#63

TM 225
FEBRUARY 1976

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This work was supported in part by the Coastal Water Research Project and in part by the U.S. Environmental Protection Agency under Grant R800962 to Dr. Donald J. Reish.

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ABSTRACT

In a study of the relative lethal and sublethal effects of two forms of chromium on Neanthes arenaceodentata, hexavalent chromium (as $K_2Cr_2O_7$) showed 96-hour and 7-day LC_{50} ranges of 2.2 to 4.3 and 1.44 to 1.89 mg/l, respectively. In a long-term (three generations, 440 days) experiment with hexavalent chromium, reproduction ceased at 0.10 mg/l, and there was a reduction in brood size at levels of 0.0125 mg/l and above. In the experiments conducted with trivalent chromium (as $CrCl_3$), in which the worms were exposed to a precipitated form of chromium ion, there was less than 5 percent mortality at 12.5 mg/l during a 7-day period. Worms that lived in the chromium precipitate during a long-term (two generations, 293 days) experiment showed no adverse effects or abnormal behavior. Thus, although hexavalent chromium reduces brood sizes at relatively low seawater concentrations (0.0125 mg/l), trivalent chromium as a precipitate in seawater had no detrimental effects on the worms at concentrations of 50.4 mg/l.

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INTRODUCTION

Trace quantities of chromium are discharged into the marine waters off southern California in industrial and municipal wastewater effluents. Although this element is known to be potentially toxic, very little is known about its chemical and biological behavior in the marine environment.

Chromium occurs naturally in ocean waters, and natural concentrations off southern California--less than 0.0002 mg/l (Jan and Young, in preparation)--are significantly lower than those in the municipal wastewaters discharged into these waters (total chromium concentrations in southern California effluents can average as high as 0.9 mg/l; however, this input is lowered to 0.009 mg/l if one assumes an immediate dilution of 100 to 1 through diffuser ports; Mitchell and McDermott 1975). Most ocean sediments show much higher chromium levels than ocean waters. Chromium concentrations ranging from 4 to 93 mg/dry kg have been recorded for Pacific Ocean sediment samples (Robertson et al. 1972), and concentrations up to 870 mg/dry kg have been reported for sediments surrounding a wastewater discharge site (Sherwood 1975). The large differences in chromium concentrations in various oceanic regimes may be due, in part, to the chemical behavior of chromium in the marine environment.

Chromium occurs in several oxidation states, the most common of which are hexavalent and trivalent. Each of these forms has different chemical properties in the ocean environment that can evoke distinct biological responses. Hexavalent chromium is quite soluble in water, is a strong oxidizing agent (although it can be reduced to the trivalent form in a reducing environment), and can elicit toxic responses in some animals. Trivalent chromium, on the other hand, is the more stable ion and often forms insoluble complexes that are biologically inert (Mertz 1969). Although a few studies have documented toxic effects induced by trivalent chromium, investigations dealing with various complexed trivalent forms have not been undertaken. Besides the toxic effect, there is some evidence that an unidentified form of trivalent chromium plays a beneficial role in some metabolic cycles (National Research Council 1974; Mertz 1969).

Because of the varied responses elicited by different chromium forms, the oxidation state of chromium compounds appears to have

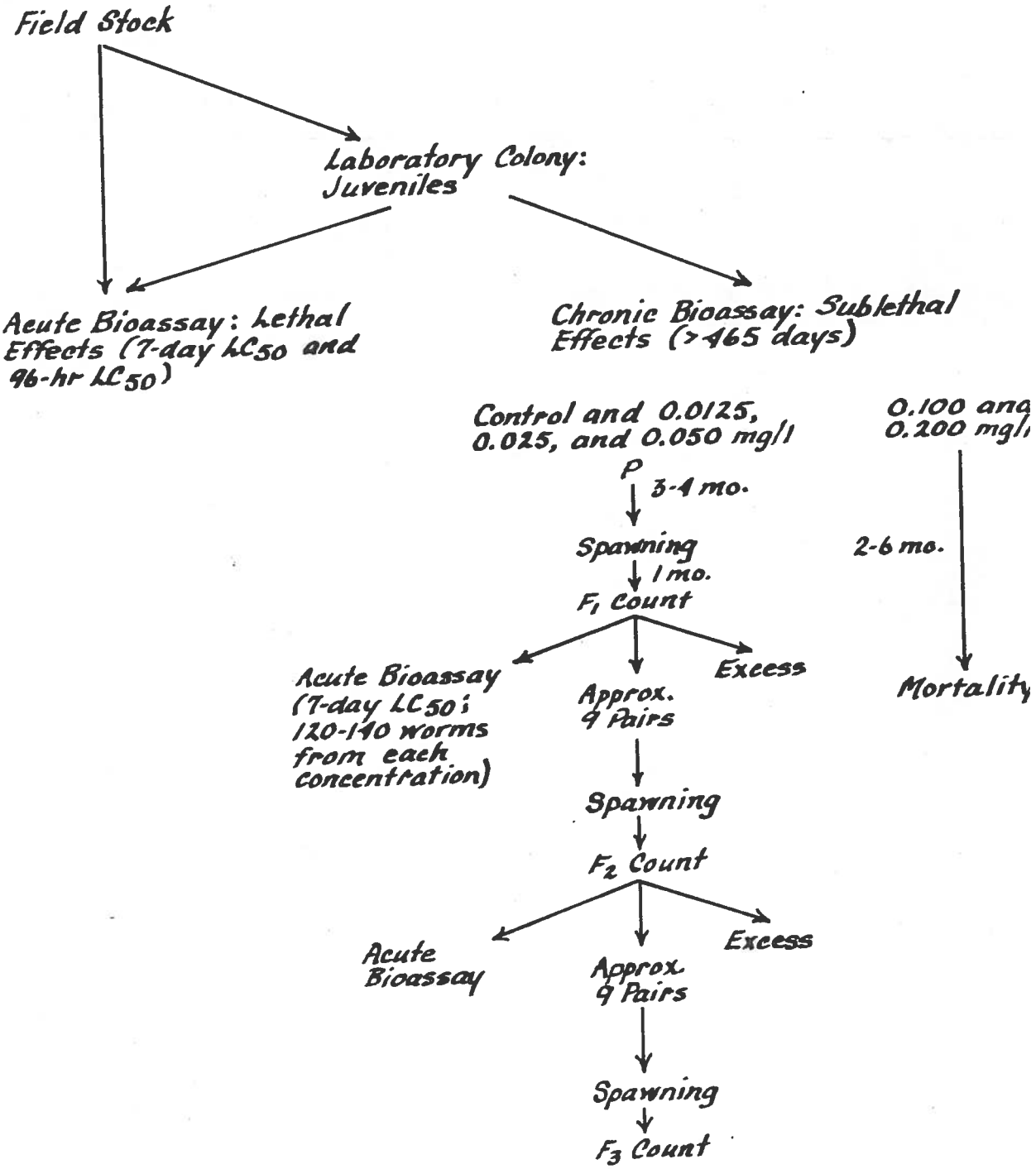

Figure 1. Experimental plan for tests of the toxicity of hexavalent chromium to Neanthes arenaceodentata.

an important bearing on the results of toxicity studies. In the past, total chromium rather than specific oxidation states of the metal has been the prime concern of investigators: As a result, values for total chromium form the basis of most previous data records and also the present wastewater discharge standards. Although ranges of acute toxicity have been reported for a number of marine species (Appendix A, Tables A-5 and A-6), it is difficult to compare the results of these studies because of the different experimental formats. However, the data indicate that, although chromium is less toxic than mercury and copper, hexavalent chromium is more toxic than zinc, lead, and cadmium. (It should be noted that much of the work done on freshwater species is not applicable to the marine situation.)

Thus, several major issues emerge: In what forms is chromium biologically active? At what levels are observable effects induced in marine biota? And what environmental levels can be considered safe with respect to the health of this biota?

During 1974, the Coastal Water Research Project initiated a program to investigate the chemical and biological behavior of chromium in the marine environment. In one phase of this program (Oshida and Reish 1975), we explored the biological effects of both hexavalent and trivalent forms of chromium on a species of polychaetous annelid, Neanthes arenaceodentata. Polychaetes are important members of the marine food web and constitute a large portion of the benthic communities in southern California coastal waters. Laboratory experiments were designed to investigate chromium effects on this worm: The objectives were to determine sublethal levels and effects on the reproductive cycle, to ascertain if there is acclimation or adaptive response in succeeding generations, and to measure accumulation levels in body tissues.

An overview of the experimental plan for hexavalent chromium is given in Figure 1. Chemical analyses were performed in conjunction with our experiments to gain concurrent information on the chemical behavior of the chromium compounds used. The results of both the biological and chemical aspects of the study are summarized in this technical memorandum. Appendix A contains the results of the literature review, and Appendix B contains details of the biological, chemical, and statistical procedures used in the study.

GENERAL PROCEDURES AND SHORT-TERM EXPERIMENTS

The nereid polychaete Neanthes arenaceodentata* was chosen for this study because it has a short life cycle, is easy to culture under laboratory conditions, and is available in southern California coastal waters. Experimental work was done both with the Reish strain of N. arenaceodentata, which was maintained at California State University, Long Beach, and with specimens collected from seawater near the mouth of the San Gabriel River a few days prior to experimentation.

In the short-term phase of the experiments, we performed 96-hour and 7-day tests on stock laboratory juveniles, field adults, and offspring of adults raised in chromium solutions. The results of these tests allowed the estimation of levels of chromium causing mortality and abnormal behavior. As much of the toxicity work done with chromium on marine invertebrates by other investigators was also short-term, the LC_{50} values for N. arenaceodentata gave a basis for comparison with other animals. The 7-day tests also allowed us to compare the chromium sensitivities of laboratory and field popultions. This was necessary for the justification of the laboratory animals as species representatives in chromium experiments.

Results from the short-term tests were used to project a concentration range for use in long-term experiments designed to investigate sublethal effects of chromium on succeeding generations of N. arenaceodentata. In addition, these tests allowed for easy assessment of possible changes in chromium sensitivities in the succeeding generations of worms raised in seawater with enhanced levels of chromium.

In each short-term test, one worm specimen was placed in a 500-ml Erlenmeyer flask with 100 ml of toxicant solution, and the flask was tightly closed. Twenty replications were made at each concentration; a series of flasks for control specimens was also set up. The worms were not fed during these tests.

Each specimen was examined daily to see if it was alive, able to build and live in mucus tubes, and exhibiting normal movement. The lack of blood pulses in the easily visible dorsal blood vessel was the criterion for death. At the end of the prescribed

*Neanthes arenaceodentata (Moore) [= Nereis (Neanthes) acuminata Ehlers fide Day 1973; = Neanthes caudata delle Chiaje fide Pettibone 1963].

experimental period, the number of survivors in each chromium concentration was noted, and the level at which 50 percent of the animals died (LC_{50}) was determined according to the procedures outlined by Litchfield and Wilcoxon (1949).

The control specimens displayed normal behavior in the short-term toxicity tests. Laboratory conditions did not inhibit their ability to build and live in mucus tubes. Healthy worms usually built and remained in tubes located at the air/water interface. Worms adversely affected by chromium displayed a regular pattern of behavior prior to death. Their first reaction was to move from the interface region toward the bottom of the flasks. This movement downward appeared to be coupled with their inability to build mucus tubes, which in turn severely limited their ability to remain in contact with the substratum. These worms were then seen floating lethargically near the bottom until they reached a point where jerky, contorting and curling movements started; after this, the worms went through a stage of inactivity prior to death. At times, they died with their proboscis everted, but with no regularity.

The results of the short-term tests are summarized in the following sections. Appendix B contains a description of the laboratory colony and details on the sampling and laboratory procedures.

SHORT-TERM TESTS, HEXAVALENT CHROMIUM

Ninety-six-hour and 7-day tests were performed with hexavalent chromium as potassium dichromate, $K_2Cr_2O_7$. The chromium ranges for each test varied within the limits of 0.31 and 5.0 mg/l, with four to six levels within each range.

The 96-hour LC_{50} values for hexavalent chromium ranged from 2.2 to 4.3 mg/l. A comparison of the 7-day LC_{50} ranges for stock juveniles (1.44 to 1.89 mg/l) with the value for field worms (1.48 mg/l) indicated that there were no significant differences in the chromium sensitivities of the two populations. Thus, the polychaetes already in laboratory culture were judged to be fair species representatives for the examination of chromium effects.

The offspring that had been spawned and raised in chromium solutions in the long-term experiment were tested to see whether or not the 7-day LC_{50} values changed from one generation to the next due to the presence of hexavalent chromium. Two filial generations (F_1 and F_2) were tested. All LC_{50} levels for the F_1 and F_2 generations raised in chromium were within the range of LC_{50} values for stock juveniles kept in seawater (Table 1).

SHORT-TERM TESTS, TRIVALENT CHROMIUM

We initiated a short-term study of the toxicity of trivalent chromium in the form of chromic chloride, $CrCl_3$. The toxicant range was to be from 0.195 to 50.0 mg/l dissolved chromium.

Table 1. 7-day LC_{50} values for hexavalent chromium on Neanthes arenaceodentata.

Test Animals	7-Day LC_{50} (mg/l) Mean	95% Confidence Limits*
Field specimens	1.48	-**
Laboratory colony		
Parental generation	1.46	-**
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 are for F_1; 7-day LC_{50} test for F_2 was not performed.

However, we found that, at the proposed chromium levels, most of the chromium was precipitated in a compound that settled to the bottom of the flasks. There was also a general lowering of the seawater pH with the increased addition of chromic chloride. Specimens of N. arenaceodentata were added to these precipitate/solution flasks and observed.

No significant mortality was seen in the flasks with concentrations of 0.195, 0.78, 3.12, and 12.5 mg/l trivalent chromium, and the pH values in these flasks remained between 7.0 and 7.8. However, there was total mortality within 24 hours in the flasks with 50.0 mg/l concentration, and the pH in these flasks had dropped to 4.5. The low pH was believed to be responsible for the deaths in the 50.0 mg/l concentration because, in the subsequest long-term experiment, worms in 50.4 mg/l solution with the pH adjusted to 7.8 to 8.1 showed no adverse effects.

DETERMINATION OF SUBLETHAL EFFECTS

A series of long-term experiments was conducted to investigate the consequences of chromium exposure to the reproductive rate, egg-laying capacity, maturation rate, and normal behavioral patterns of Neanthes arenaceodentata. Two sets of experiments were initiated, one using hexavalent chromium and the other trivalent chromium.

Similar procedures (described in Appendix B) were established for both sets of experiments so that biological and chemical comparisons could be made. Laboratory colony specimens were used; each specimen had 30 to 40 setigers and was about 1 cm in length. Twenty-two 1-gallon jars, each containing 3 liters of the appropriate solution and one worm, were set up for each chromium concentration; a series of control jars was also established. Twenty jars were designated for biological observations of the worms, and two jars were used for water quality monitoring (monitoring procedures are described in Appendix B). The pH was maintained between 7.8 and 8.0, the dissolved oxygen above 75 percent saturation, the ammonium ion below 0.5 mg/l, the temperature at 20° ± 0.6°C, the nitrites below 0.5 mg/l, and the chromium concentration within 10 percent of its prescribed experimental value. To keep the water quality within these levels, the solutions were changed every 3 weeks. Dried green algae (Enteromorpha crinita) was added weekly as a food source.

Biological observations were made two to five times a week to determine mortality, tube-building capabilities, behavior, and feeding activity. When the sex of the worms could be established (19 to 55 days from the start of the experiment), the males were paired with females in their respective chromium concentration. Not all worms could be paired because of unequal sex ratios among the twenty worms in each concentration. These paired and unpaired specimens were observed one to five times a week for deaths, feeding behavior, tube-building, egg-laying, and brood incubation. The females naturally died after spawning, leaving the males to incubate the eggs. After incubation had continued for about 23 days, the adult males were removed, and each was placed alone in a jar containing the same chromium concentration. The offspring (F_1 generation) in each jar were counted approximately 1 week after the male had been removed. The offspring were allowed to grow to 30 to

Table 2. Mean times to spawning (egg to spawning adult) for three generations of Neanthes arenaceodentata in hexavalent chromium.

Concentration (mg/l)	Generation	Time to Spawning (Days)* Mean	95% Confidence Limits**
Control	P	112	79-145
	F_1	153	142-164
	F_2	129	118-140
0.0125	P	100	70-130
	F_1	130	118-140
	F_2	132	120-144
0.0250	P	90	77-103
	F_1	138	120-157
	F_2	124	110-138
0.050	P	123	86-160
	F_1	111	87-124
	F_2	118	105-131

*For P generation, time is from Day 0 of the experiment.
**Two-tailed test.

Table 3. Number of young per brood for three generations of Neanthes arenaceodentata spawning in hexavalent chromium.

Concentration (mg/l) and Generation		No. of Pairs	No. of Females that Laid Eggs	No. of Young/Brood Mean	95% Confidence Limits*
Control	P	9	8	255	104-406
	F_1	9	9	292	145-439
	F_2	9	8	273	99-364
0.0125	P	10	9	133	59-208
	F_1	10	10	258	146-370
	F_2	10	10	190	102-277
0.0250	P	10	10	146	88-204
	F_1	9	9	164	94-234
	F_2	9	7	151	87-215
0.050	P	9	5	78	39-117
	F_1	10	7	59	15-104
	F_2	10	10	111	33-189
0.100	P	9	0		
0.200	P	2	0		

*Two-tailed test.

40 setigerous segments, at which time 22 were set up in the same manner used with the parent generation. All offspring remained in their respective chromium concentrations for the duration of the long-term experiment. This procedure was repeated for the F_2 generation.

SUBLETHAL EFFECTS OF HEXAVALENT CHROMIUM

The long-term hexavalent chromium experiment involved chromium concentrations of 0.0125, 0.025, 0.050, 0.100, and 0.200 mg/l.

During the experiment, the normal development of eggs (oocytes) within the coelomic cavity of the female worms was apparent in specimens at all exposure concentrations. However, at the time of pairing (Day 55), those worms in the two highest concentrations (0.1 and 0.2 mg/l) were displaying abnormal jerking and twisting movements. These specimens lacked the ability for coordinated movement, which made pairing questionable and the prolonged lateral contact between male and female necessary for spawning impossible.

Spawning occurred in all but the two highest concentrations (Table 2). At the 0.2 mg/l concentrations, 50 percent of the worms had died on Day 59, long before the time when spawning should have begun. At the 0.1 mg/l concentration, no spawning occurred, but most of the worms were still alive after the time when spawning should have occurred (there was 50 percent mortality on Day 184 at this concentration). Thus, exposure to 0.1 mg/l hexavalent chromium caused inhibition of spawning without causing death.

As the chromium concentration increased from the control value (less than 0.001 mg/l) to 0.05 mg/l, number of young per brood decreased; mean number of young per brood is shown in Table 3.

SUBLETHAL EFFECTS OF TRIVALENT CHROMIUM

A long-term experiment on the toxicity of 50.4 mg/l trivalent chromium as chromic cloride, $CrCl_3$, was set up in much the same manner as the long-term hexavalent experiment. As mentioned earlier, in our experiments, most of the chromic chloride added to the seawater formed a blue-gray precipitate (presumably chromic hydroxide, $Cr(OH)_3$), which settled to the bottom of the 1-gallon jars. Sufficient chromic chloride was added to the seawater to make the mixture (total, dissolved, and undissolved) in each jar 50.4 mg/l. Analysis showed that less than 0.020 mg/l of chromium was actually dissolved in the water (not filterable on a 0.1-micron filter), which meant that over 99.9 percent of the trivalent chromium was undissolved. The addition of chromic chloride caused the pH of the seawater to drop; for this reason, sodium hydroxide, NaOH, was added to readjust the pH to that of normal seawater (7.8 to 8.1). The worms were added and

Table 4. Mean times to spawning (egg to spawning adult) for two generations of Neanthes arenaceodentata in trivalent chromium precipitate.

Solution	Generation	Time to Spawning (Days)* Mean	95% Confidence Limits**
Control	P	73	68-79
	F_1	159	138-181
$Cr(OH)_3$ Precipitate	P	91	77-105
	F_1	151	125-178

*For P generation, time is from Day 0 of the experiment.
**Two-tailed test.

Table 5. Number of young per brood for two generations of Neanthes arenaceodentata spawning in trivalent chromium precipitate.

Solution and Generation	No. of Pairs	No. of Females That Laid Eggs	No. of Young/Brood Mean	95% Confidence Limits*
Control				
P	9	9	153	77-230
F_1	9	8	348	221-474
$Cr(OH)_3$ Precipitate				
P	9	9	186	111-261
F_1	8	6	248	51-445

*Two-tailed test.

allowed to live among the precipitate. Worms were also set up in clean seawater (no sediment) as controls. All experimental animals were observed and fed in the same manner as in the hexavalent chromium experiment. After the initial generation (P) had spawned, the procedure was repeated with the offspring.

Worms placed in the solutions with the chromic chloride precipitate readily built tubes on the bottom of the jars, even though this meant prolonged contact with the undissolved trivalent chromium. Worms were observed ingesting the blue-gray precipitate as well as excreting blue-gray fecal pellets. This indicates that they were exposed to the trivalent chromium internally as well as externally. N. arenaceodentata exposed to the precipitate showed no significant alterations in behavior or mortality throughout the 293-day experimental period when compared to the control worms. Also, the presence of the trivalent chromium did not significanly change the spawning time or the brood sizes, as shown in Tables 4 and 5.

CHEMICAL MONITORING OF EXPERIMENTS

The dualistic approach of simultaneously observing both chemical and biological behavior of a trace element has proven to be informative and necessary. By closely monitoring several chemical and physical indicators (chromium concentration, pH, dissolved oxygen, ammonium-nitrogen, nitrites, salinity, and temperature) and frequently replacing the chromium and control solutions in long-term experiments, controlled conditions were ensured (details on procedures are given in Appendix B). Temperature, dissolved oxygen, ammonium-nitrogen, nitrites and salinity showed only minimal fluctuations during the course of the experiments. The pH was directly influenced by high concentrations of chromic chloride but otherwise also remained stable (Appendix B, Table B-1). It was our intention to keep the chemical and physical parameters as constant as possible to ensure that the biological results of the experiment were directly attributable to the chromium concentration and form.

In the Neanthes arenaceodentata experiments, several corollary chemical experiments were undertaken to investigate the behavior of chromium compounds. Atomic absorption analyses of the solutions were performed to precisely measure total chromium concentrations at the beginning and end of several short-term experiments. Our findings showed that the hexavalent chromium concentrations remained nearly constant for the 7-day period, and that the chromium was not measurably adsorbed onto the glass containers. Measurements were also made at weekly intervals during the first 2 months of the long-term hexavalent experiment. As these chromium concentrations also remained relatively constant (±10 percent), the sampling frequency was extended, and henceforth, concentrations were measured every 3 weeks when water solutions were renewed (samples were taken before and after each water change). The data for the chromium concentration monitoring are presented in Appendix B, Table B-1).

In addition to the routine chromium monitoring mentioned above, water samples were also taken from the long-term hexavalent experiment and analyzed for hexavalent, trivalent, and total chromium to determine if there were shifts in oxidation state under experimental conditions after a 3-week period. Such shifts would not have been detected in our routine monitoring of total chromium concentrations. Our results showed that the hexavalent form was stable at all experimental concentrations

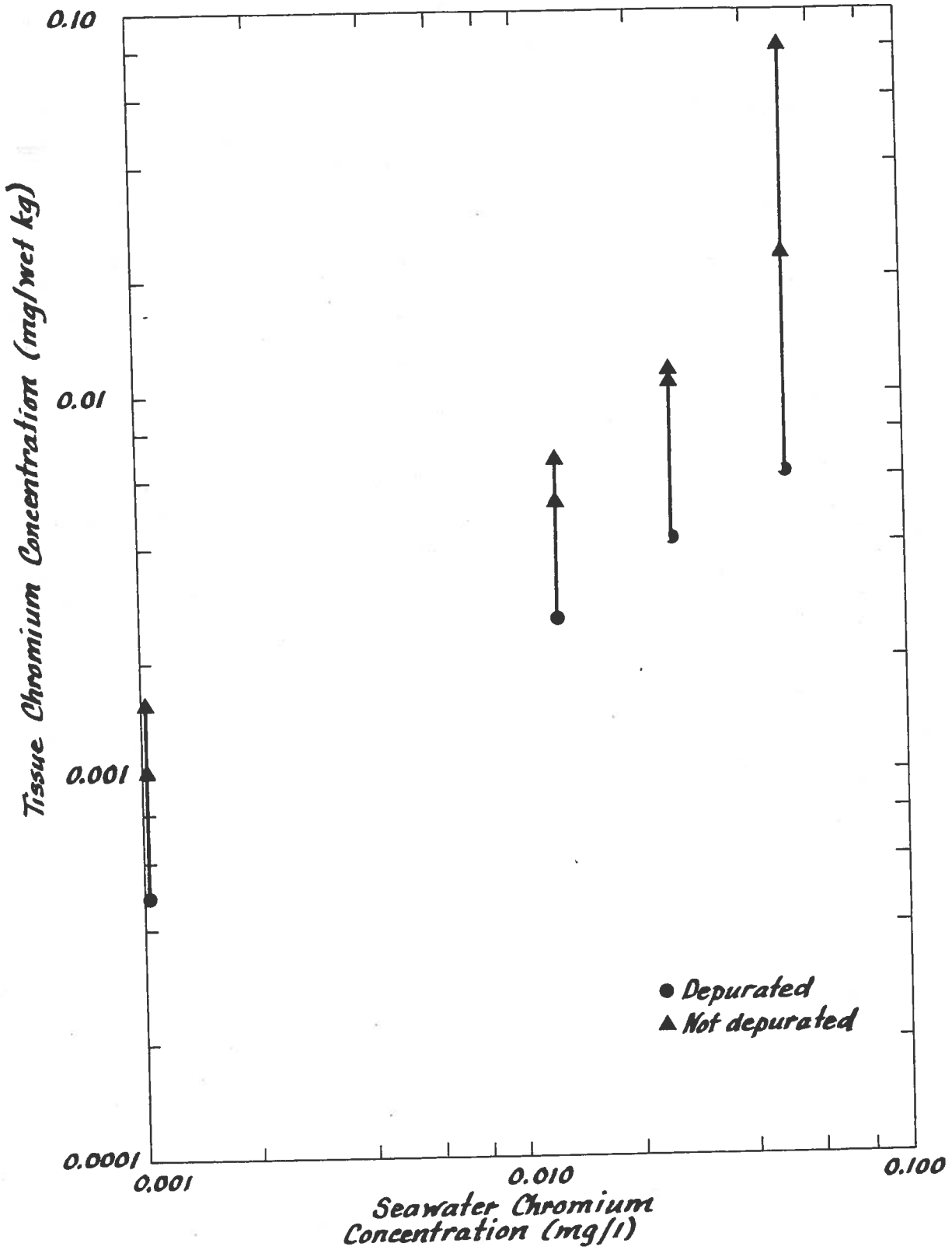

Figure 2. The relationship between chromium concentration of seawater and the body tissue concentration of chromium in Neanthes arenaceodentata.

(Appendix B, Table B-3), and there was no evidence to suggest significant shifts from one oxidation state to another.

The Project is now beginning investigation of the chromium levels in the tissues of Neanthes arenaceodentata exposed to hexavalent or trivalent forms. The results to date indicate a trend of increased levels of chromium in the body tissue of worms with exposure to increased hexavalent chromium concentrations (Figure 2). The worms exposed to a chromium level of 0.05 mg/l in the long-term experiment had tissue concentrations of chromium (6.20 to 81.7 mg/wet kg) about one order of magnitude higher than the control worms (0.486 to 1.55 mg/wet kg) exposed to less than 0.001 mg/l total chromium.

Most studies of the chromium levels of marine invertebrate tissues revealed tissue concentrations of chromium many times higher than the surrounding waters (Appendix A, Table A-6). More tests on bioaccumulations, uptake rates, and loss rates of chromium are being carried out on Neanthes arenaceodentata to identify the form or forms of chromium that are incorporated into the tissues, to correlate tissue levels with behavioral and reproductive responses already observed, and to identify the level of "no response."

DISCUSSION

The Project's toxicity experiments show that chromium in the hexavalent form (as dichromate) is responsible for both lethal and sublethal responses in Neanthes arenaceodentata (Table 6). The short-term LC_{50} experiments using field and laboratory colony worms indicate that these two populations do not show a significant difference in chromium tolerance. Additional LC_{50} tests on two successive N. arenaceodentata generations raised in solutions with enhanced chromium concentrations showed no significant changes in acute chromium sensitivity. A comparison of the lethal levels established for this worm and chromium toxicity results reported elsewhere (Appendix A, Tables A-5 and A-6) indicate that N. arenaceodentata is a particularly sensitive organism.

In the long-term study with hexavalent chromium (which lasted 440 days and covered the parental and three filial generations), a major sublethal effect has been observed. This effect was the reduction in brood sizes as the chromium concentrations increased. When we applied Dunnett's (Steel and Torrie 1960) procedure of comparing all treatment means with a control mean in a Model I analysis of variance (Appendix B) to our data, we found statistically significant ($p \leq 0.05$) effects at all chromium concentrations (0.0125 and above) with the parental generation, and significant effects at 0.05 mg/l in the F_1 and F_2 generations. Cardwell,* who is well acquainted with our toxicology work, believes there is a possibility that exposure to hexavalent chromium over multiple generations results in increased tolerance. To pinpoint the level of "no effect," he has suggested using more replicates at more concentrations in the control to 0.025 mg/l range.

We now know the chromium range that causes the sublethal effect of reduced brood size. Yet the exact mechanism of chromium action causing such a reaction is not well understood. Our results may reflect (1) alterations in the egg-laying capacities of the adult females such as differences in muscle sloughing, eleocyte development, oocyte growth rate and oocyte number (Davis and Reish 1975), (2) changes in the fertilization capabilities of the gametes, and/or (3) acute chromium sensitivities of the developing young, which modify maturation and survival in the time between the spawning of the adults and our counting of the young.

In contrast, we have not found any abnormal biological effects in the experiments using trivalent chromium (as chromic chloride). The presence of the trivalent chromium precipitate in the long-

*Dr. Rick Cardwell, Washington State Department of Fisheries.

Table 6. Toxic levels of hexavalent chromium on *Neanthes arenaceodentata*, California wastewater emission standards for total chromium, and marine environment levels of total chromium.

	Chromium Concentration
Pacific Ocean sediments	4-93 mg/dry kg
Effect on *Neanthes arenaceodentata*	
96-hour LC_{50}	2.2-4.3 mg/l
7-day LC_{50}	1.44-1.89 mg/l
59-day LC_{50}	0.20 mg/l
Cessation of spawning	0.10 mg/l
Reduction in brood size	
F_2	0.050 mg/l
F_1	0.050 mg/l
P	0.0125 mg/l
Southern California effluent (average)	
Prior to dilution	0.020-0.9 mg/l
After 100:1 dilution	0.0002-0.009 mg/l
California emission standards	
Not more than 10% of the time	0.010 mg/l
Not more than 50% of the time	0.005 mg/l
Southern California surface waters	0.0002 mg/l

term experiment neither increased nor decreased the normal mortality rate, maturation time required for spawning, or number of young per brood. This finding seems paradoxical in light of the substantial documentation that it is the trivalent form that is the most active in biochemical cycles (Mertz 1969), although it does lend support to the premise that trivalent chromium is much le toxic to marine invertebrates than hexavalent chromium (Chipman 196 The behavior of different chromium forms in the marine environment probably accounts for the variance in chromium toxicity.

A better understanding of the chemical dynamics of chromium has been gained in the last year. Analyses of seawater and sediment samples taken in southern California waters indicate that chromium is present in nearshore surface waters at a level of 0.0002 mg/l, and that most of this chromium is in the hexavalent state (Jan and Young, in preparation). Presumably, as hexavalent chromium undergoe wastewater treatment, the majority of it is reduced to the trivalent form in the presence of high organic loads. As it is discharged into the ocean, a small fraction remains in the hexavalent state, but most of it is in the trivalent state and is associated with particulate matter. These particles settle out of the water column and accumulate on the ocean floor, while the dissolved hexavalent chromium, which appears to be relatively stable, remains in the water column. Further details of this work are given in Appendix A.

The trivalent state appears to be of concern with respect to bottom-dwelling and bottom-feeding animals. Tests of the biological activity of chromium must be continued to answer these important questions:

- In what instances is chromium biologically available to benthic marine organisms; in what forms (hexavalent, trivalent, dissolved, undissolved), at what concentration levels, and by what routes (e.g., through external contact, through ingestion)?
- Once trivalent chromium is bound to particulate matter, can the process be reversed, naturally, to convert it back to the hexavalent form; if so, under what conditions? Can competing ligands or chelating agents challenge the stability of the trivalent chromium/particulate bond and thus alter the availability of the element?
- At what levels do hexavalent and trivalent chromium compounds have "no effect" on the health of the marine biota.

During the next year, we will continue to assess the behavior and effects of chromium compounds, closely coordinating our biological experiments with those of our chemistry staff to obtain a more comprehensive understanding of this trace element. Projected biological experiments include (1) continuing the Neanthes arenaceodentata hexavalent experiment at a lower range of concentrations to ascertain limits of "no effect," (2) testing tolerances of different phyla by conducting both hexavalent and trivalent experiments on echinoderms, molluscs, and crustaceans, and (3) testing the biological activity of different forms of trivalent chromium (e.g., particulate-bound trivalent chromium and chelated forms).

SUMMARY

The effects of hexavalent chromium, as $K_2Cr_2O_7$, and trivalent chromium, as $CrCl_3$, on survival and reproduction were tested under laboratory conditions, using the polychaete Neanthes arenaceodentata, with the following results:

1. For hexavalent chromium, the 96-hour LC_{50} ranged from 2.2 to 4.3 mg/l, and the 7-day LC_{50} ranged from 1.44 to 1.89 mg/l.
2. A long-term experiment to study the sublethal effects of hexavalent chromium (three generations, 440 days) showed that reproduction ceased at 0.100 mg/l, and there was a reduction in brood size at 0.0125 to 0.050 mg/l.
3. Tissue analysis of worms used in the long-term hexavalent chromium experiment revealed a trend of higher tissue concentrations of chromium with exposure to seawater solutions with higher hexavalent chromium concentrations.
4. The 96-hour and 7-day LC_{50} levels could not be effectively generated for trivalent chromium using chromic chloride. The trivalent chromium formed hydroxide complexes and precipitated out of solution while, at the same time, lowering the pH of the seawater solution. The trivalent chromium hydroxide complexes may not have been biologically available to the polychaetes.
5. No adverse effects on the worms in a long-term experiment (two generations, 293 days) were caused by the presence of the trivalent chromium precipitate (50.4 mg/l, with the pH adjusted to 7.8 to 8.1) in seawater, even though the worms were in direct contact with the precipitate.
6. Monitoring water quality parameters (including chromium concentration) and replacement of toxicant solutions at regular intervals was necessary to minimize biological stress not directly attributable to chromium.

ACKNOWLEDGMENTS

We wish to express our appreciation to Mr. Tsu-Kai Jan, who performed the numerous chromium analyses, and Ms. Jean Wright, who assisted in the experimental design and water quality monitoring. Additional thanks are due Drs. David Young (Coastal Water Research Project), Rick Cardwell (Washington State Department of Fisheries), and James Morgan (California Institute of Technology), as well as Mrs. Diana Vermillion (California State University, Long Beach).

A committee composed of Coastal Water Research Project staff members and headed by Mr. Willard Bascom guided this study.

This work was supported in part by the Southern California Coastal Water Research Project and in part by the U.S. Environmental Protection Agency under Grant R800962 to Dr. Donald J. Reish, California State University, Long Beach.

Appendix A
LITERATURE REVIEW

GENERAL PERSPECTIVES: CHROMIUM IN THE ENVIRONMENT

Chromium occurs naturally in the form of chromite, $FeOCr_2O_3$; it ranks fourth in abundance among 29 elements of biological importance in the earth's crust (chromium is more abundant than cobalt, copper, zinc, molybdenum, lead, nickel, and cadmium).

General information on the distribution of chromium in the environment is given in Table A-1; Table A-2 summarizes its distribution in the marine environment. The biologically important oxidation states of chromium and the sites and routes of its biological activity are summarized in Tables A-3 and A-4, respectively. (The information on these tables is adapted from Mertz 1969 and National Research Council 1974.)

BEHAVIOR OF CHROMIUM IN WASTEWATERS

The chromium level in southern California ocean waters has been found to be 0.0002 mg/l, of which 75 percent is hexavalent (Jan and Young, in preparation). This level is much lower than the highest average concentration--0.9 mg/l--in the municipal wastewater effluents discharged to these waters (Mitchell and McDermott 1975). However, this comparison of wastewater and background seawater values may be misleading. Assuming a 100-to-1 dilution of the effluent at the point of discharge, the chromium concentration drops to 0.009 mg/l. Fifteen percent (0.00135 mg/l) of this chromium is dissolved (filterable through a 0.45-micron filter) and 85 percent (0.00765 mg/l) is associated with particulate matter. A test on primary effluent has shown that 15 percent of the particle-associated chromium is released in 1 hour; the rate of release is then much reduced, and only 2 percent is released in the next 2 weeks (Chen and Hendricks 1974). Therefore, the maximum amount of dissolved chromium directly in the discharge plume would approach 0.0026 mg/l (0.00135 mg/l originally dissolved plus 0.0013 mg/l released from particulate). This value will decrease substantially as the effluent moves out of the discharge site and is further diluted; eventually the chromium level will reach the background value of 0.0002 mg/l.

THE EFFECTS OF CHROMIUM ON MARINE INVERTEBRATES

The results of the Project's review of literature on tests of the toxicity of chromium to marine invertebrates and analyses for chromium in the body tissues of these animals are given in Tables A-5 and A-6, respectively.

THE EFFECTS OF TRACE METALS ON POLYCHAETES

The results of the Project's review of literature on tests of the toxicity of trace metals to polychaetes and analyses for metals in the body tissues of these animals are given in Tables A-7 and A-8, respectively.

Table A-1. Distribution of chromium in the environment.*

Material	Chromium Content	
	Average	Range
Continental crust (µg/g)	125	80-200
Igneous rocks		2-3,400
Shales and clays	120	30-590
Soils	40	10-150
Phosphorites	300	30-3,000
Coals	20	10-1,000
Plants on land (µg/g)	0.23	
Food (µg/g)		
Meats	0.13	0.03-0.27
Fish	0.02	0.01-0.02
Vegetables	0.18	0-3.62
Grains	0.13	0-0.52
Cereals	0.22	0.05-0.23
Fruits	0.01	0-0.20
Water (mg/l)		
Rivers		0.0007-0.084
Drinking water**	0.00043	
Air (µg/cu m)		
Urban		<0.002-0.12
Rural	<0.01	

*After Mertz (1969) and the National Research Council (1974).
**California state standard for drinking water is 0.050 mg/l as hexavalent chromium.

Table A-2. Distribution of chromium in the marine environment.

Material	Chromium Content	
	Literature[a]	Coastal Water Project
Plants (µg/dry g)	1.000	
Plankton (µg/dry g)	3.500	
Brown algae (µg/dry g)	1.300	
Seawater (mg/l)	<0.001	
Japan	0.00004-0.00007	
England	0.00013-0.00025	
Irish Sea	0.00046	
Southern California		<0.001[b]
Southern California waste-water effluent (mg/l)		0.0002-0.18[c]
Sediments (mg/dry kg)		
Pacific Ocean	4-93[d]	
Palos Verdes, California		886

a. Adapted from National Research Council (1974). b. 75% is in the hexavalent form. c. Assumes immediate dilution of 100:1; California state standard is 0.005 mg/l not more than 50% of the time and 0.01 mg/l not more than 10% of the time. d. Adapted from Robertson et al. (1972).

Table A-3. Biologically important oxidation states of chromium.

Oxidation State	Characteristics
Hexavalent chromium	• Soluble in seawater • Readily reduced to trivalent chromium in the presence of organic matter* • Toxic to animals
Trivalent chromium	• Not very soluble in seawater • Forms stable complexes with both organic and inorganic matter* • Biological activity depends on form of complex and site of interaction

*Forms of chromium bound to organic matter are not well understood.

Table A-4. Routes and sites of biological activity of chromium.

External sites of entry
- Skin
- Mucous lining of nose and mouth

Internal sites of entry
- Lungs (via air)
- Tissues (via intestinal tract)
- Blood, circulatory system

Chemical activity of chromium
- Hexavalent can be absorbed, depending on pH; trivalent is poorly absorbed
- Hexavalent is reduced to trivalent subcutaneously

Implications of chromium
- Hexavalent has corrosive effects on skin and mucous linings and can cause ulcerated tissues
- Hexavalent can enter the circulatory system. It penetrates red blood cells, is reduced to trivalent form, and binds with hemoglobin
- Trivalent does not normally enter the circulatory system but, upon direct injection, binds to plasma and does not penetrate erythrocytes
- Trivalent binds with proteins and nucleic acids; probably functions in enzyme activity. It has also been implicated as a requirement for glucose metabolism as "glucose tolerance factor"

Table A-5. Review of literature on tests of the toxicity of chromium to marine invertebrates.

Test Animal	Test Type	Effective Concentration (mg/l)	Reference
Capitella capitata (polychaete), laboratory colony			Reish et al. 1975
Adult	Cr^{+6} as CrO_3		
	96-hr LC_{50}	5.0	
	28-day LC_{50}	0.28	
Trochophore larvae	Cr^{+6} as CrO_3		
	96-hr LC_{50}	8.0	
Neanthes arenaceodentata (polychaete), laboratory colony			
Adult	Cr^{+6} as CrO_3		
	96-hr LC_{50}	>1.0	
	28-day LC_{50}	0.55	
Juvenile	Cr^{+6} as CrO_3		
	96-hr LC_{50}	>1.0	
	28-day LC_{50}	0.7	
Neanthes arenaceodentata (polychaete), laboratory colony	Cr^{+6} as $K_2Cr_2O_7$		Mearns 1974 Author concluded that there is a definite need to closely monitor water quality parameters.
	EC_{50} (ability to build mucus tubes)		
	96-hr	0.2-0.3	
	7-day	0.1	
	LC_{50}		
	96-hr	3.0-4.0	
	7-day	1.4-1.7	
Hermione hystrix (polychaete)	Cr-51 as $CrCl_3$ added to bottom silt		Chipman 1966 The addition of Cr-51 to seawater in the form of $CrCl_3$ resulted in the formation of radioactive particles and adsorption of the Cr-51 to surfaces. The addition of Cr-51 as $Na_2Cr_2O_4$ resulted in an ionic solution in the seawater. When H. hystrix was exposed to the trivalent Cr-51 present in the bottom silt, it took up some radioactivity, but there was no accumulation within the body of the worm. H. hystrix readily took up Cr-51 added as $Na_2Cr_2O_4$ but accumulation was a slow process, and only small amounts were accumulated over 19 days. The uptake of chromate for seawater was a passive process.
	Whole body uptake	-	
	Cr-51 as $Na_2Cr_2O_4$ dissolved in seawater		
	Whole body uptake	-	

Table A-5 continued

Test Animal	Test Type	Effective Concentration (mg/l)	Reference
Nereis virens (polychaete), western shore, Southampton, Great Britain	Cr^{+6} as $Na_2CrO_4 \cdot 10\ H_2O$		Raymont and Shields 1963 In a tracer experiment using Cr-51, authors found both gut and body wall to be sites of Cr absorption. Considerable absorption occurred in the absence of food intake. Blood vessels showed high Cr levels, which might indicate transport of Cr from the gut and body wall to the blood vessels. The data suggested that a threshold toxicity level for C. maenas was not sharply defined.
	5 wk (no mortality)	0.5	
	3-wk LC_{50}	1.0	
Carcinas maenas (crab), western shore, Southampton, Great Britain	Cr^{+6} as $Na_2CrO_4 \cdot 10\ H_2O$		
	12 days (no mortality)	40	
	3-wk LC_{50}	60	
Artemia salina (brine shrimp), dried eggs	24-hr LC_{50}		Okubo and Okubo 1965 The data suggested that the concentration of the pollutant that has no adverse effect upon the embryonic development of sea urchins and bivalves may often be considered about equal to the safe concentration for inshore marine fishes. The eggs of sea urchins and bivalves share various common features: Fertilization takes place externally, there is no special protective outer shell, and the process of completion of the first stage of growth, whether by pluteus or D-type larvae is similar. Authors concluded that these points of similarity seem to be the reason for the very similar results.
	Cr^{+6} as $Cr_2(SO_4)_3$	40	
	Cr^{+6} as K_2CrO_4	70	
Sesarma hematocheir zoea (crab), Yutsubo, Kanagawa Prefecture, Japan	24-hr LC_{50}		
	Cr^{+6} as $Cr_2(SO_4)_3$	56	
	Cr^{+6} as K_2CrO_4	200	
Balanus amphitrite albicostatus (barnacle), adult, Yutsubo, Kanagawa Prefecture, Japan	Cr^{+6} as $Cr_2(SO_4)_3$, 1-hr test for cessation of cirri movement	3.2	
Anthocidaris crassipina (urchin), Yutsubo, Kanagawa Prefecture, Japan	24-hr test for normal development from egg to pluteus		
	Cr^{+6} as $Cr_2(SO_4)_3$		
	Not affected	3.2	
	Affected	10	
	Cr^{+6} as $K_2Cr_2O_7$		
	Not affected	3.2	
	Affected	10	
Hemicentrotus pulcherrimus (urchin), Enoshima, Kanagawa Prefecture, Japan	72-hr test for normal development from egg to pluteus		
	Cr^{+6} as $Cr_2(SO_4)_3$		
	Not affected	-	
	Affected	<1.0	
	Cr^{+6} as $K_2Cr_2O_7$		
	Not affected	3.2	
	Affected	10	

Table A-5 continued

Test Animal	Test Type	Effective Concentration (mg/l)	Reference
Mytilus edulis (mussel), Omori District, Tokyo, Japan	48-hr test for normal development from egg to straight-hinged larva Cr^{+6} as $Cr_2(SO_4)_3$ Not affected Affected Cr^{+6} as $K_2Cr_2O_4$ Not affected Affected	 3.2 10 3.2 10	Okubo and Okubo 1965 continued
Crassostrea gigas oyster, Yutsubo, Kanagawa Prefecture, Japan	24-hr test for normal development from egg to straight-hinged larva, C^{+6} as $K_2Cr_2O_4$ Not affected Affected	 3.2 10	
Mytilus edulis (mussel), coast of New Hampshire Mya arenaria (clam), coast of New Hampshire	Test on filtration rate in Cr^{+3} as $CrCl_3$ added to natural and artificial sediments Test on filtration rate in Cr^{+3} as $CrCl_3$ added to natural and artificial sediments		Capuzzo 1974 Exposure of both the mussel and the clam to Cr concentrations comparable to those found in the upper reaches of the Piscataqua River resulted in a reduction in filtration rates. The toxicity of the Cr was dependent on the nature of uptake by the bivalves. The mussel takes up chromium by diffusion and ingestion of particulate matter. The clam seems to take up chromium only by diffusion. The reduction in filtration rates was due to an impairment of the ciliary mechanism of the gill.
Crassostrea virginica (oysters), embryos, laboratory colony	EC_{50} (development to straight-hinged larval stage), Cr^{+3} as $CrCl_3$	10.3	Calabrese et al. 1973 Authors found that the trivalent Cr did not generate consistent results, and the metal toxicity given was only valid for the inorganic salt tested. Results do not take into account the form the metal may have taken in solution.
Rangia cuneata (brackish water clam), Lower Neches River, southeast Texas	96-hr LC_{50}, Cr^{+6} as $K_2Cr_2O_4$ Salinity 1 °/oo Salinity 5.5 °/oo Salinity 22 °/oo	 0.21 14.0 35.0	Olson and Harrel 1973 Authors recommend further testing, preferably long-term chronic experiments.

Table A-6. Review of literature on analyses of total chromium in body tissues of marine invertebrates.

Species	Sample	Concentration	Reference
FIELD SURVEYS			
Haliotis rufescens (abalone), California			Anderlini 1974
Shelter Cove	Gill	0.58 mg/dry kg	Analyses for Cr in abalone collected between Shelter Cove and La Jolla, California, by divers revealed no correlation between the tissue concentration of Cr and the size of the animal. Cr was not detectable in foot muscles, and levels in other tissues varied considerably. The highest levels were in the digestive glands. Samples from Long Beach had Cr concentrations two to three times higher than those from other locations, which suggested a contaminant source. Water and sediment levels of Cr at the sampling sites were not recorded. For further information, see Anderlini 1973.
	Mantle	1.8 mg/dry kg	
	Digestive gland	2.4 mg/dry kg	
Mendocino	Gill	0.62 mg/dry kg	
	Mantle	0	
	Digestive gland	1.4 mg/dry kg	
Santa Cruz	Gill	1.11 mg/dry kg	
	Mantle	2.4 mg/dry kg	
	Digestive gland	2.4 mg/dry kg	
Long Beach	Gill	4.4 mg/dry kg	
	Mantle	13.0 mg/dry kg	
	Digestive gland	13.2 mg/dry kg	
La Jolla	Gill	1.80 mg/dry kg	
	Mantle	2.6 mg/dry kg	
	Digestive gland	1.4 mg/dry kg	
Patella vulgata (limpet), Israel	Soft parts	9.7-23.2 mg/dry kg	Navrot et al. 1974
	Shell	9.3-10.4 mg/dry kg	Limpets were taken along the shore between the sewage pipe at Tel-Aviv and Dor. The content of the carbonate shell material of all specimens was the same; however, the Cr concentrations in the soft parts decreased with distance from the sewage pipe. Authors suggest using *Patella vulgata* as a biological monitor of coastal water trace elements.
Crassostrea virginica (oyster), Mobile Bay, Alabama	Soft tissue	0.35 mg/wet kg	Kopfler and Mayer 1973
	Seawater	<0.0001-0.009 mg/l	The Cr concentration in Mobile Bay oysters was approximately the same as that in Atlantic coast oysters (Pringle et al. 1968). Although the concentration of Cr was 10^2 to 10^4 times that of the seawater, there was poor correlation between the two sets of data.
Acmaea digitalis (gastropod), California			Graham 1972
Fort Point, San Francisco	Shell	<5.7 mg/dry kg	Using atomic absorption spectrophotometry, author determined Cr levels in seven species of Mollusca from eleven regions along the
	Soft parts	24.2 ± 4.0 mg/dry kg	
Whites Point, Los Angeles	Shell	<5.7 mg/dry kg	
	Soft parts	7.1 ± 0.2 mg/dry kg	

Table A-6 continued

Species	Sample	Concentration	Reference
Mytilus californianus (mussel), California			Graham 1972 continued
Half Moon Bay	Shell	<5.7 mg/dry kg	California coast from San Francisco Bay to Los Angeles. Shells and soft portions of whole bodies were analyzed separately; water and sediment values were not determined. Cr levels were relatively high in soft parts of *A. digitalis* from Fort Point and *Tegula funebralis* from Fisherman's Wharf. Author suggests that these levels reflect contamination from automobiles at Fort Point and the presence of small craft in Monterey Harbor.
	Soft parts	1.5 mg/dry kg	
Whites Point, Los Angeles	Shell	<5.7 mg/dry kg	
	Soft parts	7.8 ± 0.8 mg/dry kg	
Carmel Bay	Shell	14.2 ± 2.0 mg/dry kg	
	Soft parts	6.1 ± 0.0 mg/dry kg	
Mytilus edulis (mussel), California			
Coyote Point, San Mateo Co.	Shell	<5.7 mg/dry kg	
	Soft parts	5.8 ± 2.2 mg/dry kg	
Monterey Wharf	Shell	<5.7 mg/dry kg	
	Soft parts	<1.5 mg/dry kg	
Elkhorn Slough, Moss Landing	Soft parts	<1.5 mg/dry kg	
Whites Point, Los Angeles	Shell	<5.7 mg/dry kg	
	Soft parts	7.6 ± 0.1 mg/dry kg	
Protothoca staminea (clam), Elkhorn Slough, Moss Landing, California	Shell	<5.7 mg/dry kg	
	Soft parts	<1.5 mg/dry kg	
Thais emarginata (gastropod), California			
Fisherman's Wharf, Monterey	Shell	<5.7 mg/dry kg	
	Soft parts	<1.5 mg/dry kg	
Mussel Point, Pacific Grove	Shell	10.5 ± 2.6 mg/dry kg	
Tegula funebralis (gastropod), California			
Fisherman's Wharf, Monterey	Shell	15.8 ± 0.2 mg/dry kg	
	Soft parts	12.2 ± 1.7 mg/dry kg	
Mussel Point, Pacific Grove	Shell	14.4 ± 0.1 mg/dry kg	
Point Pinos, Pacific Grove	Shell	14.2 ± 0.4 mg/dry kg	
	Soft parts	<1.5 mg/dry kg	
Carmel Bay	Shell	10.4 ± 1.3 mg/dry kg	
	Soft parts	3.5 ± 0.1 mg/dry kg	
Point Sur, Monterey Co.	Shell	<5.7 mg/dry kg	
	Soft parts	2.7 ± 0.3 mg/dry kg	
Whites Point, Los Angeles	Shell	<5.7 mg/dry kg	
	Soft parts	6.3 ± 0.5 mg/dry kg	
Ostrea sinuata (oyster), Tasman Bay, New Zealand	Mantle	<3 mg/dry kg	Brooks and Rumsby 1965
	Gill	<3 mg/dry kg	Spectrographic determinations of Cr and 11 other elements were made on 3 species of New Zealand bivalves. Although analyses were performed on whole animals excluding shells, only
	Muscle	<3 mg/dry kg	
	Striated muscle	<3 mg/dry kg	

Table A-6 continued

Species	Sample	Concentration	Reference
Ostrea sinuata continued	Kidney	<3 mg/dry kg	Brooks and Rumsby 1965 continued the data showing the Cr content of specific organs and sediment are shown here (the whole animal samples still contained the gut contents, which may have influenced the results). In all cases, the tissues had lower Cr levels than the sediments. Goldberg's (1957) data for Cr values in seawater were used so that direct comparison of tissue levels and water levels at the collection site could not be made.
	Heart	9 mg/dry kg	
	Shell	<3 mg/dry kg	
Pecten novae-zealandiae (scallop), Tasman Bay, New Zealand	Mantle	<3 mg/dry kg	
	Gill	145 mg/dry kg	
	Muscle	<3 mg/dry kg	
	Kidney	17 mg/dry kg	
	Foot	8 mg/dry kg	
	Gonad	<3 mg/dry kg	
	Shell	<3-10 mg/dry kg	
Mytilus edulis aoteanus (mussel), Tasman Bay, New Zealand	Mantle	<3 mg/dry kg	
	Gill	10 mg/dry kg	
	Muscle	<3 mg/dry kg	
	Foot	<3 mg/dry kg	
	Gonad	<3 mg/dry kg	
	Shell	<3 mg/dry kg	
	Sediment	307 mg/dry kg	
	Seawater	0.0005 mg/l	
Cyanea capillata (coelenterate), Continental shelf, northeast U.S.	Whole body	<1 mg/dry kg	Nicholls et al. 1959 The results of spectrographic analyses of several species of zooplankton indicate that standardization techniques with greater analytical precision are needed. Cr levels greater than 1 mg/kg were only found in copepods. Authors conclude that, for any given chemical element, there will eventually be found at least one species of plankton capable of concentrating it to a spectacular degree.
Limacina retroversa (pteropod), Continental shelf	Whole body	<1 mg/dry kg	
Beroë cucumis (ctenophore), Continental shelf	Whole body	<1 mg/dry kg	
Ommastrephes illicebrosa (squid), Continental shelf	Whole body	<1 mg/dry kg	
Centropages typicus and C. hamatus (copepods), Continental shelf	Whole body	260 mg/dry kg	
Euphausia krohnii (euphausid), Continental shelf	Whole body	<1 mg/dry kg	
Sagitta elegans (chaetognath), Continental shelf	Whole body	<1 mg/dry kg	
Salpa fusiformis (thaliacean), Continental shelf	Whole body	<1 mg/dry kg	
Asterias rubens (starfish)	Whole body without internal organs	0.02 µg/dry g	Noddack and Noddack 1939 (seen in Fukai and Broquet 1965) These data are not comparable to Fukai and Broquet because of differences in preparation.
Brissopsis lyrifera (urchin)	Shell	0.02 µg/dry g	
Stichopus tremulus (sea cucumber)	Whole body without gut	0.9 µg/dry g	

Table A-6 continued

Species	Sample	Concentration	Reference
Tripneustes esculenta (urchin), Punta Higuero, Puerto Rico	Testis	19.91 µg/wet g	Stevenson et al. 1964 Authors conclude that animals have the capabilities of concentrating Cr, although the tissue levels were not directly correlated with Cr levels in the local seawater. Instead Goldberg's (1957) data was taken as the seawater Cr level in Punta Higuero in 1964. Only one animal representing each gorgonian species was used, and there were only four specimens of T. muricatus.
Echinometra lucunter (urchin), Punta Higuero, Puerto Rico	Testis	43.20 µg/wet g	
Ophiothrix suensoni (brittle star), Punta Higuero, Puerto Rico	Whole body	24.17 µg/wet g	
Gorgonians, Punta Higuero, Puerto Rico			
Eunicia Sp. 1	Whole body	16.90 µg/wet g	
Eunicia Sp. 2	Whole body	19.00 µg/wet g	
Eunicia Sp. 3	Whole body	20.90 µg/wet g	
Pseudapterygorgonia sp.	Whole body	16.00 µg/wet g	
Tectarius muricatus (gastropod), Punta Higuero, Puerto Rico	Soft tissue Shell	68.22 µg/wet g 43.52 µg/wet g	
Hermione hystrix (polychaete), Roquebrune Bay, Mediterranean Sea	Whole body	8.1 µg/dry g	Fukai and Broquet 1965 Most of the specimens were collected off the southern coasts of France and Monaco. Authors conclude that the high content of Cr in polychaetes might be connected with the behavior of Cr in the substratum, in spite of the fact that the surface contamination may be considered to be the main cause of high Cr content in the sediments. Surface water contamination may account for high concentrations of Cr in zooplankton (particulate matter was taken in the net hauls for the plankters). Small crustaceans have large surface-to-volume ratios, which probably facilitates adsoption.
Aphrodite aculeata (polychaete), Menton Bay, Mediterranean Sea	Whole body	14.7 µg/dry g	
Acartia clausi (copepod), Roquebrune Bay, Mediterranean Sea	Whole body	1.5 µg/dry g	
Crangon crangon (shrimp), off Sète, France	Carapace Soft parts	0.3 µg/dry g 0.04 µg/dry g	
Parapenaeus longirostris (shrimp), off Sète, France	Carapace Soft parts	0.5 µg/dry g 0.11 µg/dry g	
Pisa nodipes (crab), Cap d'Ail, Mediterranean Sea	Whole body	1.1 µg/dry g	
Carcinus maenas (crab), Monaco	Soft parts	0.4 µg/dry g	
Eriphia verrucosa (crab), Monaco	Carapace Soft parts	0.4 µg/dry g 0.7 µg/dry g	
Paralithodes kamtschatica (king crab), Alaska	Meat	0.09 µg/dry g	
Astropecten irregularis (starfish), Roquebrune Bay, Mediterranean Sea	Whole body	1.2 µg/dry g	
Ophiura texturata (brittle star), Menton Bay, Mediterranean Sea	Whole body	0.8 µg/dry g	

Table A-6 continued

Species	Sample	Concentration	Reference
Holothuria forskalii (sea cucumber), Monaco	Whole body without gut	0.3 µg/dry g	Fukai and Broquet 1965 continued
Stichopus regalis (sea cucumber), Roquebrune Bay, Mediterranean Sea	Whole body without gut	1.1 µg/dry g	
Mytilus edulis (mussel), Monaco	Soft parts	1.0 µg/dry g	
Tapes descussatus (clam), Sète, France	Soft parts	1.6 µg/dry g	
Venus verrucosa (venus shell), Sète, France	Soft parts	0.5 µg/dry g	
Gryphaea angulata (oyster), Arcachon, France, Atlantic Ocean	Soft parts	0.5 µg/dry g	
Pecten jacobaeus (scallop), Normandy, France, Atlantic Ocean	Soft parts	1.1 µg/dry g	
Patella intermedia (limpet), Cap Martin, Mediterranean Sea	Shell Soft parts	0.07 µg/dry g 0.8 µg/dry g	
Murex trunculus (gastropod), Cap Martin, Mediterranean Sea	Soft parts	0.9 µg/dry g	
Eledone moschata (octopus), Monaco	Whole body	0.12 µg/dry g	
Loligo vulgaris (squid), Biscary Bay, France, Atlantic Ocean	Soft parts	0.07 µg/dry g	
Phytoplankton, Narragansett Bay, Rhode Island			Phelps et al. 1975
Upper Bay	Whole body Sediment	73.3 µg/dry g 320 µg/dry g	The results suggest that the body tissue levels of Cr observed in these organisms are determined by the food and feeding activity of the animal rather than by direct uptake from the water. Cr appears to be primarily associated with the particulate, inert system and does not appear in the biological system beyond the secondary consumer level in the food web. Authors suggest that acute bioassays based on uptake of Cr from the water column appear to produce unrealistic results. The values given for M. mercenaria are means of sample means.
Lower Bay	Whole body Sediment	41.3 µg/dry g 56.7 µg/dry g	
Mercenaria mercenaria (clam), Narragansett Bay, Rhode Island			
Upper Bay	Soft parts Sediment	17.3 µg/dry g 320 µg/dry g	
Lower Bay	Soft parts Sediment	4.6 µg/dry g 56.7 µg/dry g	

Table A-6 continued

Species	Sample	Concentration	References
Pherusa affinis (polychaete), Narragansett Bay, Rhode Island			Phelps et al. 1975 continued
Upper Bay	Whole body	38.04 µg/dry g	
	Sediments	320 µg/dry g	
Lower Bay	Whole body	23.8 µg/dry g	
	Sediments	56.7 µg/dry g	
LABORATORY EXPERIMENTS			
Crassostrea virginica (oyster), West Loch, Pearl Harbor, Hawaii			Preston 1971 In an experiment to see if the oyster accumulated the radionuclide Cr-51 more readily by absorption or ingestion, test animals were exposed to 50 µc of hexavalent Cr-51 as $Na_2{}^{51}CrO_4$ dissolved in 1 liter of seawater; a second group of test animals was exposed to the same amount of the same form of Cr-51 associated with Chlamydomonas cells fed to them. Both exposure were 110 hr long. Author found that more Cr-51 was accumulated by direct absorption than by ingestion. The ultimate distribution of Cr-51 in the oyster tissues was nearly uniform (the adductor muscle had less than the other tissues).
Crassostrea virginica (oyster), North Carolina northward to Maine			Shuster and Pringle 1969 Authors found that Cr is accumulated in the body tissues in three relatively distinct phases. The first phase was one of rapidly increasing rates of Cr uptake; in the second phase, the rate of uptake slowed; and in the third phase, uptake rate was constant.
0.05 mg Cr^{+3}/1 as $Cr(NO_3)_3$			
4-7 days	Soft tissue	0.12 mg/wet kg	
10 wk	Soft tissue	3.04 mg/wet kg	
20 wk	Soft tissue	6.28 mg/wet kg	
0.10 mg Cr^{+3}/1 as $Cr(NO_3)_3$			
4-7 days	Soft tissue	0.53 mg/wet kg	
10 wk	Soft tissue	6.40 mg/wet kg	
20 wk	Soft tissue	10.87 mg/wet kg	
Tapes decussatus (clam), Sète, France, Mediterranean Sea			Chipman 1966 Author investigated the uptake and accumulation of Cr-51 by a detrital and particle-feeding clam when the radionuclide was added to seawater in different chemical and physical forms (in trivalent form as $CrCl_3$ and as $CrCl_3$ chelated with EDTA, in hexavalent form as $NaCr_2O_4$). The addition of Cr-51 as trivalent $CrCl_3$ resulted in precipitation, with the greater part of the radionuclide present as filterable particles. On the 3rd day of the experiment, over 50% of the unfilterable Cr-51 originally present was still present. In spite of this,

Table A-6 continued

Species	Sample	Concentration	Reference
			Chipman 1966 continued there was no evidence of continued uptake or accumulation of Cr-51 by the clams. Cr-51 in trivalent form was not accumulated in *T. decussatus* tissues: This inability to accumulate trivalent Cr seems to be universal among marine animals. When the trivalent Cr-51 was added as an EDTA chelate, all but 0.25% of the radioactivity was not filterable. Although there was a very slight Cr uptake into the clam shells and a little more by the soft tissues, there was no accumulation of the Cr-51 to values above the seawater concentration in 13 days. The addition of Cr-51 as hexavalent $NaCr_2O_4$ to seawater resulted in a solution containing radioactive ions. Typically, when clams were immersed in this solution, Cr-51 rapidly entered and accumulated in the tissues. The rate of increase slowed after the first few days; but by that time, the rate of accumulation had become rather constant and continued without change for a long time. The amount of Cr and Cr-51 entering the clams was related to concentration of the element in the seawater, accumulation apparently being a passive process. The radionuclide was tightly bound in tissues, and biological retention time of the radioactivity was long when the worms were returned to natural seawater.

Table A-7. Review of literature on tests of the toxicity of trace metals to polychaetes.

Test Animal	Metal and Type of Test	Effective Concentration (mg/l)	Reference
Capitella capitata, laboratory colony			Reish et al. 1975 Of the metals tested (including chromium), mercuric chloride was always the most toxic to both polychaete species, followed by copper sulfate. Chromic oxide or zinc sulfate were usually the next most toxic, followed by lead acetate and cadmium chloride. The toxicity values given are valid only for those forms of inorganic salts tested, and do not take into account the form the metals may have taken in seawater solution.
Adults	Mercuric chloride		
	96-hr LC_{50}	>0.1	
	28-day LC_{50}	0.1	
	Copper sulfate		
	96-hr LC_{50}	0.2	
	28-day LC_{50}	0.2	
	Zinc sulfate		
	96-hr LC_{50}	3.5	
	28-day LC_{50}	1.25	
	Lead acetate		
	96-hr LC_{50}	6.8	
	28-day LC_{50}	1.0	
	Cadmium chloride		
	96-hr LC_{50}	7.5	
	28-day LC_{50}	0.7	
Trochophore larvae	96-hr LC_{50}		
	Mercuric chloride	0.014	
	Copper sulfate	0.18	
	Zinc sulfate	1.7	
	Lead acetate	1.2	
	Cadmium chloride	0.22	
Neanthes arenaceodentata, laboratory colony			
Adults	Mercuric chloride		
	96-hr LC_{50}	0.022	
	28-day LC_{50}	0.017	
	Copper sulfate		
	96-hr LC_{50}	0.3	
	28-day LC_{50}	0.25	
	Zinc sulfate		
	96-hr LC_{50}	1.8	
	28-day LC_{50}	1.4	
	Lead acetate		
	96-hr LC_{50}	>10	
	28-day LC_{50}	3.2	
	Cadmium chloride		
	96-hr LC_{50}	12.0	
	28-day LC_{50}	3.0	

Table A-7 continued

Test Animal	Metal and Type of Test	Effective Concentration (mg/l)	Reference
Neanthes arenaceodentata continued			Reish et al. 1975 continued
Juveniles	Mercuric chloride		
	96-hr LC_{50}	0.1	
	28-day LC_{50}	0.09	
	Copper sulfate		
	96-hr LC_{50}	0.3	
	28-day LC_{50}	0.14	
	Zinc sulfate		
	96-hr LC_{50}	0.9	
	28-day LC_{50}	0.9	
	Lead acetate		
	96-hr LC_{50}	>7.5	
	28-day LC_{50}	2.5	
	Cadmium chloride		
	96-hr LC_{50}	12.5	
	28-day LC_{50}	3.0	
Capitella capitata, laboratory colony, larvae	Induction of abnormal larvae		Reish et al. 1974 Abnormal (bifurcated) larvae were induced in the presence of copper and zinc. Two generations were required to induce abnormal larvae in zinc, and one generation in copper. The frequency of occurrence of the abnormality was less than 1 percent. The affected larvae were unable to settle. No abnormal larvae appeared in the controls.
	Copper sodium citrate	0.01	
	Zinc sodium citrate	0.05	
Phyllodoce maculata, Cramond, Firth of Forth, Scotland	Hydrated copper sulfate		McLusky and Phillips 1975 Authors suggest that the rate of uptake, rather than the amount of Cu accumulated, may be the lethal factor. Worms placed in concentrations of 0.12 mg/l and above immediately reacted by wriggling and producing mucus. There was a linear relationship between the Cu content of the worm tissues and the period of exposure to the experimental solution. No plateau effect was observed, and accumulation seemed to take place at a steady rate.
	4-day LT_{50}	0.12	
	7-day LT_{50}	0.10	
Ophryotrocha labronica	Cupric sulfate		Saliba and Ahsanullah 1973 The data showed slight decreases in growth rates with increased Cu concentrations above 0.025 mg/l. O. labronica were unable to acquire an increased tolerance to the
	Growth rate inhibition	0.025	
	No reproduction	0.1	

Table A-7 continued

Test Animal	Metal and Type of Test	Effective Concentration (mg/l)	Reference
			Saliba and Ahsanullah 1973 continued toxic effects of Cu through acclimatization at sublethal levels. The actual measurement of the dissolved Cu in solution was not given.
Ophryotrocha labronica, Bay of Biscay, Great Britain	Mercuric chloride, 0.5-hr LT_{50}	1.0	Brown and Ahsanullah 1971 Of six metals tested, Hg was the most toxic to O. labronica, followed by Cu, Zn, Cd, Fe, and Pb. The biological activities of the citrated forms of copper and zinc were not explained; however these forms showed relatively high toxicity values.
	Copper sodium citrate, 4.5-hr LT_{50}	1.0	
	Zinc sodium citrate, 13.0-hr LT_{50}	1.0	
	Cadmium sulfate, 410-hr LT_{50}	1.0	
	Ferrous sulfate, 500-hr LT_{50}	1.0	
	Lead nitrate, +600-hr LT_{50}	1.0	
	Copper sodium citrate, suppression of growth rate	0.05	
Nereis virens, obtained from bait dealer (were probably from Maine)	Cadmium chloride, 96-hr TL_{50}	11.0	Eisler 1971 Author concluded that a 96-hr study is too short to permit determination of the toxicity of cadmium to aquatic species.
Spirorbis lamellosa, Port Hacking, New South Wales, Australia	2-hr LC_{50}		Wisely and Blick 1967 Results suggest that Hg as chloride is most toxic to these species, followed by Cu as citrate and Zn as citrate. However, the death rate of larvae in some Cu solutions was similar to that of larvae in comparable solutions not containing Cu but similar in pH. Therefore, the effects of lowered pH need to be taken into account in assessing mortality rates. The toxicants added were assumed to be totally dissolved, although no measurements for dissolved Cu, Hg, or Pb were taken.
	Mercuric chloride	7.0×10^{-7}*	
	Copper sodium citrate	8.0×10^{-6}*	
	Zinc sodium citrate	7.5×10^{-5}*	
Galeolaria caespitosa, Port Hacking, New South Wales, Australia	2-hr LC_{50}		
	Mercuric chloride	6.0×10^{-6}*	
	Copper sodium citrate	4.5×10^{-5}*	

*Units are moles per liter.

Table A-8. Review of literature on analyses for trace metals in body tissues of polychaetes.

Species	Metal and Type of Sample	Concentration	Reference
Nereis diversicolor, South Devon and Cornwall, Great Britain			Bryan 1971 Worms that had depurated their gut contents were used for tissue analyses. Results suggest that N. diversicolor is able to regulate concentrations of some metals in its body tissues when sediment concentrations of these metals change. This paper includes a general section on the effects of metals in seawater.
Plym Estuary	Total Zn		
	Whole body	34 mg/wet kg	
	Sediments	228 mg/dry kg	
	Total Cu		
	Whole body	9 mg/wet kg	
	Sediments	49 mg/dry kg	
	Total Pb		
	Whole body	0.7 mg/wet kg	
	Sediments	56 mg/dry kg	
Tamar Estuary	Total Zn		
	Whole body	36 mg/wet kg	
	Sediments	528 mg/dry kg	
	Total Cu		
	Whole body	22 mg/wet kg	
	Sediments	409 mg/dry kg	
	Total Pb		
	Whole body	1.1 mg/wet kg	
	Sediments	230 mg/dry kg	
Nereis diversicolor, Devon and Cornwall, Great Britain			Bryan and Hummerstone 1971 The concentration of Cu in N. diversicolor was roughly related to the total concentration of Cu in the sediment. In contrast, the Zn concentration in worm tissues remained markedly constant despite wide variation in environmental levels, and appeared to be accurately regulated. It also seemed that some worms were able to survive in polluted areas because they had developed a tolerance to the toxic effects of Cu; this tolerance was neither readily lost by exposure to a "clean" environment nor readily gained by nontolerant worms. The Cu uptake over the body surface appeared to be important as much of the Cu was deposited in the epidermis of the body wall and in parts of the nephridia. The chemical form of the Cu in the tissues was not known. Animals that had depurated their guts were used in the tissue analyses.
Plym Estuary	Total Cu		
	Whole body	28 µg/dry g	
	Sediments	41 µg/dry g	
	Total Zn		
	Whole body	199 µg/dry g	
	Sediments	339 µg/dry g	
	Total Pb		
	Whole body	5.9 µg/dry g	
	Sediments	44 µg/dry g	
Dart Estuary	Total Cu		
	Whole body	22 µg/dry g	
	Sediments	44 µg/dry g	
	Total Zn		
	Whole body	163 µg/dry g	
	Sediments	140 µg/dry g	
	Total Pb		
	Whole body	5.9 µg/dry g	
	Sediments	44 µg/dry g	
Avon Estuary	Total Cu		
	Whole body	33 µg/dry g	
	Sediments	52 µg/dry g	
	Total Zn		
	Whole body	176 µg/dry g	
	Sediments	99 µg/dry g	
	Total Pb		
	Whole body	3.4 µg/dry g	
	Sediments	35 µg/dry g	

Table A-8 continued

Species	Metal and Type of Sample	Concentration	Reference
Nereis diversicolor continued			Bryan and Hummerstone 1971 continued
Camel Estuary	Total Cu		
	Whole body	31 µg/dry g	
	Sediments	73 µg/dry g	
	Total Zn		
	Whole body	155 µg/dry g	
	Sediments	122 µg/dry g	
	Total Pb		
	Whole body	0.7 µg/dry g	
	Sediments	21 µg/dry g	
Tamar Estuary	Total Cu		
	Whole body	106 µg/dry g	
	Sediments	436 µg/dry g	
	Total Zn		
	Whole body	166 µg/dry g	
	Sediments	578 µg/dry g	
	Total Pb		
	Whole body	5.8 µg/dry g	
	Sediments	299 µg/dry g	
Tiddy and Tamar Estuaries	Total Cu		
	Whole body	257 µg/dry g	
	Sediments	591 µg/dry g	
	Total Zn		
	Whole body	185 µg/dry g	
	Sediments	532 µg/dry g	
	Total Pb		
	Whole body	4.9 µg/dry g	
	Sediments	287 µg/dry g	
Restronguet Creek	Total Cu		
	Tissues		
	Whole body	1,142 µg/dry g	
	Parapodia	840 µg/dry g	
	Body wall	514 µg/dry g	
	Gut	182 µg/dry g	
	Whole segments	566 µg/dry g	
	Sediments	3,020 µg/dry g	
	Total Zn		
	Whole body	194 µg/dry g	
	Sediments	2,237 µg/dry g	
	Total Pb		
	Whole body	3.5 µg/dry g	
	Sediments	359 µg/dry g	
Diopatra cuprea*	Total Zn; whole body		Cross et al. 1970
	Tissue	87 µg/dry g	Comparison of the relationship of zinc
	Seawater	0.7 µg/l	levels in body tissues and sediments to
	Sediments	0.34-1.9 µg/dry g	the type of feeding employed by each poly-
			chaete species indicated that the elements

*Menhaden Shoals and Core Creek, near Beaufort, North Carolina.

Table A-8 continued

Species	Metal and Type of Sample	Concentration	References
Chaetopterus variopedatus*	Total Zn, whole body		Cross et al. 1970 continued
	Tissue	100 µg/dry g	were not partitioned entirely between the surface-feeders and the subsurface-feeders. Polychaetes could regulate zinc concentrations in their tissues to some degree. In estuarine areas where sediment feeders are abundant, they may affect the rate of exchange of some elements between sediment and water.
	Seawater	0.7 µg/l	
	Sediments	0.34-1.9 µg/dry g	
Amphitrite ornata*	Total Zn, whole body		The numbers here were taken from the original graphs and figures. The body tissue values are median values. There was no mention that the worms had emptied their gut contents before their tissues were analyzed. Water values are from unfiltered seawater.
	Tissue	78 µg/dry g	
	Seawater	0.7 µg/l	
	Sediments	0.34-1.9 µg/dry g	
Nereis sp.*	Total Zn, whole body		
	Tissue	82 µg/dry g	
	Seawater	0.7 µg/l	
	Sediments	0.34-1.9 µg/dry g	
Glycera americana*	Total Zn, whole body		
	Tissue	164 µg/dry g	
	Seawater	0.7 µg/l	
	Sediments	0.34-1.9 µg/dry g	
Arabella iricolor*	Total Zn, whole body		
	Tissue	143 µg/dry g	
	Seawater	0.7 µg/l	
	Sediments	0.34-1.9 µg/dry g	
Polynoid Sp. 17**	Total Zn, whole body		Phelps 1967
	Station 6		The most notable difference between Stations 1 and 6 was in the quantities of Fe in the sediments. Levels at Station 6 were twice those found at Station 1. Zn was not detected in the sediments at either location. Author believes that, among the polychaetes at least, composition by feeding types is an important consideration in the partitioning of stable elements within the benthic community. It appears that the less dependent an organism becomes on sediment as a source of nutrients, the lower its concentration of stable elements. Differences in feeding mechanisms and habits between groups of polychaetes offer the most obvious clue to differences in trace element content within the benthic communities. Selective
	Tissue	55 µg/dry g	
	Sediments	Not detected	
Polynoid Sp. 30**	Total Zn, whole body		
	Station 6		
	Tissue	59 µg/dry g	
	Sediments	Not detected	
Ceratonereis sp.**	Total Zn, whole body		
	Station 1		
	Tissue	845 µg/dry g	
	Sediments	Not detected	
	Station 6		
	Tissue	125 µg/dry g	
	Sediments	Not detected	

*Menhaden Shoals and Core Creek, near Beaufort, North Carolina.
**Añasco Bay, Puerto Rico.

Table A-8 continued

Species	Metal and Type of Sample	Concentration	Reference
Protoaricia sp.*	Total Zn, whole body		Phelps 1967 continued deposit-feeders concentrated Fe to the apparent exclusion of Zn; nonselective deposit-feeders concentrated Zn to the apparent exclusion of Fe.
	Station 1		
	Tissue	1,239 µg/dry g	
	Sediments	Not detected	
	Station 6		
	Tissue	370-1,554 µg/dry g	
	Sediments	Not detected	
Sigambra (nr.) tentacula*	Total Zn, whole body		
	Station 1		
	Tissue	2,490 µg/dry g	
	Sediments	Not detected	
	Station 6		
	Tissue	80 µg/dry g	
	Sediments	Not detected	
Agloaphamus sp.*	Total Zn, whole body		
	Station 1		
	Tissue	2,961 µg/dry g	
	Sediments	Not detected	
	Station 6		
	Tissue	159 µg/dry g	
	Sediments	Not detected	
Cirratulid sp.*	Total Fe, whole body		
	Station 1		
	Tissue	42.8 mg/dry g	
	Sediments	26.7 mg/dry g	
Ampharetid sp.*	Total Fe, whole body		
	Station 1		
	Tissue	41.0 mg/dry g	
	Sediments	26.7 mg/dry g	
Armandia maculata*	Total Fe, whole body		
	Station 6		
	Tissue	30.6 mg/dry g	
	Sediments	55.5 mg/dry g	
Paraprionospio sp.	Total Fe, whole body		
	Station 1		
	Tissue	0.4 mg/dry g	
	Sediments	26.7 mg/dry g	
	Station 6		
	Tissue	24.6 mg/dry g	
	Sediments	55.5 mg/dry g	

*Añasco Bay, Puerto Rico.

Table A-8 continued

Species	Metal and Type of Sample	Concentration	Reference
Capitella sp.*	Total Fe, whole body		Phelps 1967 continued
	Station 6		
	Tissue	22.1 mg/dry g	
	Sediments	55.5 mg/dry g	
Magelona sp.	Total Fe, whole body		
	Station 1		
	Tissue	0.2 mg/dry g	
	Sediments	26.7 mg/dry g	
LABORATORY EXPERIMENTS			
Nereis virens, Weston Shore, Southampton, Great Britain	Cupric sodium citrate		Raymont and Shields 1963 The data show marked increases in body wall and gut Cu concentrations. It was also found that the rate of accumulation was directly related to Cu concentrations. The rate was proportional to the size of the worm and was temperature-dependent.
	48-hr exposure		
	Control		
	Body wall	27 mg/dry kg	
	Gut	19 mg/dry kg	
	0.2 mg/l		
	Body wall	140 mg/dry kg	
	Gut	87 mg/dry kg	
	96-hr exposure		
	Control		
	Body wall	25 mg/dry kg	
	Gut	16 mg/dry kg	
	0.2 mg/l		
	Body wall	266 mg/dry kg	
	Gut	128 mg/dry kg	
Phyllodoce maculata, Cramond, Firth of Forth, Scotland	Hydrated copper sulfate ($CuSO_4 \cdot 5 H_2O$), whole body, 4 days, 0.12 mg/l	244.29 mg/dry kg	McLusky and Phillips 1975 There was a linear relationship between the Cu content of worm tissues and the period of exposure. As the exposure time increased, the amount of Cu accumulated in the tissues increased. No plateau was observed.

Appendix B

GENERAL PROCEDURES

BIOLOGICAL PROCEDURES

Laboratory Colony

The laboratory population of Neanthes arenaceodentata from which the Project obtained specimens for toxicity tests has been maintained for 11 years (over 30 generations) in the laboratory of Dr. Donald Reish, California State University, Long Beach. The orginal worms were collected from the Los Angeles Harbor. The juvenile worms used in our experiments were about 1 cm in length and had 30 to 40 setigerous segments.

Collection of Field Specimens

The field specimens used in the experiments were taken from a site at the mouth of the San Gabriel River at low tides of less than +0.15 m. Muddy sediments still covered by approximately 0.3 m of water were collected and sifted through a wire mesh screen. The worms were caught in the screen, removed with a No. 2 camel hair artist's paint brush and placed in a bucket containing seawater. The worms were brought to the laboratory and kept in individual 100- by 20-mm petri dishes containing seawater. The adults from the field used in the short-term experiment were about 2 cm in length and had over 40 setigerous segments.

Food for Test Animals

The N. arenaceodentata used in the long-term experiments were fed Enteromorpha crinita taken from the Colorado Lagoon, Long Beach, during a high tide. The algae was rinsed in lagoon water several times and then dried in the sun. The dried algae was then kept in closed jars until used.

The algae was analyzed by atomic absorption spectrophotometry and found to contain 5.2 ± 0.8 mg/dry kg total chromium.

Cannibalism Among Test Animals

N. arenaceodentata are often cannibalistic to members of their own sex. In a preliminary experiment using trivalent chromium, we placed two worms in each gallon jar rather than the customary

one. This arrangement led to cannibalism between worms of the same sex, and the experiment was terminated after 37 days. In all other experiments, only one worm was placed in each gallon jar until the time when the worms could be sexed and paired.

Pairing

As there is no morphological difference between immature male and female N. arenaceodentata, we used the characteristic hostility between members of the same sex to differentiate the sexes and pair animals in the long-term experiment. A characteristic fighting reaction takes place when two members of the same sex encounter each other: Each worm everts its proboscis and exposes its jaws; the two animals then lock jaws or attempt to bite each other.

Opposite sexes do not elicit this fighting response; in fact, the tendency is for a male and female to remain in close contact with each other along their lateral surfaces.

To pair worms, we placed two worms in a petri dish containing the respective chromium concentration, and observed them for fighting or pairing reactions. This procedure was followed until the maximum number of pairs at each concentration were formed.

Counting Number of Young per Brood

To determine the number of young produced by each pair, two-thirds of the solution in each gallon jar containing young worms was decanted into another clean gallon jar; the worms and the remaining solution were placed in a white enamel pan. The worms were then removed from the pan individually with a pipette, counted, and placed in the gallon jar with the solution. Worms were removed from algal clumps by teasing the algae with forceps. Muc of the work was done with the aid of a dissecting microscope.

Preparation of Worms for Tissue Analysis

Male worms used in the long-term experiments were removed after completion of the brood incubation, weighed, and frozen for analysis of chromium in the body tissues. Worms designated "depurated" had been held in filtered (0.45-micron) seawater for 48 hours prior to weighing and freezing. The "nondepurated" worms were just rinsed with filtered seawater prior to weighing and freezing. It is possible that some chromium adhered to body surfaces, although care was taken to thoroughly clean the animal prior to freezing and spectrophotometric analysis.

CHEMICAL PROCEDURES

Seawater Source

Seawater for these experiments was obtained from Marineland of the Pacific, Palos Verdes, California, and stored in a 2,650-liter tank in the laboratory at California State University, Long Beach. All seawater used in short-term experiments was passed through a 0.45-micron millipore filter. Seawater used in long-term experiments was filtered through No. 1 Whatman filter paper.

Glassware

All glassware was scrubbed clean with a brush or scouring cloth, rinsed with 10 percent hydrochloric acid, rinsed twice with warm tapwater, rinsed twice with deionized/distilled water, and air-dried prior to use in the experiments.

Toxicant Dilution

For the hexavalent chromium experiments, a stock solution of 1,000 mg/l chromium was prepared using $K_2Cr_2O_7$; dilutions were then made according to dilution factors used for the individual experiments.

To make the trivalent chromium precipitate ($Cr(OH_3)$) concentration for the long-term experiment, 30 g of $CrCl_3$ were mixed in seawater, and enought NaOH was added to yield a pH of normal seawater with a total volume of 950 ml; the mixture was then magnetically stirred to get an even distribution of the precipitate throughout the solution. A syringe was used to transfer 15 ml of this mixture to the 3 liters of seawater for each replication.

Water Changes During the Long-Term Experiments

Fresh toxicant solutions were made up in 50- to 100-liter quantities. The "old" water in each gallon jar was siphoned out using glass tubing and aquarium air hoses. The new solutions were likewise siphoned in. The slow siphoning was necessary to avoid disturbing the worms. Ninety-nine percent of the hexavalent chromium solution in each gallon jar was removed and replaced at each water change; 50 percent of the solution was removed and replaced at each water change in the trivalent chromium experiment.

Water Quality Monitoring

The water quality in the test solutions was monitored during the experiments using the following procedures:

- Dissolved oxygen was measured with a Beckman Fieldlab TM oxygen analyzer (No. 100800).

- Nitrite concentrations were measured with an Ecolife pH and nitrite saltwater test kit.
- pH levels were monitored with a Beckman Zeromatic SS-3 pH meter equipped with a 39402 C-3U pH probe.
- Salinity values were obtained using the Argentometric method for chloride analysis (Standard Methods 1971).
- Ammonium-nitrogen concentrations were monitored using the Solorzano adaptation of the phenolhypochlorite methods (Zadorojny et al. 1973).
- Chromium values were obtained by the atomic absorption spectrophotometric method. Samples analyzed for hexavalent chromium were prepared using methyl iso-butyl ketone. Trivalent chromium samples were extracted by inducing coprecipitation with ferric hydroxide ($Fe(OH)_3$) and dissolving the sample in a 5 percent nitric acid (HNO_3) solution. Tissues were prepared using nitric acid digestion.

The temperature during the experiments ranged from 19.4° to 20.6°C; nitrite values were always zero. Data on the other parameters are summarized in Tables B-1 and B-2.

The results of a 3-week test of the stability of the oxidation state of the potassium dichromate used in the hexavalent chromium experiments are given in Table B-3.

STATISTICAL PROCEDURES

The Project's long-term tests for sublethal responses to hexavalent chromium in Neanthes arenaceodentata revealed that, as chromium concentrations increased, the number of young per brood decreased. The statistical procedures that showed this decrease to be significant are given in Figures B-1 through B-3.

Table B-1. Water quality measurements, long-term toxicity tests on *Neanthes arenaceodentata* using $K_2Cr_2O_7$ (three generations) and $CrCl_3$ precipitate (two generations).

Chromium Concentration (mg/l)	No. of Samples	Means: Before Water Change	Means: After Water Change	Means: All Samples ± Std. Dev.	Range
DISSOLVED OXYGEN (mg/l)					
$K_2Cr_2O_7$					
Control	106	-	-	7.1 ± 0.18	6.5-7.4
0.0125	106	-	-	7.1 ± 0.16	6.8-7.4
0.025	106	-	-	7.1 ± 0.17	6.6-7.4
0.05	106	-	-	7.1 ± 0.15	6.7-7.4
0.1	58	-	-	7.1 ± 0.17	6.5-7.4
0.2	43	-	-	7.1 ± 0.19	6.5-7.4
$CrCl_3$ precipitate					
Control	46	-	-	7.0 ± 0.20	6.7-7.4
Precipitate	45	-	-	7.0 ± 0.17	6.7-7.4
pH LEVELS					
$K_2Cr_2O_7$					
Control	107	-	-	7.9 ± 0.13	7.5-8.2
0.0125	107	-	-	7.9 ± 0.12	7.6-8.3
0.025	107	-	-	7.9 ± 0.11	7.7-8.2
0.05	107	-	-	7.9 ± 0.11	7.6-8.2
0.1	60	-	-	8.0 ± 0.14	7.6-8.2
0.2	45	-	-	8.0 ± 0.12	7.7-8.2
$CrCl_3$ precipitate					
Control	38	-	-	7.9 ± 0.08	7.7-8.1
Precipitate	39	-	-	7.9 ± 0.08	7.8-8.1
SALINITY (°/oo)					
$K_2Cr_2O_7$					
Control	44	33.7	33.5	33.6 ± 0.24	33.4-33.8
0.0125	44	33.8	33.5	33.7 ± 0.24	33.4-34.9
0.025	43	33.7	33.5	33.6 ± 0.18	33.4-34.5
0.05	43	33.7	33.5	33.6 ± 0.17	33.4-34.3
0.1	24	33.8	33.5	33.7 ± 0.18	33.4-34.0
0.2	18	33.9	33.5	33.7 ± 0.26	33.5-34.6
$CrCl_3$ precipitate					
Control	13	33.6	33.5	33.6 ± 0.05	33.5-33.6
Precipitate	13	33.6	33.5	33.6 ± 0.05	33.5-33.6

Table B-1 continued

Chromium Concentration (mg/l)	No. of Samples	Means: Before Water Change	Means: After Water Change	Means: All Samples ± Std. Dev.	Range
AMMONIUM-NITROGEN (mg/l)					
$K_2Cr_2O_7$					
Control	42	0.15	0.03	0.11 ± 0.23	0-1.38
0.0125	43	0.20	0.04	0.11 ± 0.24	0-1.56
0.025	42	0.28	0.06	0.13 ± 0.39	0-2.57
0.05	43	0.15	0.07	0.11 ± 0.16	0-0.78
0.1	26	0.08	0.03	0.05 ± 0.08	0-0.38
0.2	19	0.09	0.05	0.08 ± 0.07	0.01-0.26
$CrCl_3$ Precipitate					
Control	11	0.24	0.11	0.16 ± 0.17	0-0.48
Precipitate	11	0.11	0.14	0.19 ± 0.20	0-0.58
TOTAL CHROMIUM* (mg/l)					
$K_2Cr_2O_7$					
Control	9	-	-	0.001 ± 0.002	<0.001-0.007
0.0125	18	0.013	0.013	0.013 ± 0.002	0.011-0.015
0.05	9	0.055	0.052	0.054 ± 0.004	0.052-0.058
0.2	5	-	-	0.205 ± 0.004	0.198-0.208
$CrCl_3$ Precipitate					
Control	7	-	-	<0.001	-
Filtrate	13	0.006	0.003	0.004 ± 0.003	<0.001-0.012

*The first 2 months of data indicated relative stability in hexavalent chromium concentrations. Thus, for the duration of the experiment, only the 0.0125 mg/l solution was monitored routinely. As we used a serial dilution procedure, beginning with the highest concentration, any cumulative error would be most apparent at the 0.0125-mg/l level.

Table B-2. Water quality measurements, short-term toxicity tests on Neanthes arenaceodentata using $K_2Cr_2O_7$.

	No. of Samples	Mean ± Std. Dev.	Range
DISSOLVED OXYGEN (mg/l)			
Day 0	25	7.0 ± 0.18	6.8-7.4
Day 7	25	7.0 ± 0.17	6.7-7.4
pH LEVEL			
Day 0	25	7.8 ± 0.08	7.7-7.9
Day 7	25	7.6 ± 0.14	7.2-7.8
SALINITY (°/oo)			
Day 0	4	33.4 ± 0.27	33.0-33.6
Day 7	-		
AMMONIUM-NITROGEN (mg/l)			
Day 0	25	0.02 ± 0.03	0-0.10
Day 7	25	0.33 ± 0.38	0-1.40

Table B-3. Three-week test of the stability of the oxidation state of the potassium dichromate used in toxicity tests on Neanthes arenaceodentata.

Nominal Concentration (mg/l)	Total Chromium (mg/l)	Percent Trivalent	Percent Hexavalent
Day 0			
Control	<0.001	0	100
0.0125	0.013	0	100
0.025	0.029	0	100
0.05	0.05	1	99
Day 21			
Control	<0.001	31	69
0.0125	0.013	1	99
0.025	0.031	<1	99
0.05	0.052	1.5	98

Part A. Number of young per brood

	Concentration (mg/l)			
	Control	0.0125	0.025	0.050
Pair 1	223	82	98	67
Pair 2	236	349	222	128
Pair 3	355	170	233	58
Pair 4	0	107	36	49
Pair 5	412	0	219	89
Pair 6	302	159	150	
Pair 7		129	198	
Pair 8		59	96	
Pair 9		144	63	
Mean	255	133	146	78

Part B. Anova table for data in Part A.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	93,938.87	3	31,312.96	3.44
Error	227,716.58	25	9,108.66	
Total	321,655.45	28		

Part C. Dunnett's Test (Model I)

1. $S_{\bar{d}} = \sqrt{\frac{2(\text{Error Mean Square})}{\text{No. of replicates/treatment}}} = \sqrt{\frac{2(9,108.66)}{29/4}}$

   $= 50.13$

2. $d' = t(\text{Dunnett})S_{\bar{d}} = 2.17(50.13) = 109$

3. Control - d' = 255 - 109 = 146

4. As the mean numbers of young per brood in the 0.0125, 0.025, and 0.05 mg/l hexavalent chromium concentrations were less than or equal to 146, these values are significantly lower than the control value at $p \leq 0.05$.

Figure B-1. Statistical tests for number of young per brood in the parental generation (P) of Neanthes arenaceodentata exposed to hexavalent chromium.

Part A. Number of young per brood

	Concentration (mg/l)			
	Control	0.0125	0.025	0.050
Pair 1	578	102	70	109
Pair 2	246	229	172	61
Pair 3	235	326	317	122
Pair 4	234	479	165	17
Pair 5	141	160	217	0
Pair 6	597	229	125	20
Pair 7	332	546	22	86
Pair 8	265	186	134	
Pair 9	0	47	255	
Pair 10		276		
Mean	292	258	164	59

Part B. Anova table for data in Part A.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	259,725.97	3	86,575.32	4.50
Error	595,956.32	31	19,224.40	
Total	855,682.29	34		

Part C. Dunnett's test (Model I)

1. $S_{\bar{d}} = \sqrt{\frac{2(\text{Error Mean Square})}{\text{No. of replicates/treatment}}} = \sqrt{\frac{2(19{,}224.40}{35/4}}$

   $= 66.29$

2. $d' = t(\text{Dunnett})S_{\bar{d}} = 2.15(66.29) = 142.52$

3. Control - d' = 292 - 142 = 150

4. As the mean numbers of young per brood in the 0.0125 and 0.025 mg/l hexavalent chromium concentrations are greater than 150, these concentrations are "safe." 0.05 mg/l is "unsafe."

Figure B-2. Statistical tests for number of young per brood in the first filial generation (F_1) of Neanthes arenaceodentata exposed to hexavalent chromium.

Part A. Number of young per brood

	Concentration (mg/l)			
	Control	0.0125	0.025	0.050
Pair 1	147	244	298	81
Pair 2	281	180	97	332
Pair 3	155	85	125	226
Pair 4	661	213	110	1
Pair 5	393	154	115	201
Pair 6	341	215	167	15
Pair 7	85	487	144	42
Pair 8	123	184		92
Pair 9		71		16
Pair 10		62		106
Mean	273	190	151	111

Part B. Anova table for data in Part A.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatment	123,672.23	3	41,224.08	2.42
Error	527,590.46	31	17,019.05	
Total	651,262.69	34		

Part C. Dunnett's test (Model I)

1. $S_{\bar{d}} = \sqrt{\frac{2(\text{Error Mean Square})}{\text{No. of replicates/treatment}}} = \sqrt{\frac{2(17,019.05}{35/4}}$

   $= 62.37$

2. $d' = t(\text{Dunnett})S_{\bar{d}} = 2.15(62.37) = 134$

3. Control - d' = 273 - 134 = 139

4. As the mean number of young per brood in the 0.0125 and 0.025 mg/l hexavalent chromium concentrations are greater than 139, these concentrations are "safe." 0.05 mg/l is "unsafe."

Figure B-3. Statistical tests for number of young per brood in the second filial generation (F_2) of Neanthes arenaceodentata exposed to hexavalent chromium.

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