Philip S. Oshida and Jean L. Wright

ACUTE RESPONSES OF MARINE INVERTEBRATES TO CHROMIUM

During the past year, we have been testing the toxicity of chromium against local marine invertebrates. We have used both static and flow-through seawater systems in our effort to determine relative sensitivities to chromium of a shrimp, a brittle star, and a sipunculid worm. Limited but informative experiments were conducted with hexavalent chromium as potassium dichromate ($K_2Cr_2O_7$) and trivalent chromium as chromic chloride (CrCl3).

HEXAVALENT CHROMIUM

The brittle star, *Ophiothrix spiculata*, is common to southern California waters and is often found in large aggregations on the sea bottom or on floating material, such as algae holdfasts. A characteristic of the local brittle star fauna is that, although it is found in many shallow subtidal areas along this sector of California, it is rarely present near municipal wastewater outfalls. The *0. spiculata* for this study were collected from shallow (less than 15 m) waters near Santa Catalina and Santa Cruz Islands.

The brittle stars (0.6 to 1.0 cm in disc diameter) have been maintained in laboratory aquaria at 20°C for 2 months. Three sides of each aquarium were covered with a polyethylene/ polypropylene mesh (0.8-sq-cm openings), which allowed the animals to climb off the bottom and hold onto the vertical surfaces. The stars were fed frozen brine shrimp.

In the hexavalent chromium experiment, ten brittle stars were placed in each of five seawater-filled gallon jars lined with mesh. The animals were allowed to acclimate for 24 hours in the aerated seawater prior to the addition of $K_2Cr_2O_7$. The Cr^{+6} concentrations were 3.75, 7.5, 15.0, and 30.0 mg/liter; a control (less than 0.001 mg Cr/liter) was also maintained. After 24 hours of exposure, brittle stars in all but the control solutions showed arm fragmentation and an inability to climb off the bottom. After 72 hours, 80 percent of the stars in the lowest concentration, 3.75 mg/liter, and 100 percent in the others had died, while the control animals showed total survival and no fragmentation. The animals were not fed during the experiment.

Unlike *Ophiotrix spiculata*, the penaeid shrimp, *Sicyonia ingentis*, shows an affinity for outfall areas. *S. ingentis* normally found at depths of about 180 meters in the southern California area. However, this species also tends to be found in shallower water (90 meters) in areas influenced by wastewater discharge. Our experimental shrimp (mean wet weight: 16.2 gm; mean carapace length: 3.0 cm) were taken in trawls from 91 meters near the Palos Verdes Peninsula. The shrimp were maintained for 2 weeks prior to experimentation in a 30-gallon laboratory container at 12°C and provided with aeration. In the experiment, a 20-gallon aquarium, connected to a flow-through seawater/toxicant pump was set up for each of the five concentrations (control; 15, 30, 45, and 60 mg Cr^{+6}/liter) . Seven shrimp were added to each aquarium and monitored for a week for abnormal behavior and mortality. The water flow was maintained at a constant rate so that the volumetric residence time was about 24 hours and the water temperature was a constant 12°C.

After 96 hours, none of the shrimp in 60 mg/liter were alive, one was alive in 45 mg/liter, 4 were alive in 30 mg/liter, and all were alive in 15 mg/liter and the control. This would indicate that the 96-hour 50 percent survival limit would be between 30 and 45 mg/liter. There was no evidence of abnormal behavior or molting during the experiment. At the conclusion of the 7-day study, no shrimp were alive in 45 or 60 mg/liter and only one was alive in 30 mg/liter. Six of the seven survived in 15 mg/liter and all of the control specimens were living. A 7-day 50 percent lethal concentration would be between 15 and 30 mg/liter.

In another experiment, with a similar setup but lower concentrations (control; 0.22, 0.67, 2.0, and 6.0 mg/liter) of hexavalent chromium, it took 18 days for 50 percent of the shrimp to die in 6.0 mg/liter. None of the other concentrations showed 50 percent mortality in 60 days. *Sicyonia ingentis* from this experiment will be analyzed for chromium uptake.

Sipunculid worms (*Themiste* sp., 8 to 10 cm in length), were collected under rocks in fine sand during low tide at Point Dume. The worms were in aggregations of 10 to 30 individuals, which made them easy to collect. They were maintained at 20°C in the laboratory for 2 weeks prior to experimentation. These animals adjusted well to laboratory conditions by burying in silica sand and feeding on dried green algae, *Enteromorpha crinita.*

Four hexavalent chromium concentrations (1.1, 3.3, 10.0, and 30.0 mg/liter) plus a control solution (less than 0.001 mg/liter) were used in the experiment. Each chromium solution was put into a 5-gallon aquarium that had 3 to 4 cm of silica sand on the bottom. The silica sand substratum allowed the animals to bury themselves. Four worms were added to each aquarium. It took 11 days, 19 days, and 52 days for two worms to die in each of 30, 10, and 3.3 mg/liter respectively. There was no mortality after 80 days in 1.1 mg/liter or the control.

TRIVALENT CHROMIUM

Two experiments were conducted using trivalent chromium (as CrCl3) as the potential toxicant. In one study, the brittle star, *Ophiothrix spiculata*, was the test animal; the other involved the spinuculid, *Themiste* sp. Each experiment was set up in .such a manner that half the specimens were exposed to sediments (silica sand or coarse gravel) spiked with trivalent chromium precipitate and half were exposed to uncontaminated sediment. The spiked sediment was produced by adding a given amount of $CrCl_3$ to a beaker of seawater and readjusting the pH to 7.9 or 8.0 using sodium hydroxide. The contents of the beaker were then added to the seawater in the experimental container, and the $Cr(OH)_3$ precipitate was allowed to settle onto the sediment. There were 850 milligrams of chromium per kilogram of sediment. Solutions in all these toxicity studies were provided with aeration.

In the sipunculid experiment, four 1-gallon jars were set up with spiked trivalent chromium sediment and four jars served as controls. Two animals were placed in each container. After 70 days, there were no adverse or abnormal effects generated by the presence of trivalent chromium. All animals were still alive, and their tentacular activity was easily observed.

The brittle star experiment involved two 5-gallon aquaria that contained spiked sediment and two that contained uncontaminated sediment. One aquarium from each group had the polyethylene/polypropylene mesh on three sides to allow the animals to live off the bottom. In the other aquarium, which did not have the mesh, the brittle stars were forced to live on the sediment. The two different approaches allowed us to observe and differentiate responses caused by dissolved or suspended chromium (animals above the bottom) and responses caused by the physical properties of chromium directly in contact with the brittle stars on the bottom.

After 2 weeks, there had been no adverse effects caused by the trivalent chromium in either of the aquaria. In fact, all the brittle stars were behaving normally. They ate frozen brine shrimp twice during the 2-week period. The experiment is continuing.

DISCUSSION

These experiments allowed us to determine the relative sensitivities of some marine invertebrates to chromium. At levels of 1 to 50 mg/liter, or about 7,000 to 333,000 times background concentration (0.00015 mg/liter), hexavalent chromium (as $K_2Cr_2O_7$) was lethal to marine animals in 4 to 7 days. The brittle star, *Ophiothrix spiculata*, which is characteristically absent from wastewater discharge areas, showed that it is more sensitive to hexavalent chromium than some species of fish (Sherwood 1975), shrimp and sipunculids. The first observable reaction of the brittle stars was the casting off, or autotomy, of portions of the arms, followed by death (the brittle stars did not survive long enough to regenerate their arms, if indeed they could

have). It appears that *0. spiculata* may be as sensitive to hexavalent chromium as the marine annelid, *Neanthes arenacedentata* (Oshida et al. 1976). The 96-hour 50 percent mortality concentrations for *N. arenaceodenta* and *0. spiculata* are 2.2 to 4.3 mg/liter and less than 3.75 mg/liter, respectively.

The shrimp, *Sicyonia ingentis*, was much more tolerant than either the annelid or brittle star, as it was able to survive for 60 days in a hexavalent chromium concentration of 2.0 mg/liter. The hard carapace may help protect the animal by inhibiting absorption of chromium into the body tissues.

The *Themiste* sp. appeared to prolong survival by breathing less often (accomplished by maximizing the time interval between branchial tentacle extensions). This response was noticed but not quantified. Worms in higher chromium concentrations did not seem to extend the branchial tentacles as often as did those in the control solution or lower concentrations.

In this experiment, and in previous work with *Neanthes arenaceodentata*, we have not observed any adverse effects attributable to trivalent chromium (as $Cr(OH)_3$ precipitate). It appears that this form of trivalent chromium is not harmful to marine invertebrates and is probably not biologically available.

Our studies indicate that adult *Sicyonia ingentis* and *Themiste* sp. appear to be insensitive to chromium at the low levels usually found in the ocean. However, *Ophiothrix spiculata* and *Neanthes arenaceodentata* are sensitive and, hence, may be effective bioassay animals for the study of chromium toxicity. Of the animals examined, the brittle star and polychaete were the most sensitive to chromium and revealed this sensitivity in anomalous behavior and reproduction. At the present time, we have an experiment in progress to test the effects of hexavalent chromium on *N. arenaceodenta* at levels (0.0025 to 0.040 mg/liter) near the California wastewater discharge standard.

We would like to thank Jack Word, Brad Myers, and Ed Motola of the Project for their suggestions in experimental design and assistance in animal identification and collection of specimens.

REFERENCES

Oshida, P.S., A.J. Mearns, D.J. Reish, and C.S. Word. 1976. The effects of hexavalent and trivalent chromium on *Neanthes arenaceodentata* (Polychaeta: Annelida). TM 225, Coastal Water Research Project, El Segundo, Calif.

Sherwood, M.J. 1975. Toxicity of chromium to fish. In 1975 Annual Report. Coastal Water Research Project, El Segundo, Calif., pp. 61-62.