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Acute and Chronic Toxicities of Boric Acid to *Daphnia magna* Straus

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Boron salts naturally occur in low concentrations in most unpolluted waterways. The average concentration for boron in surface waters has been reported to range from 0.001 mg/L (LIVINGSTON 1963) to 0.1 mg/L (KOPP and KRONER 1970; SPRAGUE 1972). Concentrations rarely exceed 0.5 mg/L, but have been measured as high as 5.0 mg/L. In some geographical areas such as the American Southwest, boron does occur in concentrations in surface waters used for irrigation that have been shown to be toxic to plants of commercial importance. The critical concentration of boron in irrigation waters used on sensitive crops such as citrus is 0.3 to 1.0 mg/L (WILCOX 1960). The discovery of the phytotoxic properties of boron compounds to citrus has led in part to the investigation of their toxicities to aquatic organisms including algae (GERLOFF 1968; ANTIA and CHENG 1975) and fish (WALLEN et al. 1957; ALABASTER 1957). Lacking, however, are investigations of the toxicity of boron-containing compounds to the common aquatic microcrustacean, Daphnia magna Straus. This species is commonly used in toxicity tests as a test organism. In addition it represents a member of an important trophic level that serves as a food source for many fish species. This report details the acute and chronic toxicities of boric acid to Daphnia magna.

MATERIALS AND METHODS

A 48-hr static acute test and a 21-day static renewal chronic toxicity test were conducted in the Environmental Safety Department's laboratory at the Procter and Gamble Company, which is controlled for light (16 hr illumination) and temperature (20 + 2°C). The dilution water used in the studies was a carbon-filtered, well water which has been described previously (MAKI and BISHOP 1979).

Test Organism: Daphnia magna used in both studies were obtained from a stock culture. The culture water was identical to that used in the toxicity tests. On the day preceding test initiation, reproductively mature adults were isolated. The young produced by these adults were removed and used in a test within 24 hours. Daphnia were not fed during the acute tests but were fed daily during the chronic toxicity test. The daily diet consisted of 0.15 ml of a 5:1 mixture of Ralston Purina Trout Chow and dehydrated alfalfa, both certified pesticide-free, in

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deionized water. The diet was supplemented with 1 ml of an alga suspension containing approximately 20 million cells of the green alga, Selenastrum capricornutum Printz, per ml of AAP medium (U.S. EPA 1971).

Test Material: Stock solutions in both tests were prepared by adding analytical grade boric acid (FISHER SCIENTIFIC CO. FAIRLAWN, N.J.) to distilled water. No solvent was used. The appropriate amount of the stock solution was then added to the dilution waters. In both tests, there were five test concentrations and a control. The test concentrations in the chronic study were verified using the curcumin method (APHA 1975) and are reported as mg/L boron. The effect concentrations represent mean-measured, control-corrected values. Boron in the control averaged 0.17 mg/L which is not unusually high for a well water such as that used in the studies. The 48-hr LC_{50} value derived in the acute study was based on nominal levels of the test concentrations.

Acute Toxicity Test: The acute toxicity of boric acid was determined in a 48-hr static test that followed the guidelines of the U.S. EPA (1975). There was no water renewal or aeration during testing. For each test concentration five daphnids (<24 hr old) were randomly placed into 200 ml of test water in 250 ml glass beakers. Mortality was recorded daily and the 48-hr LC_{50} and associated 95% confidence interval were determined by probit analysis (FINNEY 1971). The pH, dissolved oxygen, water hardness and water temperature of the test waters were determined following standard practices (APHA 1975) at the beginning and end of the test for one test chamber in the control and in the low, middle and high test concentrations.

Chronic Toxicity Test: A static renewal procedure was followed in conducting the chronic toxicity test. Ten 250 ml glass beakers containing 200 ml of the test solution were used for each test concentration. Of the ten beakers, seven contained one daphnid and three contained five organisms. The seven beakers with one daphnid were used for observations on survival, growth and reproduction while daphnids in the remaining beakers provided information on adult survival only. For the control there were 20 beakers, six containing five daphnids and 14 containing one daphnid. The control contained more test chambers in order to provide more data for statistical purposes.

The test duration was 21 days during which the test solutions in all test chambers including the control were renewed three times weekly usually on Monday, Wednesday and Friday. Prior to renewal, the young produced by adults in the three beakers containing five organisms were discarded. Surviving adults in these test chambers were enumerated and retained. Surviving adults and juveniles in the remaining seven beakers were enumerated after which juveniles and dead adults were discarded. The surviving adults in all test chambers were then transferred to test chambers containing fresh test solutions of the same

concentration to which the daphnids were previously exposed. The test chambers were rinsed with deionized water prior to renewal with 200 mls of fresh test solution.

Water samples were collected for analytical verification of boron from one test chamber in the control and in each of the test concentrations. One set of water samples was collected from the "old" test solutions and one set was collected also from the "new" test solutions at the time of water renewal. The pH, dissolved oxygen, water hardness and water temperature were also determined at this time for test waters in the same beakers from which waters were analyzed for boron.

Adult survival and number of young were recorded daily for each test chamber. Mortality data was analyzed by probit analysis (FINNEY 1971) to derive the 21-day LC_{50} value and associated 95% confidence interval. T-tests were used to analyze differences in mean brood sizes and in daphnid length. Length of the adults (precision to nearest 0.1mm) surviving 21 days of exposure were determined from the apex of the helmet to the base of the spine. All statistical procedures followed ZAR (1974).

RESULTS

Water Chemistry: During the chronic toxicity test, the water chemistries of the test waters varied but not to an extent that was thought to have affected the results. The pH ranged from 7.1 to 8.7 units. The dissolved oxygen exceeded 9 mg/L (> 90% saturation) in all cases. Mean water temperature was 19.2 (range = 18-21) ^{o}C and water hardness averaged 166 (range = 135 - 217) mg/L as $CaCO_3$.

Acute Toxicity: The 48-hr LC_{50} value determined in the static acute test was 226 mg/L boron (95% confidence interval = 200 - 246 mg/L) and the no kill concentration was <200 mg/L.

Chronic Toxicity: The measured boron concentrations in the test waters exceeded 95% of the corresponding nominal values. The boron concentrations in the renewed test solutions (time 0) and for the same test solutions prior to the next water renewal which was approximately 48 hours later were not significantly different.

The 21-day LC_{50} value determined in the chronic test was 53.2 mg/L boron (95% confidence interval = 44.1 - 64.5 mg/L). This LC_{50} value was based on the 100%, 32% and 14% adult mortality observed in the 106, 53 and 27 mg/L boron test waters, respectively. Control mortality was 9%. A summary of the effects of boron on daphnid length and reproduction appears in TABLE 1. No data on these parameters appears for those daphnids exposed to the highest test concentration of 106 mg/L boron since 100% mortality occurred prior to reproduction (Figure 1).

TABLE 1

Effects of boron on the length and reproductive performance of Daphnia magna during 21 days of exposure. * = significant difference from control at .05 level.

Mean Boron Conc.	Mean Length in mm[1] (± 1 S.D.)[2]	Days to First Reproduction	Mean Brood Sizes (± 1 S.D.)
Control	3.9 (.23)	9	32 (15.7)
6	3.8 (.23)	10	29 (18.9)
13	4.0 (.12)	10	23 (16.0)*
27	3.9 (.18)	10	22 (16.4)*
53	3.5 (.40)*	10	16 (12.7)*
106	---------	----	---------

1 of surviving adults at test termination
2 Standard Deviation

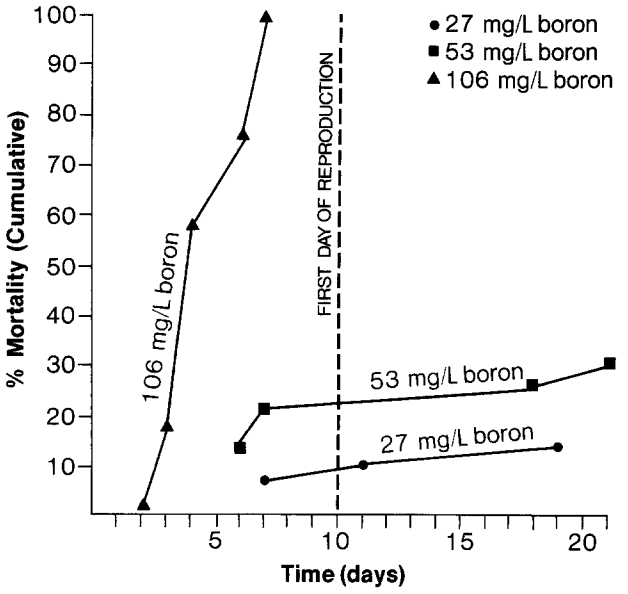

Figure 1: Mortality curves for daphnids exposed to the three highest boron concentrations in the 21-day static renewal chronic toxicity test. Boron concentrations represent mean-measured levels.

Mean brood size and total young produced were the most sensitive reproductive parameters monitored in this study. Boron

concentrations averaging 53 mg/L reduced length of adult daphnids (TABLE 1) when compared to length of those organisms in the control ($P<0.05$). The mean brood sizes were reduced in the test tanks containing on the average 13 mg/L boron or more ($P<0.05$). As an example, the average brood size at 53 mg/L boron was 50% less than that in the control. Consequently, the total number of young produced for 21 days progressively declined with boron concentrations $\geq$ 13 mg/L (Figure 2). The number of offspring produced by daphnids reared in waters containing 53 mg/L boron was approximately 70% lower (267) than that in the control. Based upon the most sensitive parameters, the no observed effect concentration (NOEC) was 6 mg/L boron and the no effect concentration was between 6 and 13 mg/L.

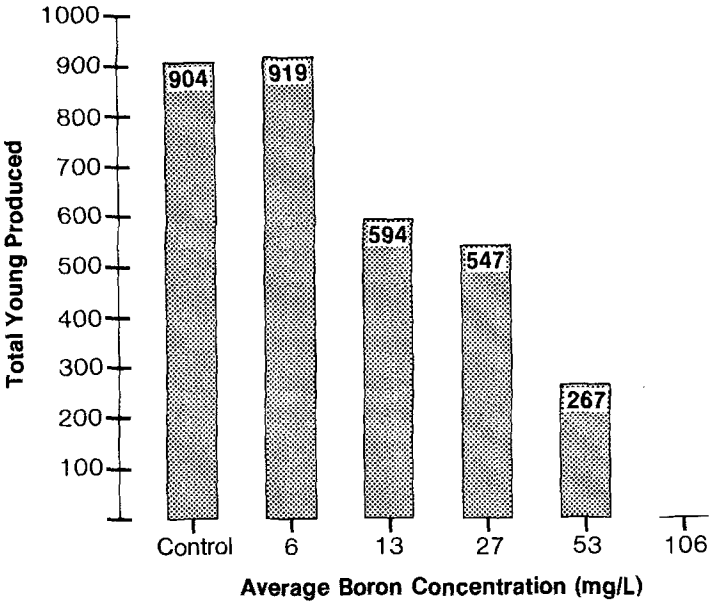

Figure 2: The total number of offspring produced during a 21-day static renewal chronic toxicity test. Boron concentrations represent mean-measured levels. The value for total young in the control represents the mean for the two sets of test chambers used.

The time to first reproduction was not significantly affected by test concentrations (TABLE 1). In all treatments, the time to first reproduction was 10 days after test initiation except in the control where it was 9 days.

DISCUSSION

The toxicities of boron to aquatic animals have been reported to exceed 2,500 mg/L in most cases (MANN 1973); however, lower toxicity levels have been observed. The 96-hr LC_{50} value for

the mosquito fish, Gambusia affinis, was found to be 980 mg/L (WALLEN et al. 1957) and 339 mg/L was the 48-hr LC_{50} value to rainbow trout (ALABASTER 1957). In long-term exposures, the toxicity of sodium metaborate to coho salmon, Onchorhynchus kisutch, was 113 mg/L (283-hr LC_{50}) and the toxicity in salt water to yearling coho was 12 mg/L (THOMPSON et al. 1976). Boron concentrations (in boric acid) of 0.001 to 0.1 mg/L in reconstituted laboratory water were observed to reduce the survival and to impair the development of rainbow trout embryos (BIRGE and BLACK 1977).

The toxicities of boron to aquatic organisms other than fish have also been determined. ANTIA and CHENG (1975) found boric acid at boron concentrations of 5 to 10 mg/L to be nontoxic to 19 marine algae species; however, levels of 10 to 50 mg/L were thought to cause shifts in population composition. Effects of boron on growth of green algae, Chlorella, were observed between 50-100 mg/L (GERLOFF, 1968). In the only study found detailing the toxic effects of boron on aquatic invertebrates, larval mosquitoes, Anopheles quadrimaculus, were sensitive to 50 mg/L boric acid (9 mg/L boron) with only 2% surviving to the adult stage (FAY 1959).

Conclusion

In summary, the effect concentrations of boron to daphnids as determined in this study are similar to those observed for algae and for mosquito larvae and are within the wide range of toxic levels reported for fish. The toxic concentrations reported in this laboratory study are well above the average boron concentrations reported for U.S. surface waters and also above the maximum boron concentration found in U.S. surface waters by Kopp and Kroner (1970) which was 5.0 mg/L.

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