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# Acute Toxicity of Sodium Molybdate Dihydrate (Molyhibit 100) to Selected Saltwater Organisms

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Sodium molybdate dihydrate ($NaMoO_4 \cdot 2H_2O$), manufactured by Amax Inc. as Molyhibit 100, is used in the manufacture of molybdate-chrome-orange pigments and numerous molybdophosphoric acid organic pigments. It is also used as a condensation catalyst for phthalocyanine pigments, as an accelerator in phosphate conversion coating baths, as a soil additive for plant growth, development, and nitrogen fixation. Additionally sodium molybdate has numerous applications as a broad base corrosion inhibitor (Vukasovich 1987, personal communication).

The recent development of various applications has introduced the possibility of exposing estuarine and marine organisms to concentrations of molybdenum (Mo) that would not naturally be found in these environments. Although some data can be found on the toxicity of Mo to a number of freshwater and terrestrial organisms (Birge 1978, Goettl and Davies 1977), the effects on a limited number of saltwater species has been reported by even fewer investigators (Abbott 1977; Mitchell et al. 1985; Anderson 1986).

Results of acute toxicity of Molyhibit 100 as molybdenum to the pink shrimp, mysid shrimp, sheepshead minnow, and the American oyster are reported here in an effort to enhance the data base.

## MATERIALS AND METHODS

The pink shrimp, Penaeus duorarum, were obtained from Biotec Inc., Pensacola, Florida, 13 days prior to the initial testing. The shrimp weighed an average of 1.5 g. The salinity was adjusted over a 3-day period to 25 ppt and held for 10 days prior to testing. The acclimation temperature was $20^{\circ}C \pm 2^{\circ}C$. During the acclimation period the mortality was less than 6%. The Pennaeus were not fed during the 96-h testing period.

The mysid shrimp, Mysidopsis bahia, were cultured at a local commercial supplier and received at the laboratory 48 h prior to initial testing. The mysids were maintained in accordance with procedures in US Environmental Protection Agency (1978). The

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salinity and temperature were 27 ppt and 20°C±2°C respectively. During this period no mortality was observed. The Mysidopsis were fed brine shrimp during the 96-h test period.

The sheepshead minnows, Cyprinodon variegatus, were obtained from a local commercial supplier 12 days prior to initial testing. The fish averaged 15 mm in length and 0.4 g. The salinity was adjusted to 25 ppt over a 2-day period and held for 10 days prior to testing. The acclimation temperature was 20°C±2°C. During this period the mortality was less than 5 percent. The Cyprinodon were not fed during the 96-h test period.

The American oysters, Crassostrea virginica, were obtained from local waters and received at the laboratory 12 days prior to the start of initial testing. During acclimation they were maintained at a salinity of 20 ppt, a temperature of 20°C±2°C and fed a corn starch-nutrient solution twice a day (25 g of cornstarch dissolved in 100 mL of filtered test water). The holding water was changed every other day. The oysters were prepared for the bioassay immediately before initiation of the test by grinding the periphery of the shell to remove approximately 3-5 mm, leaving a smooth blunt edge. During the 96-h test period, the Crassostrea were fed identically as in the acclimation period.

The test water for the pink shrimp, sheepshead minnow, American oyster was artificial sea water prepared by dissolving ocean salts (Instant Ocean) in deionized water. The test water for the mysids was natural sea water filtered through 0.5 um.

The test material, sodium molybdate dihydrate (Molyhibit 100) was supplied by Climax Molybdenum Division, Amax, Inc. It was a white granular solid. Appropriate amounts of material from lots #4049K were dissolved in sea water of the same salinity as the acclimation water to produce the test solution for the pink shrimp and the mysid shrimp. Material from lot #4016L was used for the preparation of test solutions for the American oyster and the sheepshead minnows.

The test chambers for the pink shrimp and sheepshead minnows were all glass silicon sealed aquaria containing 17 L and 18 L respectively.

The test chambers for the mysid shrimp were 1-L wide-mouth glass bottles. The test chambers for the American oyster were glass aquaria 50 cm long, 30 cm wide, and 15.5 cm high. One end of each aquarium had a U-shaped cut in the top of the glass wall reaching to a depth of 12 cm above the tank bottom. The cut acted as a weir, allowing the test solution to overflow into a reservoir, where it was pumped up into the opposite end of the test chamber. This allowed for continual current to be maintained over the test organisms. A grid was displayed to allow for equal distribution of the oysters and identification of individuals. All of the test chambers were placed in a climate controlled room prior to and during the toxicity test.

The test solution concentrations were selected by evaluation of results from a 48-h range finding test. Test solutions were prepared approximately 30 min prior to the initiation of the test by dissolving the appropriate amount of Molyhibit 100 into the respective sea waters. The actual concentration of molybdenum after the initial preparation was determined by atomic absorption spectroscopy and subsequently adjusted to the desired concentration.

Table 1. Test setup and conditions for exposure of various saltwater species to molybdenum, $20^{o}C \pm 2^{o}C$.

| | S | V | #/Ch | #/C | g/L | Exposure Range mg/L[1] |
|---|---|---|---|---|---|---|
| Penaeus duorarum | 25 | 17 | 10 | 20 | .88 | 500-3000 |
| Mysidopsis bahia | 27 | 1 | 10 | 20 | <.25 | 500-4000 |
| Cyprinodon variegatus | 25 | 18 | 10 | 20 | .22 | 500-4000 |
| Crassostrea virginica | 20 | 22.7 | 20 | 20 | ND | 500-4000 |

[1]Concentration given in mg molybdenum (Mo)/L of test water.
ND = Not Determined.
S = Salinity of test solution in ppt (parts per thousand).
V - Volume of test chambers in liters.
#/Ch = number of organisms per test chamber.
#/C = number of organisms per test concentration.
g/L = loading given in g/L of organism in test water.

Each of the 96-h static toxicity tests were set up as presented in Table 1. The pH and dissolved oxygen (DO) were determined at 24-h intervals beginning with the initiation of the test. The pH remained constant in all test chambers throughout the 96 h. The DO in the Cyprinodon and Pennaeus test chambers began to decline after 24 h. Slow bubble filtered air was used in these chambers in order to maintain the DO at a level above 60% saturation. The test solutions in the Crassostrea setup were completely exchanged with freshly prepared test solutions every 24 h.

Based on the mortality results of the Cyprinodon, Mysidopsis and Penaeus tests, 96-h LC50s and 95% confidence limits were

calculated using the computer program of Stephan (1977). The following statistical methods were used: binomial probability, moving average, and probit.

The oysters were removed from the test chambers at 24-h intervals and new shell growth of each oyster was measured to the nearest .0025 cm with a vernier caliper. The effect of exposure of the oysters to molybdenum was determined by comparing shell deposition of the control organisms to that of the exposed organisms. The percent reduction in growth for each exposure concentration was calculated using Equation 1:

$$\% \text{ Reduction} = \frac{\left|\text{Mean shell growth of exposed oysters} - \text{mean shell growth of control oysters}\right|}{\text{mean shell growth of control oysters}} \times 100$$

The EC50 for the oyster study was calculated by graphic and linear regression techniques. The EC50 that was determined is the concentration at which a population would be expected to exhibit an average of 50% reduction in shell deposition.

## RESULTS AND DISCUSSION

Shortly after initiation of the tests, a noticeable white precipitate began to form in all test chambers with concentrations of molybdenum above 1500 mg/L. Atomic absorption analysis throughout the test period verified that the concentration of molybdenum was not altered by more than 5% by this event. Although the precipitate did appear to physically stress the organisms by interfering with their respiration, supplemental testing with filtered test solution provided congruent test results.

Due to the nature of this data and the requirements of the probit program, the "goodness of fit probability" was not acceptable for the use of this method. The results calculated by the moving average and the binomial probability are presented in Table 2.

Table 2. Calculated 96-h LC-50 values for various saltwater species exposed to Molyhibit 100 as mg/L Mo

| Species | Statistical Method (95% confidence limits) | |
|---|---|---|
| | Binomial Probability | Moving Average |
| *Penaeus duorarum* | 1909 (1500-2500) | 1849 (1677-2071) |
| *Mysidopsis bahia* | 1205 (1000-2000) | 1045 ( 857-1230) |
| *Cyprinodon variegatus* | 3057 (2000-4000) | 2587 (2268-3022) |

The results of the shell deposition study with Crassostrea virginica are presented in Table 3. The 96-h EC50 values calculated for the oysters exposed to Molyhibit 100 by the graphic and linear regression methods are 1375 mg/L Mo and 1849 mg/L Mo respectively.

Table 3. Calculated percent reduction in mean shell deposition of Crassostrea virginica exposed to molybdenum

| Concentration Mo (mg/L) | # of Live Organisms 96 h | Mean Shell Deposition (cm) | Percent Reduction* |
|---|---|---|---|
| 0 | 20 | .127 ± .076 | -- |
| 500 | 19 | .104 ± .102 | 18 |
| 1000 | 20 | .079 ± .051 | 38 |
| 2000 | 20 | .071 ± .025 | 44 |
| 3000 | 19 | .025 ± .025 | 80 |
| 4000 | 14 | .003 ± .005 | 98 |

*See Equation 1

Some of the literature available attempts to discuss the toxicity of molybdenum based on the assay of complex matrices such as mine tailings (Mitchell et al. 1985; Anderson et al. 1986). Comparison of these papers' findings to the data presented here is of no relevance since many constituents are found in the leachate at concentrations known to have toxic effects on the test organisms.

However, where chemical analyses of the leachates are performed to determine the constituents that are present and these constituents are then selected for individual testing such as reported by Birge (1978) for coal flyash, some comparison data may be produced.

Birge (1978) tested 22 elements that were found to be present in the coal flyash leachate for toxicity to various developmental stages of trout, goldfish and toads and reported the LC50s for Mo to be 0.73, 60.0, 0.96 ppm respectively. Although these values appear to be somewhat low, Birge found only two (Mn, W) of the 22 elements to be less toxic than Mo to the most sensitive test organism.

In extensive studies with trout involving long term egg, dietary and environmental exposure Goettl and Davies (1976, 1977) reported "no effect" concentrations of 18.46 mg/L, 1000 ug/g and LC50 of 1320 mg/L respectively. Other researchers (Easterday and Miller 1963) have reported LC50s for the freshwater bluegill of 1320 mg/L Mo. The results of this study (LC50 of 2587 mg/L Mo) indicates that the saltwater fish Cyprinodon variegatus is more tolerant of high Mo concentration than the freshwater species reported previously.

A 48-h LC50 of 1018 mg/L Mo was reported by Abbott (1977) for the marine shore crab Carcinus maenas. No other significant data could be found for saltwater species.

The toxicity exhibited by Molyhibit 100 in this study indicates that it is of minor concern with respect to the impact on the marine environment. On an environmental basis, molybdenum corrosion inhibitors could offer a favorable alternative to the other heavy metal compounds presently in use.

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