

Toxicity of cadmium to free-living marine and brackish water nematodes (*Monhystera microphthalma*, *Monhystera disjuncta*, *Pellioditis marina*)

G. Vranken, R. Vanderhaeghen & C. Heip

Marine Biology Section, Zoology Institute, State University of Ghent, Ledeganckstraat 35, B-9000 Ghent, Belgium

ABSTRACT: Cadmium toxicity to 3 marine and brackish-water nematodes (*Monhystera microphthalma*, *M. disjuncta*, *Pellioditis marina*) was estimated under static tests. These nematodes are very resistant to cadmium poisoning. LC 50 values are extremely time-dependent; an exposure time of 96 h underestimates the degree of toxicity. For *M. microphthalma* LC 50 values decreased from 23.6 ppm after 96 h to 5.4 ppm after 312 h. For *M. disjuncta* LC 50 values were 21.2 and 18.4 ppm after 192 and 264 h respectively. *P. marina* was much more tolerant to cadmium in comparison with the monhysterids and had LC 50 values of 90.5 ppm at 120, and of 77.0 ppm at 192 h. It is argued that MEC (minimum effective concentrations) values are ecologically more meaningful than LC 50 values, and that the former levels can be used as indicators of intolerable thresholds in the field. MEC values based on mortality and on a developmental assay in which success in attaining the adult stage was studied, turned out to be very similar. The developmental assay takes less time than the survival assays and the slope of the response curve can be used as a parameter for describing species sensitivity.

INTRODUCTION

In the last decade, numerous studies were published on effects of heavy metals on marine invertebrates. Throughout these studies one frequently encounters problems of application and evaluation of acute toxicity tests. A serious problem concerns the time limitation of these tests. Some studies (e.g. Brown & Ahsanullah 1971, Best & Morita 1983) indicate that for certain heavy metals the conventional 96 h tests are too short to determine acute toxicity effects adequately. Ahsanullah et al. (1981) suggest that even a period of 14 d is insufficient for completing meaningful acute lethal tests of cadmium, zinc and copper on the marine shrimp *Callinassa australiensis*. Nimmo et al. (1977) report for pink shrimp *Penaeus duorarum* a decrease in LC 50 values from 3.5 to 0.72 mg Cd l⁻¹ after 4 and 30 d respectively. In a test on grass shrimp *Palaemonetes vulgaris*, the same authors found a significant decrease in LC 50 values from 0.76 to 0.12 mg Cd l⁻¹ after 4 and 29 d respectively. Hoppenheit & Sperling (1977) used experimental periods of 30 wk to trace lethal effects of

cadmium on populations of the harpacticoid copepod *Tisbe holothuriae*. These values show that median lethal threshold concentrations for some metals are time-dependent. However, prolongation of experiments does not fit the requirements for a routine standardized laboratory toxicity test.

It is generally admitted that whereas studies on lethal toxicities offer a quantifiable measure of heavy metal toxicity, they also hide valuable information on sub-lethal effects manifesting themselves in changed population dynamics. Possible effects on generation time, metal accumulation in consecutive generations, impairment of reproduction etc. at relatively low test concentrations is overlooked when using these acute lethal toxicity procedures. Additional arguments against the use of acute lethal test data for evaluating environmentally 'acceptable' concentrations have been presented by Brown (1981).

A more realistic approach to obtaining 'safe' concentrations is the determination of minimal effect levels (instead of LC 50 values). Minimal effects concentrations (MEC) are concentrations at which the first

significant differences in the test criterion used appear, when compared to blanks. Thus MEC values depend on the criterion used, and this criterion should be a sensitive and ecologically meaningful parameter, e.g. the life-cycle events of the test species.

In this report, we study the toxicity of cadmium to 3 marine and brackish-water nematodes. We determine LC 50 values in consecutive experimental periods and compare these with MEC values based on juvenile mortality and development time.

MATERIAL AND METHODS

Test-species and food-organisms. The 3 nematode species used are bacterivorous. The cosmopolitan *Monhystera disjuncta* is a non-selective deposit-feeder (Gerlach & Riemann 1973). It was isolated from the 'sluice dock' of Ostend (Belgium), a marine lagoon near the coast. *M. microphthalma* is a brackish-water bacterivorous non-selective deposit-feeder. It was isolated from the 'Dievengat', a poly-mesohaline pond, situated in a polder in northwestern Belgium. *Pellioiditis marina* is a euryhaline and bacterivorous species (Tietjen et al. 1970). It too was isolated from the 'Dievengat' (consult Vranken & Heip 1983 for an agnotobiotic culture method). Adults of the 3 species have an individual wet weight of 0.5 µg. The total length of the juveniles used to start the experiments varied between 300 and 400 µm in *M. disjuncta* and *M. microphthalma*, and between 400 and 450 µm in *P. marina*. The 3 test species were grown in monoxenic bacterial cultures. An isolate (previously labeled as ISC₂) belonging to the *Alteromonas haloplanktis* rRNA branch was used as food-organism. This strain possesses an exceptionally high nutritional value for all 3 nematode-species (Vranken et al. 1984). The bacterial strain was isolated from the sluice dock of Ostend. Purification was done according to the streak plate method on marine nutrient-agar. DNA-rRNA hybridization (De Ley & De Smedt 1975) was used to identify the strain ISC₂. Bacterial cultures were grown separately either on solid medium (marine nutrient agar) or liquid medium (marine heart infusion broth), thus sim-

plifying the culture medium of Vranken et al. (1984). Addition of a sterol mixture (see below) turned out to be indispensable for completing the life-cycles in all 3 test-species.

Cultures. Temperature, salinity and pH were kept constant at favourable intensities for laboratory tests. *Pellioiditis marina* and *Monhystera disjuncta* were cultivated at 17 °C and 30 ‰S; *M. microphthalma*, at 25 °C and 20 ‰S. In all tests the pH ranged between 7 and 7.5. The nematodes were cultured in a nutrient-poor culture-medium (0.5 % bacto-agar) in small vented Petri dishes (Ø: 35 mm) to which the bacterial food was added in a central-ring-shaped excavation in the agar (Fig. 1). This technique has several advantages: (1) Bacterial growth is limited due to the lack of nutrients, resulting in a clear and easily observable culture; (2) axenization procedures can be restricted to a minimum because the development of other bacteria (contaminants) is very limited; (3) reproducibility of the tests is greatly enhanced, and mortality during pre-adult stages is nearly zero in the control.

Food preparation. Erlenmeyer flasks (100 ml) were filled with 50 ml heart infusion broth suspended in artificial seawater according to Dietrich & Kalle (1957) and then sterilized. The medium was inoculated with the bacterial strain (ICS₂ – *Alteromonas haloplanktis*) under a horizontal laminar air flow bench (NUAIRE-201 – Table Model). The culture was incubated for 48 h at 20 °C in a Heraeus-incubator and rotated in a rotary shaking machine (INFORS – AG-CH-4103) at 125 rpm. Bacterial growth was measured with a Vitatron UPM-Universal Photometer measuring the absorbance at 546 nm. Bacterial cells were harvested by centrifugation at 6000 rpm for 15 min. The pellet was resuspended in sterile artificial seawater and added to the cultures.

Nematode culture medium. Small vented Petri dishes (Ø 35 mm) were filled with 4 ml 0.5 % bacto-agar (Difco) in artificial seawater to which 0.2 ml of a sterol mixture was added (containing 10 µg ml⁻¹ each of: cholesterol, stigmasterol, ergosterol, 7-dehydrocholesterol, and β-sitosterol). After the agar cooled down, a central ring-shaped excavation (Ø: 15 mm) was made into the culture medium by pushing the top

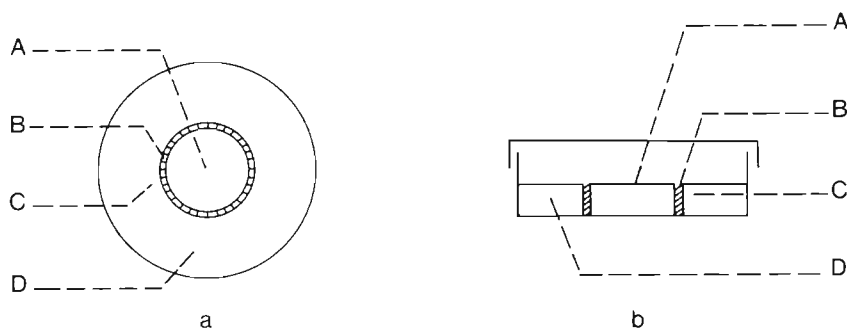


Fig. 1. Scheme of starting a standardized nematode culture. (a) Top view; (b) side view. A: central zone; B: ring-shaped excavation where bacterial suspension is added; C: zone with lower bacterial concentrations due to agar-diffusion and bioturbation; D: zone in which the axenized nematodes are inoculated

of a sterile test tube to the bottom of the Petri dish. This excavation was then filled with ± 0.05 ml of bacterial suspension.

Axenization of nematodes. With a treatment of 1000 IU penicillin and 1 mg ml⁻¹ streptomycin during 20 h in 0.5 % agar, all 3 test species could be axenized satisfactorily. *Monhystera microphthalma* and *Pellioiditis marina* had been successfully kept in monoxenic stock cultures for several months. In *M. disjuncta* egg mortality increased considerably when kept too long in monoxenic cultures; hence stock cultures of *M. disjuncta* were kept under agnotobiotic conditions, and axenization became necessary for starting monoxenic tests.

Toxicity tests. A stock solution of 1000 ppm Cd²⁺ was prepared in artificial seawater using CdCl₂ · 2½H₂O, and diluted to final concentrations of 1, 2.5, 7.5, 10 and 25 ppm Cd²⁺ for *Monhystera microphthalma*; 1, 5, 10 and 25 ppm Cd²⁺ for *M. disjuncta*; 1, 5, 10, 25, 50, 60, 70, 80 and 100 ppm Cd²⁺ for *Pellioiditis marina*. Monospecific bacterial cells (ISC₂) were grown at the same Cd concentrations as the tests, before they were added to the cultures. Acute Cd toxicity to this bacterial strain occurred between 25 and 50 ppm, so that toxicity tests above 25 ppm Cd were carried out using bacterial cells grown at 25 ppm Cd. Test organisms were first grown monoxenically on a diet of metal-free cells of *Alteromonas haloplanktis* until the age of 2.5 d (*P. marina*), 5.5 d (*M. microphthalma*) or 4.5 d (*M. disjuncta*). At this age juveniles were transferred to the different Cd concentrations.

Each concentration consisted of 4 replicates in which ca 110 nematodes were tested. For *P. marina* the control consisted of 8 replicates containing a total of 229 individuals. For *M. disjuncta* we used ca 60 individuals per concentration; the control consisted of 5 replicates (93 nematodes); from these, 4 replicates were monitored daily to estimate mean development time. For *M. microphthalma* the control consisted of 2 separate experimental sets, with 4 replicates each; 1 set was used to determine development time. For *M. microphthalma* the control consisted of 2 separate experimental sets, with 4 replicates each; 1 set was used to determine development time. Observations on mortality were made once daily. Criteria for death were inactivity and failure to respond after stimulation with a needle.

RESULTS

LC 50 and MEC

Homogeneity of mortality rates obtained in the different Cd concentrations was investigated by G_H-statistic (Sokal & Rohlf 1981), computed for final mortality-response. In only 2 of the 21 series conducted (2.5 and 7.5 ppm Cd, *Monhystera microphthalma*) did heterogeneous responses occur in the different replicates (0.001 < P < 0.01). This may have been the result of uncontrolled pH-fluctuations. In later experiments Tris buffer (0.5 mM) was used to compensate for similar difficulties.

Table 1. Minimal effect concentrations (MEC-values) and LC 50 values of nematodes for Cd²⁺, obtained over different incubation periods. Values in brackets following MEC values: mortalities in percentages. MEC values were computed at the P = 0.05 level with the G-test (Sokal & Rohlf, 1981); 95 % confidence limits around LC 50 calculated according to Sokal & Rohlf (1981); b and r²: slope and coefficient of determination of the regression of mortality (arc sin $\sqrt{\text{proportion}}$) against the Cd²⁺ concentrations; n: number of points used in the regression

Species	Exposure time (h)	G/q	MEC (ppm Cd ²⁺)	LC 50 (ppm Cd ²⁺)	95 % CI (ppm Cd ²⁺)	b (± SE)	r ²	n
<i>M. microphthalma</i>	72	101***	25 (24)	—	—	—	—	—
	96	224***	7.5 (8)	24	20.6–26.6	1.93 (± 0.112)	0.93	24
	144	495***	7.5 (26)	13	10.4–15.1	3.28 (± 0.190)	0.93	24
	240	663***	1 (6)	6	4.4–8.1	6.79 (± 0.663)	0.85	20
	312	757***	1 (6)	5	4.2–6.8	8.03 (± 0.550)	0.92	20
<i>M. disjuncta</i>	96	57***	25 (22)	—	—	—	—	—
	144	110***	10 (10)	—	—	—	—	—
	192	199***	10 (15)	21	17.0–26.2	2.53 (± 0.247)	0.91	12
	264	253***	10 (26)	18	13.9–23.5	2.83 (± 0.299)	0.90	12
<i>P. marina</i>	72	43***	60 (4)	—	—	—	—	—
	96	171***	50 (9)	—	—	—	—	—
	120	388***	50 (14)	91	76.0–109.0	0.74 (± 0.095)	0.73	24
	192	609***	50 (14)	77	66.6–88.4	1.02 (± 0.096)	0.84	24

*** P < 0.001

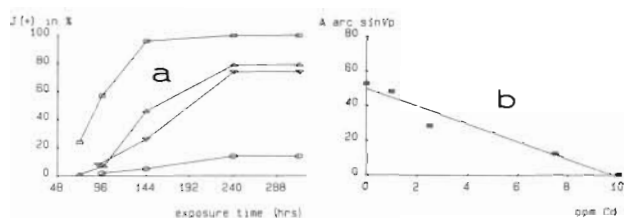


Fig. 2. *Monhystera microphthalma*. (a) Cumulative mortality (%) for different Cd concentrations: 2.5 ppm (O); 7.5 ppm (▽); 10 ppm (Δ); 25 ppm (□), at different exposure times (h). (b) Number of adults (%) after 72 h (= time period within which ca 50 % of the nematodes reached adulthood in the control)

For *Monhystera microphthalma* Fig. 2a shows the percentage mortality at the 4 highest Cd concentrations at 5 different observation times. Table 1 gives the G/q statistics (Sokal & Rohlf 1981), which test whether juvenile mortality is significantly influenced by the concentrations applied. These calculations demonstrate that at each observation time there exists a pronounced influence of Cd ($P < 0.001$). The minimal effect concentration (MEC values in Table 1) declines sharply from 25 ppm Cd after 72 h to 1 ppm Cd after 240 h exposure. At 25 ppm Cd, the highest Cd level tested for *M. microphthalma* mortality increased from 24 % after 72 h to 100 % after 240 h, whereas at the smallest concentration (1 ppm Cd) mortality varied between 0 % after 72 h and 6 % after 312 h.

LC 50 values were calculated for each exposure time by inverse prediction from the regression of mortality, transformed into angles (arc sin \sqrt{p} transformation) against Cd concentration. The slopes of these regressions with their standard errors (SE) are shown in Table 1. For *Monhystera microphthalma* the regressions for the different incubation times are highly sig-

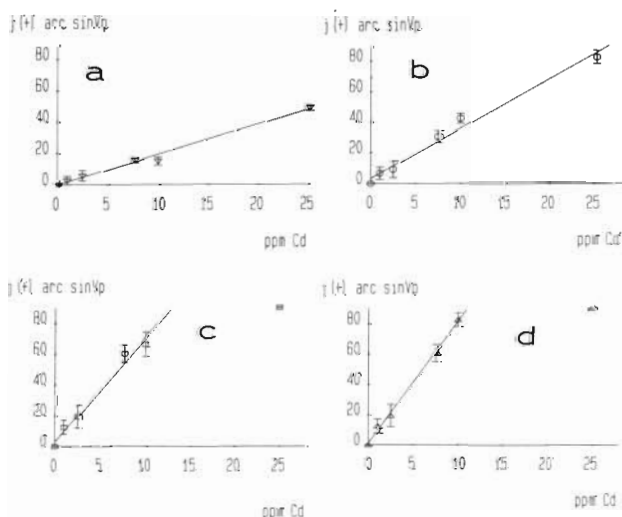


Fig. 3. *Monhystera microphthalma*. Juvenile mortality after 96 h (a); 144 h (b); 240 h (c); 312 h (d). Bars: standard errors of 4 simultaneous experiments

nificant ($P < 0.001$) (Fig. 3) with coefficients of determination (r^2) ranging between 0.93 (144 h) and 0.85 (240 h). LC 50 values decrease from 23.6 ppm Cd after 96 h to 6.2 ppm Cd after 240 h. A further but less substantial decrease of LC 50 was observed as incubation time increased from 240 h to 312 h. The values of the slopes (b in Table 1) may be considered a measure of sensitivity of the test-organism to the range of the toxicant. High b's indicate high and fast mortalities; small b's, insensitivity accompanied with small mortalities. For *M. microphthalma* b values increase from 1.93 after 96 h to 8.03 after 312 h. However b values are not independent from each other since they were calculated from the same experimental set. Therefore b values obtained for different exposure times cannot be compared statistically. Nevertheless, a 4-fold increase of b is noted with *M. microphthalma*. This shows clearly that Cd toxicity is highly dependent on exposure time (Fig. 3). LC 50 obtained after 96 h dramatically underestimates the toxicity of this particular metal.

Table 1 also shows the results obtained with the other test species. For *Monhystera disjuncta* a highly significant influence of Cd ($P < 0.001$) was noted after each observation time. MEC after 96 h was 25 ppm Cd; at this concentration 22 % of the juveniles died after 96 h (Fig. 4 c). After 264 h mortality at this concentration increased to 77 % (Fig. 4 c). At the other 3 observation times MEC remained at 10 ppm Cd. At 10 ppm Cd, mortality rose from 2 % after 96 h, a value not different from the blank, to 26 % after 264 h (Fig. 4 c). LC 50 values for *M. disjuncta* were 21.2 ppm after 192 h, and 18.4 ppm after 264 h. The data set obtained after 144 h does not encompass the LC 50.

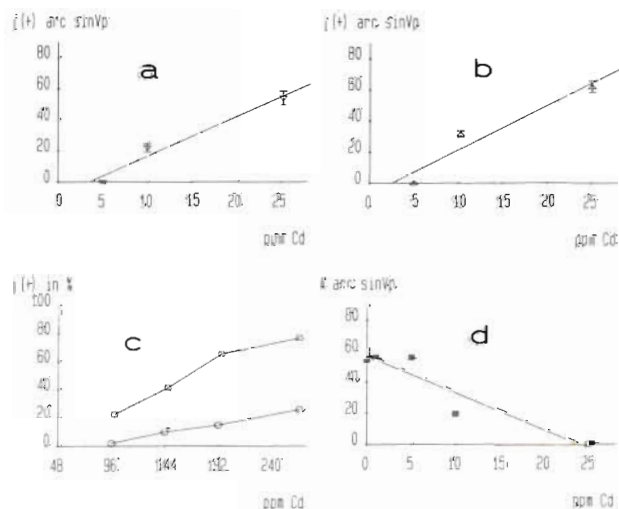


Fig. 4. *Monhystera disjuncta*. (a) Juvenile mortality after 192 h. (b) Juvenile mortality after 264 h. Bars: standard errors of 4 simultaneous replicates. (c) Cumulative mortality (%) for different Cd concentrations: 10 ppm (O); 25 ppm (□), at different exposure times (h). (d) Number of adults (%) after 120 h

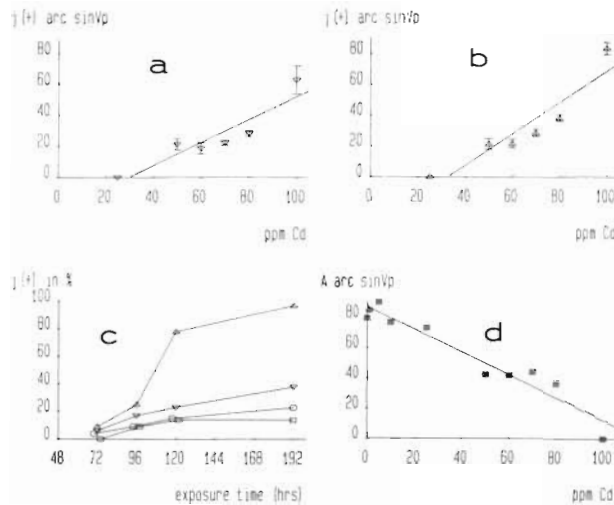


Fig. 5. *Pellioditis marina*. (a) Juvenile mortality after 120 h. (b) Juvenile mortality after 192 h; intervals in (a) and (b): standard errors of 4 replicates. (c) Cumulative mortality (%) for different Cd concentrations: 50 ppm (\square); 70 ppm (\circ); 80 ppm (∇); 100 ppm (Δ), at different exposure times (h). (d) Number of adults (%) after 72 h

For *Pellioditis marina* (Fig. 5c), mortality was 8 % at 100 ppm Cd after 72 h and 97 % at 100 ppm after 192 h. After 72 and 96 h we could not estimate the LC 50. LC 50 values after 120 and 192 h were 90.5 and 77 ppm Cd. Again, there is obvious increase in sensitivity as indicated by the b value (Fig. 5a, b) which increased from 0.74 after 120 h to 1.02 after 192 h. MEC was 60 ppm Cd after 72 h, and 50 ppm for the other periods (Table 1).

Development to adulthood

A developmental assay (Table 2), in which the percentages of individuals attaining the adult stage are compared with the control, was obtained at the time when at least 50 % of the individuals reached the adult stage in the blank. In *Monhystera microphthalma* and

Table 2. Minimal effect Cd concentrations at the $P = 0.05$ level (G-test) obtained from a developmental assay of nematodes in which the successfulness of attaining the adult stage is compared with the blank. Values in brackets: percentage of adult individuals (φ and δ) obtained after the exposure time indicated

Species	Exposure time (h)	G/q	MEC (ppm Cd ²⁺)	Control
<i>M. microphthalma</i>	72	346***	2.5 (23)	(63)
<i>M. disjuncta</i>	120	194***	10 (11)	(67)
<i>P. marina</i>	72	725***	50 (45)	(97)

*** $P < 0.001$

Pellioditis marina this percentage was monitored ca 72 h after initiation; in *M. disjuncta* it took 120 h before 67 % of the 4.5 d old juveniles reached adulthood in the blank. MEC obtained in this assay are listed in Table 2. For all 3 species, the decline in the percentages of individuals attaining adulthood (A) is a linear function of Cd concentration (C).

This can be described by the following equations:

M. microphthalma:

$$A = 49.74 - 5.09 C \quad (r^2 = 0.95; n = 5) \quad (\text{Fig. 2b}) \quad (1)$$

M. disjuncta:

$$A = 57.06 - 2.41 C \quad (r^2 = 0.88; n = 5) \quad (\text{Fig. 4d}) \quad (2)$$

P. marina:

$$A = 86.89 - 0.75 C \quad (r^2 = 0.93; n = 10) \quad (\text{Fig. 5d}) \quad (3)$$

Development times

Table 3 compiles mean development times (pooled sexes) for different Cd levels. While in *Monhystera disjuncta* and *Pellioditis marina* only the higher concentrations caused a prolongation of development time, in *M. microphthalma* even the lowest concentration (1 ppm) led to a significant increase in the developmental period (T_{\min}).

Table 3. Development time (T_{\min}) of pooled sexes in nematodes, with 95 % confidence interval (CI) in parentheses at different Cd²⁺ concentrations (ppm). N: number of adults (φ and δ pooled). Experiments were started with 5.5 d old juveniles of *Monhystera microphthalma*, 4.5 d old juveniles of *M. disjuncta*, and 2.5 d old *Pellioditis marina*. Time zero: moment of egg-deposition

Species	Cd ²⁺ (ppm)	T_{\min} (\pm CI) (d)	N
<i>M. microphthalma</i>	Control	8.9 (\pm 0.20)	113
	1	9.6 (\pm 0.35)	98
	2.5	9.6 (\pm 0.28)	86
	7.5	9.7 (\pm 0.35)	26
	10	14.5 (\pm 11.37)	3
	25	—	—
<i>M. disjuncta</i>	Control	9.6 (\pm 0.25)	61
	1	9.4 (\pm 0.24)	67
	5	9.4 (\pm 0.25)	59
	10	11.7 (\pm 0.72)	27
	25	—	—
<i>P. marina</i>	Control	5.2 (\pm 0.07)	229
	1	4.9 (\pm 0.09)	116
	5	5.0 (\pm 0.09)	114
	10	5.0 (\pm 0.11)	112
	25	5.6 (\pm 0.05)	107
	50	6.4 (\pm 0.24)	88
	60	6.0 (\pm 0.12)	94
	70	6.0 (\pm 0.16)	80
	80	6.0 (\pm 0.16)	64
	100	7.2 (\pm 1.40)	3

DISCUSSION

Sensitivity to heavy metals

The experimental procedure described in this study is completely new, very simple to work with, and results in homogeneous responses both in regard to mortality rates and development time. Our results document that the 3 nematode species tested exhibit large differences in tolerance to cadmium exposure. They all are relatively insensitive to Cd intoxication, hence high Cd levels are required to induce a 50 % death rate. Using 192 h LC 50 as criterion, *Monhystera microphthalma* can be classified as the most sensitive species; it is 2 times more susceptible to Cd than *M. disjuncta*, and 8 times more sensitive than *Pellioditis marina*. Acute lethal toxicity levels (LC 50) for the latter species are 90 ppm Cd after 120 h and 77 ppm Cd after 192 h. Similarly high LC 50 values have been recorded for other nematodes: LC 50 for the second juvenile stage (J2) of *Panagrellus silusiae* varied between 36 ppm Cd (24 h) and 5.85 ppm (72 h) (Haight et al. 1982); Feldmesser & Rebois (1966) report 48 h LC 50 between 35 and 40 ppm for mixed populations of *Panagrellus* and *Rhabditis*; Samoiloff et al. (1980) found no decrease in survival rates of *P. redivivus* at 1.12 ppm, the lowest concentration causing a significant reduction in survival was 11.2 ppm Cd.

Several marine organisms exhibit similarly high resistance levels to Cd intoxication: in fiddler crabs *Uca pugilator* acute toxicity levels ranged from ca 10 ppm (240 h) to 50 ppm (96 h) (O'Hara 1973, cited in Bryan 1976). The harpacticoid copepod *Nitocra spinipes* (Bengtsson 1978) exhibits intermediate sensitivities with an 96 h LC 50 of 1.8 ppm Cd. The hydroids *Laomedea loveni* (Theede et al. 1979) and *Eirene viridula* (Karbe 1972) are particularly sensitive to Cd exposures; their LC 50 values range from 3 to 80 ppb (168 h), and from 100 to 300 ppb Cd, respectively ('akute Schädwirkungen'). The freshwater *Daphnia magna* is also very sensitive compared with the nematodes; it has a 48 h LC 50 of 65 ppb Cd (Biesinger & Christensen 1972). The brine shrimp *Artemia* sp. has a 168 h LC 50 of 50 ppm Cd (Brown & Ashanullah 1971).

These examples indicate considerable differences between taxa. Such differences may result from differences in permeability, uptake mechanisms, and/or rates of Cd accumulation (George & Coombs 1977, Coombs & George 1978, George et al. 1978). They may also be attributed to the presence or absence of Cd binding proteins (metallothioneins) that possess a Cd detoxication and immobilization function (Olafson & Thompson 1974, Lee et al. 1977), or to the capacity for storing metals in granules and vesicles, thus sequester-

ing the metal, and hence eliminating or at least reducing the toxic impact of the metal (Coombs & George 1978).

It has been reported repeatedly that Cd toxicity drops with increasing salinity (see Borgmann 1983 for a review). Such reduction of Cd toxicity at higher salinities has been attributed to increased complexation and increased competition with calcium and/or magnesium (Borgmann 1983). Phillips (1976) and George et al. (1978), working with the mussel *Mytilus edulis*, found a decrease in Cd uptake as salinity increases. Therefore, a certain amount of the differences in Cd tolerance reported here between the 3 nematode species may have been induced by differences in culture conditions: *Monhystera microphthalma* was kept at 20 ‰, the other 2 species at 30 ‰.

The medium we used is relatively poor in nutrients and composed of bacto-agar (a highly purified agar), enriched with a mixture of 5 sterols. The agar might selectively bind metals and hence reduce their toxicity. Concerning the complexation of Cd by sterols, no information was found in the literature. Ramamoorthy & Kushner (1975) reported that in comparison with Hg^{2+} , Pb^{2+} and Cu^{2+} , very little Cd^{2+} is bound by microbial growth media. Casamino-acids, not used in our medium, were the most active binding substances. In fresh water, the free cadmium ion Cd^{2+} is the dominant Cd form (Gardiner 1974, Bryan 1984), whereas at 20 and 30 ‰ (as in our experiments) Cd is almost completely complexed and present in the form of chloride complexes (Sunda et al. 1978). As Cd toxicity is a function of free Cd ion concentration (Sunda et al. 1978, Bryan 1984), the low Cd toxicity to the species we studied, especially in comparison with freshwater species, is comprehensible. Presumably, chloride complexation is more important in our medium than organic complexation.

Field observations

A high resistance of nematodes to environmental stress, especially due to heavy metals, is known from the field; in some polluted areas nematodes are the major survivors (Van Damme & Heip 1977). Among the nematodes, especially the non-selective deposit feeders (to which the 3 tested species belong) are extremely pollution resistant. In the Southern Bight of the North Sea, a complete compositional shift within the nematode community has been observed from 'healthy' stations, possessing a diverse nematode community composed of all feeding types, to pollution stressed 'diseased' environments, inhabited by a 'poor' nematode community composed almost completely of

non-selective deposit feeders. Such structural impoverishment has been correlated with heavy metal pollution (Heip et al. 1984). These observations corroborate the acute toxicity levels reported in this paper; therefore, the high LC 50 values observed in our assays are not artefacts due to the optimal culture conditions employed.

Effects of exposure time

We can draw 2 additional important conclusions: (1) 96 h LC 50 values dramatically underestimate the toxicity of Cd; (2) LC 50 values are very time-dependent. In *Monhystera microphthalma* we found a 4-fold reduction of LC 50 by extending the exposure time from 96 h to 312 h. In *M. disjuncta* and *Pellioiditis marina*, 96 h LC 50 values were not determinable; in the first-named species LC 50 values were reduced from 28 ppm at 144 h to 18.4 ppm at 264 h. A similar time-dependency of LC 50 has been reported in the literature. For the nematode *Panagrellus silusiae* Cd LC 50 was reduced from 111 ppm after 24 h, to 13.2 ppm for an exposure time of 72 h (Haight et al. 1982); LC 50 for the grass shrimp *Palaemonetes vulgaris* was reduced from 760 ppb to 120 ppb Cd by extending the exposure time from 96 h to 29 d (Nimmo et al. 1977), and Best & Morita (1983) reported that for the asexual planarian *Dugesia dorocephala* the 5 d LC 50 was in the 2 to 3 ppm range; the 12 d LC 50, less than 0.4 ppm Cd.

Possible effects of starvation

As exposure time increases, death caused by Cd-induced starvation has to be considered. In the course of our experiments, the intestines of well-fed juveniles of both *Pellioiditis marina* and *Monhystera disjuncta* changed colour. Initially, the intestine was darkly coloured; after some time it turned very pale with a typical spotted pattern. These changes were less obvious in *M. microphthalma*. This indicates to us that some individuals stop, or at least reduce, their food uptake. Hence, some deaths may have been caused by starvation. Mudry et al. (1982) made analogous observations on *Panagrellus silusiae*; some individuals (numbers decreasing with experimental time) continue to feed in the presence of heavy metals, whereas in most of them pharyngeal pumping was completely suppressed, resulting in inactivation of normal feeding. Mudry et al. (1982) considered the cuticle as a barrier preventing the passage of heavy metals. However, Lopez et al. (1979) calculated that pumping rates in the brackish water nematode *Adoncholaimus*

thalassophygus were too low to explain the observed uptake of ^{14}C -glucose and they proposed cuticular absorption as a possible mechanism for the uptake of dissolved matter. Other studies (Samoiloff 1973, Howell 1983) indicate that the nematode cuticle is highly active. The outer part (outer cortical layer) of the 3-layered nematode body wall consists of secreted nematode collagen (Bird 1971, Lee & Atkinson 1976) containing disulphide and sulphydryl groups which provide binding possibilities for heavy metals (Howell 1983). Consequently, this structure may play a role in the uptake of heavy metals. Furthermore Mudry et al. (1982) used only very high metal levels ($\text{Cd} = 100 \text{ mg l}^{-1}$); hence it remains uncertain whether the same 'all-or-none' feeding behaviour exists at lower heavy-metal levels. At present, no information is available on whether this starvation behaviour depends on some threshold level.

Effects of cadmium on bacterial food

The nematodes were fed bacteria previously grown in different Cd concentrations. Growth inhibition of the bacterium occurred between 20 and 50 ppm Cd. Similar results have been obtained by Doyle et al. (1975) who studied the influence of Cd on 6 microorganisms commonly found in the intestinal tract of man. Two species, *Escherichia coli* and *Bacillus cereus*, grew well at 40 and $80 \mu\text{g Cd ml}^{-1}$, whereas the growth of the other species was depressed. *Alteromonas haloplanktis*, the food organism used in our experiments, is a Gram-negative bacterium and in comparison with Gram-positive bacteria, appears to be more resistant (Babich & Stotzky 1977, Jakubczak et al. 1981). High resistance with the Gram-positive bacterium *Staphylococcus aureus* is plasmid mediated (Novick & Roth 1968). The penicillinase phasmids carried separate loci for resistance to inorganic ions, among them cadmium. Such resistant plasmid-carrying cells possess a mechanism for preventing Cd uptake (Summers & Silver 1978). For example, in *E. coli* which shows a similar Cd resistance to that of *A. haloplanktis*, Cd uptake is very low (Doyle et al. 1975, Mitra et al. 1975). Consequently, Cd passage to bacterial feeders may be limited. In contrast, Patrick & Loutit (1976) reported that bacteria were able to concentrate Cr, Cu, Mn, Fe, Pb and Zn (Cd was not studied) from sediment and an artificial growth medium, and that tubificids – when fed bacteria containing metals – showed increased metal concentrations. Similar results have been obtained by Berk & Colwell (1981) who studied the transfer of mercury through a marine microbial food web. The 2 bacterial species used, *Vibrio* sp. and *Pseudomonas*

sp., accumulated considerable amounts of mercury. A magnification factor of more than 250 times has been observed with *Pseudomonas* sp. Ciliates feeding on the bacteria accumulated the mercury, whereas copepods fed labelled ciliates contained less mercury than the ciliates. As the toxicity and the uptake of cadmium is highly differential among the bacterial species studied (Doyle et al. 1975, Babich & Stotzky 1977), it is rather difficult to speculate upon Cd accumulation by *A. haloplanktis*. Nevertheless, it is clear that microorganisms accumulate Cd and hence pass it to higher trophic levels.

What are toxic levels?

We observed no developmental inhibition in *Pelioditis marina* and *Monhystera disjuncta* in the 0 to 10 ppm Cd range; higher Cd levels caused only small increases in development time. In *M. microphthalma*, a different response was observed; compared to the blank, all test concentrations caused a slight developmental retardation. The degree of inhibition is too low to assume large changes in food uptake.

Besides starvation and high resistance, Popham & Webster (1979) reported several structural changes of Cd exposed nematodes *Caenorhabditis elegans*. Using electron microscopy, they observed modifications of mitochondria in intestine and oesophagus, disrupted cytosomes, shorter microvilli of the intestinal cells, and the formation of nuclear inclusions in the oesophageal cells. Such modifications preclude death.

The inappropriateness of death as a criterion for determining environmentally safe concentrations is well known (e.g. Samoiloff et al. 1980). Only at 11.2 ppm Cd did these authors observe a significantly decreased survival, compared to the control. Using fecundity as a criterion, Samoiloff (1980) observed a significant reduction at as little as 11.2 ppb. According to Reish & Carr (1978) reproductive suppression in 2 polychaetes (*Ctenodrilus serratus*, *Ophryotrocha diadema*) was 2 orders of magnitude less than the 96 h LC 50. Death is too slow and too rough a criterion.

While LC 50 tests remain a useful and quick criterion for toxicity testing and for the effect ranking of chemical agents, these tests are not sensitive enough to determine minimum effective concentrations, i.e. concentrations inducing detrimental effect, not present in healthy individuals and leading to health impairment (Reish & Carr 1978). A compromise is to use death as a rough ranking criterion, but to consider the lowest concentration which induces a negative response significantly different from the control as a concentration intolerable to the environment. For the 3 nematode species tested by us these MEC concentrations are very high.

Other tests

For a short-term test with higher sensitivity than the traditional LC 50 test we developed an assay in which we monitored the number of adults at the time when ca 50 % of the nematodes reached adulthood in the control. These numbers represent 2 different response-types: mortality and developmental inhibition. The MEC values obtained from such experimental design are very similar to those recorded in mortality experiments, but this test takes considerably less time. Another promising feature is that the response curves of the 3 species show basically the same pattern. Hence, comparison of the slopes obtained for different species may yield additional information on the species' sensitivity to pollutant stress. Further investigations with other metals and other nematode species are necessary to corroborate the present findings.

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