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EFFECTS OF CHROMIUM ON REPRODUCTION IN POLYCHAETES

Chromium is discharged into the marine waters off southern California in industrial and municipal wastewater effluents. During the past few years, the Project has been investigating the effects of chromium in the marine environment. Our major goals have been (1) to identify the forms of chromium that are biologically available, (2) to determine the level at which chromium has an observable effect on marine biota, and (3) to determine the environmental levels of chromium that can be considered safe with respect to the health of this biota.

As previously reported, our tests include monitoring the survival, behavior, and reproduction of *Neanthes arenaceodentata*, a local marine worm, exposed to several concentrations and forms of chromium. Our initial long-term experiments, which were partially reported last year, were carried out at Dr. Donald J. Reish's polychaete laboratory at California State University, Long Beach. We observed the effects of two forms of chromium (hexavalent and trivalent) on the maturation of individual juveniles, the pairing of males and females, the laying of eggs, and the development of the embryos to young worms over several generations. Both these previous experiments lasted many days (440 days for hexavalent and 293 days for trivalent) and were conducted with six to ten replicate pairs of polychaetes per concentration. Both experiments involved the use of individual 1-gallon glass jars as containers for each pair, and dried algae, *Enteromorpha crinita*, as a food source. The water quality (pH, total ammonia, nitrites, dissolved oxygen, temperature and chromium concentration) was monitored and controlled within specified limits.

The long-term hexavalent chromium experiment involved toxicant concentrations of 0.0125, 0.025, 0.050, 0.1, and 0.2 mg/liter. Although oocytes developed within the coelom of the female *N. arenaceodentata* at all concentrations, the worms in the two highest concentrations displayed abnormal jerking and twisting movements. These animals lacked the ability for coordinated movement, which made pair formation questionable, and the prolonged lateral contact between a female and male conducive to spawning impossible. Spawning and embryo development occurred in all but the two highest concentrations (Table 1). At the 0.2 mg/liter level, 50 percent of the

worms had died by Day 59, which was long before spawning should have begun. No spawning occurred in 0.1 mg/liter, but most of the worms were still alive after the time when spawning should have occurred. Thus, exposure to hexavalent chromium at a level of 0.1 mg/liter produced total inhibition of spawning in this polychaete species without causing death.

As reported last year, it was evident that brood sizes in the parental (P) and filial (F) generations of *N. arenaceodentata* decreased as the hexavalent chromium concentrations increased. This year, with the completion of the experiment and application of statistical analysis (Dunnett's Test, Steel and Torrie 1960), we have found that there was a significant reduction in brood sizes at 0.0125 mg/liter (and above) in the P_1 generation, and at 0.05 mg/liter in the F_1 and F_2 generations (Table 1). The results of this experiment suggested that the level of "no effect" for *N. arenaceodentata* not spawned and raised in elevated levels of hexavalent chromium is near 0.0125 mg/liter, with elevation by perhaps a factor of 4 for generations spawned in elevated concentrations.

Short-term tests were carried out in conjunction with this study to determine if there were differences in the chromium sensitivities of (1) laboratory and field populations and (2) succeeding generations of worms raised in seawater with enhanced levels of chromium. The results showed that the 7-day LC5Q values (the concentrations at which 50 percent of the worms had died in 7-days) for field and laboratory stocks were not significantly different (Table 2). Furthermore, the 7-day LC_{50} values did not significantly change from one generation of worms spawned and raised in hexavalent chromium to the next (Table 2): All the LC_{50} levels for the F_1 and F_2 generations raised in chromium were within the range of LC5Q values for stock juveniles kept in seawater. The worms showed no indications of adaptive changes in acute chromium sensitivity (LC_{50}) for two generations of exposure.

Worms from the long-term experiment were analyzed for body burdens of chromium. In collaboration with George Alexander (University of California at Los Angeles), F_1, F_2, and F_3 *Neanthes arenaceodentata* were depurated for 72 hours in clean seawater, freeze-dried and analyzed for whole body chromium levels by optical emission spectroscopy. Parental generation worms were not depurated prior to analysis. The tissues of worms from the P_1, F_1, and F_2, generations that had completed incubation of their respective offspring, as well as sexually immature worms from the F_3 generation, were analyzed. Worms of a specific concentration (e.g., 0.0125 mg/liter and a specific generation (e.g., F_1) were composited with worms of the same concentration and generation, freeze-dried, and analyzed as one sample Freeze-drying allowed us to determine that water accounts for about 76 percent of the total wet weight of the worms.

The results (Figure 1) show the chromium levels present in the body tissues to be proportional to the chromium levels in the toxicant solutions. Among worms from almost every concentration, -each successive generation has a higher body level of chromium than the previous generation. We have not

determined the specific mechanisms causing successive generations to have higher levels of tissue chromium than the previous ones.

A long-term experiment with trivalent chromium (as chromic chloride, $CrCl_3$) was set up in much the same manner as the long-term hexavalent chromium study. Sufficient $CrCl_3$ was added to the seawater to make the total dissolved and undissolved chromium concentration in each jar 50.4 mg/liter; the pH was then readjusted to that of normal seawater (7.8 to 8.1) with sodium hydroxide. Most of the chromic chloride added to the seawater formed a blue-gray precipitate, presumably $Cr(.OH)_3$, which settled to the bottom of the jars. Tsu-Kai Jan of the Project analyzed samples from the experiment using atomic absorption spectroscopy. His results showed that, at the adjusted pH, less than 0.02 mg/liter of chromium was actually dissolved (filterable through a 0.1-micron filter) in the water. Worms placed in the precipitate solutions readily built tubes on the bottoms of the jars, even though this meant prolonged contact with the undissolved chromium. They were also observed ingesting the blue-gray precipitate and excreting blue-gray fecal material, and thus were exposed both internally and externally to the trivalent chromium. There were no significant alterations in behavior, mortality, brood size or spawning time (Table 3). This experiment was concluded after 293 days (two worm generations).

In January, we initiated another long-term experiment with hexavalent chromium and *Neanthes arenaceodentata* to further investigate the chromium level that has "no effect" on *N. arenaceodentata* reproduction. The chromium concentrations selected (0.0025, 0.005, 0.01, 0.02, and 0.04 mg/liter) emphasized levels between the control values (less than 0.001 mg/liter) and the concentration at which there was a statistically significant reduction in brood size in the P-i generation of the prior hexavalent chromium experiment (0.0125 mg/liter).

The experimental design was modified so that we could test 20 to 30 pairs of worms in each concentration (the first experiment involved 6 to 10 pairs per concentration). Ninety juvenile worms were added to each of six 76-liter (20-gallon) aquaria filled with their respective test solutions. There was natural die-off, probably due to crowding and cannibalism (Table 4). After 45 days in the test solutions, the remaining males and females were paired. Each pair of worms was placed in a glass tube (which was 43 mm in diameter, 80 mm long, and covered on both ends by a polyester mesh with 0.5 sq mm openings) and added to the aquaria. The isolation of pairs in tubes eliminated cannibalism. The water quality was monitored weekly, and test solutions were changed every 1 to 3 weeks.

Twenty-three to 29 pairs in each concentration spawned (Table 4); at this time, 13 to 20 of the broods in each concentration have been counted. The experiment will continue until offspring are produced by the Fi generation.

The results of this second long-term experiment may differ from those obtained in the initial experiment because the conditions of the'two experiments were not identical. When the mean brood sizes of the available control generations in the first (P_1, F_1, and F_2) and second (P_1) experiments are

compared, the coefficient of variation of the means is less than 15 percent. The mean brood sizes for the experiment in progress are reported in Table 4. Brood size in one concentration (0.02 mg/liter) was significantly larger than the control when tested with Dunnett's procedure (Steel and Torrie 1960). With the continued counting of the broods, more subtle changes in brood sizes due to chromium may become more obvious.

At this time, any assessment of the results of the two long-term hexavalent chromium toxicity tests has to be made with reservations, as the second experiment is still in progress. It appears that, in general, a larger number of young are produced by each pair in the more recent experiment. This change in offspring numbers may reflect the changes in experimental procedure. A comparison of the levels of statistically significant effects of hexavalent chromium on *Neanthes arenaceodentata* for the first and second experiments shows the effective levels to be 0.0125 mg/liter and 0.020 mg/liter, respectively. The effective level for the second study may change as more broods are counted.

We feel that *Neanthes arenaceodentata* is a reliable test animal and warrants more use in bioassays. We will continue to study the behavior and effects of chromium, closely coordinating our biological experiments with those of our chemistry staff to obtain a more comprehensive understanding of this trace element. Alan Mearns, David Young, Cindy Word, Tsu-Kai Jan and Jean Wright provided valuable assistance in setting up and monitoring experiments. The completed work on *Neanthes arenaceodentata* is described in a recent publication, TM 225, available at no charge from the Project.

REFERENCES

Steel, R.G.D., and J.H. Torrie. 1960. Principles and procedures of statistics. New York: McGraw-Hill.

Table 1. Results of 440-day test of the toxicity of hexavalent chromium to *Neanthes arenaceodentata.*

Effect	Chromium Concentration (mg/liter)*					
	Control	0.0125	0.025	0.05	0.1	0.2
P_1 Generation						
50% mortality with no eggs laid	–	–	–	–	Day 184	Day 59
Oocyte development in coelom	Yes	Yes	Yes	Yes	Yes	Yes
Number of pairs that spawned	6	9	9	5	0	0
Mean time to spawning (days)	112	100	90	123		
Mean brood size	255	133	146	78		
Total offspring	1,528	1,199	1,315	391		
F_1 Generation						
50% mortality with no eggs laid	–	–	–	–		
Oocyte development in coelom	Yes	Yes	Yes	Yes		
Number of pairs that spawned	9	10	9	7		
Mean time to spawning (days)	153	130	138	111		
Mean brood size	292	258	164	59		
Total offspring	2,628	2,580	1,477	415		
F_2 Generation						
50% mortality with no eggs laid	–	–	–	–		
Oocyte development in coelom	Yes	Yes	Yes	Yes		
Number of pairs that spawned	8	10	7	10		
Mean time to spawning (days)	129	132	124	118		
Mean brood size	273	190	151	111		
Total offspring	2,186	1,895	1,056	1,112		

*6 to 10 pairs were kept in each concentration.

Table 2. Short-term test of the toxicity of hexavalent chromium to *Neanthes arenaceodentata.*

Test Animals	7-Day LC_{50} (mg/l)	
	Mean	95 Percent Confidence Limits*
Field Specimens	1.48	–**
Parental Generation	1.44–1.89	–**
Filial Generations		
Control Specimens†	1.78	1.54–2.05
Specimens Raised in Chromium Solutions		
0.0125 mg/l		
F_1	1.77	1.66–1.88
F_2	1.75	1.59–1.99
0.025 mg/l		
F_1	1.70	1.44–1.99
F_2	1.65	1.51–1.79
0.05 mg/l		
F_1	1.67	1.56–1.79
F_2	1.69	–**

*Two-tailed test.

**95% confidence limits were not generated as there were too few data points.

†Data for F_1; 7-day LC_{50} for F_2 was not performed.

Table 3. Results of 293-day test of the toxicity of trivalent chromium to *Neanthes arenaceodentata.*

Effect	Control	Chromium Precipitate
P_1 Generation		
50% mortality with no eggs laid	–	–
Oocyte development in coelom	Yes	Yes
Number of pairs that spawned	9	9
Mean time to spawning (days)	73	91
Mean brood size	153	186
Total offspring	1,380	1,676
F_1 Generation		
50% mortality with no eggs laid	–	–
Oocyte development in coelom	Yes	Yes
Number of pairs that spawned	8	8
Mean time to spawning (days)	159	151
Mean brood size	348	248
Total offspring	2,781	1,488

Table 4. Preliminary results (Day 182) of a test of the toxicity of hexavalent chromium to *Neanthes arenaceodentata.***

	Chromium Concentration (mg/l)					
Effect, P_1 Generation	Control	0.0025	0.005	0.01	0.02	0.04
Initial number (Day 0)	90	90	90	90	90	90
Number alive Day 7	72	79	76	78	77	79
Pairing (Day 45)						
Number alive	54	58	53	67	67	59
Ratio, males to females	29:25	31:27	24:29	43:24	38:29	36:23
Number of pairs	24	25	24	24	29	23
Number that have laid eggs (Day 182)	22	25	24	23	28	21
Number of broods counted (Day 182)	20	17	15	13	14	13
Brood size						
Mean	346	262	387	351	597	249
Standard deviation	226	169	226	223	265	172

*Experiment is still in progress.

Figure 1. Concentrations of chromium in the body tissues of *Neanthes arenaceodentata* exposed to hexavalent chromium in long-term toxicity test.