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A SAFE LEVEL OF HEXAVALENT CHROMIUM FOR A MARINE POLYCHAETE

Chromium is released to the marine environment in industrial and municipal wastewater effluents. The potential effects of such inputs make it important to understand the response of sensitive marine animals to elevated chromium levels. In the past year, we have con-ducted experiments to determine the maximum seawater concentration of hexavalent chromium that is safe for a marine polychaete, *Neanthes arenaoeodentata*, and to investigate the possible association between tissue accumulations of chromium in the polychaete and alterations in its reproduction (specifically, in number of offspring). This effort is part of a 3-year study of the effects of low levels of chromium on this animal.

By mid-1976, we had completed a series of long-term experiments that suggested that trivalent chromium was not toxic to *N. avenaoeodentata*, but that, at concentrations in the range of 12.5 to 50 pg/liter, hexavalent chromium began to cause detrimental effects on reproduction. During the past year, we completed an additional series of experiments involving six hexavalent chromium concentrations below 50 pg/liter (four of which were lower than 12.5 pg/liter). In the new experiments, our sample size was increased from between 5 and 10 pairs of animals to 20 to 30 pairs. The results indicate that a level of 29.9 pg/liter of dissolved hexavalent chromium is safe for *Neanthes avenaceodentata.*

Chromium occurs naturally in ocean waters, and the background concentrations of dissolved chromium off southern California are near 0.19 pg/liter, of which 76 percent is hexavalent and 24 percent is trivalent (Jan and Young 1976). Chromium commonly enters municipal and industrial sewage in plating and tanning operation wastes, in waters given chromate-corrosion-inhibition treatment, and in aluminum anodizing wastes (National Research Council 1974). The average total chromium concentrations in some southern California effluents can be as high as 900 pg/liter. An average of 2.5 percent of the discharged chromium is in the dissolved hexavalent state; assuming an immediate initial dilution of 100:1, the concentration of dissolved chromium in the ocean near the diffuser ports would be about 0.22 pg/liter. The remainder of the chromium in effluent is trivalent and/or particulate (the toxicity of chromium attached to particles is not known).

Although there is some evidence that a form of chromium is a trace nutrient and plays a beneficial role in glucose metabolism (National Research Council 1974), previous data had shown that high concentrations of hexavalent chromium were acutely toxic to a variety of marine animals, such as fish (Sherwood 1975), polychaetes (Reish et al. 1975), echinoderms (Okubo and Okubo 1965), and crustaceans (Oshida and Wright 1976). However, these concentrations were often over four orders of magnitude above the hexavalent chromium levels found in ocean waters. The goal of the work described here was to investigate the effects of chromium at concentrations closer to natural seawater levels; our preliminary efforts were described in a technical memorandum (Oshida et al. 1976). Other laboratory work on intermediate levels of chromium include Reish's (1977) experiments with polychaetes and the work of Martin et al. (1975) with mussels.

Among animals previously used in chromium toxicity tests at the Coastal Water Research Project were the shrimp *Sicyonia ingentis*, a sipunculid (*Themiste sp*.), the brittle star *Ophiothrix spiculata* and *Neanthes arenaceodentata*. *N. arenaoeodentata* had the lowest 7-day LC50 value for hexavalent chromium and thus was selected for further long-term tests. This polychaete is a recommended marine bioassay organism (Standard Methods 1976).

PROCEDURES

Young polychaetes were obtained from a laboratory culture, and 90 were exposed to each of six hexavalent chromium concentrations ranging from 1 to 38.2 pg/liter in 76-liter aquaria (Table 1). The polychaetes were fed algae (*Enteromorpha crinita*) and allowed to grow for 45 days, at which time the females and males were paired. Each pair was then placed in a glass cylinder covered on both ends by polyester mesh, and the cylinder was returned to the appropriate aquarium. Each pair of worms constructed and occupied a single tube made out of mucus, algae, and fecal material.

The polychaetes were observed one to five times a week for time of spawning and feeding behavior. On the average, spawning occurred 90 days after pairing. After spawning, the females died naturally within 48 hours; the males remained in the mucous tubes to incubate the eggs. At this time, each glass tube was removed to a 1-gallon jar that contained 3 liters of the particular chromium concentration in which the adult had been raised. The offspring were counted soon after the first young worms emerged from the tube. After being counted, the young worms were put in a 19-liter aquarium that contained the same toxicant solution to which their parents had been exposed.

The adult males that had been incubating the brood were placed in petri dishes containing normal seawater and allowed to depurate for 72 to 120 hours. These animals were then frozen, freeze-dried, and analyzed for trace elements by George Alexander (Department of Nuclear Medicine, University of California at Los Angeles), using arc-emission spectroscopy.

After all the young worms had been counted, the entire procedure was repeated for the first filial generation (F_1, using 90 offspring from each of the six chromium concentrations. Throughout the entire experiment, polychaetes from a particular concentration were never exposed to any other concentration. The experiment covered two complete generations and lasted 309 days. Water quality was maintained within certain specific limits throughout the experiment (Table 1), and the chromium levels were monitored at regular intervals.

When working at low toxicant levels, both increases and decreases in brood size might be expected. For this reason, a two-tailed Dunnett's test (Steel and Torrie 1960) for comparing all treatment means with a control mean in a Model-1 analysis of variance was used to investigate significant differences ($p < 0.05$) in numbers of offspring caused by hexavalent chromium. The null hypothesis was that hexavalent chromium does not alter brood size in *N. arenaceodentata*. Data from previous bioassays (Oshida 1976; Oshida et al. 1976), in which a one-tailed Dunnett's test was used, have been reexamined using the two-tailed test, which appears to be more appropriate.

RESULTS

In this experiment, the worms fed normally at all concentrations. Oocytes developed within the coelomic cavity of all female polychaetes, and normal fighting and pairing behavior was evident at all concentrations. Two effects could be measured at the highest level: Uptake and brood size reduction.

Although there were no significant reductions in brood size among the parental (P) generation polychaetes exposed to chromium, the number of young per brood produced in 16.6 pg/liter was significantly higher than that produced by the control polychaetes (Table 2, Figure 1).

The polychaetes in the F_1 generation reacted differently than the P generation animals exposed to the same chromium levels—the F_1 generation animals exposed to 38.2 µg/liter showed a significant decrease in mean brood size (Table 2, Figure 2).

The tissue levels of chromium in P generation *N. arenaoeodentata* are summarized in Table 3 and Figure 1. There was very significant regression ($p \leq 0.01$) between the concentrations of chromium in the polychaete tissues (y) and the levels of chromium to which the animals were exposed (x). Polychaetes exposed to higher levels of hexavalent chromium had proportionally higher levels of chromium in their tissues. The line for regression between the exposure concentrations and tissue levels for this generation was:

$$y = 1060.4x - 1778.1.$$

The tissue concentrations of chromium in the F_1 poly-chaetes are summarized in Table 3 and Figure 2. Again, there was a significant regression between the polychaete tissue levels of chromium and the hexavalent chromium exposure concentrations. The regression line for this generation was:

$$y = 734.5x + 4588.7.$$

The tissue concentrations for both generations are reported on a dry-weight basis. The dry material accounted for 19.9 percent of the total weight of the polychaetes (based on 18 samples; the standard error was 0.35 percent).

DISCUSSION

The quantities of chromium accumulated by *N. arenaceodentata* were in proportion to the exposure concentration (Figures 1 and 2). Chipman (1966) found a similar pattern when he monitored chromium uptake by another marine polychaete, *Hermione hystrix*, in a 19-day experiment. He suggested that the greater uptake of chromium at higher seawater concentrations indicated that uptake by *H. hystrix* was a passive process and that accumulation within the organism might have been the result of the binding of chromium with body proteins.

Raymont and Shields (1963) used Cr as a radioactive tracer for monitoring chromium absorption in the polychaete, *Nereis virens*. They found the gut and body walls to be sites of the largest amount of chromium absorption; the parapodial region also had increased concentrations of chromium, probably because the body wall is thin in this area. They also found evidence that chromium could be transported via the body wall and gut wall to the small blood vessels.

Although there are undoubtedly specific differences in reactions to hexavalent chromium exposure, the general explanations for chromium uptake by Chipman (1966) and Raymont and Shields (1963), coupled with the data in Table 3 and Figures 1 and 2, suggest that *Neanthes arenaoeodentata* was accumulating chromium in proportion to the ambient water concentration and that most of the accumulated chromium was located in the body wall, gut wall, and parapodial regions.

Another sublethal response to hexavalent chromium was a significant reduction in the number of offspring from F_1 polychaetes that had been spawned and maintained in seawater with a chromium concentration of 38.2 pg/liter (Table 2, Figure 2). The polychaetes raised similarly in the other concentrations (2.6 to 16.6 pg/liter) did not show reductions in the numbers of their offspring. Thus, it appears that there was a level of chromium between 16.6 and 38.2 µg/liter above which the number of offspring was reduced. This result corresponded with information from a previous study of three generations of *N. arenaceodentata* exposed to hexavalent chromium (Oshida et al. 1976) in which there was a significant reduction in the number of offspring above a level that was between 25 and 50 µg/liter.

Although this experiment resulted in no acute effects of hexavalent chromium to individual *N. arenaceodentata*, the work revealed chronic effects, which were reduction in the numbers of offspring and bioaccumulation of chromium in the tissues.

There does not appear to be a direct relationship between the levels of chromium in the tissues of test animals and decrease in mean brood size. The F_1 generation polychaetes exposed to the 16.6 µg/liter concentration had accumulated 22,200 µg/dry kg in their tissues (Table 3). This concentration of chromium in the tissues was at least 110 times greater than the concentration in the control polychaetes (less than 200 µg/dry kg), which were exposed to less than 1 µg of chromium per liter of seawater. Yet there was no alteration in behavior nor reduction in number of offspring in the worms at exposure to 16.6 µg/liter. The F_1 polychaetes exposed to 38.2 µg/liter had tissue levels of chromium of 30,300 µg/dry kg, and their mean number of offspring per brood was significantly reduced. The evidence presented for the F_1 polychaetes exposed to 16.6 and 38.2 µg/liter suggests that there is a threshold body burden level between 22,200 and 30,300 µg/dry kg above which there is a significant reduction in brood size. However, this suggestion is not valid as P generation polychaetes exposed to 38.2 µg/liter had tissue levels of chromium in excess of 40,500 µg/dry kg, and there were no effects on brood size. *N. arenaceodentata* has shown the ability to accumulate chromium in its body tissues to levels many times above normal and still be able to live and reproduce without displaying any adverse effects. There is no direct evidence that chromium accumulation levels affect reproduction in this polychaete.

Results from the previous study (Oshida et al. 1976; three generations) as well as results from this experiment (two generations) were used to calculate a "safe" level of dissolved hexavalent chromium in seawater for *Neanthes arenaceodentata* with respect to the number of offspring produced. Dunnett's procedure for comparing all treatment means with a control mean (Steel and Torrie 1960) was used to calculate the level of significant enhancement and reduction of the number of offspring (e.g.. Figure 3) for each of the five generations. Using a two-tailed Dunnett's test, the mean brood size of the polychaetes in a particular test concentrations must be equal to or less than the mean brood size at the level of significant reduction to demonstrate that the test concentration had an effect ($p \leq 0.05$). An equation for the line connecting the number-of-offspring values for the second experiment F_1 generation at the 16.6 and 38.2 µg/liter concentrations was determined, and the chromium concentration at the level of significant reduction was calculated for this line. This procedure was repeated for the P generation in this study and for each of the three generations in the previous studies because the level of significant reduction was different in each experiment (in working with the data from the previous experiments, an equation for the line connecting the brood size values for all generations at the 25 and 50 µg/liter concentrations was determined). The chromium concentrations at the levels of significant reduction for each respective generation are presented in Table 4.

The mean concentration at the level of significant reduction for all five experimental generations was 37.7 µg/liter. Water quality monitoring during the experiments had shown that the chromium concentrations in the experimental containers decreased in 8- to 21-day intervals; this factor had to be taken into account in the determination of a safe level. The mean percentage of chromium lost between solution renewals was 20.6. This percentage was subtracted from the mean concentration at the level of significant reduction. The resulting value—a concentration of 29.9 µg/liter—takes into account possible losses of hexavalent chromium between solution renewals and is a conservative estimate of the "safe" level of dissolved hexavalent chromium in seawater for *Neanthes avenaceodentata*. When evaluating the effects of hexavalent chromium on *N. avenaceodentata*, it should be noted that the maximum safe level of exposure is far above the California emission standards of 5 µg/liter (which, upon discharge, is immediately diluted more than 100:1); thus, present California law has a probable safety factor for this animal of nearly 1,000.

There is a large gap between the hexavalent chromium concentrations that cause acute effects and those that cause chronic effects. To try to find relationships between the chronic and acutely toxic levels, we have made estimates of the application factor—the ratio between the "maximum allowable toxicant concentration" and the "incipient lethal level" (Standard Methods 1976). Given the maximum allowable hexavalent chromium concentration for *Neanthes arenaceodentata* of 29.9 µg/liter, and an incipient LC50 for this animal of between 100 and 200 µg/liter (Oshida et al. 1976), the application factor appears to be between 0.3 and 0.15. It must be recognized that application factors vary with test organisms and toxicants.

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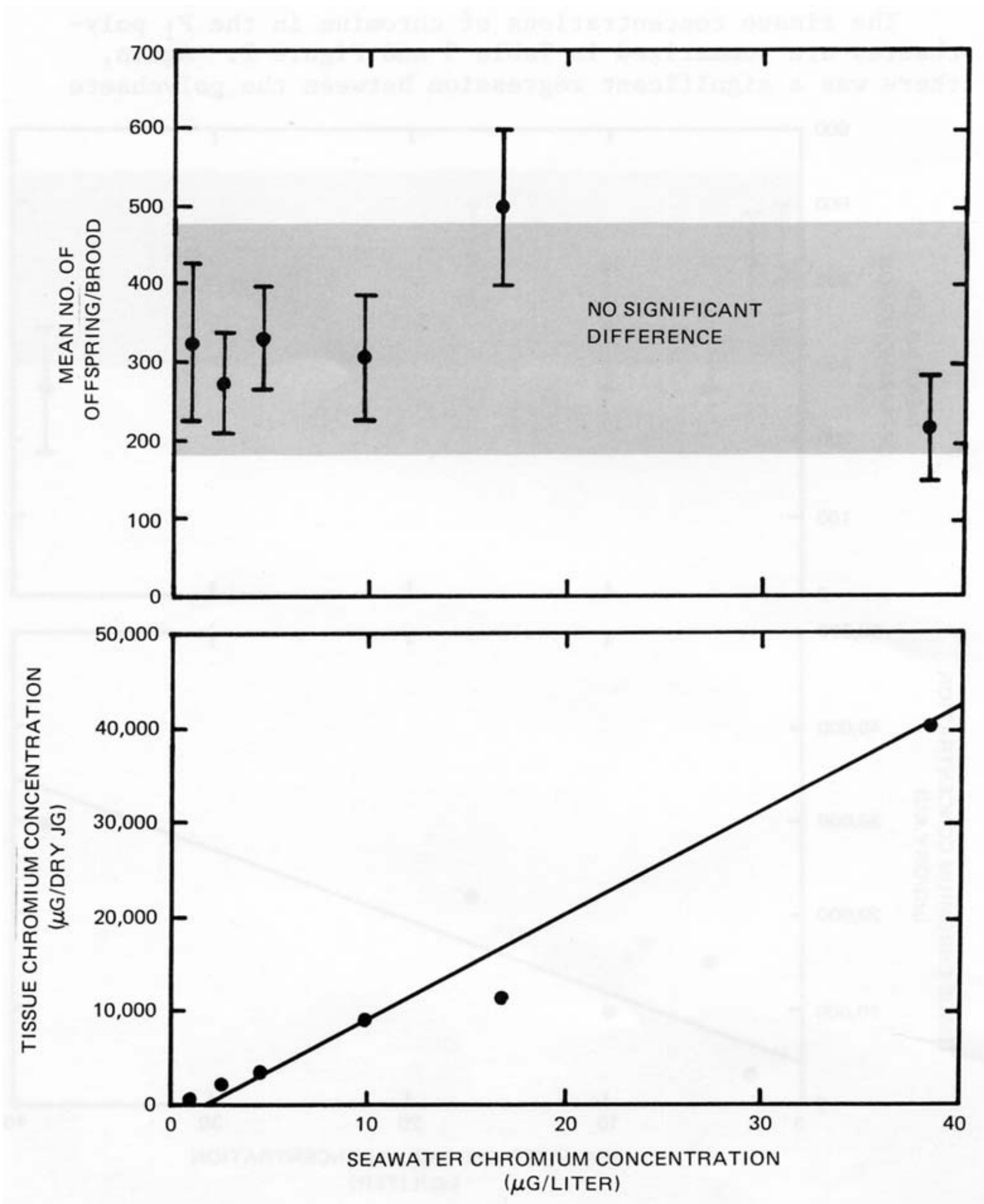

Figure 1. The relationship of polychaete tissue concentration, mean number of offspring per brood, and chromium exposure concentration for the parental generation of *Neanthes arenaceodentata*. Values are means ± 95 percent confidence limits.

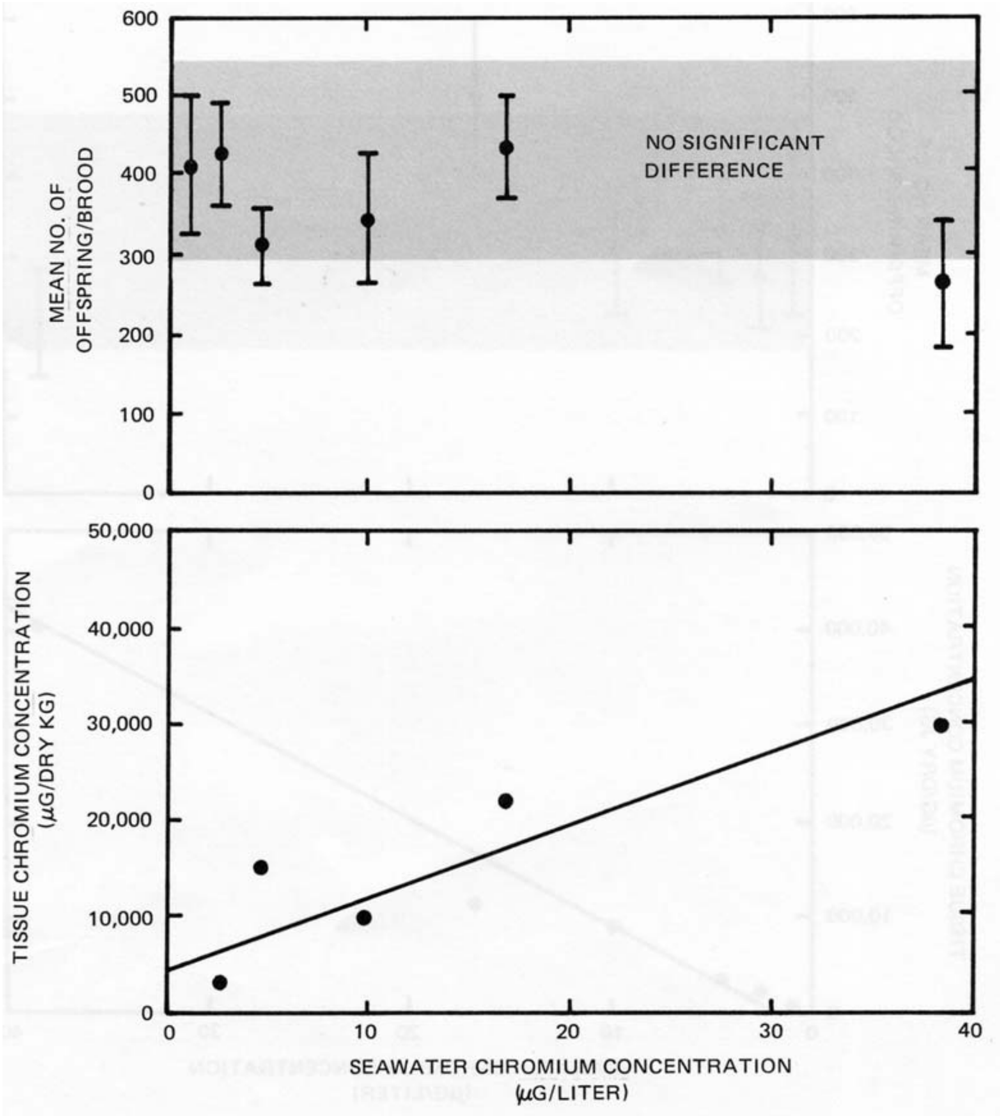

Figure 2. The relationship of polychaete tissue concentration, mean number of offspring per brood, and chromium exposure concentration for the F_1 generation of *Neanthes arenaceodentata*. Values are means ± 95 percent confidence limits.

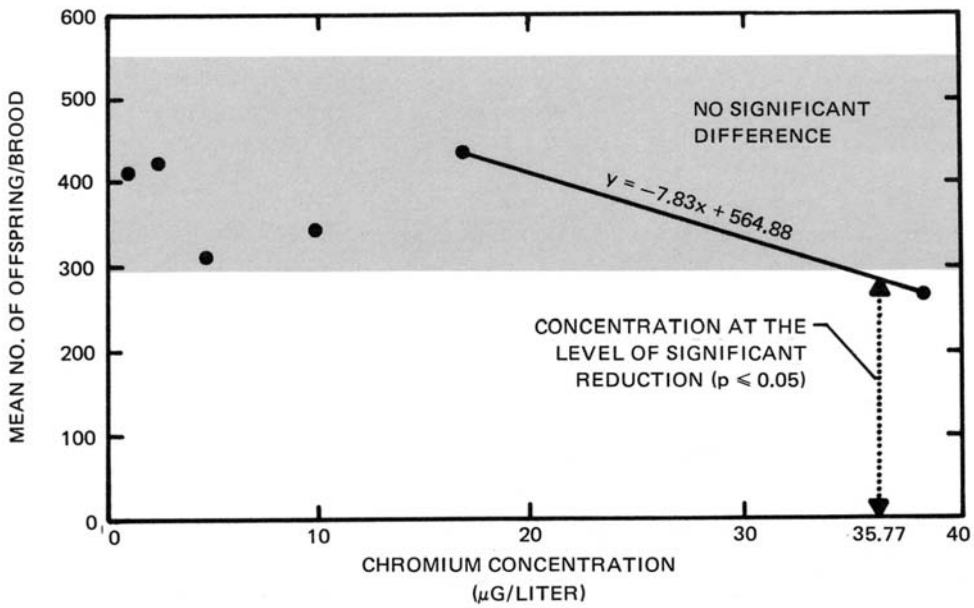

Figure 3. An example of the method used to determine the chromium concentrations at the level of significant reduction in brood size (given in Table 4). The example illustrated is for the F_1 generation brood size data.

Table 1. Design of 309-day experiment exposing marine polychaetes to hexavalent chromium.

Seawater	Natural seawater from Marineland of the Pacific.
Toxicant	Hexavalent chromium as potassium dichromate ($K_2Cr_2O_7$: analytical reagent grade) at concentrations of <1 μg/liter (control) and 2.6, 4.5, 9.8, 16.6, and 38.2 μg/liter.
Containers	76-liter, aerated aquaria; one aquarium, containing 50 liters of solution, per toxicant concentration.
Test Organism	*Neanthes arenaceodentata* (Moore) • Initial number per concentration: 90. • Initial size: ~1 cm in length, 30 to 40 setigers. • Source: Laboratory colony, originally from Los Angeles Harbor. • Food: Dried green algae, *Enteromorpha crinita.* • Number of pairs per concentration at Day 45: 19 to 29.
Polychaete Pair Cylinders	Glass cylinders 8.0 cm in length and 4.3 cm in diameter, covered on both ends with polyester netting with 0.5-mm-square mesh.
Water Quality Guidelines	• Temperature: 20 to 21° C. • pH: 7.9 to 8.2. • Ammonia: <0.5 mg/liter. • Salinity: 33.3 to 34.0 parts per thousand. • Dissolved oxygen: Constant aeration provided.

Table 2. Numbers of offspring per brood for two generations of *Neanthes arenaceodentata* exposed to hexavalent chromium.

Chromium Concentration (μg/liter)	No. of spawning pairs	Mean	Standard Error	95 Percent Confidence Limits[a]
Parental generation (P)				
Control	22	323.3	48.9	221.6–425.0
2.6	25	273.9	31.6	208.7–339.1
4.5	29	329.1	33.2	261.1–397.1
9.8	22	303.3	39.0	222.2–384.4
16.6	28	494.7[b]	48.4	395.4–594.0
38.2	22	213.4	32.8	145.2–281.6
First filial generation (F_1)				
Control	20	411.7	41.7	324.4–499.0
2.6	26	426.8	31.6	361.7–491.9
4.5	20	310.7	23.6	261.3–360.1
9.8	19	346.7	39.0	264.8–428.6
16.6	28	434.9	31.7	369.8–500.0
38.2	20	265.8[c]	39.0	184.2–347.4

a. Two-tailed test.

b. Using a two-tailed Dunnett's test, there was a significant ($p \leq 0.05$) increase when compared with the control mean of the same generation.

c. Using a two-tailed Dunnett's test, there was a significant ($p \leq 0.05$) decrease when compared with the control mean of the same generation.

Table 3. Body tissue concentrations of total chromium in two generations of *Neanthes arenaceodentata* that had been exposed to various levels of hexavalent chromium.

Exposure Concentration (μg/liter)	No. of Samples	Tissue Concentration (μg/dry kg) Mean	Standard Error
Parental generation (P)			
Control (<1.0)	3	400	152
2.6	3	2,000	58
4.5	3	3,470	203
9.8	2*	8,950	50
16.6	3	11,030	928
38.2	3	40,570	3,357
First filial generation (F_1)			
Control (<1.0)	3	<200**	–
2.6	3	3,370	133
4.5	3	15,030	1,017
9.8	3	9,830	694
16.6	3	22,200	1,290
38.2	3	30,300	1,186

*One sample was contaminated during processing.

**All three samples had chromium concentrations below the limit of detectability.

Table 4. The concentrations at the levels of significant reduction for five generations of *Neanthes arenaceodentata* that have been exposed to hexavalent chromium.

Study*	Generation	Level of significant reduction (μg/liter)
Present	P	41.0
Present	F_1	35.8
Oshida et al.	P	31.5
Oshida et al.	F_1	34.0
Oshida et al.	F_2	46.3

mean: 37.7 μg/liter

standard error: 2.64 μg/liter

n: 5

*Data on three generations were from Oshida et al. (1976); data on the remaining two were generated in this study.