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Toxicity of Methylmercury to *Daphnia pulex*

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Recognition of the seriousness of polluting our environment with mercury intensified when it was established that under anaerobic conditions mercury ions can be methylated to form methylmercury (Wood 1985), a highly toxic and significantly more mobile form of mercury. Methylmercury is bioconcentrated in aquatic organisms in the food chain by direct uptake from the water and biomagnified by ingestion of contaminated foods.

While toxicity of methylmercury to humans and mammals has received much attention, toxicity to aquatic organisms has not been sufficiently examined (D'ltri 1972; Miller & Clarkson 1971; Hartung and Dinman 1972).

Toxic effects of methylmercury on Daphnia pulex were determined, including estimates of acute mortality, and chronic net reproduction (R_o) survivorship (1) and growth rate. Estimates of LC_{50} and EC_{50}, as well as accumulated effects over three generations were established.

Thus we have provided a comparison of chronic toxic effects of mercury derived from using the lifetable approach (Seber 1973) with simple effects based on intrinsic growth rates. We have also shown that substantial reductions occur in EC_x from one generation to the next.

MATERIALS AND METHODS

Acute tests (LC_{50}) employed D. pulex [The EPA clone is a cross D. pulex x D. pulicaria (P. Hebert pers. comm.] neonates (<24 hr old). Test water was filtered with Whatman qual. 1 filter paper. Ten animals were placed in

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100 mL test water at 20° C. Treatment involved five concentrations of methylmercury (chloride) prepared without solvents, with five replicates each.

Lifetable experiments utilized D. pulex neonates. Test water was again filtered lake water. Experiments ran for 30 days. Four concentrations of methylmercury (chloride) (10ng/L, 100 ng/L, 500 ng/L and 1000 ng/L) were used in experimental treatments. Neonates were placed in 40 mL test water (50 mL beakers) with 20 replicates at 20° C. A photoperiod of 16 hr light/8 hr dark was used. Yeast-alfalfa fish-chow suspension was added for food. Progeny were counted and removed daily. Test water was changed every three days.

For growth rate experiments the initial size (L_o) was taken by average size of 20 animals (<24 hr old) randomly chosen from about 200. Treatment concentrations of methylmercury were 0 ng/L (control), 100 ng/L, 500 ng/L, 1000 ng/L and 1500 ng/L. Twenty test animals were raised in filtered lake water(100 mL) with added food suspension as a food resource and the test water was changed every two days. Final size (L_2) was the average size of 20 animals after seven days growth in test water. Size measurements were made from tip of the helmet to base of the tail spine.

The LC_{50}'s and EC_{25}'s and EC_{50}'s (as shown in Table 3) were determined by linear regression (Statistix 3.5 c). Treatment effects on R_o (Table 2) were determined using an unbalanced ANOVA (Statistix 3.5).

Concentrations of methylmercury for acute tests were 1000-45000 ng/L and for chronic tests were 10-1000 ng/L, which were higher than the concentration of natural water [general environmental levels of methylmercury are 8 ng/L (Wood 1985)] but very much lower than those concentrations which cause mercury poisoning in humans [0.2-1.0 ppm (Grant 1971)].

The test animals (D. pulex) were taken from an EPA clone; test water was collected from Lake Calhoun, Minneapolis. These test conditions were closer to nature than reference water made by addition of artificial ions to distilled water.

Males occurred while using Daphnia lifetables for toxicity tests (Table 1). This biological phenomenon is normal. Young males should be removed at the onset to avoid discarding experiments.

Table 1. The number of males occurring in test groups (N=20)

Methylmercury concentration (ng/L)	f_o	f_1	f_2
0	0	0	2
10	0	0	0
100	0	1	2
500	0	2	1
1000	0	2	-

RESULTS AND DISCUSSION

The concentration of methylmercury affecting survival of Daphnia magna was higher (260-870 ng/L) (Biesinger et al. 1982) than that for D. pulex reported here. Likewise, the EC_{50} for reduction in reproductive rate was higher (1140 ng/L).

The LC_{50}s were 31205 ng/L (24 hr), 5700 ng/L (48 hr) and 1805 ng/L (96 hr). The lethal concentrations of methylmercury illustrate the reduced sensitivity of acute tests.

Experimental results from lifetables are summarized in Table 2 (a,b,c). Treatments from 10 ng/L to 1000 ng/L methylmercury caused gradual reduction in age-specific birth rates (m_x) and net reproductive rate (R_o). Much more pronounced effects were observed at concentrations greater than 100 ng/L.

With regard to the effects of methylmercury on net reproduction, an obvious break between 100 and 1000 ng/L was significant ($p<0.95$ - $p<0.001$) (Figure 1). The critical concentration for methylmercury was between 10-100 ng/L (Table 2c). The effective concentration (EC_{50}) calculated from R_o was 707 ng/L (f_o), 336 ng/L (f_2), 229 ng/L (f_2) (Table 3). Effective concentration (EC_{50}) declined from generation 1(f_o) to generation 3 (f_2). The ratio R_o (treatment)/R_o (control) shows clearly the effects of various concentrations of methylmercury by generation (Figure 2).

Some animals died before the age of reproduction (8 days) in all treatments (Table 4); however, the percentage dead gradually increased with increasing methylmercury concentration. The LC_{25} values (8 days) were 794 ng/L(f_o), 275 ng/L(f_1), and 331 ng/L(f_2) (Table 5).

Table 2. Lifetable, providing estimates for natality (m_x) and estimates of net reproduction ($R_o = l_x m_x$) of Daphnia pulex exposed to methylmercury for 30 days.

Concentration (ng/L)	N	l_x	m_x	+SD	R_o	+SD
a) f_o generation						
0	20	1.0	119.5	7.4	119.5	16.1
10	20	1.0	116.9	7.1	116.9	10.3
100	20	1.0	110.8	6.3	110.7	13.5
500	20	0.94	**91.9	6.8	*86.5	12.9
1000	20	0.64	***63.9	10.6	***40.6	5.7
b) f_1 generation						
0	20	0.97	98.5	9.3	98.4	11.9
10	20	0.97	94.5	9.9	92.4	14.9
100	19	0.93	85.1	12.6	79.3	15.6
500	18	0.66	***58.6	4.9	38.7	8.4
1000	18	0.58	***43.9	8.1	***25.5	6.0
c) f_2 generation						
0	18	0.95	134.6	5.1	127.4	16.8
10	20	0.95	111.6	10.2	106.0	14.7
100	18	0.85	115.0	5.7	*98.7	13.2
500	19	0.80	***33.7	8.1	***25.5	6.0

* $0.01<p<0.05$
** $p<0.01$
*** $p<0.001$

Both the LC_{50} for methylmercury and the EC_{50} for reduction in reproductive rate were lower (87-114 ng/L) than previously reported.

Table 3. EC_{25} and EC_{50} derived from R_o of Daphnia pulex exposed to methylmercury.

	f_o	f_1	f_2	mean
EC_{25}	380	131	110	207
EC_{50}	707	336	229	424

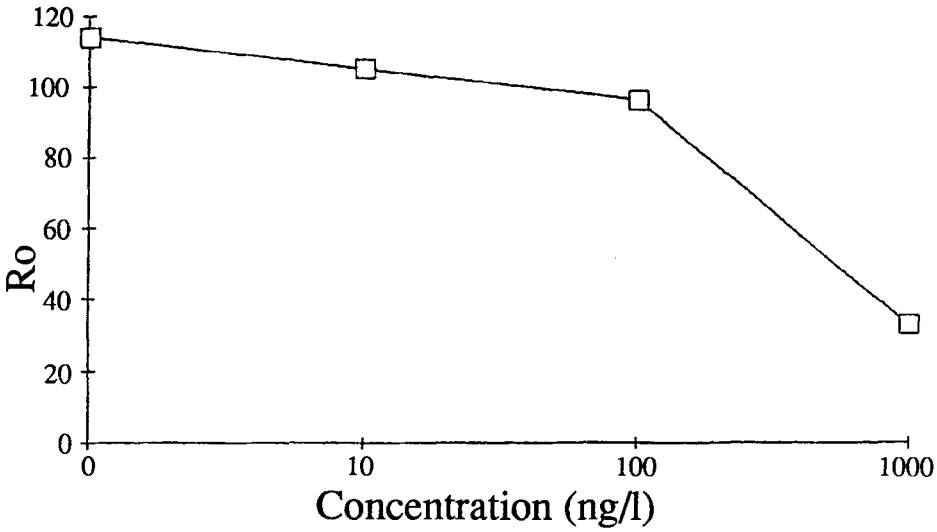

Figure 1. The relationship between R_o and concentration of methylmercury

Table 4. Percent death of Daphnia pulex before age of reproduction (8 days, when exposed to methylmercury (ng/L).

Methylmercury concentration	f_o	f_1	f_2
0	0	0	5
10	0	0	5
100	0	15	10
500	5	30	30
1000	35	35	-

Table 5. Values of LC_{25} for reproduction (8 days) with concentration of methylmercury (ng/L).

	f_o	f_1	f_2	mean
LC_{25}	794	275	331	467

Daphnia intrinsic growth rates (Table 6a) declined gradually with increasing methylmercury concentration. EC_{25} of 1258 ng/L was much higher than the critical concentration observed in lifetable experiments (207 ng/L). The sensitivity of growth tests (r) is therefore less than lifetable tests (R_o).

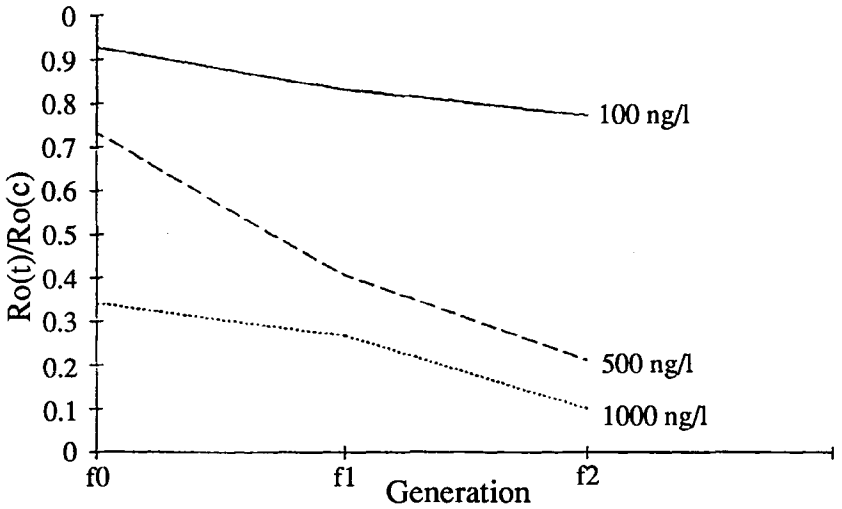

Figure 2. Ratios of R_o(treatment)/R_o(control) for each generation.

Table 6a. Intrinsic growth rate [$r=\ln (l_1/L_o/t_2-t_o)$], as influenced by methylmercury concentration. [N=number of animals, (t_2-t_o) =7 days].

Treatment methylmercury concentation (ng/L)	N	r (replicate$_1$)	r (replicate$_2$)	r (replicate$_3$)
0	20	0.106	0.098	0.082
100	20	0.109	0.098	0.082
500	20	0.091	0.087	0.079
1000	20	0.084	0.082	0.072
1500	20	0.070	0.060	0.058

L_o = starting size
L_1 = final size

Table 6b. The value of EC_{25} (derived from growth rate) with concentrations methylmercury (ng/L).

Groups	1	2	3	mean
EC_{25}	1148	1310	1318	1258

Using chronic estimates of mortality and reproduction, we have established that chronic effects occurred at lower concentrations (580-794 ng/L) than acute effects [LC_{50}=5700 ng/L (48h)]. The acute/chronic ratio was 9.7:1. The US-EPA has suggested (R. Carlson, pers. Comm.) a probable ratio of 25:1. The determination of LC_x and EC_x with methylmercury on D. pulex will provide

a bioassay with which to estimate the concentration of methylmercury in natural waters over the world. Such techniques will be eagerly applied in China.

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