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EFFECTS OF HEXAVALENT CHROMIUM ON SEA URCHIN EMBRYOS AND BRITTLE STARS

A goal of the Project's investigations into the chronic effects of exposure to chromium on marine animals has been to discover and use as test animals the species that are most sensitive to the hexavalent form of this chemical. Because it is not feasible to perform chronic toxicity tests on a large number of species, we have used species that proved to be most sensitive in acute toxicity tests. In last year's annual report, we gave the preliminary results of experiments involving several species of invertebrates common off southern California; at that time, a species of brittle star, *Ophiothrix spiculata*, appeared to be as acutely sensitive to hexavalent chromium as the polychaete, *Neanthes arenaceodentata*—the species that had been shown to be most sensitive in previous comparisons. Further work, completed in the past year and described here, has revealed that the two species have very similar acute sensitivities (as indicated by 7-day LC50 values) to hexavalent chromium.

Tests of the effects of hexavalent chromium on the development of sea urchin embryos are also described in this article. Many animals appear to be most sensitive to environmental contaminants (e.g., chemicals) during the embryological and juvenile stages of their life cycles. Our tests were designed to identify the levels of hexavalent chromium that interfere with the early embryological stages of development in the sea urchin, *Strongylocentrotus purpuratus*. The results indicate that there is a concentration between 2.9 and 29 mg hexavalent chromium/liter that causes a 50 percent reduction in the numbers of normally developing embryos. Other scientists testing the toxicity of chromium to oysters and mussels and other species of echinoderms have reported similar results.

BRITTLE STARS

Brittle stars were considered as suitable subjects for chromium toxicity tests for several reasons, including the fact that they are found in many shallow subtidal areas along the southern California coast but are rarely present at similar depths in areas of municipal wastewater discharge. The species selected, *Ophiothrix spiculata*, is common to southern California waters and is often found attached to subsurface drifting material such as algae holdfasts or

in large aggregations on the sea bottom. The *0. spiculata* used in this study were collected from shallow (9 to 11 meters) water near Santa Catalina Island and maintained in laboratory aquaria at 20°C for 2 months prior to being used in the experiment. During this 2-month period, the animals were fed live brine shrimp.

In the experiment, 5 brittle stars were placed in each of 20 seawater-filled 3.8-liter jars that were lined with a polyethylene/polypropylene mesh (0.8-sq-cm openings), which allowed them to climb off the bottom and cling to the vertical surfaces. The brittle stars were allowed to acclimate for 24 hours in aerated seawater prior to the addition of the hexavalent chromium. Five toxicant concentrations were tested (less than 0.01 (control), 1.05, 1.38, 1.67, and 2.10 mg/liter), and there were four replicates of each concentration.

The experiment lasted for 7 days, during which time the animals were not fed. The dissolved chromium concentrations were measured at the start and end of the experiment using the analysis described in Standard Methods (1976), and the brittle stars were checked daily for mortalities. The 7-day LC50 was calculated using the techniques outlined by Litchfield and Wilcoxon (1949).

There were no mortalities in the control jars, and the 7-day LC50 value was 1.7 mg/liter (the upper and lower 95 percent confidence limits were 1.95 and 1.48 mg/liter, respectively).

The 7-day LC50 value for *0. spiculata* is almost an order of magnitude lower than those obtained for two other animals (the sipunculid, *Themiste* sp., and the shrimp, *Sicyonia. ingentis*) in previous experiments, but it is very similar to the values obtained for *Neanthes arenaceodentata* (1.44 to 1.89 mg/liter), indicating that the two species have similar acute sensitivities to chromium. Until this test, *N. arenaceodentata* had been the most sensitive of the animals used at the Project in acute tests. It would be useful to know if *0. spiculata* and *N. arenaceodentata* also exhibit similar chronic symptoms when exposed to low concentrations of the chemical—at this time, there is little evidence to support or deny the hypothesis that animals with similar acute responses to a toxicant also exhibit similar chronic effects at like toxicant levels. Until further testing is conducted with *0. spiculata*, *N. arena-ceodentata* remains the marine animal whose responses to chromium are best known to us as well as the most acutely sensitive to hexavalent chromium of the animals we have tested.

SEA URCHIN EMBRYOS

Within the last year, we have used sea urchin embryos in tests of the toxicity of several substances; other investigators have also utilized the synchronously dividing sea urchin eggs and embryos as test organisms in a variety of toxicity tests (Hagstrom and Lonning 1973; Kobayashi 1971). In the experiment described here, we focused on the effects of hexavalent chromium on survival and development of the fertilized egg through the early pluteus larva stage. At 17°C, development to the pluteus stage occurs within 48 hours for our experimental species, *Strongylocentrotus purpuratus*, which makes for a short but valuable toxicity test.

The gametes used in our toxicity test were spawned from adult urchins collected subtidally near Flatrock on the Palos Verdes Peninsula. The adult urchins were brought to the laboratory and maintained at 12 C in a flow-through seawater system for 4 days before they were induced to spawn by injection of 0.5 to 1 milliliter of 0.5-molar potassium chloride. The gametes were collected and fertilized in a manner similar to that described by Hinegardner (1967). The eggs were fertilized at one time in the same beaker. After fertilization, the eggs were aliquoted into four nearly equal portions, each of which was then transferred to one of the four hexavalent chromium toxicant solutions (less than 0.01 (control), 0.3, 2.9 and 29 mg/liter). The eggs were then aerated and agitated at 17°C for the next 48 hours. Samples of the developing eggs were taken at 2, 4, 19, 22, 24, 26, 28, 46, and 48 hours past fertilization. Immediately after sampling, these eggs and embryos were preserved in 2 percent buffered formalin; later, over 300 eggs from each sample were examined. At the same time that these samples were taken, the densities of the eggs in each of the solutions were measured by taking four to six 20-microliter samples and counting the number of eggs in each sample. The density measurements were to account for egg mortality and decomposition, which might have been overlooked in the routine monitoring of morphological development.

In the routine checking of the experiment, the dissolved hexavalent chromium levels were measured by the method described in Standard Methods (1976).

The initial density measurements revealed that there were about 1.7 to 1.9 million eggs per liter in each solution; there was no evidence of density changes during the experiment. Examination of the developing eggs and embryos revealed that over 95 percent of the embryos in the 29 mg/liter solution had stopped developing and were inactive by the nineteenth hour past fertilization. The embryos in the other two toxicant solutions were behaving similarly to those in the control. At 48 hours, only the embryos in the 29 mg/liter concentrations had shown an adverse response to the chromium. The control, 0.3, and 2.9 mg/liter solutions contained 94.5, 94.2, and 92.4 percent pluteus larva, respectively, after 48 hours, and the intermediate samplings (between 22 and 28 hours past fertilization) showed that development was nearly synchronous in these solutions. Thus, the level of hexavalent

chromium that causes a 50 percent reduction in normally developing embryos within 48 hours of fertilization appears to be between 2.9 and 29 mg/liter.

This result is similar to that reported by Okubo and Okubo (1965), who found that embryos of the sea urchin *Anthocidoris crassispina* were affected by hexavalent chromium at a concentration between 3.2 and 10 mg/liter. In that same study, the same levels of hexavalent chromium were also shown to affect the development of embryos of the bay mussel (*Mytilus edulis*). It appears that sea urchin and bivalve embryos have similar sensitivities to hexavalent chromium when they are in their pre-pluteus and pre-D-cell larval stages, respectively. Other evidence of this similarity has been reported by Osborn et al.,* who found that 4.3 mg/liter of hexavalent chromium in seawater causes a 50 percent decrease in the number of embryos of the oyster, *Crassostrea gigas*, that reach the D-cell larval stage in 48 hours.

These 48-hour toxicity tests on embryos and eggs are valuable because they provide a quick and easy method for testing acute toxicity. However, in this experiment, only the sea urchin embryos exposed to the highest hexavalent chromium concentration exhibited an adverse reaction. This was somewhat of a surprise, as the eggs undergo a critical process—invagination—during the initial 48-hours past fertilization, and we thought that, at this stage, they might easily be affected by abnormally high levels of chromium. In the future, it would be useful to expose adult sea urchins to low levels of hexavalent chromium and monitor their reproductive potential and the morphological development of any offspring. Such an experiment might reveal chronic effects caused by the low-level exposure to hexavalent chromium.

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