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THE TOXICITY OF MERCURY ON THE FREE-LIVING MARINE NEMATODE MONHYSTERA DISJUNCTA BASTIAN, 1865

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ABSTRACT

The influence of the mercury compounds Hg_2Cl_2, $HgCl_2$, and CH_3HgCl as measured by egg mortality, preadult mortality, and development time, was studied on the marine nematode Monhystera disjuncta. A significant influence on egg, juvenile, and preadult mortality was observed for Hg_2Cl_2 and $HgCl_2$, while CH_3HgCl did not cause egg mortality. It was concluded that the juvenile mortality is the most sensitive index for chronic stress. No apparent changes in the development time occurred. The acute toxicity of the above mentioned mercury compounds, measured as the 96 h LC50-values, were calculated for both adult females and males.

KEYWORDS

Marine ecotoxicology, Methods, Mercury, Nematodes, Egg mortality, Monhystera disjuncta, Development time.

INTRODUCTION

The significance of free-living nematodes in marine sediments has been recently emphasized by several authors (Platt and Warwick, 1980 ; Heip et al., 1982). Marine nematodes are widely distributed, with total densities ranging from 10^7 worms.m^{-2} in estuaries and salt marshes, to 10^5.m^{-2} in the deep sea (Heip et al., 1982). Hence, nematodes are important in terms of biomass, an average yearly biomass of 0.18 g C.m^{-2} was recorded for the Belgian coast by Heip et al. (in press), and as potential food organisms for animals of higher trophic levels (Laserre et al., 1976 ; Platt and Warwick, 1980). Indirectly nematodes also stimulate nutrient regeneration (Findlay and Tenore, 1982), bacterial productivity (Tenore et al., 1977 ; Gerlach, 1978 ; Tietjen, 1980), and organic decomposition (Gerlach, 1977). Nematodes also greatly influence the physical characteristics of the sediments they are inhabiting by bioturbation (Cullen, 1973) and their mucus-secreting behaviour (Riemann, 1974 ; Riemann and Schrage, 1978).

In spite of the ecological significance of the marine nematodes, experimental ecotoxicological studies on the viability and other responses of these organisms in relation to exposure to heavy metals or other toxicants, are, however, almost unexisting. In order to fill this gap, a cosmopolitan free-living marine (estuarine) nematode Monhystera disjuncta Bastian, 1865 was selected for the present study as a suitable test species (for ecotoxicological testing see also Vranken et al., 1984).

The developmental biology of the species was described by Gerlach and Schrage (1971). Chitwood and Murphy (1964) reported on its embryonic development and behaviour under culturing conditions. Alongi and Tietjen (1980) investigated its competitive ability in relation to bacterivorous and herbivorous nematodes, and Tietjen (1980) drew up a carbon budget for adult females.

In the present study, the toxic effects of three mercury compounds (Hg_2Cl_2, $HgCl_2$, and CH_3HgCl) on various aspects of the life history of M. disjuncta were studied. The aim of these experiments was to select the life-cycle parameters best suitable for ecotoxicological tests under gnotobiotic conditions. The test medium was chemically defined (Vranken et al., 1984) but the food source was a mixture of unidentified bacteria isolated from the Sluice Dock in Ostend, Belgium.

MATERIALS AND METHODS

M. disjuncta was isolated from the Sluice Dock in Ostend (for cultivation, maintenance, and habitat description, see Vranken et al., 1984). The medium used for the bioassays was a 0.4 % bacto-agar made with Dietrich and Kalle's (1957) artificial seawater enriched with 1 % $Na_2SiO_3.9H_2O$ (15 $g.l^{-1}$), 1 % amino acid solution (for composition see Vranken et al., 1984) and 1 % Provasoli-Walne (1:5) modified nutrient medium (Ukeles, 1976 ; Vlasblom pers. commun.). The assays were performed at 20 °C, 30 ‰ salinity, and a pH of 7.5. The food consisted of a mixture of unidentified bacteria isolated from the Sluice Dock. The density of this live food was not controlled but no food limitation occurred throughout the course of the experiments.

The influence of different concentrations of three mercury compounds (Hg_2Cl_2, $HgCl_2$, and CH_3HgCl) on the following life-history characteristics was studied :

- egg mortality, e(+) : mortality occurring during the embryonic phase, from the egg laying till the hatching of the larva ;
- juvenile mortality, j(+) : mortality during postembryonic development, from the moment the worm leaves the egg till it reaches adulthood ;
- preadult mortality, (+) : mortality before adulthood, e(+) and j(+) represented together in one figure ;
- development time (= minimum generation time, T_{min}) : period between identical stages of successive generations, approximately the sum of the embryonic and postembryonic period ;
- adult mortality (M) during a test period of 96 h.

The assays to determine the preadult mortality were started with 105 eggs per concentration, for both Hg_2Cl_2 and CH_3HgCl. The eggs were transferred in small lumps of agar to fresh culture media. This manipulation had no effect on the egg viability. For $HgCl_2$, five females per replica were allowed to deposit eggs for 24 h. Then the females were removed and the eggs counted. Their total number ranged from 72 (10 ppb) to 435 (control). The use of this method was discontinued because of the large discrepancies in the number of eggs produced in the various experiments. From the start of the experiment onwards, the number of freshly hatched juveniles, dead juveniles, and unhatched eggs was recorded during daily controls. After a time period T_{min}, new adults were removed routinely. For the blank of $HgCl_2$,

six simultaneous replicates were run. At 25 ppb and 50 ppb Hg^{2+} ($HgCl_2$) one replicate was lost and in one replicate at 2.5 ppm Hg^{+} (Hg_2Cl_2) not a single female developed into the adult stage (Table IV). At 25 ppb Hg^{2+} (CH_3HgCl) only one replicate, and at 10 ppb Hg^{2+} (CH_3HgCl) only two replicates yielded adult females. In the other experiments, the female juveniles died before adulthood. The 96 h LC50 tests were carried out with worms approximately 12-days-old. For Hg^{2+} (in CH_3HgCl) five replicates containing ten females and ten males each were exposed to each of the six concentrations ranging from 25 to 150 ppb. For the other two compounds only four replicates with 20 adults were used and six test concentrations, rangingfrom 250 to 1 500 ppb Hg^{2+} (compound $HgCl_2$), and from 30 to 35 ppm Hg^{+} (compound Hg_2Cl_2). The concentrations mentioned refer only to the mercury ions and not to the compounds.

The 96 h LC50 values for the adults were calculated according to the Reed-Muench method, and respective confidence limits were estimated with Pizzi's formula (Woolf, 1968). The median lethal concentrations (LC50) for the other life stages, e(+), j(+) and (+), were computed by inverse prediction, using a linear least square regression of the survival percentage, corrected for control response (Hewlett and Plackett, 1979), and transformed to angles (arcsine transformation), against concentrations (arithmetic scale). Both logit and probit analyses resulted in some cases in very high Pearson's statistics, indicating that these fittings were unsatisfactory in some cases. The confidence limits for these median lethal concentrations were estimated, by a method described by Sokal and Rohlf (1981).

RESULTS

In Table I the egg mortality e(+), juvenile mortality j(+), and preadult mortality (+), at different concentrations of Hg_2Cl_2, $HgCl_2$, and CH_3HgCl are presented, as well as the G-statistic adjusted by Williams' correction (q) (Sokal and Rohlf, 1981). These statistic tests demonstrate whether or not egg mortality is independent of the heavy metal concentration. The different concentrations tested, are arrayed according to an increasing mortality rate (figures in parenthesis). Responses (mortalities) underscored by the same line represent non-significant within sets of concentrations at the $P < 0.05$ confidence level.

Table I. Life history data of M. disjuncta. e(+) = egg mortality, j(+) = juvenile mortality, and mortality form egg deposition until adulthood (+) at 20 °C, 30 ‰ of salinity, and pH = 7.5 for different concentrations of Hg^{+} in Hg_2Cl_2, Hg^{2+} in $HgCl_2$ and CH_3HgCl, the % mortalities in parenthesis, the G/q statistic and an unplanned test (see text for explanation)

Hg compound	Life stage	G/q	Concentration of mercury and % mortality (in parenthesis)								
Hg^{+}	e(+)	107 ***	0(15)	1(17)	5(26)	2.5(29)	10(57)	15(67)			
in Hg_2Cl_2	j(+)	307 ***	0(0)	1(0)	5(0)	2.5(24)	10(84)	15(100)			
(ppm)	(+)	369 ***	0(15)	1(17)	5(26)	2.5(46)	10(98)	15(100)			
Hg^{2+}	e(+)	781 ***	25(2)	50(7)	10(24)	0(32)	100(35)	2(41)	250(72)	500(78)	1 000(93)
in $HgCl_2$	j(+)	143 ***	2(0)	25(0)	250(1)	0(2)	10(2)	100(5)	50(12)	500(33)	1 000(100)
(ppb)	(+)	797 ***	25(2)	50(18)	10(25)	0(33)	100(38)	2(41)	250(73)	500(85)	1 000(100)
Hg^{2+}	e(+)	62 ***	0(15)	75(26)	100(30)	500(35)	10(44)	50(54)	25(55)		
in CH_3HgCl	j(+)	522 ***	0(0)	10(0)	25(62)	50(96)	75(100)	100(100)	500(100)		
(ppb)	(+)	437 ***	0(15)	10(44)	25(83)	50(98)	75(100)	100(100)	500(100)		

* $0.01 < P \leqslant 0.05$
** $0.001 < P \leqslant 0.01$
*** $P \leqslant 0.001$

The figures in Table I demonstrate that in all cases there was a significant influence of the mercury concentration ($P < 0.001$) on the magnitude of the recorded effect. For Hg_2Cl_2 a very clear pattern was observed in which only the egg and juvenile mortality rates at 2.5 ppm were higher than at 5 ppm, and hence also for the two-pooled preadult mortality. The aberrant position of 2.5 ppm Hg^+ (Hg_2Cl_2), especially for the juvenile mortality may be attributed to the heterogeneity among replicates ($P < 0.001$, Table II). For $HgCl_2$ a relatively high non-hatching percentage

Table II. Goodness of fit test (G_H-statistic) to locate heterogeneity among replicates (Sokal and Rohlf, 1981). e(+) = egg mortality, j(+) = juvenile mortality. The series with complete homogeneous response (either 100 % mortality or survival) are labeled only with ns, df = 2 except for data marked c and °

Compound	Concentration	e(+)		j(+)	
Hg^+ in Hg_2Cl_2	Control	7.543	*		(ns)
	1 ppm	0.403	(ns)		(ns)
	2.5 ppm	1.126	(ns)	21.902	***
	5 ppm	17.633	***		(ns)
	10 ppm	10.409	**	19.940	***
	15 ppm	2.749	(ns)		(ns)
Hg^{2+} in $HgCl_2$	Control	64.993[c]	***	8.824[c]	(ns)
	2 ppb	4.249	(ns)		(ns)
	10 ppb	27.817	***	1.034	(ns)
	25 ppb	0.384°	(ns)		(ns)°
	50 ppb	16.494°	***	47.524°	***
	100 ppb	17.685	***	4.940	(ns)
	250 ppb	100.512	***	2.806	(ns)
	500 ppb	25.445	***	24.169	***
	1 000 ppb	13.157	**		(ns)
Hg^{2+} in CH_3HgCl	Control	7.543	*		(ns)
	10 ppb	92.987	***		(ns)
	25 ppb	0.541	(ns)	40.265	***
	50 ppb	14.851	***	5.465	(ns)
	75 ppb	2.733	(ns)		(ns)
	100 ppb	7.187	*		(ns)
	500 ppb	1.584	(ns)		(ns)

[c] df = 5
° df = 1
* $0.01 < P < 0.05$
** $0.001 < P < 0.01$
*** $P < 0.001$

(32 %) was recorded, hence only the higher test concentrations (250, 100, 1 000 ppb) induced significantly different egg responses. For the juvenile mortality a similar pattern was noted. Only the higher toxicant levels, were significantly different form the control except for a concentration of 250 ppb which caused less mortality than the control. For this compound a high variability among replicates existed, particularly for the egg mortality series, in which six of the nine sets were made up of highly heterogeneous replicates ($P < 0.001$). For the juvenile mortality, on the contrary, the variability among replicates was considerably less. The highest heterogeneous set for this life-history stage was observed at 50 ppb Hg^{2+} ($HgCl_2$) and all mortality occurred within one replicate. However, the responses generated in the different replicates between 0 and 250 ppb Hg^{2+} ($HgCl_2$) were homogeneous, clearly indicating that these concentrations encompassed the nontoxic concentrations range for this particular nematode species. The preadult mortality pattern matched the egg mortality response indicating that the latter interfered with the pooled preadult mortality.

For CH_3HgCl, no distinct relationship existed between the hatching probability and the different levels of mercury. This resulted in a non-significant regression (coefficients not shown in Table III). With increasing doses of CH_3HgCl on the contrary, a distinct effect on the juvenile and preadult mortality was noted.

In Table III a compilation of the results listed in Table I are given, namely the regression of mortality (M) during the different life stages, against concentration (C) ; $M = a + bC$, with a and b being constants. Other included statistics are the coefficient of determination (r^2), the F-statistic testing the significance of regression, and the standard errors of a and b (in parenthesis).

From this analysis it is clear that a linear relationship (Fig. 1, 2, and 3) exists between the mortality response and the concentration (and not the logarithm of the dose). In all cases the regression of the juvenile mortality against the concentration provided the highest coefficients of determination, the highest F-statistics, and consequently the smallest confidence limits for the median lethal concentration. From the foregoing it can be concluded that the juvenile responses are more straight forward, whereas the egg-mortality responses are not always very comprehensible. Studying preadult mortality as a whole can therefore result into similar not very coherent observations.

Table III. Compilation of the results for M. disjuncta listed in Table I. M = regression of response mortality (in arc sin proportion on the mercury concentration (C) applying the least-squares linear regression : M = a + bC in which a and b are constants with the standard error in parenthesis, e(+) = egg mortality, j(+) = juvenile mortality, (+) preadult mortality. The confidence limits of the LC50 values are calculated according to Sokal and Rohlf (1981)

Mercury compound	Life stage	a	b	r^2	F	LC50	95 % Confidence limits
Hg^{+} (Hg_2Cl_2)	e(+)	10.00 (± 4.42)	2.94 (± 0.52)	0.91	31.5*	11.9 ppm	4.7 - 24.1 ppm
	j(+)	9.95 (± 4.64)	5.46 (± 0.51)	0.98	114.8**	6.4 ppm	0.5 - 12.2 ppm
	(+)	8.42 (± 11.85)	5.83 (± 1.40)	0.85	17.3*	6.3 ppm	0.0 - 20.7 ppm
Hg^{2+} ($HgCl_2$)	e(+)	21.70 (± 8.54)	0.05 (± 0.02)	0.78	10.9*	425 ppb	0 - 4 392 ppb
	j(+)	4.56 (± 5.29)	0.08 (± 0.01)	0.95	58.0**	505 ppb	107 - 975 ppb
	(+)	20.37 (± 6.64)	0.07 (± 0.01)	0.91	32.2*	336 ppb	0 - 912 ppb
Hg^{2+} (CH_3HgCl)	j(+)	6.01 (± 9.64)	1.24 (± 0.23)	0.91	29.4*	31 ppb	0 - 80 ppb
	(+)	35.90 (± 9.43)	0.79 (± 0.20)	0.88	15.4ns	11.6 ppb	0 - 261 ppb

* $0.01 < P \leq 0.05$
** $0.001 < P \leq 0.01$
*** $P \leq 0.001$
ns not significant (P = 0,059)

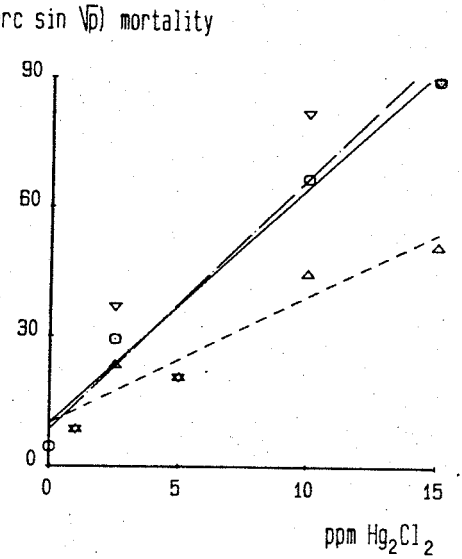

Fig. 1. Mortality of M. disjuncta during three different life-history stages, e(+) = egg mortality (Δ----Δ), j(+) = juvenile mortality (j+) (o—o), (+) = preadult mortality (▽—·—▽) at different concentrations of Hg^+ in Hg_2Cl_2.

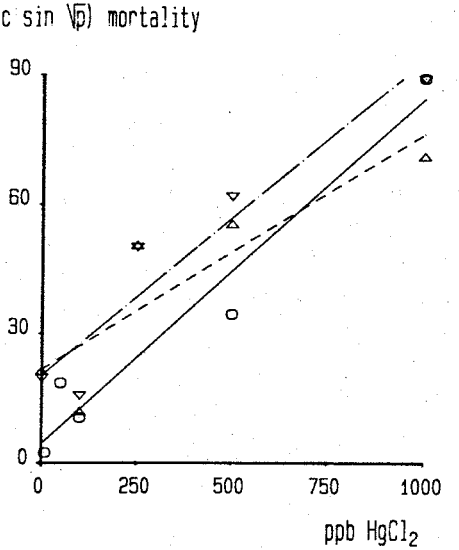

Fig. 2. Mortality of M. disjuncta during three life-history stages, e(+) = egg mortality (Δ----Δ), j(+) = juvenile mortality (o——o), (+) = preadult mortality (▽—·—▽) at different concentrations of Hg^{2+} in $HgCl_2$.

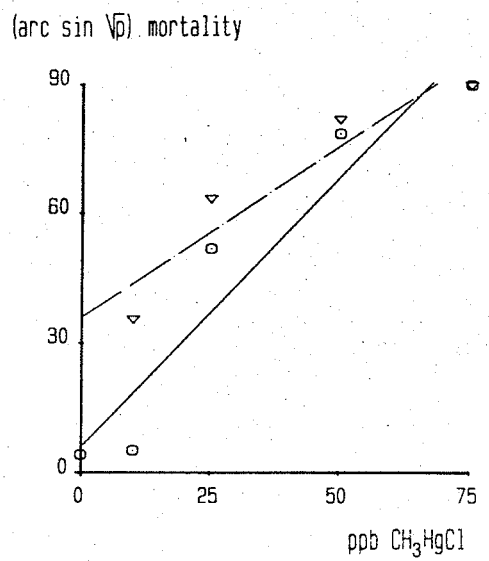

Fig. 3. Mortality of M. disjuncta during two different life-history stages, j(+) = juvenile mortality (o——o), (+) = preadult mortality (▽—·—▽) at different concentrations of Hg^{2+} in CH_3HgCl.

As a second test criterion the duration of the development time from egg to adulthood was chosen. In Table IV the results obtained with female nematodes are presented. The statistical analyses of the data with a two-level nested ANOVA led to the following conclusions : in all cases significant heterogeneity among replicates existed ($0.01 < P < 0.05$ for Hg_2Cl_2, $P < 0.001$ for $HgCl_2$, and $0.001 < P < 0.01$ for CH_3HgCl), no difference occurred between development time at the various test concentrations of mercury, except for Hg_2Cl_2 ($0.01 < P < 0.05$), for which, however, no prolongation at higher mercury doses could be observed (GT_2 intervals Table IV).

From the calculations of the GT_2 intervals it can be concluded that none of the T_{min} scores at the highest concentrations were significantly different from the blanks. It must also be noted that the development times at 2.5 ppm Hg^+ (Hg_2Cl_2), 250 ppb Hg^{2+} ($HgCl_2$), and 10 ppb Hg^{2+} (CH_3HgCl) are significantly longer than those realized in the blanks. At least for $HgCl_2$ and CH_3HgCl, this seems contradictory with the overall nested ANOVA. This is, however, not true since the differences between the replicates were taken into account in the ANOVA, whereas in the GT_2 computations only the

pooled data, not considering the variability among the replicates, were used. The regressions of T_{min} per replicate, transformed into logarithms, against the logarithms of the mercury concentrations, were also calculated for each mercury compound tested. This resulted in three non-significant regressions with Fs = 0.558 (n = 12) for Hg_2Cl_2, Fs = 0.219 (n = 23) for $HgCl_2$, and Fs = 0.585 (n = 6) for CH_3HgCl. The slopes (b) of these regressions were b = 0.0328 (SE = 0.0439) for Hg_2Cl_2, b = 0.0050 (SE = 0.0106) for $HgCl_2$, and b = 0.0261 (SE = 0.0341) for CH_3HgCl. These computations corroborated the higher ANOVA results and they unmistakenly show that the development time remained unaltered as the mercury concentrations increased.

Table IV. The development time from egg to adulthood of female M. disjuncta. T_{min} = mean development time of females with standard deviations in parenthesis at 20 °C, 30 ‰ salinity and pH = 7.5 at different mercury concentrations in three different compounds, n = the number of experiments, N = the number of females, and Gabriel's 95 % GT_2 comparison intervals (Sokal and Rohlf, 1981), T_{min}'s with intervals which do not overlap are significantly different

Mercury compound	Concentration	T_{min} (days)	95 % GT_2	n	N
Hg^+ in Hg_2Cl_2 (ppm)	0	9.4 (± 1.35)	8.96 – 9.85	3	34
	1	9.0 (± 1.03)	8.61 – 9.35	3	48
	2.5	11.5 (± 1.67)	10.68 – 12.32	3	10
	5	10.3 (± 1.65)	9.84 – 10.66	3	39
	10	9.0		3	1
	15	–		3	–
Hg^{2+} in $HgCl_2$ (ppb)	0	10.2 (± 1.35)	9.90 – 10.41	6	170
	2	10.2 (± 1.74)	9.50 – 10.81	3	26
	10	9.5 (± 1.56)	8.98 – 10.11	3	35
	25	9.7 (± 1.31)	9.40 – 10.06	2	103
	50	9.7 (± 1.26)	9.47 – 10.00	2	161
	100	9.9 (± 1.52)	9.59 – 10.21	3	120
	250	11.9 (± 2.60)	11.41 – 12.41	3	47
	500	11.2 (± 1.89)	10.29 – 12.07	3	14
Hg^{2+} in CH_3HgCl (ppb)	0	9.4 (± 1.35)	8.94 – 9.85	3	34
	10	11.3 (± 1.70)	10.64 – 12.02	3	15
	25	9.5 (± 2.00)	8.53 – 10.54	3	7
	50	–		3	–
	75	–		3	–
	100	–		3	–
	500	–		3	–

Table V. Acute toxicity of three mercury compounds at 20 °C, and 30 ‰ salinity, and pH 7.5, to adult female and male M. disjuncta, expressed as 96 h LC50 values calculated with the Reed-Muench method and 95 % confidence intervals according to Pizzi (Woolf, 1968)

Mercury compound	Sex	96 h LC50	95 % confidence intervals
Hg^{+} in Hg_2Cl_2	M	32.3 ppm	32.0 - 32.6 ppm
	F	32.2 ppm	31.9 - 32.6 ppm
Hg^{2+} in $HgCl_2$	M	834 ppb	745 - 923 ppb
	F	762 ppb	667 - 858 ppb
Hg^{2+} in CH_3HgCl	M	96 ppb	88 - 103 ppb
	F	97 ppb	88 - 105 ppb

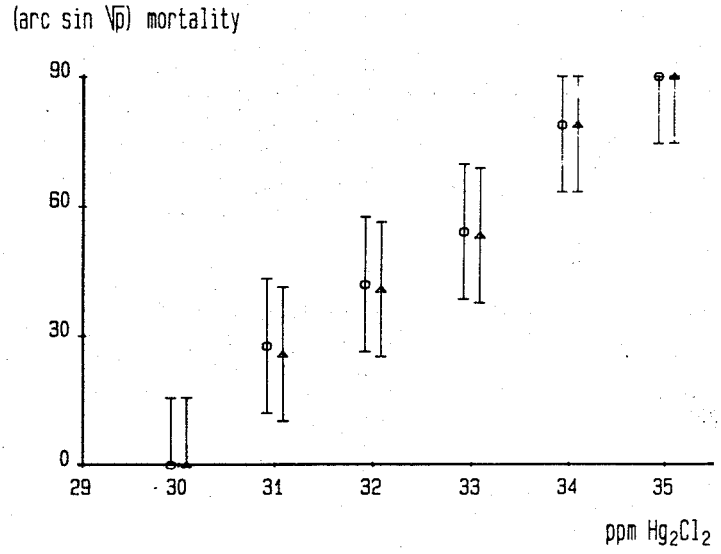

Fig. 4. 95 % comparison intervals (T-method) for the mean adult mortality (96 h) for females (O) and males (△) M. disjuncta at different concentrations of Hg^{+} in Hg_2Cl_2. Means with not-overlapping intervals are significantly different.

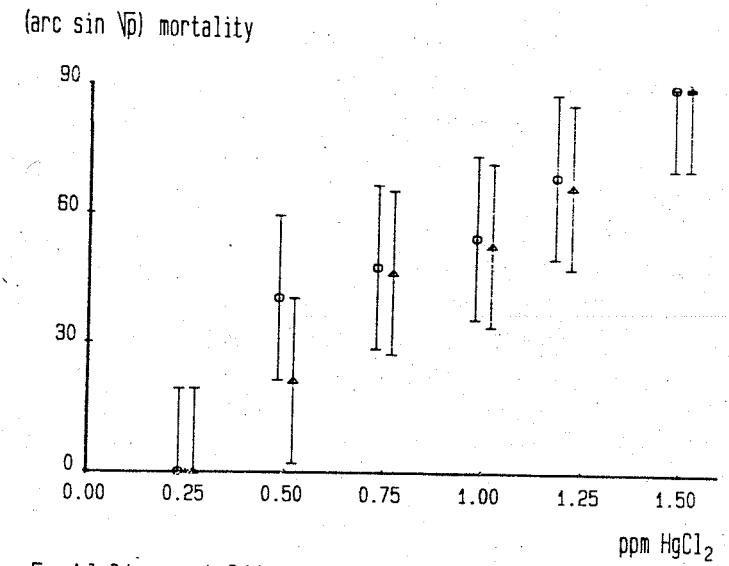

Fig. 5. Adult mortality of M. disjuncta. 95 % comparison intervals (T-method) for the mean adult mortality (96 h) for females (O) and males (Δ) at different concentrations of Hg^{2+} in $HgCl_2$. Means with not-overlapping intervals are significantly different.

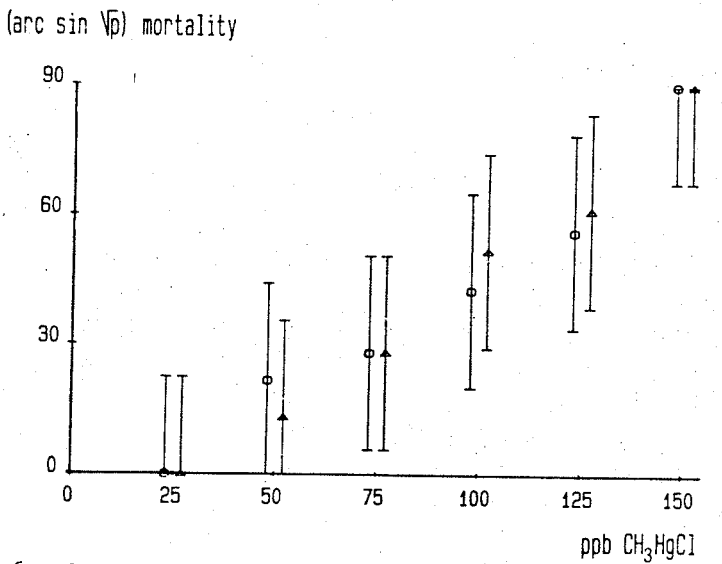

Fig. 6. Adult mortality of M. disjuncta. 95 % comparison intervals (T-method) for the mean adult mortality (96 h) for females (O) and males (Δ) at different concentrations of Hg^{2+} in CH_3HgCl. Means with not-overlapping intervals are significantly different.

To summarize it may be concluded that ecotoxicological tests based on the inhibition of the development are inappropriate for this monhysterid species, due to the all-or-nothing response pattern for this particular life-history parameter.

Comparative acute toxicity levels with 12-days-old females and males (20 °C, 30 ‰ salinity) expressed as 96 h LC50 are summarized in Table V. From a two-way ANOVA (percentages transformed to angles) it may be concluded that for the three mercury compounds studied, highly significant differences ($P < 0.001$) between the mortality responses are induced at the different test concentrations. An unplanned test to locate the heterogeneity among responses is shown in Fig. 4, 5, and 6 for the respective mercury compounds (with the T-method 95 % comparison intervals around the means were calculated ; means with intervals which do not overlap were significantly different ($P < 0.05$) (Sokal and Rohlf, 1981)). The lowest concentrations inducing significantly different responses were 32 ppm Hg^{+} (Hg_2Cl_2) for both sexes ; 500 ppb Hg^{2+} for the females, and 750 Hg^{2+} ($HgCl_2$) for the males ; 125 and 100 ppb Hg^{2+} (CH_3HgCl) respectively for females and males. Both sexes exhibited the same susceptibility. These 96h LC50 values also demonstrate the higher resistance of adults when compared to the embryonic and postembryonic stages.

DISCUSSION

The present study on M. disjuncta revealed a differing susceptibility of this nematode species to the three mercury compounds tested and a difference in sensitivity for the various life-history stages. In terms of toxicity ranking it was noted that for females mercuric (II) chloride and methyl mercury chloride were approximately 40 and 300 times more toxic than mercuric (I) chloride. For the juveniles these relative discrepancies are somewhat less marked, the differences namely 13 times for mercuric (II) chloride, and 200 times for methyl mercury chloride still remained noteworthy.

Similar results have been obtained in earlier investigations summarized by Bryan (1976). The widest range in response was noted for the species which was estimated to be the most impermeable to metals, namely the brine shrimp (Artemia salina), whereas for the barnacle (Elminius modestus) and

the copepod (Acartia clausi), these differences were less pronounced. In the case of Artemia salina the higher toxicity of the organic mercury compound has been related to its higher lipid-solubility.

The greater sensitivity of the juveniles as compared to adult worms points juveniles mortality out as a suitable test criterion for bioassays, even more reliable than mortality rates in other stages, especially because the egg-mortality pattern, as shown for methyl mercury chloride, is not properly understood at the present time. Generally, the eggs are known to be more resistant than juvenile and adult nematodes (Khan and McFadden, 1980), probably due to the fact that the egg shell is the only nematode structure containing chitin (Bird, 1971 ; Lee and Atkinson, 1976). It is therefore less permeable to some substances as compared to the highly metabolically-active cuticle (Samoiloff, 1973 ; Bird, 1980). This multilayered structure exhibits several features of cell membranes, resulting almost certainly into the incorporation of heavy metal ions (Howell, 1983 ; Simkiss, 1983).

In addition to surface adsorption by the cuticle, metal uptake through the gut is considered as a possibility (Howell, 1982, 1983). For both routes of uptake (and loss) this author emphasized the role of mucus secretion (acid-mucopolysaccharide) as well by the caudal as by the pharyngeal gland. With the mucus, particulate material (bacteria, diatoms, detritus particles), and dissolved organic nutrients are collected, and it has been suggested that nematodes are feeding on these substances (Riemann and Schrage, 1978). Whether feeding occurs through particulate material or through dissolved nutrients, it is clear that the significant accumulation of heavy metals in the secreted mucus (Howell, 1982) influences their uptake.

Even though the actual importance of the two proposed pathways of heavy metal accumulation is unknown, one can conclude from the present experiments that the test nematodes exhibited a high capacity to withstand stress caused by exposure to mercury compounds. The toxicity of Hg^{2+} ($HgCl_2$) to M. disjuncta, expressed as 96 h LC50 (Tables III, V), is considerably lower than for other marine organisms as reviewed by Stebbing (1976). Connor (1972) reported LC50 values ranging between 1 to 3.3 ppb (48 h LC50) for Ostrea edulis larvae and Bengtsson (1978) 230 ppb (96 h LC50) for the harpacticoid copepod Nitocra spinipes. Data recorded for two related marine (Brackish) free-living nematodes Monhystera microphthalma and Diplolaimelloides bruciei (Mexican strain) (Bogaert et al., 1984) correspond

better with the toxicity levels obtained in the present study. These authors observed a 100 % juvenile mortality at 640 ppb Hg^{2+} (3.2×10^{-6} mol.l^{-1}) for both nematode species, whereas a similar concentration, as calculated from the coefficients shown in Table III results in 70 % juvenile mortality for M. disjuncta. The observed discrepancy can be attributed to interspecific differences and (or) to the experimental procedure used. Monhystera microphthalma and Diplolaimelloides bruciei were cultured in fluid conditions whereas M. disjuncta was grown in a more solid substrate (bacto-agar). Samoiloff (1980) considers the latter substrate unsatisfactory for ecotoxicological studies because the agar might selectively bind the toxic agent. The three nematode species used in the present assays are, however, bottom-inhabiting organisms and therefore, we considered agar as a suitable substrate for laboratory experiments including bioassays.

In comparison with Panagrellus redivivus, a nematode species extensively used by Samoiloff and co-workers (Samoiloff et al., 1980, 1983), M. disjuncta exhibits a somewhat greater sensitivity. For the former species a significant reduction in larval survival was observed at 10^{-6} mol.l^{-1} (200 ppb Hg^{2+}) methyl mercury chloride, 10^{-5} mol.l^{-1} (2 ppm Hg^{2+}) methyl mercury chloride resulted in 100 % mortality, whereas for M. disjuncta a significant reduction in juvenile mortality was noted at 25 ppb CH_3HgCl, and a 100 % juvenile occurred at 75 ppb CH_3HgCl. Mercuric chloride produced a significant decrease in the survival of P. redivivus at 10^{-4} mol.l^{-1} (20 ppm Hg^{2+}) whereas 1 ppm Hg^{2+} caused a 100 % mortality of larval M. disjuncta.

Since nematodes molt four times before reaching adulthood Samoiloff et al. (1980) were able to enhance the sensitivity of the test by using the successfulness of completing a particular molt as an indicator of the inhibitoring action of specific agents. This attractive method is, however, not generally applicable to test the action of a wide variety of chemicals. For example no prolongation of the development time (T_{min}) of M. disjuncta was observed in the present experiments. An "all-or nothing" response pattern was noted ; individuals developing until adulthood reached this stage in the same time as did worms grown in the blank. It was also noted that all the individuals unable to reach sexual maturity in the same span, died. Whether or not this response is typical for M. disjuncta can presently not be answered, and more research in needed. Results similar to those presented in this study, have, however, been obtained by Haight et al. (1982) with the nematode Panagrellus silusiae. These authors found that mercury was ineffective in blocking growth at 10 mg.l^{-1} mercury ($HgCl_2$),

whereas the adult LC50 was 5.11 $mg.l^{-1}$ mercury. Other scientists (Bogaert et al., 1984) obtained similar results, observing in some cases no developmental effect at all.

From the present assays, it was concluded that the tested nematode species exhibit a relative large capacity to withstand physiological stress. This has also been observed in nature under some polluted conditions, nematodes can be extremely dominant and sometimes the only surviving animals (Van Damme and Heip, 1977 ; Heip et al., 1984). Monhystera disjuncta belongs to the 1B feeding group (non-selective deposit feeders) according to Wieser's (1953) classification and members of this feeding group are extremely insensitive to pollution and capable to tolerate high levels of contaminations, especially heavy metals (Heip et al., 1984). Representatives of the 2A feeding type (herbivores), on the contrary, are very sensitive to pollution, and are sometimes completely replaced by the 1B types. In order to assess the influence of heavy metal pollution on the entire nematode community, it is therefore recommended to select representative members of the four main feeding types (Wieser, 1953) for ecotoxicological testing.

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