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THE ACUTE, AQUATIC TOXICITY OF SELECTED MINERAL PARTICLES

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Graphite fibers, nickel-graphite fibers, polycrystalline iron whiskers, and graphite Micro-260 (M-260) were tested to determine their toxicities to Daphnia magna. The graphite fibers and nickel-graphite fibers were ground with a mortar and pestle before testing. The M-260 had an EC_{50} of 80.6 mg/L for four pooled replicates. None of the other compounds caused any deaths at concentrations up to 100 mg/L. All the bioassays were 48-hr acute studies. A 96-hr growth inhibition assay using Ankistrodesmus falcatus was performed with the graphite. No inhibition of A. falcatus was observed; however, the growth of the alga was enhanced by the two lowest test concentrations.

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PREFACE

The work described in this report was authorized under Project No. 1L161611A552, Smoke/Obscurant Munitions. The work began in September 1985 and was completed in October 1986.

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THE ACUTE, AQUATIC TOXICITY OF SELECTED MINERAL PARTICLES

1. INTRODUCTION

Several mineral particles have been tested to determine their acute, aquatic toxicities. The tested particles were graphite fibers, polycrystalline iron whiskers, nickel-graphite fibers, and synthetic graphite Micro-260 (M-260). Each compound was tested against the cladoceran, Daphnia magna (D. Magna); in addition, particles derived from the graphite fibers were tested using the algal species, Ankistrodesmus falcatus (A. falcatus).

2. METHODS AND MATERIALS

The graphite and nickel-graphite fibers were obtained from the American Cyanamid Company (Wayne, NJ). Polycrystalline iron whiskers were supplied by HDS Fibers, Incorporated (Charlottesville, VA). Graphite M-260 was supplied by The Asbury Graphite Mills, Incorporated (Asbury, NJ).

Because none of the compounds was soluble in water, mechanical methods were used to make uniform suspensions. The graphite and nickel-graphite fibers were ground to a fine powder consistency with a mortar and pestle and placed in a small polycarbonate tube. Diluent was added to the tube, and a suspension was created by placing the capped tube in an ultrasonic water bath. The polycrystalline iron whiskers were not ground but simply placed in a small volumetric flask with diluent. The flask was then immersed in an ultrasonic water bath until the fibers were separated. The mixture was swirled to create a suspension. Because it was already a powdery compound, the M-260 was added to diluent in a polycarbonate tube and placed in the ultrasonic water bath. The studies were conducted with serial dilutions of the stock suspensions. All the tests performed conformed to the current guidelines of the American Society of Testing and Materials.[1]

First instar D. magna, reared from at least third-generation adults, were cultured as described by Goulden et al.[2] Ten neonates were placed in each 250-mL glass beaker containing 100 mL of sample. Two replicates of each concentration were used in every test performed. The diluent was public drinking water that had been passed through particulate filters, aged for at least 48 hr, autoclaved, aerated, and hardened[3] with vitamins added.[4] A 20 $\pm$ 1 ^{o}C temperature and 16:8 hr light-dark cycle

were employed throughout testing. Water hardness of the diluent was in the range of 125-140 ppm calcium carbonate.

Algal growth inhibition tests were performed to determine the IC_{50} (concentration that inhibits growth 50% as compared to control) of the graphite particles using the microalga, A. falcatus. The alga was obtained from Carolina Biological Supply Company (Burlington, NC) and maintained on 1% Difco-Bacto agar slants. Cells were transferred via sterile inoculation to a semiflow-through apparatus containing T83MV culture medium with vitamins added.[4] Test chambers were 500-mL Ehrlenmeyer flasks with ground glass stoppers. Serial dilutions of the suspended graphite fibers (prepared in T83MV medium by the technique described above) were added to the Ehrlenmeyer flasks along with the test organism at a concentration of approximately 3.0×10^4 cells/mL for a total volume of 100 mL. Three replicates of controls and the varying test concentrations were used. Test suspensions were incubated at 20.0 ± 1.0 °C with a 12:12 light/dark cycle. The concentrations tested were 0.01, 0.1, 10.0, and 100.0 mg/L.

3. RESULTS

EC_{50} was not calculated for the synthetic graphite, nickel-graphite, or the polycrystalline iron whiskers because no deaths had occurred at any concentration tested. The highest concentration tested for the graphite and nickel-graphite formulations was 100 mg/L, while the highest concentration of iron whiskers was 250 mg/L. The four replicate tests of the M-260 graphite yielded a pooled EC_{50} of 80.6 mg/L. The results of the separate replicates and pooled value, as determined by a probit analysis program [Communication, with C.E. Stephan, U.S. Environmental Protection Agency (USEPA), Duluth, MN] are shown in the Table.

The growth of A. falcatus was not inhibited by any of the concentrations of the graphite tested. The analysis of variance, assuming equal variances, did show a significant ($p < 0.05$) enhancement of growth at test concentrations of 0.01 and 0.1 mg/L (Figure).

Table. EC_{50} Calculations for D. magna Exposed to Graphite Micro-260.

Replicate	EC_{50} (mg/L)
1	90.8 (46.9-271.2)
2	105.3 (0-infinity)
3	61.4 (35.3-105.9)
4	85.2 (54.1-151.1)
All pooled	80.6

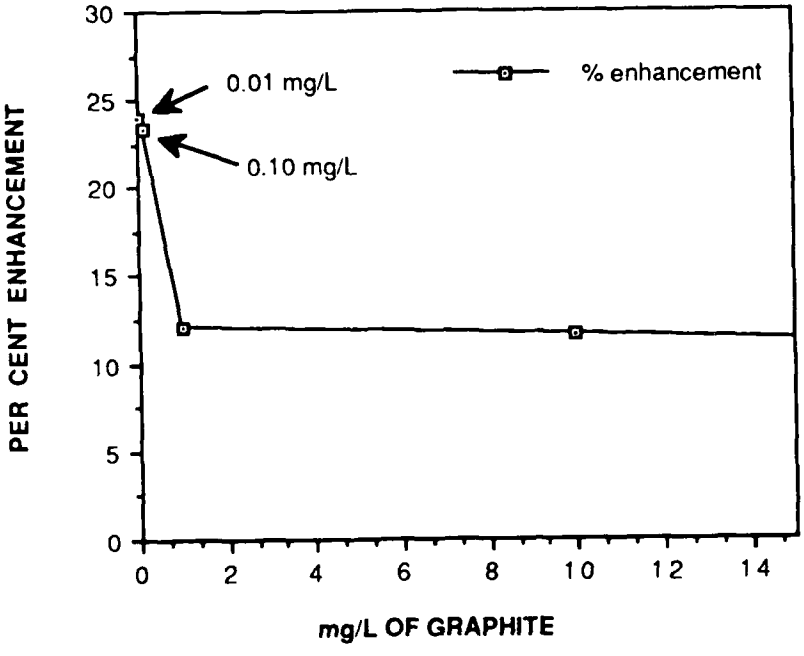

Figure. Enhancement of A. falcatus by the Addition of Graphite.

4. DISCUSSION

Because graphite is primarily carbon, a high toxicity was not anticipated. Mechanical damage from possible ingestion of the particles was not expected to result in significant toxicity based on prior experimentation with similarly prepared suspensions of silica and titanium dioxide.[5] Also, the particle size obtained by the grinding process was not small enough to allow the particles to remain in suspension for more than a few minutes. M-260 did remain in suspension for a longer time. This increased availability of the test material for the daphnids may have contributed to its higher toxicity. Another factor could have been the presence of contaminants such as iron in the M-260. Iron was found in the compound when a proton-induced x-ray emission (PIXE) analysis was performed (Communication, G.M. Thomson, U.S. Army Ballistic Research Laboratory, Aberdeen Proving Ground, MD). No comparisons can be made because no PIXE analysis was performed on any of the other compounds.

The enhancement of the growth of A. falcatus at low concentrations may indicate that the compound is providing additional nutrients. This suggests that the graphite could contribute to eutrophication of a water system when available at very low concentrations. This conclusion was supported by data obtained from a Standardized Aquatic Microcosm experiment using the M-260 and conducted after these acute studies.[6]

Comparisons of the toxicities of these compounds and the brass particles previously tested revealed that all the formulations tested in this study were much less toxic than the brass.[5,7] The brass exhibited EC_{50} values of 17.7-30.9 µg/L when tested with daphnids, three orders of magnitude greater than the M-260. Similarly, brass was also toxic to A. falcatus (IC_{50} = 0.316 mg/L), whereas the graphite displayed no toxicity at concentrations up to 100.0 mg/L.

A previously tested aluminum compound caused no growth inhibition of A. falcatus at concentrations up to 100.0 mg/L. The toxicity to daphnids was not as well-defined but was within the same order of magnitude as the M-260 [Communication, M.V. Haley, U.S. Army Chemical Research, Development and Engineering Center (CRDEC), Aberdeen Proving Ground, MD].

5. CONCLUSION

Particles derived from the graphite and nickle-graphite fibers, as well as the polycrystalline iron whiskers, showed no acute toxicity to D. magna at concentrations below 100.0 mg/L. The graphite fiber particles did not inhibit growth of the algal species tested. These compounds would be assigned a score of 1 to 3 according to a draft scoring criteria for aquatic toxicity published by USEPA.[8] The scoring is on a scale from 0 to 9 with a score of 9 being most toxic. A score between 4 and 5 would be assigned to the M-260 based on the daphnid data. In comparison, the brass discussed above would be assigned a score of 9 for both daphnids and algae. The data suggests that use of the compounds tested under this project would pose much less risk to the environment than brass when used with appropriate concern and control.

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