129.0–243.1)	120.2 (90.1–160.3)
Plectus acuminatus	398.0 (322.0–492.4)	132.8 (98.2–179.7)	107.0 (82.4–138.9)
Acrobeloides buetschlii	885.2 (701.1–1117.0)	838.6 (541.7–1298.5)	527.4 (395.0–704.2)
Cephalobus persegnis	174.6 (134.3–227.1)	105.9 (79.1–141.9)	82.9 (58.3–117.8)
Rhabditis species	270.2 (215.4–339.0)	170.4 (132.5–219.1)	125.3 (97.4–161.2)
Caenorhabditis elegans	262.0 (224.8–305.4)	134.6 (105.3–172.1)	130.9 (104.1–164.5)
Diplogasteritus species	37.3 (29.3–47.7)	29.4 (23.5–36.7)	29.4 (23.5–36.7)
Tylenchus elegans	>800 (96 h)		
Aphelenchus avenae	>800 (96 h)		

Each compartment was filled with 0.9 ml of water containing toxicant. Samples of 0.1 ml of the multi-species suspension, containing 10–50 adult individuals of each species, were taken and suspended in each compartment. The toxicity tests were carried out with six to eight concentration steps, the ratio between successive concentrations being 1.8. The concentrations used differed between each species due to differences in sensitivity observed in preliminary range-finding tests. Overall, the concentrations ranged from 7 μmol/L to 2 mmol/L for cadmium and 0.1 μmol/L to 38 μmol/L for pentachlorophenol. Mortality, which was recognizable by the decayed bodies, of the species was recorded after 24, 48, 72, or 96 h. Dead nematodes were not removed. Observations were done using an inverted microscope (magnification of 40–100).

Data Analysis

LC_{50}-values and their confidence intervals were calculated according to the trimmed Spearmann-Kärber method (Hamilton et al. 1977). Differences between LC_{50}-values were tested using a Student's t-test. It was assumed that the LC_{50}-values of the different nematode species were log-logistic distributed (Kooijman 1987), hence the cumulative frequency distribution can be written as:

$$\text{Prob}(\ln LC_{50} \leq x) = \{1 + \exp[(\alpha - x)/\beta]\}^{-1}$$

where α = sample mean LC_{50} of m species, $\beta = S_m \sqrt{3}/\pi$, and S_m = sample standard deviation (Kooijman 1987).

Results

For most species, LC_{50}-tests were conducted for 72 h, because longer exposure times resulted in increased mortality in the control groups. Table 1 shows the LC_{50}-values of cadmium for 12 species after different exposure times. There were large differences in sensitivity, with LC_{50}-values ranging from 29 to more than 800 μmol/L after 72 h. *Diplogasteritus* species was the most sensitive followed by *D. stagnalis*, *P. acuminatus*, *A. obtusicaudatus*, *T. gracilis*, *C. persegnis*, *Rhabditis* species, and *C. elegans* as intermediate and *A. buetschlii*, *T. elegans*, and *A. avenae* as insensitive. For *T. elegans* and *A. avenae* no mortality was recorded below 800 μmol cadmium/L after 96 h. There was no significant difference in sensitivity between species from the Secernentia and the Penetrantia. The LC_{50}-value decreased with time, and this decrease was most pronounced for *D. stagnalis* and *P. acuminatus*.

The LC_{50}-values for pentachlorophenol at subsequent time periods are given in Table 2. LC_{50}-values ranged from 0.5 to more than 34.5 μmol/L after 72 h exposure. *P. punctatus* appeared to be the most sensitive followed by *D. stagnalis*, *A. obtusicaudatus*, *T. gracilis*, and *T. elegans* as intermediate and *C. persegnis*, *Rhabditis* species, *P. acuminatus*, and *Diplogasteritus* species as relatively tolerant. *C. elegans*, *A. avenae*, and *A. buetschlii* were rather insensitive. The genus *Tripyla* was found in every sediment sample, although densities were too low to calculate and LC_{50}-value. It was observed, however, that some specimens were still alive at 33 μmol pentachlorophenol/L after 48 h. The LC_{50} declined most apparently with time for *P. punctatus*. Species of the subphylum Secernentia were less sensitive to pentachlorophenol as compared to the Penetrantia (t-test, $p \leq 0.05$).

The obtained toxicity data could be considered as independent trials from a log-logistic distribution. Figure 2 shows the calculated (α = 4.83, β = 0.42) and observed cumulative frequency data for cadmium, including the LC_{50}-value of 302 μmol cadmium/L in sea water for *Monhystera disjuncta* (Vranken *et al.* 1991). Figure 3 shows the calculated (α = 1.75, β = 0.66) and observed cumulative frequencies for pentachlorophenol, including the LC_{50}-value (18 μmol/L) in sea water for *M. disjuncta* (Vranken *et al.* 1991).

There appears to be no correlation between the sensitivity to cadmium and pentachlorophenol for different species. *D. stagnalis* and *A. obtusicaudatus* were intermediate sensitive to cadmium but relatively susceptible to pentachlorophenol. *A. avenae* was tolerant to both compounds whereas *C. elegans* was insensitive to pentachlorophenol but more sensitive to cadmium.

Chemical analysis revealed that the concentrations of cadmium and pentachlorophenol in the test compartments remained constant in time, so no loss due to adsorption, volatilization, or biodegradation occurred during the test. The pH measurements did not indicate a change throughout the test.

Discussion

Large variations in sensitivity to cadmium and pentachlorophenol were observed among the 12 tested nematode species.

Table 2. Acute toxicity of pentachlorophenol to nematodes in aqueous solutions. LC_{50}-values (μmol/L) of pentachlorophenol at subsequent time intervals (95% confidence intervals in brackets)

Species	Exposure time (h)		
	24	48	72
Prionchulus punctatus	6.4 (4.9–9.7)	1.1 (0.7–1.9)	0.5 (0.2–1.5)
Dorylaimus stagnalis	5.0 (3.8–7.0)	3.6 (2.9–4.6)	3.6 (2.9–4.6)
Aporcelaimellus obtusicaudatus	7.5 (3.3–17.2)	3.8 (3.5–4.2)	3.6 (3.2–4.1)
Tobrilus gracilis	2.8 (2.0–3.9)	2.6 (1.6–4.1)	1.9 (1.1–3.5)
Plectus acuminatus	19.5 (17.8–21.3)	19.1 (17.6–20.7)	18.7 (17.4–20.2)
Acrobeloides buetschlii	>34.5 (96 h)		
Cephalobus persegnis	>34.5	>34.5	9.6 (3.5–26.1)
Rhabditis species	>34.5	>34.5	9.1 (5.8–14.2)
Caenorhabditis elegans	>34.5 (96 h)		
Diplogasteritus species	26.6 (22.9–30.5)	26.4 (22.0–32.0)	25.4 (11.7–54.7)
Tylenchus elegans	13.9 (11.3–17.1)	6.4 (5.3–8.0)	4.5 (3.8–5.5)
Aphelenchus avenae	>34.5 (96 h)		

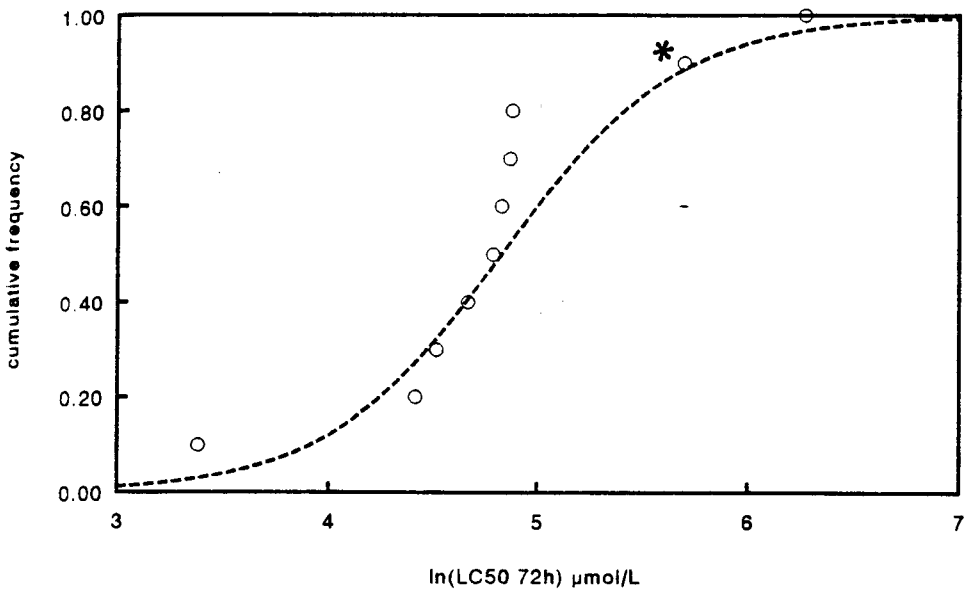

Fig. 2. Cumulative frequency distribution of ln(LC_{50}-values) for 10 nematode species exposed to cadmium. Log-logistic curve (---) and empirical values (○); * = data obtained from Vranken *et al.* (1991)

Acute toxicity levels differed with a factor > 27 (from 29 to more than 800 μmol/L) between lowest and highest LC_{50}-values for cadmium and a factor > 69 (from 0.5 to more than 34.5 μmol/L) for pentachlorophenol. The variation of LC_{50}-values among the tested nematode species could be described by a log-logistic distribution. These findings agree with the results obtained for aquatic organisms (Kooijman 1987).

Taxonomic similarities were only partly reflected in patterns of sensitivity. For pentachlorophenol all tested species of the subphylum Secernentia were significantly less sensitive than species from the subphylum Penetrantia. No consistent pattern was observed between the sensitivity to pentachlorophenol and cadmium. For example, the species *D. stagnalis* and *A. obtusicaudatus* were intermediate in sensitivity to cadmium but relatively susceptible to pentachlorophenol. *C. elegans* was insensitive to pentachlorophenol but more sensitive to cadmium. *A. avenae* was tolerant to both compounds. *T. elegans* was susceptible to pentachlorophenol but insensitive to cadmium.

The LC_{50}-values for both cadmium and pentachlorophenol decreased in time for all nematode species tested. LC_{50}-values were still declining after 72 h exposure, indicating that equilibrium between uptake and elimination had not been reached yet (Kooijman 1981). The LC_{50} decline for cadmium appears to be slower as compared to pentachlorophenol, suggesting that cadmium accumulates more slowly into the nematode body than pentachlorophenol. These results indicate that uptake and elimination rates of cadmium and pentachlorophenol differed between the various species.

There appears to be a correlation between feeding groups and pentachlorophenol sensitivity. Carnivorous, omnivorous, and plant feeding nematodes are relatively sensitive, whereas bacterial and fungal feeders are tolerant (Table 2). These findings indicate the vulnerability of particular feeding groups to pentachlorophenol contamination. Physiological research may gain insight into these apparent discrepancies.

Compared to other soil invertebrates, nematodes are relatively insensitive to cadmium (Table 3). Table 4 shows that the sensitivity of nematodes to pentachlorophenol is comparable to that of other invertebrate species.

The observed variations in sensitivity cannot be explained by differences in life-history strategies. It is often assumed, either explicitly or implicitly, that slow colonizing species (K-strate-

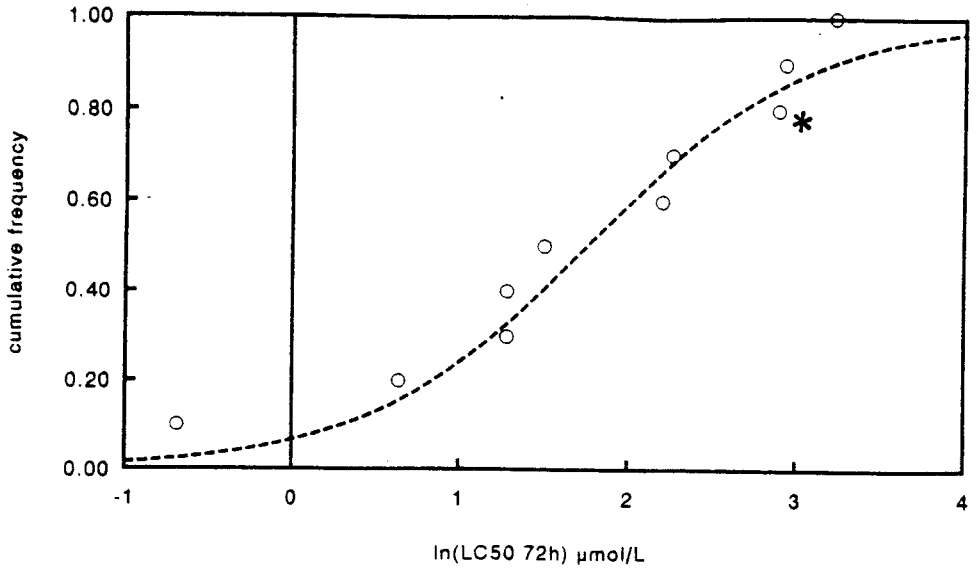

Fig. 3. Cumulative frequency distribution of ln(LC_{50}-values) for 10 nematode species exposed to pentachlorophenol. Log-logistic curve (---) and empirical values (○); * = data obtained from Vranken *et al.* (1991)

Table 3. LC_{50}-values (µmol/L) for cadmium for different soil invertebrate species including the highest compared to the lowest values in this study

Organism	Exposure time (h)	pH	LC_{50} (µmol/L)	Author
Nematoda				
T. elegans/A. avenae	96	6.0	>800	This study
Diplogasteritus species	72	6.0	29	This study
Panagrellus silusiae	24	?	480	Haight *et al.* (1982)
Monhystera disjuncta	96	7.5	302	Vranken *et al.* (1991)
Crustacea				
Parastenocaris germanica	96	6.8	20	Notenboom *et al.* (1992)
Oligochaeta				
Lumbricus rubellus	96	7.2	3	Ma (1982)
Tubifex tubifex	48	?	0.3	Brkovic-Popovic (1977)
Coelenterata				
Hydra oligactis	48	7.0	14	Slooff *et al.* (1983)

gists, *sensu lato*) are more sensitive to toxicants than opportunistic species (r-strategists, *sensu lato*) (Warwick 1986; Zullini and Peretti 1986; Wodarz *et al.* 1992). However, colonizing abilities are regulated by factors such as reproductive output, population growth rate, and density. As Boyce (1984) pointed out in a comprehensive review on r and K selection, the r/K model does not provide a causal relationship between demographic processes and environmental pertubations. Therefore, it is not surprising that we found no correlation between colonizing abilities and sensitivity to toxicants. Our results show that a rapid colonizer, *Diplogasteritus* species, was relatively more sensitive to cadmium than *A. obtusicaudatus*, a slow colonizing species. For pentachlorophenol, *T. elegans*, a fast coloniser, was equally sensitive to *D. stagnalis*, a K-strategist. These results corroborate with the findings for terrestrial arthropods (Van Straalen *et al.* 1989).

The heterogeneity of the ectodermal tissue among nematode species is assumed to play an important role in explaining the variation in acute toxicity data. Chemical compounds enter the nematode body mainly through the cuticle and ingestion does not play an important role (Castro and Thomason 1971). Low molecular weight compounds, like ethylene-dibromide and water, reside less than 1 s in *A. avenae* and *C. elegans*. The permeation of these compounds through the cuticle and hypodermata is a dynamic process which is actively controlled. Each substance enters the body at its own rate independent of the other chemical compounds, and permeation rates differ strongly among different nematode species (Castro and Thomason 1971). In addition, it was observed that the internal concentrations were generally higher than the external concentrations (Marks *et al.* 1968).

Large differences in heavy metal uptake can be observed in closely related species. Accumulation varies between species and they exhibit distinct uptake and elimination rates. Howell (1983) showed that two taxonomic closely related species, *Enoplus brevis* and *Enoplus communis*, exhibited distinct differences in heavy metal accumulation through the cuticle. A marked uptake of copper by *E. communis* was found (accumulation factor of 10.6), whereas in *E. brevis* the copper uptake was relatively smaller (accumulation factor of 5.0).

The observation that the subphylum Secernentia was less susceptible to pentachlorophenol than the subphylum Penetrantia might also be the result of differences of cuticle characteristics as such. Subphyla of nematodes can be distinguished on basis of their ectodermal characteristics (Maggenti 1981). The endo-cuticle, for instance, differs largely in thickness and col-

Table 4. LC_{50}-values (μmol/L) for pentachlorophenol for different soil invertebrate species including the highest compared to the lowest values in this study

Organism	Exposure time	pH	LC_{50} (μmol/L)	Author
Nematoda				
Caenorhabditis elegans, *Acrobeloides buetschlii*, *Aphelenchus avenae*	96 h	6.0	>34.5	This study
Prionchulus punctatus	72 h	6.0	0.5	This study
Monhystera disjuncta	96 h	7.5	18	Vranken *et al.* (1991)
Crustacea				
Parastenocaris germanica	96 h	6.8	0.14	Notenboom *et al.* (1992)
Oligochaeta				
Tubificids	24 h	7.5	1.2	Whitley (1968)
Eisenia fetida	14 days	5.6	5.67[a]	Van Gestel and Ma (1990)
Lumbricus rubellus	14 days	5.6	28.6[a]	Van Gestel and Ma (1990)

[a]Pore water values calculated from total soil concentrations using adsorption data

lagen density between the subphyla. Although there appears to be a structural pattern in cuticle and hypodermal morphology within the phylum Nematoda, further investigation is needed to ascertain these assumptions.

This paper illustrates that a large diversity exists within the phylum Nematoda with regard to sensitivity to toxicants. The response to xenobiotics seems difficult to predict and depends on the species tested and the nature of the chemical compound. This should be taken into account when assessing the hazard of chemical substances, based on toxicity tests with only few organisms (Van Straalen and Denneman 1989). Also environmental quality criteria should be based on the diversity of the physiological spectrum which exists in living organisms.

Acknowledgments. We thank H. H. B. van Megen and P. H. G. Van Koert for their technical assistance and A. M. T. Bongers, P. A. A. Loof, and H. Zell for identification of the nematode species. We also thank R. H. A. Plasterk, who kindly supplied cultures of *C. elegans*, and are much indebted to R. P. M. van Veen, M. J. van 't Hart, and C. C. Griffioen-Verschoor of the National Institute of Public Health and Environmental Protection for conducting chemical analysis. We also want to express our gratitude to N. M. van Straalen, F. J. Gommers, and two anonymous peer reviewers for correction of the manuscript and helpful suggestions. This research was granted by The Netherlands Integrated Soil Research Programme under Project No. 8981.

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