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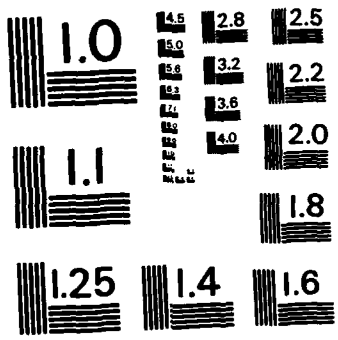

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The aquatic toxicity of a brass particulate was examined. Acute, 48-hour bioassays were performed using the water flea, Daphnia magna. Tests were conducted with uniform suspensions of uncoated brass particulate, brass particulate coated with a Teflon solution, silica, and titanium dioxide. The Teflon coating solution and the supernatant of the brass suspension (after settling of the brass) also were tested. All tests were conducted according to guidelines set forth by the US Environmental Protection Agency (EPA) and the Organization for Economic Corporation & Development Cooperation (OECD). The effective concentrations that would be lethal to 50% of a population were calculated for uncoated (20.9 ug/l) and coated (23.6 ug/l) brass particulate. The silica, titanium dioxide, and Teflon each had an EC50 of >1 gm/l. Chemical fate studies demonstrated that the brass dissociated to its ionic components of copper and zinc quickly at pH 2.0. At pH 5.0 and 6.5, the dissociation occurred too slowly to hypothesize that the observed toxicity was due to the presence of copper ions.

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19. ABSTRACT (Continued)

The data suggested that the toxicity is due to filtration by the daphnids and subsequent ingestion. EC50 determinations for the brass are nearly identical with published EC50 values for copper salts.

PREFACE

The work described in this report was authorized under Project No. 1L161611A552, Smoke/Obscurant Munitions. This work was started in September 1983 and completed in May 1984.

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TOXICITY OF BRASS PARTICULATE TO *DAPHNIA MAGNA*

1. INTRODUCTION

An enormous body of literature exists on the toxicity of heavy metals to aquatic species. Usually these heavy metals are introduced in experiments as salts. By employing this method of dosing, no consideration is made for the many instances that metals are introduced to the environment as filings, tailings, or powder. Rates of ionization, transport, sedimentation, and ingestion by filter-feeding organisms can all affect the toxicity expressed by the metal.

This study was undertaken to determine the aquatic toxicity of brass particulate and to ascertain whether the observed toxicity was simply a result of the dissociation of the brass into copper and zinc. Acute bioassays using *Daphnia magna*, microscopic examination of exposed daphnids, and investigations of the chemical fate of brass particulate were employed to gather the data.

This study is one of a series investigating the toxicity of brass particulates to aquatic organisms. Other studies include 96-hour algal growth-inhibition assays, daphnid chronic bioassays, 96-hour fish toxicity tests, and ecosystem-level experiments employing gnotobiotic microcosms.

2. METHODS AND MATERIALS

Acute 48-hour bioassays were performed on both coated and uncoated brass powder, the Teflon solution used to coat the brass, and supernatant obtained from a suspension of the particulate. The composition and specifications of the brass particulate are detailed in Table 1. Bioassays were also performed on two other compounds of similar particle size. A silica particulate, Min-U-Sil, was obtained from Pennsylvania Glass Sand Corporation, Pittsburgh, PA. The size distribution was approximately: 1 μ, 21%; 1 to 2 μ, 35%; 2 to 3 μ, 30%; 3 to 4 μ, 11%; 4 to 5 μ, 3%.[1] Titanox 1000, a titanium dioxide particulate with a particle size of 5 to 6 μ, was generously supplied by NL Chemicals, Hightstown, NY.

First instar *Daphnia magna*, reared from at least third-generation post acclimation adults, were used as the experimental animals. The culture techniques used were 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 for each test. All bioassays conformed to the current guidelines of the Organization for Economic Cooperation and Development (OECD)[3] and US Environmental Protection Agency (USEPA).[4] Public drinking water that had been passed through particulate filters, activated charcoal filters, and aged a minimum of 48 hours in a 200-gallon polyethylene holding tank was used as the diluent. A 20° ± 1°C temperature and 16:8 hour light-dark cycle were employed throughout the bioassay regime. Additionally, water hardness between 50 to 65 ppm calcium carbonate and a pH range of 6.8 to 7.0 were observed during testing.

Table 1. Physiochemical Properties of Brass Particulate*

Size (Avg diameter)	Range of thickness	Composition	
			%
1.72 μ	800-3200 Å	Cu	68.5
		Zn	27.5
		Al	0.2
		Pb	0.1
		Sb	0.1
		Palmitic acid	0.5
		Stearic acid	0.7

*Sandra Thomson, personal communication

Since neither the coated nor uncoated brass was soluble in water, an initial 1 mg/ml uniform suspension was prepared in a 16- x 152-mm polycarbonate tube with the aid of an ultrasonic water bath. Using this suspension, a stock suspension was prepared and diluted to the required test concentrations. Bioassays were performed with stock suspensions made from newly prepared initial suspensions and initial suspensions that were 24 hours old. Bioassays were performed to assess the toxicity of the water phase of the suspension by preparing a 1 mg/ml suspension as described above, allowing it to settle for 24 hours, and withdrawing an aliquot from the water phase to make a stock solution corresponding to that prepared from the original suspensions. Toxicological evaluations were performed on serial dilutions of the liquid Teflon which had a density of 1.1606 gm/l. The titanium dioxide and silica were prapared as uniform suspensions for testing.

First instar D. magna that had been swimming in a 1-mg/ml suspension of the brass for 1 hour were examined microscopically, and a photographic record was made.

Studies of the chemical fate were performed to determine the rate of dissociation of the brass to its ionic components. Aliquots of uncoated brass particulate were added to 200-ml measures of distilled water contained in 250-ml polycarbonate flasks. The flasks were adjusted to pH values of 2.0, 5.0, 6.5, and 8.5 with nitric acid and ammonium hydroxide before the addition of the brass.

and zinc (Zn^{++}) on days 1, 5, 16, and 19 post exposure with a Perkin-Elmer 460 Atomic Absorption Spectrophotometer.

3. RESULTS

Mean EC50 values,* as determined by Bliss probit analysis,[5] for suspensions of the uncoated and coated brass to *D. magna* are listed in Table 2. The values of all bioassays conducted ranged between 10 µg/l and 52 µg/l. Representative EC50 determinations for the five tests performed on fresh brass stock are displayed graphically in Figure 1. Table 2 also lists the EC50s determined for the other materials tested. The Teflon solution, silica particulate, and titanium dioxide particulate all had EC50s above 1 gm/l. The supernatant of a 250-µg/l uncoated brass suspension was not acutely toxic after 48 hours.

Table 2. The Mean EC50 of Brass, Silica, and Titanium Dioxide Suspensions and Liquid Teflon in *D. magna*

Material	Mean EC50 (µg/l)	
Uncoated brass, fresh stock	20.9	(16.4 - 26.6)*
Uncoated brass, 24-hr old stock	17.7	(14.9 - 21.0)
Coated brass, fresh stock	23.6	(20.4 - 27.3)
Coated brass, 24-hr old stock	30.9	(26.0 - 36.7)
Brass, all data, pooled	24.1	(21.9 - 26.5)
Silica	>1,000,000	
Titanium dioxide	>1,000,000	
Teflon	>1,000,000	

*95% confidence limits from probit analysis

Microscopic examination of daphnids, following exposure in the suspension, showed the organisms had no aversion to filtering the brass particles, forming a bolus and passing it to the gut. The brass in the intestine was obvious when examining the organisms under 20x magnification. Color photographs clearly showed brass in the digestive tract.

*EC50 = effective concentration that is lethal to 50% of a population.

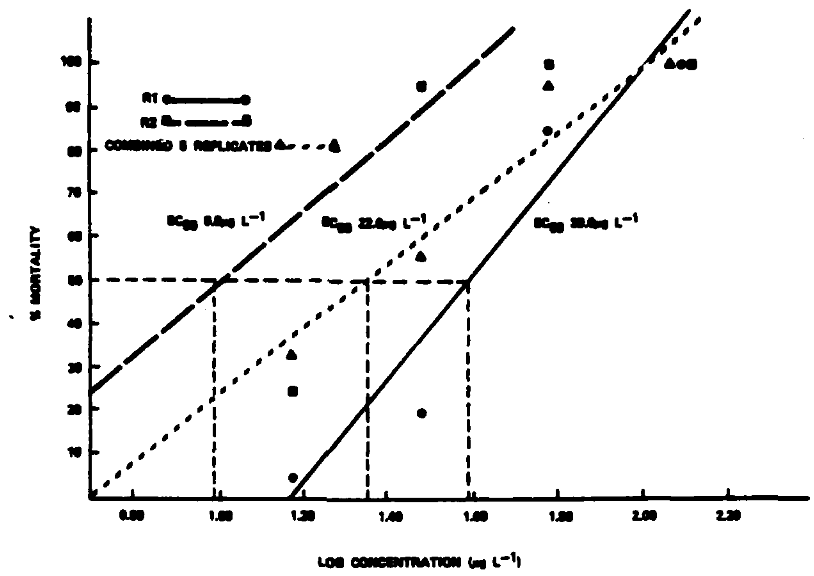

Figure 1. Representative 48-Hour EC50 Determinations for Uncoated Brass in Daphnia magna

Slopes and intercepts were determined by probit analysis.

The data generated by the chemical fate study is presented in Table 3. One day after addition, the percent recovery of zinc and copper in the pH 2.0 sample was 86% and 99%, respectively. The recoveries were significantly lower, ranging from 9% to 21%, at pH levels of 5.0 and 6.5. The lowest recovery was seen at pH 8.5. Slightly higher recoveries were observed at each successive sampling at the three higher pH levels. At day 19 post addition, zinc/copper recoveries of 33%/27% and 33%/25% were observed at pH 5.0 and 6.5, respectively.

4. DISCUSSION

The calculated EC50s for brass were nearly identical to the EC50 for copper oxide, 26 µg/l, for D. magna. Published EC50 values for zinc sulfate for D. magna were considerably higher, 1700 µg/l in soft water.[7] Comparatively, the 96-hour LC50s of

Table 3. Dissociation of Brass Particulate into Zinc and Copper

Post addition*	pH 2.0		pH 5.0		pH 6.5		pH 8.5	
	Zn^{++}/Cu^{++} mg/l^{-1}	Zn^{++}/Cu^{++} % recovery	Zn^{++}/Cu^{++} mg/l^{-1}	Zn^{++}/Cu^{++} % recovery	Zn^{++}/Cu^{++} mg/l^{-1}	Zn^{++}/Cu^{++} % recovery	Zn^{++}/Cu^{++} mg/l^{-1}	Zn^{++}/Cu^{++} % recovery
DAY 1	1.29 / 3.48	86 / 99	0.31 / 0.54	21 / 16	0.23 / 0.30	16 / 9	0.20 / < 0.1	13 / < 3
DAY 5	1.34 / 3.37	89 / 96	0.36 / 0.72	24 / 20	0.31 / 0.50	21 / 14	0.18 / < 0.1	12 / < 3
DAY 16	1.35 / 3.51	90 / 100	0.43 / 0.84	29 / 24	0.45 / 0.74	30 / 21	0.35 / < 0.1	23 / < 3
DAY 19	1.37 / 3.48	91 / 99	0.49 / 0.96	33 / 27	0.50 / 0.88	33 / 25	0.50 / < 0.1	33 / < 3

*Brass particulate (5mg/l) added to each container approximately 30% zinc/70% copper composition copper chloride and zinc chloride for yearling coho salmon have been reported as 74 μg/l and 4600 μg/l, respectively.[8] Data on the toxicity of copper and zinc in various benthic organisms showed copper more toxic than zinc for each of the six taxa examined. While most of the differences were less than an order of magnitude, copper was 600 times more toxic than zinc in Chronomous sp.[9]

Microscopic examination confirmed the hypothesis, based on particle size, that Daphnia would filter the brass. Three species of algae commonly used for daphnid food have average cell sizes that are larger than conglomerates of the brass. Chlamydomonas reinhardti and Selenastrum capricornutum have cell sizes in the range of 4 to 6μ while Ankistrodesmus falcatus ranges from 30 to 60μ in length.[10] Since the bioassays of the silica and titanium dioxide revealed no toxic effect at concentrations of 1gm/l the hypothesis that mechanical damage to the animal was responsible for the observed toxicity cannot be supported.

The fate study indicated that dissociation of the brass in the diluent to its ionic components of copper and zinc could not account for the toxicity of the brass. The most toxic components of total copper have been shown to be the free ion form (Cu^{++}) and two cationic hydroxides ($CuOH^+$, $Cu_2OH_2^{2+}$).[11] However, an insufficient quantity of the total copper existed in the test media (approximately pH 7) between 24 and 48 hours to cause the toxic effect. Since zinc is less toxic than copper and dissociates at approximately the same rate, its presence here cannot explain the acute effects.

However, the data indicated that the brass did dissociate more rapidly as the acidity of the medium increased. In a natural system with a sufficiently low pH, dissociation could be rapid enough to cause acute impacts in a short period.

The data supported the conclusion that ingestion of the particulate caused the acute effect. One possible mechanism considered was rapid dissociation of the brass caused by low pH of the digestive tract. However, the digestive tract of Daphnia is reported to have a pH of 6.8 to 7.0.[12] Further investigation to ascertain chemical characteristics of the daphnid digestive tract is warranted.

The aquatic ecosystem can be impacted by two routes of toxicity: ingestion by filter feeders and exposure to high ionic concentrations of copper in the water. The brass particulate could be distributed throughout the water column by the natural phenomena of wave motion, Langmuir currents, and seasonal turnover. Deposition of the particulate could easily affect mollusks, arthropods, and benthic macroinvertebrates. Other organisms, if unaffected by ingestion of the particulate, can be impacted by the presence of the dissociation products in the water.

The sublethal impact of copper on aquatic organisms can be as damaging to the ecosystem as lethal concentrations. At very low concentrations of copper, the reproduction of _D. magna_ has been stimulated. However, the effect at concentrations of 3.3 μg/l and above was nearly total inhibition of reproduction.[13] Mount[14] exposed fathead minnows to copper concentrations of 14 μg/l and 34μg/l to investigate survival and spawning. No effect on either was observed at the 14-μg/l concentration. At the higher concentration there was also no effect on survival, but spawning was totally inhibited.

Predicting limits for sublethal or lethal effects for a natural ecosystem from laboratory data is difficult. Reduction in pH below neutrality has been shown to increase copper toxicity significantly.[15] The ability of many anions to complex copper, thereby reducing toxicity, is well known.[16] The literature cites other cases where copper toxicity is heightened or ameliorated by existing conditions. However, the presence of copper in very low concentrations has been shown consistently to be hazardous.

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LITERATURE CITED

1. Dauber, J. H. Rossman, M. D. Pietra, G. G. Jimenez, S. A., and Daniele, R. P. Morphologic and Biochemical Abnormalities Produced by Intratracheal Instillation of Quartz Into Guinea Pig Lungs. Am. J. Pathol. 101(3), 595-607 (1980).

2. Goulden, C. E., Comotto, R. M., Hendrickson, J. A., Jr., Hornig, L. L., and Johnson, K. L. Procedures and Recommendations for the Culture and Use of Daphnia in Bioassay Studies. Am. Soc. Testing and Materials Special Technical Publication 766, pp 139-160. 1982.

3. OECD Guidelines for Testing of Chemicals. Section Z: Effects on Biotic Systems. Article 203, pp 1-12. 1981.

4. U.S. Environmental Protection Agency. EPA-660/3-75-009. Methods for Acute Toxicity Tests with Fish, Macro-Invertebrates and Amphibians. 1975.

5. Bliss, C. I. The Calculation of Dose-Mortality Curves. Ann. Appl. Biol. 22, 134-167. (1937).

6. Lewis, M. A. Effect of Loading Density on the Acute Toxicities of Surfactants, Copper, and Phenol to *Daphnia magna Straus*. Arch. Environ. Contam. Toxicol. 12, 51-55 (1983).

7. Berglind, R., and Dave, G. Acute Toxicity of Chromate, DDT, PCP, TPBS, and Zinc to *Daphnia magna* Cultured in Hard and Soft Water. Bull. Environ. Contam. Toxicol. 33, 63-68 (1984).

8. Lorz, H. W., and McPherson, B. P. Effects of Copper or Zinc in Fresh Water on the Adaptation to Sea Water and ATPase Activity, and the Effects of Copper on Migratory Disposition of Coho Salmon (*Oncorhynchus kisutch*). J. Fish. Res. Board Can. 33, 2023-2030 (1976).

9. Rehwoldt, R., Lasko, L., Shaw, C., and Wirhowski, E. The Acute Toxicity of Some Heavy Metal Ions Toward Benthic Organisms. Bull. Environ. Contam. Toxicol. 10(5), 291-294 (1973).

10. Goulden, C., and Henry, L. Daphnia Bioassay Workshop. Academy of Natural Sciences of Philadelphia. 1983.

11. Howarth, R. S., and Sprague, J. B. Copper Lethality to Rainbow Trout in Waters of Various Hardness and pH. Water Res. 12, 455-462 (1978).

12. Hasler, A. D. The Physiology of Digestion in Planktonic Crustacea. II. Further Studies on the Digestive Enzymes of (A) Daphnia and Polyphemus; (B) Diaptomus and Calanus. Biol. Bull. 72, 290-298 (1937).

13. Dave, G. Effects of Copper on Growth, Reproduction, Survival and Haemoglobin in _Daphnia magna_. Comp. Biochem. Physiol. _78C_, 439-443 (1984).

14. Mount, D. I. Chronic Toxicity of Copper to Fathead Minnows (_Pimephales promelas, Rafinesque_). Water Res. _2_, 215-223 (1968).

15. Miller, T. G., and Mackey, W. C. The Effects of Hardness, Alkalinity and pH of Test Water on the Toxicity of Copper to Rainbow Trout (_Salmo gairdneri_). Water Res. _14_, 129-133 (1980).

16. Andrew, R. W., Biesinger, K. E., and Glass, G. E. Effects of Inorganic Complexing on the Toxicity of Copper to _Daphnia magna_. Water Res. _11_, 309-315 (1977).

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