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EFFECTS OF CHROMIUM ON REPRODUCTION IN POLYCHAETES

We have conducted a number of laboratory experiments that suggest that hexavalent chromium is extremely toxic to marine polychaete worms, whereas chromium in the trivalent state is not. These tests involved monitoring the survival, behavior, and reproduction of *Neanthes arenaceodentata* (a nearshore benthic marine worm) exposed to a wide range of chromium concentrations (0.013 to 50.0 ppm). This species was chosen because it has a short life cycle (2 to 3 months), can be cultured in the laboratory, is available in southern California coastal waters, is an important member of the marine food-web, and is more sensitive to water quality conditions than other marine worms. Experimental work was done with both the Reish strain of *Neanthes arenaceodentata* that is maintained at California State University, Long Beach, and wild worms of the same species collected near the mouth of the San Gabriel River. These marine worms naturally inhabit and live in mucous tubes within marine sediments or attached to hard surfaces.

Young *N. arenaceodentata* were used in a series of short-term (7- to 14-day) and long-term (up to 10-month) toxicity tests of hexavalent chromium ($K_2CR_2O_7$ and $Cr0_3$) and tri-valent chromium ($CrCl_3$). The short-term tests were run under static conditions using one animal (e.g., 10 mg wet weight, 30 to 40 segment stage) per 100 ml of test solution in 500-ml Erlenmeyer flasks. Animals were fed a dried marine alga. *Enteromorpha crinita*. Long-term tests, involving the maturing of individual juveniles, the pairing of males and females (P_1), and the laying of eggs and hatching of young (F_1), were conducted in 3.8-liter glass jars containing 3 liters of test solution per worm or pair (adults, 20 to 30 mg, 80 to 90 segments), a system previ-ously described by Reish.

Twenty replicates were used for each concentration in both acute and long-term experiments, and additional test containers with animals were set up for monitoring water quality (pH, dissolved oxygen, salinity, temperature, ammonia, and nitrites) and toxicant concentrations. Test solutions were renewed with fresh solutions at 2- or 3-week intervals to maintain good water quality in the long-term experiments.

The overall experimental plan is summarized in the flow-diagram in Figure 1. In all, 13 acute toxicity tests and three long-term tests were completed or initiated during the past year.

The short-term tests, each involving up to 140 animals per test, were carried out to determine the effects of chromium on (1) the survival and mortality of *N. arenaceodentata* with exposure to high concentrations LC_{5O} experiments) and (2) the sublethal responses of the species, such as effects on mucus secretion and tube building (EC_{5O} experiments). We also compared the tolerance of wild worms with that of the laboratory strain and tested for the development of resistance in subsequent generations of worms raised in chromium solutions. Several chromium compounds were used in this series of tests.

Tests using hexavalent chromium showed that chromium concentrations lethal to *N. arenaceodentata* at 20°C were from 2.22 to 4.30 ppm in 4 days, from 1.44 to 1.89 in 7 days, 0.78 in 14 days, and 0.2 ppm in 56 days (Table 1). The lowest level that inhibited tube building was 0.079 ppm in 14 days. The LC5Q value obtained from the experiment using wild worms was within the range recorded for laboratory animals, indicating that the use of cultured worms does not significantly bias the results. Moreover, all LC5Q levels for the F_1 generation raised in chromium were also within the range of LC_{50}Q values set for laboratory worms; this suggests that previous exposure did not cause *N. arenaceodentata* to develop resistance to chromium in one generation.

A short-term study exposing the worms to trivalent chromium (as $CrCl_3$) was initiated; the range of concentrations desired was 0.195 to 50.0 ppm dissolved chromium. However, at these chromium levels, a blue-gray precipitate formed in the test containers; in addition, there was a general lowering of the seawater pH with the increased addition of $CrCl_3$. No significant mortality was seen at the 0.195-, 0.78-, 3.12-, and 12.5-ppm levels over a period of 3 weeks (pH values ranged between 7.0 and 7.8); however, there was total die-off within 24 hours in the 50.0-ppm concentration, which probably resulted from an extremely low pH (4.5). Thus, trivalent chromium was found to be considerably less toxic than equivalent concentrations of the hexavalent form in tests carried out under similar conditions.

EFFECTS ON BEHAVIOR AND REPRODUCTION

To assess the impact that various levels of chromium may have on the reproductive cycle of *N. arenaceodentata*, we began a series of long-term experiments using both hexavalent and trivalent forms of chromium. The data on the long-term (>250 days) effects of hexavalent chromium at concentrations of 0.0125 to 0.2 ppm on reproduction and survival are summarized in Table 2. The development of oocytes (eggs) within the coelom of females was clearly visible through the body wall in all the exposed animals and the controls. However, eggs were never laid in the two highest levels of chromium (.0.1 and 0.2 ppm). When the males and females were paired on Day 53, all but the worms in 0.2 ppm showed normal behavior. The worms at 0.2 ppm displayed abnormal jerking and twisting behavior, which prevented the "paired" specimens from remaining in contact with each other and rendered the unpaired specimens unable to move in a direct motion toward a potential mate. By Day 59, 10 of the 20 worms in this highest concentration had died.

The presence of hexavalent chromium at the lowest experimental level, 0.0125 ppm, caused a decrease in the mean brood size to a value less than one-half that of the controls (149.9 and 305.6, respectively). Brood size also decreased in the two higher concentrations in which spawning occurred, 0.025 and 0.050 ppm (137.0 and 78.2, respectively). The relative mean times required before spawning occurred were longer in the controls (117 days) and 0.05 ppm concentrations (121 days) than in the two intermediate concentrations, 0.0125 ppm (107 days) and 0.025 ppm (.90 days). Reduced spawning time may be of utmost importance as it could result in a net increase in the number of generations produced in a year and yield a significantly larger population in those animals exposed to slightly elevated chromium concentrations (0.025 ppm). This response may be the result of a metabolic stimulus provided by chromium, or a natural defense mechanism against toxicity.

Young worms (F^) were taken from the brood containers and set up under the same conditions as the parent generation 152 days after the experiment was initiated. During the next 100 days, the F^ worms in all three concentrations (0.0125, 0.025, and 0.05 ppm) and the control developed oocytes in the coelom and began to spawn; by Day 101, eight pairs in 0.0125 ppm had spawned, eight pairs in 0.025 ppm, five pairs in 0.05 ppm, and three pairs in the control. These data indicate that reproduction is inhibited at levels of 0.05 ppm hexavalent chromium. Effects at concentrations below this level require further study.

A similar long-term reproduction and survival experiment using trivalent chromium has been in progress for 107 days. There has been no significant mortality prior to spawning— only one worm (5 percent) of the original 20 has died in each of the two levels, control and precipitate. The worms living in the precipitate solutions have shown natural behavior throughout the experiment: They have been able to build mucous tubes attached to the glass on the bottom (although they had to build them among the precipitate), and they have been observed eating the Enteromorpha crinita. Upon close examination, the blue-green color characteristic of the chromium precipitate can be seen in the guts of the worms. In fact, blue-green fecal pellets are produced by the polychaetes, indicating that the worms ingest and excrete chromium—laden sediment. This exposure has not halted spawning; eight of the nine pairs in the chromium mixture have spawned, and all nine control pairs have laid eggs (Table 3). The numbers of young worms produced have not yet been determined as the embryos are still developing. This experiment is still in progress, but the data already suggest that pure trivalent chromium in seawater forms a precipitate that is not toxic to *N. arenaceodentata*.

SUMMARY

Hexavalent chromium is considerably more toxic to *N. arenaceodentata* than trivalent chromium under the conditions used in our tests. This suggests that standards based on hexavalent chromium may have more ecological significance than those based on total or trivalent chromium. Additional tests are required to further define the level of hexavalent chromium that does not affect reproduction; that level will probably occur in the range of 0.001 to 0.025 ppm.

The present experiments are continuing, and animals will be measured to determine relationships between exposure and uptake in their tissues. These results were presented at the recent annual meetings of the California Water Pollution Control Federation, and the Southern California Academy of Sciences. David Young, Cindy Word, Tsu-Kai Jan, Jean Wright and Diana Vermillion provided valuable assistance in designing, setting up, and monitoring experiments.

Figure 1. Summary of experimental plan for Neanthes arenaceodentata.

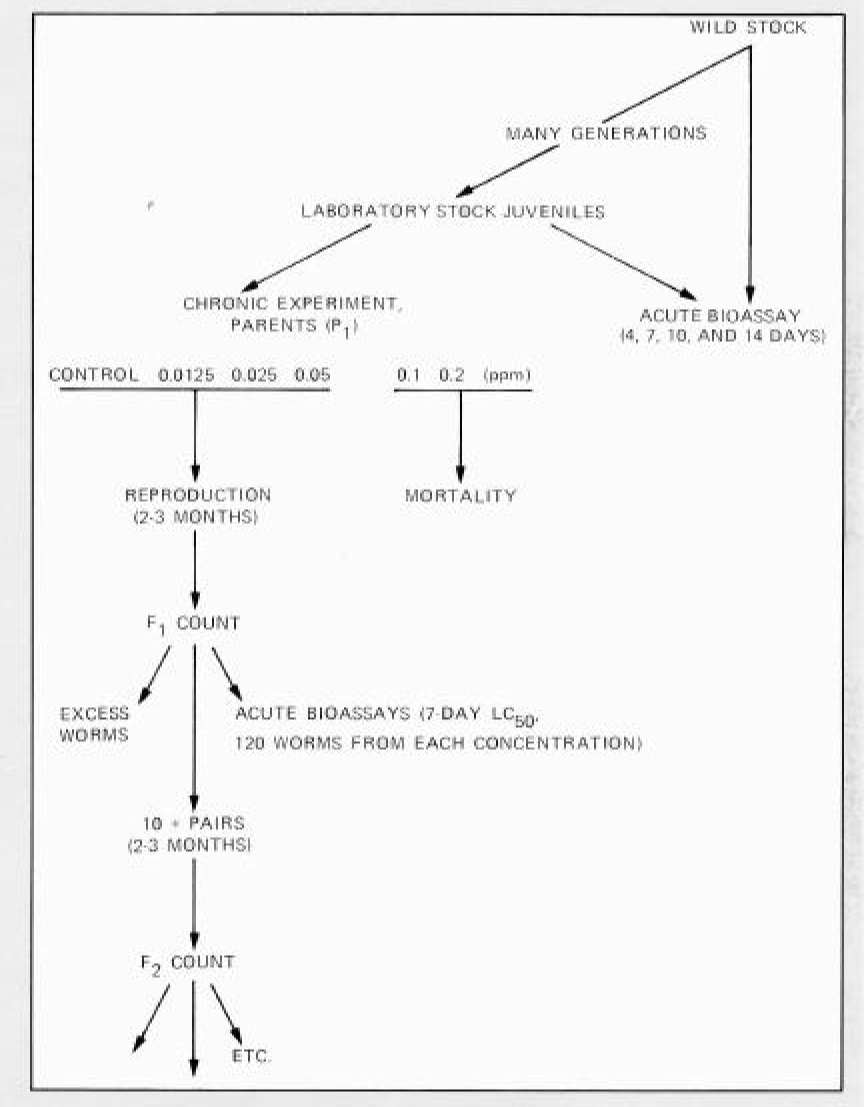

Table 1. Short-term experiments on Neanthes.

Life Stage (Origin) (ppm)	Exposure Time (Days)	Chromium Concentration (ppm)	4-day LC_{50}* (ppm)	Lack of Tube Production	Final LC_{50}
Hexavalent chromium as $K_2Cr_20_7$					
Juvenile (stock)	10	0.2-50.0	4.30		
Juvenile (stock)	14	0.06-1.0	-**	0.079	0.78
Juvenile (stock)	7	0.63-5.0	2.00	0.23	
Juvenile (stock)	7	0.67-2.8	2.80	0.23	
Juvenile (stock)	56	0.13-0.20	-**		
Juvenile (stock)	7	0.62-5.0	2.22		1.89
Juvenile (stock)	7	0.67-2.8	-**		1.44
Adults (San Gabriel R.)	7	0.31-5.0	3.23		1.48
Juveniles (Control-P_1)	7	0.31-5.0	3.45		1.78
Juveniles (0.0125 ppm-P_1)	7	1.18-2.0	-**		1.77
Juveniles (0.025 ppm-P_1)	7	0.31-5.0	3.63		1.70
Juveniles (0.05 ppm-P_1)	7	1.18-2.0	-**		1.67
Hexavalent chromium as CrO_3					
Juvenile (stock)	14	0.062-0.5	-**		-**
Trivalent chromium as $CrCl_3$					
Juvenile (stock)	14	0.195-50.0	-***		-***

*Concentration at which there was 50 percent mortality.
**No significant mortality, LC50 could not be generated.
***Mortality was probably due to low pH, not chromium.

Table 2. Long-term experiments on Neanthes in hexavalent chromium.

	Chromium Concentration (pm)					
Effect	**Control**	**0.0125**	**0.025**	**0.05**	**0.1**	**0-.2**
P_1, Day 253 (concluded						
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	5	8	10	5	0	0
Mean time to spawning (days)	117	107	90	121		
Mean brood size	305.6	149.9	137.0	78.2		
Total offspring	1,528	119	1,370	391		
F_1, Day 101 of 253 (in progress*)						
50% mortality with no eggs laid	-	-	-	-		
Oocyte development in coelom	Yes	Yes	Yes	Yes		
Number of pairs that spawned	3	8	8	5		
Mean time to spawning (days)	99.0	69.5	71.6	72.2		

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*Brood size and offspring data not yet available.

Table 3. Long term experiment on Neanthes in trivalent chromium.

Effect	**Control**	**Chromium Precipitate**
P_1, Day 107 (in progress)		
50% mortality with no eggs laid	-	-
Oocyte development in coelom	Yes	Yes
Number of pairs that spawned	9	8
Mean time to spawning (days)	73.1	85.6