Evaluation of Daphnia ambigua for Routine Aquatic Toxicity Testing at the Savannah River Site

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EVALUATION OF *Daphnia ambigua* FOR ROUTINE AQUATIC TOXICITY TESTING AT THE SAVANNAH RIVER SITE (U)

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ABSTRACT

Short-term whole effluent toxicity testing, which is currently a requirement of the U.S. EPA's National Pollutant Discharge Elimination System (NPDES), commonly uses the cladoceran species *Ceriodaphnia dubia.* Despite the advantages to using a common test species to model the toxic effects of effluents, it could be argued that toxicity test results would be more meaningful if a wider variety of test organisms were commonly used. One particular argument against *C. dubia* is that tests conducted with this species do not always reflect local, site-specific conditions. The careful selection and use of an indigenous test species would produce a more realistic model of local instream effects and would account for regional differences in water quality.

Permitted effluent discharges from Savannah River Site (SRS), a government weapons facility operated by the U.S. Department of Energy, require toxicity testing with *C. dubia*. However, water quality in these receiving streams is markedly different (lower pH and hardness) from standard laboratory water used for the culturing and testing of *C. dubia*, and it has been shown that this receiving water presents varying degrees of toxicity to *C. dubia*. Based on these results, it is possible that toxic effects observed during an effluent study could be the result of test organism stress from the dilution water and not the effects of SRS effluents. Therefore, this study addressed the substitution of *C. dubia* with an indigenous cladoceran species, *Daphnia ambigua* for routine regulatory testing at SRS. Given the indigenous nature of this species, combined with the fact that it has been successfully cultured by other investigators, *D. ambigua* was ideal for consideration as a replacement for *C. dubia*, but further study of the overall success and sensitivity of laboratory-reared *D. ambigua* was required.

This investigation determined that *D. ambigua* could be laboratory cultured with only minimal changes to established regulatory protocol and that the life-cycle characteristics of this species were conducive to traditional acute and chronic aquatic toxicity test methods. Acute toxicity tests showed that when comparing LC_{50} values for *C. dubia* and *D. ambigua*, *D. ambigua* was less sensitive to some toxicants (sodium chloride, copper sulfate, and sodium lauryl sulfate) while more sensitive to others (chlorpyrifos). Results of chronic tests with copper sulfate and sodium chloride resulted in the same NOEC/LOEC values for both species. When exposed to unaltered SRS stream water, *C. dubia* demonstrated a "toxic" response for two of the three streams tested, while reproduction for *D. ambigua* was higher in all stream samples. Acute toxicity tests with sodium chloride in stream water, generally followed the sensitivity trend noted in tests conducted with regular laboratory water.

INTRODUCTION

Federal legislation explicitly states that the discharge of toxic substances in toxic amounts is to be prohibited; therefore, the detection of toxic effects plays an important role in identifying and controlling toxic discharges to surface waters (USEPA, 1994). Since it is not economically feasible to determine the toxicity of each of the thousands of potentially toxic substances in complex effluents or to conduct exhaustive chemical analyses of effluents, the most direct and cost-effective approach is to conduct short-term whole effluent toxicity tests with aquatic organisms (USEPA, 1993). The objective of these tests is to provide a toxicity model which will estimate the "safe" or "no effect" concentration of the effluent (i.e., the concentration which will permit normal survival and propagation of aquatic life in the receiving waters).

Short-term whole effluent toxicity testing, which is currently a requirement of the U.S. EPA's National Pollutant Discharge Elimination System (NPDES), generally follows several standard protocols (USEPA, 1994; ASTM, 1993a,b) which include a description of the culture and use of the common cladoceran species *Ceriodaphnia dubia*. *C. dubia* are favored by EPA because this species is easily cultured in the laboratory and extensive data are available on the concentration-response model provided by this species (LaPoint et al., 1995).

Despite the advantages of using this popular test species, it could be argued that toxicity test results would be more meaningful if a wider variety of test organisms were commonly used. The selection of an appropriate test species is necessary for a realistic representation of the concentration-response relationship in an impacted stream. Rand and Petrocelli (1985) have suggested the following criteria for selection of an ideal aquatic toxicity test organism:

- the test species should represent a broad range of sensitivities;
- the test species should be widely available and abundant;
- the test species should be indigenous to or representative of the ecosystem receiving the impact;
- the test species should be recreationally, commercially, or ecologically important;
- the test species should be amenable to routine maintenance in the laboratory and techniques should be available for culturing;
- there should be adequate background information on a test species so that test data may be more easily interpreted.

While ubiquitous use of *C. dubia* fills most of the above criteria, tests conducted with this species do not always reflect local, site-specific conditions. The careful selection and use of an indigenous test species would produce a more realistic model of local instream effects and would account for regional differences in water quality, such as the very soft waters of the southeastern United States where *C. dubia* are not abundant.

The NPDES permit for Savannah River Site (SRS) requires toxicity testing using *C. dubia* at numerous outfalls on several receiving streams. However, water quality in these receiving streams is markedly different from standard laboratory water used for the culturing and testing of *C. dubia*. Receiving stream waters on SRS are very soft (< 20 mg/L as $CaCO_3$) and have relatively low pH values. Studies to determine if unimpacted receiving stream water from three onsite streams would support *C. dubia* have shown that the stream water itself shows varying degrees of toxicity to this species (Specht, 1994). Based on these results, it is possible that

toxicity observed during a chronic study could be the result of toxicity from the dilution water and not the effects of SRS effluents. Therefore, this study addressed the development of a toxicity test using a local, site-specific aquatic species which was presumed to be more suited for testing on the SRS and other facilities in the southeastern United States.

Daphnia ambigua was considered an ideal candidate as a site-specific test organism for routine NPDES toxicity testing at SRS. It is commonly found in waters of the southeastern United States and is abundant in waters of SRS. Hanazato (1990) has reported the maintenance of *D. ambigua* in laboratory cultures for more than five years, and a study performed by LaPoint et al. (1995) determined that *D. ambigua* can be cultured in very soft waters. However, further study of the overall success and sensitivity of this species was required. The objectives of this study were to investigate the following questions:

1. Can *D. ambigua* be cultured long-term in the laboratory under conditions which do not vary greatly from those already established by the USEPA, and are the life-cycle characteristics of this organism conducive to established toxicity testing methodology?

   Feral *D. ambigua* were obtained from local ponds to establish a laboratory population suitable for use in toxicity testing, and at the same time, a laboratory culture of *C. dubia* was initiated. It is essential that good quality test organisms be readily available throughout the year from in-house or commercial sources to meet NPDES requirements (USEPA, 1993). Although it had been shown that *D. ambigua* had been maintained in the laboratory for long periods of time (Hanazato, 1990), it was unknown if this organism could be cultured under the conditions necessary for standard NPDES toxicity testing.

2. How sensitive to toxicants is *D. ambigua* compared to *C. dubia*?

   A comparison of the sensitivity of the two species when exposed to a variety of toxicants was conducted. *C. dubia* was selected as a standard EPA-recommended test organism because of its sensitivity to a wide range of chemical toxicants. Therefore, when replacing the standard test organism as an indicator species, it is important that the proposed test organism be at least equally sensitive to the same toxicants.

3. Will *D. ambigua* perform better, in terms of mortality and reproduction, in SRS surface waters than *C. dubia*?

   Performance of the two species when exposed to surface water from three SRS streams was compared. Because the water quality of SRS streams is quite different from that of the culture water of *C. dubia*, the current test organism, a toxic response has been noted when these organisms are exposed to unaltered SRS stream water (Specht, 1994). This portion of the investigation was important in demonstrating whether or not this trend was evident in tests with the indigenous species.

METHODS

Culture Conditions

It was first necessary to determine if *D. ambigua* could be cultured in the laboratory and to determine if methods used for the culture of *C. dubia* were adequate for the successful culture of the proposed test organism. The USEPA protocol for *C. dubia* culture was altered in terms of environmental conditions and feeding requirements until a healthy reproducing population of *D. ambigua* was established in the laboratory. The final culturing protocol followed a combination of methods outlined by ASTM (1993a), USEPA (1994), and LaPoint et al. (1995) and is described below.

D. ambigua and *C. dubia* Cultures

A starting population of feral *D. ambigua* were collected from a local pond, sorted from other zooplankton species, and several individuals were selected and isolated. Once these individuals produced two to three broods for the establishment of a culture, they were permanently mounted on microscope slides and taxonomically verified.

Laboratory cultures of *D. ambigua* were maintained in 1.5 liter culture dishes in moderately hard reconstituted water (a solution of reagent-grade chemicals diluted with ultrapure water from a Nanopure® water system) prepared in the laboratory according to specifications by the U.S. EPA (USEPA, 1994). Additional "backup" cultures were maintained in water collected from Fire Pond on SRS. Culture dishes were kept in an environmental chamber with constant temperature (21°C ±2) and a photoperiod of 16 hours light and 8 hours dark. Each culture dish contained 20 to 30 individual daphnids of the same age. Water in culture dishes was renewed at least three times per week, and records were maintained for each dish. The individuals in these dishes produced the neonate daphnids used in toxicity tests. A starter culture of *C. dubia* was obtained from a laboratory supplier (Environmental Consulting and Testing, Superior, WI) and maintained in culture dishes as described above, with the exception of temperature (25°C ±2 for *C. dubia* cultures).

Once the final culture protocol was established, *D. ambigua* were also maintained in "individual cultures" for a period of 7 weeks. In these cultures, sixteen to twenty neonates of the same age were isolated into separate glass vials with 20 milliliters of culture water. These individuals were maintained until they produced at least three broods of young. This exercise provided baseline data on *D. ambigua* survival, reproduction, and culture variability when reared under laboratory conditions.

Feeding

Both species of daphnids were fed a diet consisting of algae (*Selenastrum capricornutum)* and a mixture of yeast, alfalfa, and fermented Trout Chow® as recommended by the U.S. EPA (USEPA, 1994). Four milliliters of an algal solution (cell density 7.0 X 10^7 cells/mL) were added to each culture dish when the culture water was renewed (three times per week). An equal amount of the yeast/alfalfa/trout food mixture was added at the same time. Two

milliliters of each food type were added per day between renewals. For individual cultures, 100 μL/day of each food type was added to each vial.

Food Preparation

S. capricornutum was grown in the laboratory using Wood's Hole MBL algal media (Stein, 1973) prepared with ultrapure water. Media was mixed and filter sterilized into autoclaved 1-gallon clear Nalgene® bottles prior to being inoculated with 1 to 2 milliliters of an axenic starter culture of *S. capricornutum* obtained from a laboratory supplier (Carolina Biological Supply, Burlington, NC). Algal cultures were maintained under continuous light at ambient laboratory temperature; bubbling from a filtered air supply provided continuous mixing. Algal cells were harvested (centrifuged and separated from the media) after two to three weeks when the cell density had reached approximately 8 X 10^6 cells/mL. Cell densities were determined by a hemocytometer (Stein, 1973). Once centrifuged, the algal cells formed a pellet which was resuspended in a small volume of ultrapure water. Cell density was then determined in this concentrated solution, and it was diluted to reach a final cell density of 7.0 X 10^7 cells/mL for the final "feeding" solution. The feeding solution was stored under refrigeration for up to one month.

The additional food mixture consisted of a combination of fermented trout food, powdered alfalfa, and yeast. The fermented trout food solution was prepared by adding 5 grams of trout food per liter of ultrapure water. This solution was vigorously aerated at ambient laboratory temperature for 7 days, and water lost to evaporation was replaced. After the digestion period, the solution was allowed to settle for at least 12 hours before the supernatant was separated from the solids. Powdered alfalfa was added to ultrapure water at a rate of 5 grams per liter, then stirred for a minimum of 12 hours using a magnetic stir plate. Solid material was allowed to settle for at least one hour, and the supernatant was combined with an equal volume of the trout food solution and frozen until needed. The yeast solution was prepared by mixing 5 grams of dry baker's yeast (Fleischmann's®) per liter of ultrapure water. This solution was mixed vigorously by hand then added to the thawed alfalfa and trout food mixtures with no settling period and with no freezing.

TOXICITY TESTING

Toxicity testing was carried out in two distinct phases. The purpose of the initial phase was to compare both the acute and chronic sensitivity of the two species when exposed to several toxicants in side-by-side testing scenarios. The purpose of the second phase of testing was to gauge the performance of each species in SRS stream water, with and without the addition of a toxicant.

Phase I - Sensitivity Comparison

The initial phase of testing included: 1.) 48-hour static acute toxicity tests using sodium chloride, copper sulfate, an insecticide (chlorpyrifos), and a surfactant (sodium lauryl sulfate); and 2.) three-brood chronic toxicity tests with sodium chloride and copper sulfate.

Test Compounds and Analytical Methods

Reagent-grade copper sulfate and sodium chloride were used in both acute and chronic toxicity testing. Copper and sodium in test solutions were measured using a Trace Scan ICP (Thermo Jarrell Ash, Model A-15). For the copper sulfate tests, results were statistically analyzed and reported in terms of the concentration of total copper. Measured sodium concentrations were used to calculate the actual concentration of sodium chloride in test solutions; results were analyzed and reported in terms of sodium chloride concentration. Sodium lauryl sulfate (reagent grade), used in acute tests, was analyzed as a methylene blue active substance following USEPA method 425.1 (USEPA, 1983).

Research-grade chlorpyrifos (Dursban XP, DowElanco, Indianapolis, IN) was used in acute testing. Actual concentrations in test solutions were measured using a Perkin Elmer® Q-Mass 910 benchtop GC/MS. Samples were prepared for analysis through a solid-phase microextraction technique (Webster et al., 1996) which used a polymer-coated fused silica fiber to extract the organic compound from the analyte. The fiber, coated with polydimethylsiloxane and attached to a syringe-like apparatus, was exposed to the sample for approximately 15 minutes until a portion of the organic compounds in the sample migrated to the fiber and were sorbed by the polymer coating. Once equilibrium was reached, the fiber was inserted into the injection port of the GC where the analyte was thermally desorbed and the GC/MS procedure was completed as normal.

Acute Testing

The 48-hour acute static tests were performed following test conditions described in ASTM (1993b). Neonates of each species were exposed to a control treatment and an ascending series of five or six treatment concentrations prepared by spiking dilution water with a concentrated solution of the toxicant. Range-finding tests were conducted initially to aid in the selection of the final test concentrations. Five replicates, each containing ten neonates, were prepared for each concentration; replicates consisted of 250-mL containers with 100 mL of test solution. Moderately hard reconstituted water (USEPA, 1994) served as the control solution and dilution water for these tests. Test vessels were randomly placed in environmental chambers which controlled photoperiod (16 hours light and 8 hours dark) and maintained a temperature of 25 ± 2°C for *C. dubia* and 21 ± 2°C for *D. ambigua*.

A concentrated solution of chlorpyrifos was prepared by dissolving the compound in analytical-grade methanol at a rate of 40 mg/L. A concentrated working stock solution was then prepared by spiking dilution water with an aliquot of the methanol/chlorpyrifos mixture. The solvent was present in the highest test concentration at a level of 37.5 μL/L, a concentration well below the 500 μL/L maximum recommended by ASTM (1993b) and USEPA (1985). A solvent control was prepared at this concentration (37.5 μL/L) and included with the test. All other test compounds were dissolved directly into laboratory water to produce a concentrated spiking solution.

At test initiation, basic water chemistry parameters (dissolved oxygen, temperature, pH, conductivity, alkalinity, and hardness) were measured in the control and in the highest test concentration. For all other test concentrations, dissolved oxygen, pH, conductivity, and

temperature were measured. Dissolved oxygen, pH, and temperature were also checked in surrogate test vessels at 24 and 48 hours.

Mortality served as the endpoint for these tests, and results were expressed in terms of the 48-hour LC_{50} (the toxicant concentration lethal to 50% of the test organisms). These LC_{50} values were calculated using the Trimmed Spearman-Karber Method, and this statistical analysis was conducted using the CT-TOX Multi-method Computer Program (CTDEP, 1989). The LC_{50} values and their corresponding 95% confidence limits served as an indicator of the relative sensitivities of these two species.

Samples of the test solutions were preserved and analyzed, using the methodology described above, to determine actual toxicant concentrations in test solutions. Because of the differences in toxicant behavior (e.g., the volatile nature of chlorpyrifos), some test solutions were analyzed more rigorously than others. The analytical scenarios are summarized in Table 1.

Table 1. Summary of analytical measurements conducted on test solutions during 48-hour acute toxicity testing.

Toxicant	Sampling Interval During Acute Testing
Sodium chloride	Test initiation
Copper sulfate	Test initiation; 48 hours[a]
Sodium lauryl sulfate	Test initiation
Chlorpyrifos	Test initiation; 24 hours[a]; 48 hours[a]

[a]Samples were taken from either actual test vessels or from surrogate vessels which contained an equal volume and an equal number of test organisms as the real test vessels. Actual values were calculated based on the geometric mean of all samples for a particular test concentration.

Chronic Testing

Chronic three-brood toxicity tests were conducted using sodium chloride and copper sulfate for comparison of the two species at chronic toxicity levels. These tests were conducted following guidelines presented in ASTM (1993a) using laboratory-prepared water as a control and diluent. Neonates (less than 24 hours old) of each species were exposed to a control treatment and an ascending series of treatment concentrations prepared by spiking dilution water with a concentrated solution of the toxicant. The selection of test concentrations was based on abbreviated range-finding tests. Twenty replicates, each containing one neonate, was prepared for each concentration; replicates consisted of 30-mL containers with 20 mL of test solution. Moderately hard reconstituted water (USEPA, 1994) served as the control solution and dilution water for these tests. Test solutions were renewed daily from stock solutions which were mixed on the first day of testing and stored at 4°C for the duration of the test. Test organisms were fed at a rate of 200 μL feeding solution/replicate/day. Test solutions were renewed daily, and the duration of chronic toxicity tests depended upon the species. Chronic toxicity tests were conducted until at least 60% of the control organisms had 3 broods of young. Typically, this took 7 days for *C. dubia* and 10 days for *D. ambigua*. Tests were conducted in environmental chambers which controlled photoperiod (16 hours light and 8 hours dark) and maintained a temperature of 25 ± 2°C for *C. dubia* and 21 ± 2°C for *D. ambigua*.

Basic water quality parameters were measured daily in both the renewal solutions and in 24-hour old solutions which were stored in the environmental chamber in surrogate test vessels. In the renewal solutions, dissolved oxygen, temperature, pH, conductivity, alkalinity, and hardness were measured in the control and in the highest test concentration. For all other test concentrations, dissolved oxygen, conductivity, pH, and temperature were measured. Dissolved oxygen, pH, and temperature were measured in the surrogate test vessels after 24 hours.

In order to determine the actual toxicant concentration in each test, samples of each stock solution were preserved for analysis at test initiation, the midpoint, and at the end of the test. Copper and sodium were analyzed by Trace Scan ICP as described earlier.

Mortality and reproduction served as test endpoints, and statistical analyses followed guidelines established by the USEPA (1994). Mortality in each test concentration was statistically compared to mortality in the control group by Fisher's Exact Test. If the mortality of a particular group was considered significantly different from the control, the group was dropped from further comparisons; those remaining groups were then analyzed for significant reproductive effects when compared to the control group. The reproductive response used in this analysis was the total number of young produced until either the adult's death or the end of the experiment, whichever came first. The data for each test was determined to be either parametric or nonparametric using the Chi-square test for normality and Bartlett's Test for homogeneity of variance. Test data which passed both of these tests was considered parametric, and the final statistical comparison was conducted by ANOVA and a Dunnett's t-Test. Nonparametric data was analyzed by Steel's Many-One Rank Test. These statistical tests are explained in Zar (1984) and were carried out through the TOXSTAT (Version 3.4) statistical software package (Gulley, 1994).

Phase II - Comparison in SRS Waters

Since the main purpose of this study was to evaluate the appropriateness of an indigenous test organism, it was valuable to evaluate the effects of SRS surface water on each of the two test species, both with and without the influence of a toxicant. Therefore, surface water was collected from three streams on SRS (Upper Three Runs, Pen Branch, and Fourmile Branch) at locations upstream from any outfall and used for testing.

Stream Water Reproductive Success Comparison

Initially, neonates of each species were exposed to water from each stream in a scenario similar to the one described above for chronic toxicity testing. Twenty replicates, each containing one neonate, was prepared for each water type; replicates consisted of 30-mL containers with 20 mL of test solution. Moderately hard reconstituted water (USEPA, 1994) served as the control solution and, since this was also the culture water, a basis of comparison for reproductive success if this had been an actual NPDES test. Solutions were renewed daily, and test organisms were fed at a rate of 200 μL feeding solution/replicate/day. These tests were continued until at least 60% of the control organisms had 3 broods of young.

Mortality and reproduction served as endpoints for these tests. The statistical hypothesis testing described in the preceding section and the Kruskal-Wallis Multiple Comparison Test (Zar, 1984)

were used to compare survival and reproduction of the control organisms (those in the laboratory culture water) to that of the test groups (those in the stream waters).

Acute Stream Water Comparison

A series of three 48-hour static acute tests was performed in which sodium chloride was added to water from each of the streams, and both species were tested side-by-side. Since the purpose of this set of tests was to compare the responses of the two test organisms in SRS stream water that contained a toxicant (not to repeat the measure of sensitivity), these tests were somewhat less rigorous than the acute tests described above. Only two replicates, of ten organisms each, were exposed to each test concentration, and only the concentrated spiking solutions were analyzed for actual toxicant concentrations. All other methods were the same as those explained in the preceding section. As with the other acute tests, mortality served as the endpoint for these tests, and 48-hour LC_{50} values were calculated using the Trimmed Spearman-Karber Method (CTDEP, 1989).

RESULTS AND DISCUSSION

Prior to any toxicity comparisons, there were two general factors related to this proposed test organism which had to be resolved. First, it was important to ascertain if this species could be cultured on a long-term basis following criteria similar to that already established for *C. dubia* and, therefore, generally accepted by regulatory agencies. Secondly, it had to be decided if the life cycle characteristics of the proposed test organism were appropriate for traditional acute and chronic toxicity testing.

After some trial and error, it was determined that *D. ambigua* could be cultured using established *C. dubia* protocol (USEPA, 1993; 1994) with only minimal changes. A culture temperature of 21°C produced a healthier population than those cultured at the 25°C recommended for *C. dubia*. In addition, cell density in the algal feeding solution was increased to 7.0 X 10^7 cells/mL from the 3.0-3.5 X 10^7 cells/mL established by the U.S. EPA. This new feeding solution was used successfully for both species.

D. ambigua demonstrated the ability to be cultured for a long period of time, a characteristic particularly important when using organisms which are not commonly carried by commercial laboratory suppliers and are unavailable from natural populations during certain times of the year. A reproducing population of this species was maintained from generation-to-generation over the entire 16-month period of this investigation.

This initial culturing exercise also provided an indication of the appropriateness of this species as a test organism in terms of life cycle and reproductive success under laboratory conditions. *D. ambigua* mass cultures regularly produced adequate numbers of neonates for use in toxicity tests. When cultured individually, it was determined that *D. ambigua* would produce 3 broods of young within ten to eleven days, and therefore, could be used in standard 3-brood toxicity tests with a three to four day extension of the test period established in accepted regulatory protocol (USEPA, 1994). Individual cultures also showed a higher overall variance in *D. ambigua* reproductive data (compared to *C. dubia*) indicating that a higher number of replicates (20 as opposed to the recommended 10) would be a conservative choice for chronic testing.

Another overall disadvantage of the proposed test species was the organism's tendency to float in test solutions. This required careful handling during test solution renewal and was somewhat more time consuming than working with *C. dubia*. Furthermore, this led to the use of glass test vessels as opposed to disposable plastic which enhanced the tendency of floating organisms to stick to the sides.

Phase I - Sensitivity Comparison

Effluents generally contain a wide variety of toxicants, and test compounds were selected to reflect this variety. Toxicants chosen for this investigation included a metal (copper), an inorganic salt (sodium chloride), a surfactant (sodium lauryl sulfate), and an organic pesticide (chlorpyrifos). While this study did not account for possible synergistic or antagonistic effects of these toxicants, it did provide a simple model for the expected effects of common toxicant classes upon each test species in actual NPDES testing.

Results of the 48-hour acute toxicity tests are summarized in Table 2 (see Appendix 1 for details). In the side-by-side tests with sodium chloride, copper sulfate, and sodium lauryl sulfate, *D. ambigua* were less sensitive (i.e., had a higher LC_{50}) than *C. dubia*. The opposite, however, was true for the insecticide, chlorpyrifos, where the LC_{50} for *C. dubia* was somewhat higher than that for *D. ambigua* (0.056 µg/L and 0.035 µg/L, respectively). For each of the toxicants, the 95% confidence intervals for the two species did not overlap, suggesting a reliable difference in toxicant sensitivity at the time of testing.

Table 2. Forty-eight hour LC_{50} values for *D. ambigua* and *C. dubia* exposed to sodium chloride, sodium lauryl sulfate, copper, and chlorpyrifos. Values in parenthesis are 95% confidence limits.

Toxicant	48-hour LC_{50}	
	C. dubia	*D. ambigua*
Sodium chloride (mg/L)	1.59 (1.52-1.67)	2.00 (1.81-2.20)
Copper (µg/L)	4.16 (3.70-4.69)	6.53 (6.17-6.92)
Sodium lauryl sulfate (mg/L)	1.26 (1.14-1.39)	2.44 (2.23-2.67)
Chlorpyrifos (µg/L)	0.056 (0.054-0.059)	0.035 (0.032-0.037)

The chronic 3-brood toxicity test with sodium chloride demonstrated that both species were relatively equal in chronic sensitivity to these toxicants. The No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) for *C. dubia* in sodium chloride were 0.44 and 0.85 g/L, respectively (Table 3). The *C. dubia* NOEC/LOEC were based upon a combination of both the mortality and reproduction endpoints. NOEC/LOEC values for *D. ambigua* were identical to those of *C. dubia*; however, they were based solely on the mortality endpoint (Tables 4 and 5). In other words, those test concentrations without significant mortality did not show any significant reproductive effects, but mortality was significant at lower concentrations than for *C. dubia*.

Chronic toxicity testing with copper also demonstrated that the chronic sensitivity of the two species was about the same (Tables 3, 6, and 7). Both species had NOEC/LOEC values of 19 and 27 µg/L, respectively, and unlike the sodium chloride test, these values were based on a combination of mortality and reproduction for both *C. dubia* and *D. ambigua*. See Appendix 2 for a more detailed presentation of chronic toxicity test data.

Table 3. Summary of the 3-brood chronic toxicity tests with *C. dubia* and *D. ambigua* when exposed to sodium chloride and copper sulfate.

Toxicant	*C. dubia*		*D. ambigua*	
	NOEC	LOEC	NOEC	LOEC
Sodium chloride	0.44 g/L	0.85 g/L	0.44 g/L	0.85 g/L
Copper	19 µg/L	27 µg/L	19 µg/L	27 µg/L

Table 4. Results of chronic 3-brood toxicity with *Ceriodaphnia dubia* in sodium chloride. Shaded rows represent the NOEC/LOEC concentrations.

Test Concentration (g NaCl/L)	Daily Survival (%)								Number of offspring per female		
	1	2	3	4	5	6	7	Sig.	N	Mean	Sig.
Control	100	100	100	100	100	100	100	–	20	23.6	–
0.21	100	100	100	100	100	100	100	–	20	22.0	–
0.44	100	100	100	100	100	100	100	–	20	21.8	–
0.85	100	100	100	100	100	100	100	–	20	14.8	Yes[a]
1.3	100	100	100	100	100	95	95	–	20	12.3	Yes[a]
1.7	100	100	95	90	90	85	85	–	20	0.5	Yes[a]
2.2	100	10	10	0	0	0	0	Yes[b]	20	0	b

[a]Reproduction significantly reduced (α=0.05) when compared to the control group using Steel's Many-One Rank Test.
[b]Survival significantly reduced (α=0.05) when compared to the control group using Fisher's Exact Test. These groups are not considered during the statistical comparison of reproduction.

Table 5. Results of chronic 3-brood toxicity test with *Daphnia ambigua* in sodium chloride. Shaded rows represent the NOEC/LOEC concentrations.

Test Concentration (g NaCl/L)	Daily Survival (%)											Number of offspring per female		
	1	2	3	4	5	6	7	8	9	10	Sig.	N	Mean	Sig.
Control	100	100	100	100	100	100	100	90	90	90	–	20	15.1	–
0.21	100	100	100	100	100	100	100	95	90	85	–	20	15.1	–
0.44	100	100	100	100	100	100	100	100	95	95	–	20	13.1	–
0.85	100	100	100	90	90	70	70	65	65	50	Yes[a]	20	3.1	a
1.3	100	100	75	60	30	30	30	30	30	30	Yes[a]	20	0.25	a
1.7	100	100	100	0	0	0	0	0	0	0	Yes[a]	20	0	a
2.2	100	75	10	10	10	10	10	10	10	10	Yes[a]	20	0	a

[a]Survival significantly reduced (α=0.05) when compared to the control group using Fisher's Exact Test. These groups are not considered during the statistical comparison of reproduction.

Table 6. Results of chronic 3-brood toxicity with *Ceriodaphnia dubia* in copper sulfate. Shaded rows represent the NOEC/LOEC concentrations.

Test Concentration (µg Cu/L)	Daily Survival (%)								Number of offspring per female		
	1	2	3	4	5	6	7	Sig.	N	Mean	Sig.
Control	100	100	100	100	100	100	100	--	20	28.4	--
19	100	100	100	100	100	100	95	--	20	26.2	--
27	100	100	100	100	100	90	90	--	20	23.5	Yes[a]
33	100	100	100	100	100	60	60	Yes[b]	20	17.0	b
39	100	100	100	100	100	50	50	Yes[b]	20	11.8	b
49	100	100	100	95	95	65	65	Yes[b]	20	0.4	b
53	100	100	100	100	100	100	100	--	20	2.4	Yes[a]

[a]Reproduction significantly reduced (α=0.05) when compared to the control group using Steel's Many-One Rank Test.
[b]Survival significantly reduced (α=0.05) when compared to the control group using Fisher's Exact Test. These groups are not considered during the statistical comparison of reproduction.

Table 7. Results of chronic 3-brood toxicity test with *Daphnia ambigua* in copper sulfate. Shaded rows represent the NOEC/LOEC concentrations.

Test Concentration (µg Cu/L)	Daily Survival (%)											Number of offspring per female		
	1	2	3	4	5	6	7	8	9	10	Sig.	N	Mean	Sig.
Control	100	100	100	100	100	100	100	100	100	95	--	20	19.7	--
19	100	100	100	100	100	100	100	100	100	100	--	20	16.2	--
27	100	100	100	100	100	100	100	100	95	95	--	20	14.2	Yes[a]
33	100	100	100	100	100	100	100	100	100	100	--	20	7.5	Yes[a]
39	100	100	100	100	100	65	65	65	65	65	Yes[b]	20	3.5	b
49	100	100	100	90	90	55	55	55	55	55	Yes[b]	20	2.6	b
53	100	100	100	100	90	55	45	45	45	45	Yes[b]	20	0.2	b

[a]Reproduction significantly reduced (α=0.05) when compared to the control group using ANOVA and Dunnett's t-Test.
[b]Survival significantly reduced (α=0.05) when compared to the control group using Fisher's Exact Test. These groups are not considered during the statistical comparison of reproduction.

Phase II - Species Comparison in SRS Waters

Various NPDES permits for SRS require the use of receiving stream water as a diluent during routine toxicity testing; therefore, it was considered beneficial to demonstrate the reproductive success and sensitivity to toxicants of each species when exposed to stream water from several sources on SRS. Also, the use of actual onsite surface water presented a more realistic model of site-specific conditions and subsequent organism response. The results of Phase II are presented in this section, and a more detailed presentation of the test data is given in Appendix 3.

When exposed to the three SRS stream waters (Pen Branch, Four Mile Branch, and Upper Three Runs) in the chronic 3-brood study, *C. dubia* showed a significant reduction in reproduction in two of the three streams tested, when compared to reproductive rates in the laboratory control (Table 8). In contrast, *D. ambigua* reproductive rates increased in the stream waters when compared to the laboratory control group. Mortality was not a significant factor for either species during this study.

This exercise demonstrated the reproductive success which could be expected from each species in SRS surface water and showed whether or not significant differences existed merely from exposure to the surface water without the addition of a toxicant. The response of *C. dubia* to the stream water could be attributed to the differences in site-specific water quality versus that of the traditional laboratory culture water (Table 9). Surface water in southeastern streams such as Four Mile Branch and Upper Three Runs are characterized by low hardness and pH, possibly accounting for the "toxic" response of *C. dubia* when exposed to this water. Water from Pen Branch, which has a somewhat higher pH and hardness, had no apparent negative effect on *C. dubia* during this 7-day period. Since moderately hard reconstituted water is the culture water recommended for *C. dubia* by the USEPA (1994), it is used in most commercial laboratories. The *C. dubia* reproductive effects demonstrated by this test are indicative of what may happen in actual NPDES tests which require the use of SRS surface water as a control and diluent. Stated simply, chronic effects noted in effluent tests from this site may be due to the dilution water itself, not because of any particular toxicant.

Table 8. Results of the 3-brood stream water reproductive success study performed with laboratory water and surface water from three streams on SRS.

Water Type	Percent Survival		Mean Number of Young per Female (N=20)	
	C. dubia	*D. ambigua*	*C. dubia*	*D. ambigua*
Control (laboratory water)	100	100	32.2	18.3
Pen Branch	100	100	32.4	24.8
Four Mile Branch	100	100	9.8[a]	23.6
Upper Three Runs	90	100	24.3[a]	24.5

[a]Reproduction significantly reduced ($\alpha = 0.05$) when compared to the control group using Steel's Many-One Rank Test.

Table 9. Summary of water quality parameters measured on daily renewal solutions for the stream water reproduction study. Values are expressed as means, ranges are presented in parentheses, N=10.

Parameter	Water Type			
	Laboratory Reconstituted Water	Pen Branch	Four Mile Branch	Upper Three Runs
pH	8.22 (8.15-8.30)	7.64 (7.56-7.72)	5.63 (5.47-5.80)	6.53 (6.33-6.76)
Hardness (mg/L as $CaCO_3$)	67.1 (58-72)	18.6 (16-22)	7.4 (6.0-8.0)	7.4 (6.0-10.0)
Alkalinity (mg/L as $CaCO_3$)	59.2 (56-62)	25.4 (24.0-26.0)	5.5 (4.0-6.0)	3.8 (3.0-4.0)
Conductivity (μs/cm)	245.6 (239-251)	54.1 (53.1-54.7)	25.5 (24.9-25.7)	13.3 (13.1-13.5)
Dissolved Oxygen (mg/L)	8.54 (8.34-8.75)	8.36 (8.24-8.67)	8.36 (8.24-8.54)	8.26 (8.16-8.42)

Furthermore, results of this study are consistent with a similar study, Specht (1994), which took water from these same three streams and documented water quality and *C. dubia* reproductive success over a period of 11 months. Specht (1994) found that survival and/or reproduction was impaired when *C. dubia* was cultured in water from these three streams over an extended period of time.

D. ambigua, on the other hand, adapted much more easily to the change in water chemistry from the laboratory culture water to the SRS stream water. When the data from the *D. ambigua* test was statistically analyzed (Kruskal-Wallis Multiple Comparison Test; Zar, 1984), reproduction in those groups exposed to stream water was significantly greater than that of the control group.

Table 10 summarizes the results of 48-hour acute tests with sodium chloride in each of the three stream waters. The results of the Four Mile Branch and Upper Three Runs tests mirrored those of the original sodium chloride sensitivity comparison conducted in laboratory water; *D. ambigua* were less sensitive to the toxicant than *C. dubia*. There was a general decrease in LC_{50} values for both species when compared to laboratory water, but this is to be expected due to the lower hardness of the stream water. In water from Pen Branch (the stream with a somewhat higher hardness and pH), the LC_{50} for *C. dubia* was slightly higher than that for *D. ambigua* (1.54 and 1.40, respectively), but overlapping confidence limits make this difference negligible. It is difficult to determine whether the difference in sodium chloride LC_{50} values for the two species, particularly in Upper Three Runs and Four Mile Branch, is due to an effect from the dilution water or just general differences in species sensitivity.

Table 10. Forty-eight hour toxicity test results for *D. ambigua* and *C. dubia* exposed to sodium chloride using water from three SRS streams as a diluent. LC_{50} concentrations are expressed in terms of g/L. Values in parenthesis are 95% confidence limits.

SRS Stream Water Diluent	48-hour LC_{50}	
	C. dubia	*D. ambigua*
Upper Three Runs	0.15 (0.13-0.17)	0.58 (0.53-0.64)
Pen Branch	1.54 (1.47-1.61)	1.40 (1.31-1.50)
Four Mile Branch	0.37 (0.33-0.43)	0.63[a] (0.45-0.89)
Laboratory Water (copied from Table 2 for comparison)	1.59 (1.52-1.67)	2.00 (1.81-2.20)

[a]LC_{50} and confidence limits calculated by the Binomial Method due to the nature of the data (i.e., no partial mortalities in test concentrations; see Appendix 3).

SUMMARY

This investigation determined that *D. ambigua* can be cultured following basically the same accepted guidelines for *C. dubia*, and the life cycle characteristics of the proposed test organism make it an ideal candidate for NPDES testing. Disadvantages of this species include some difficulty in handling and a greater reproductive variance over *C. dubia*; however, these problems are relatively easy to overcome and are not particularly cost prohibitive.

Acute testing showed that *D. ambigua* were less sensitive to sodium chloride, copper, and sodium lauryl sulfate compared to *C. dubia*, but were also more sensitive to the insecticide, chlorpyrifos. Chronic testing with sodium chloride and copper showed toxicant sensitivities which were relatively equal between the two species. This is not surprising since *C. dubia* chronic tests are conducted at a higher temperature, resulting in greater toxicant uptake, while *D. ambigua* tests are conducted for a longer period of time (10 days versus 7 days). This apparently results in a "balance" of chronic toxicant sensitivity between the two species.

When exposed to unaltered surface water from three SRS streams (Upper Three Runs, Pen Branch, and Four Mile Branch), *C. dubia* demonstrated negative reproductive effects in water from two of these streams, Upper Three Runs and Four Mile Branch. At the same time, reproduction for *D. ambigua* actually increased significantly in all three streams when compared to the culture water control. When a toxicant (sodium chloride) was tested using these stream waters, there was an overall decrease in LC_{50} values for both species compared to tests with laboratory water (presumably because of the lower dilution water hardness). As with the general sensitivity tests (those performed in laboratory water), *D. ambigua* was somewhat less sensitive to the toxicant when tested in two of the three streams, but LC_{50} values were similar in the test with water collected from Pen Branch.

In conclusion, this study indicated that *D. ambigua* would be a reasonable replacement for *C. dubia* for site-specific testing at SRS. While testing with *D. ambigua* may result in a loss of the overall acute sensitivity (compared to *C. dubia*) to some toxicants, there was also an indication of increased sensitivity to others (e.g., chlorpyrifos). In chronic tests, which are the actual tests used to determine NPDES compliance at SRS, the two species showed equal sensitivity. This potential "trade off" may provide a more realistic model of actual instream effects at SRS. Also, when testing in receiving stream water, the use of *D. ambigua* may eliminate the potential of observed effects caused by vast differences in dilution water characteristics versus those of the culture water. Using this species will avoid this uncertainty and still provide a dependable level of protection for indigenous aquatic fauna.

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APPENDIX 1

ACUTE TOXICITY TEST DATA:

GENERAL SENSITIVITY OF *D. ambigua* VS. *C. dubia* EXPOSED TO

1. SODIUM CHLORIDE
2. COPPER SULFATE
3. SODIUM LAURYL SULFATE
4. CHLORPYRIFOS

APPENDIX 1 A

ACUTE TOXICITY TEST DATA:

D. ambigua AND *C. dubia* EXPOSED TO SODIUM CHLORIDE

APPENDIX 1 A - ANALYTICAL CHEMISTRY DATA

Nominal and actual concentrations of sodium chloride in solutions used for acute toxicity testing. Actual concentrations of sodium were measured on initial test solutions and are applicable to the testing for both species. Values were determined by Trace Scan ICP.

Nominal NaCl Concentration (g/L)	Measured Na Concentration (g/L)	Measured Na minus Background Na Concentration[1] (g/L)	Actual NaCl Concentration[2] (g/L)
0.5	0.213	0.188	0.479
1	0.370	0.345	0.879
1.5	0.526	0.501	1.27
2	0.728	0.703	1.79
2.5	0.913	0.888	2.26

[1]Background Na concentration = 0.0248 g/L.
[2]Concentration of NaCl was calculated using the following formula:
[NaCl] = ([Na]/22.9) * 58.35

APPENDIX 1 A

Forty-eight hour mortality observations on *C. dubia* in the sodium chloride toxicity test.

Actual Sodium Chloride Concentration (g/L)	Replicate										Total	
	1		2		3		4		5			
	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	10	0	10	0	10	0	50
0.479	0	10	0	10	0	10	0	10	1	9	1	49
0.879	0	10	0	10	0	10	0	10	1	9	1	49
1.27	1	9	2	8	0	10	1	9	0	10	4	46
1.79	6	4	8	2	8	2	8	2	6	4	36	14
2.26	10	0	10	0	10	0	10	0	10	0	10	0

48-hour LC_{50} = 1.59 g/L (95% confidence limits = 1.52 - 1.67 g/L).

Forty-eight hour mortality observations on *D. ambigua* in the sodium chloride toxicity test.

Actual Sodium Chloride Concentration (g/L)	Replicate										Total	
	1		2		3		4		5			
	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive
Control	1	9	1	9	0	10	0	10	0	10	2	48
0.479	0	10	0	10	0	10	1	9	0	10	1	49
0.879	1	9	0	10	1	9	2	8	1	9	5	45
1.27	1	9	1	9	2	8	3	7	1	9	8	42
1.79	4	6	2	8	3	7	2	8	2	8	13	37
2.26	10	0	8	2	7	3	7	3	6	4	38	12

48-hour LC_{50} = 2.00 g/L (95% confidence limits = 1.81 - 2.20 g/L).

Summary of statistical analysis for the *C. dubia* 48-hour acute test with sodium chloride.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 5-29-97
SAMPLE: Sodium chloride (g/L)
DURATION: 48 hours
SPECIES: Ceriodaphnia dubia

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	1.606	1.270	1.790	0.520
MAA	1.477	1.377	1.596	0.219
Probit	1.541	*****	*****	*****
Spearman-Karber	1.591	1.516	1.669	0.153

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 2.00%
LC50: 1.591
95% Lower confidence: 1.516
95% Upper confidence: 1.669

BINOMIAL METHOD

Concentration (g/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.48	50	1	2	0.450D-11
0.88	50	1	2	0.4530D-11
1.27	50	4	8	0.2231D-07
1.79	50	36	72	0.1301D+00
2.26	50	50	100	0.8882D-13

The binomial test shows that 1.27 and 1.79 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.8699 percent. An approximate LC50 for this data set is 1.606.

RESULTS USING MOVING AVERAGE

Span	G	LC50	95% Confidence limit	
4	.0173	1.48	1.38	1.60

RESULTS CALCULATED BY PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT
8	158.4853	******	.000

A probability of 0 means less than 0.001

Slope = 8.66
95% Confidence limits: -100.32 And 117.63

LC50= 1.54
95% Confidence limits: 0 and + infinity

LC1 = .83
95% Confidence limits: 0 and + infinity

Summary of statistical analysis for the *D. ambigua* 48-hour acute test with sodium chloride.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 5-29-97 DURATION: 48 hours
SAMPLE: sodium chloride SPECIES: Daphnia ambigua

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	2.001	1.790	2.260	0.470
MAA	1.991	1.842	2.213	0.370
Probit	1.938	****	****	****
Spearman-Karber	2.000	1.814	2.204	0.390

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 24%
LC50: 2.000
95% Lower confidence: 1.814
95% Upper confidence: 2.204

BINOMIAL METHOD

Concentration (g/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.48	50	1	2	.4530D-11
0.88	50	5	10	.2105D-06
1.28	50	8	16	.5818D-04
1.79	50	13	26	.4681D-01
2.26	50	38	76	.1529D-01

The binomial test shows that 1.79 and 2.26 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.9379 percent. An approximate LC50 for this data set is 2.001.

RESULTS USING MOVING AVERAGE

Span	G	LC50	95% Confidence limit	
2	.0951	1.99	1.84	2.21

RESULTS CALCULATED BY PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT
4	0.0951	7.403	0.007

Since the probability is less than 0.05,
Results calculated using the probit method
probably should not be used.

SLOPE = 6.82
95% CONFIDENCE LIMITS: -35.01 AND 48.64

LC50= 1.94
95% CONFIDENCE LIMITS: 0 AND + INFINITY

LC1 = .88
95% CONFIDENCE LIMITS: 0 AND + INFINITY

TEST: Sodium chloride test with *C. dubia*
Start Date: 5/27/97

Initial Water Chemistry

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	7.73	8.26	24.8	0.224	62	64
0.479	8.55	8.25	24.0	1.140		
0.879	9.03	8.20	24.1	20.800		
1.27	7.97	8.16	24.1	2.940		
1.79	8.31	8.15	24.1	3.860		
2.26	8.22	8.20	24.3	4.750	62	66

24-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.18	8.46	24.2
0.479	8.33	8.45	24.5
0.879	8.06	8.41	24.9
1.27	8.08	8.39	24.5
1.79	8.25	8.40	24.2
2.26	8.15	8.39	24.8

48-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.31	8.45	24.0
0.479	8.16	8.46	24.2
0.879	8.28	8.42	24.3
1.27	8.10	8.38	24.4
1.79	7.86	8.41	24.0
2.26	8.21	8.44	24.1

TEST: Sodium chloride test with *D. ambigua*
Start Date: 5/27/97

Initial Water Chemistry

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	7.97	8.24	21.1	0.240	62	64
0.479	8.23	8.25	20.8	1.153		
0.879	8.34	8.19	21.1	2.080		
1.27	8.01	8.20	21.3	2.950		
1.79	7.86	8.21	21.4	3.650		
2.26	8.11	8.20	20.9	4.480	62	66

24-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.70	8.24	21.0
0.479	9.06	8.27	20.2
0.879	8.54	8.24	19.7
1.27	8.74	8.21	19.7
1.79	8.72	8.21	20.7
2.26	8.61	8.21	20.9

48-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.88	8.34	20.2
0.479	8.79	8.29	20.0
0.879	8.66	8.26	20.1
1.27	9.16	8.23	20.3
1.79	9.25	8.21	20.0
2.26	9.16	8.20	20.1

APPENDIX 1 B

ACUTE TOXICITY TEST DATA:

D. ambigua AND *C. dubia* EXPOSED TO COPPER SULFATE

APPENDIX 1 B - ANALYTICAL CHEMISTRY DATA

Nominal and actual concentrations of copper in solutions used for acute toxicity testing. Actual concentrations of copper were measured on initial and final test solutions for each test species. Values were determined by Trace Scan ICP.

Test Species	Nominal Cu Concentration (μg/L)	Measured Cu Concentration in Initial Test Solutions[1] (μg/L)	Measured Cu Concentration in Final Test Solutions (μg/L)	Mean of Initial and Final Cu Concentrations (μg/L)
C. dubia	1	1.9	2.1	2.00
	2	3	3.1	3.05
	4	5.6	5.9	5.75
	6	8.3	8.4	8.35
	10	13.3	12.8	13.1
	17	22.4	22.5	22.4
D. ambigua	1	1.9	1.9	1.90
	2	3	2.8	2.90
	4	5.6	5.6	5.60
	6	8.3	8.6	8.45
	10	13.3	13.3	13.3
	17	22.4	21.1	21.8

[1]Background Cu concentration was less than the detection limit (1.0 μg/L).

APPENDIX 1 B

Forty-eight hour mortality observations on *C. dubia* in the copper sulfate toxicity test.

Actual Copper Concentration (µg/L)	Replicate										Total	
	1		2		3		4		5			
	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	1	9	1	9	0	10	2	48
2.0	3	7	0	10	3	7	3	7	0	10	9	41
3.05	5	5	0	10	2	8	3	7	0	10	10	40
5.75	9	1	10	0	9	1	6	4	6	4	40	10
8.35	10	0	9	1	10	0	10	0	10	0	49	1
13.05	10	0	10	0	10	0	10	0	10	0	50	0
22.45	10	0	10	0	10	0	10	0	10	0	50	0

48-hour LC_{50} = 4.16 µg/L (95% confidence limits = 3.70 - 4.69 µg/L).

Forty-eight hour mortality observations on *D. ambigua* in the copper sulfate toxicity test.

Actual Copper Concentration (µg/L)	Replicate										Total	
	1		2		3		4		5			
	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive
Control	1	9	0	10	0	10	0	10	0	10	1	49
1.9	0	10	0	10	0	10	1	9	1	9	2	48
2.9	0	10	2	8	0	10	0	10	0	10	2	48
5.6	0	10	1	9	2	8	1	9	2	8	6	44
8.45	9	1	10	0	10	0	10	0	10	0	49	1
13.3	10	0	10	0	10	0	10	0	10	0	50	0
21.8	10	0	10	0	10	0	10	0	10	0	50	0

48-hour LC_{50} = 6.53 µg/L (95% confidence limits = 6.17 - 6.93 µg/L).

Summary of statistical analysis for the *C. dubia* 48-hour acute test with copper.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 6-16-97 DURATION: 48 hours
SAMPLE: copper SPECIES: Ceriodaphnia dubia

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	4.188	3.050	4.750	2.700
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	4.163	3.699	4.865	0.986

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 18.00%
LC50: 4.163
95% Lower confidence: 3.699
95% Upper confidence: 4.685

BINOMIAL METHOD

Concentration (µg/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
2.00	50	9	18	.2807D-03
3.05	50	10	20	.1193D-02
5.75	50	40	80	.1193D-02
8.35	50	49	98	.4530D-11
13.05	50	50	100	.8882D-13
22.45	50	50	100	.8882D-13

The binomial test shows that 3.05 and 5.75 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.9976 percent.

AN APPROXIMATE LC50 FOR THIS DATA SET IS 4.188

MOVING AVERAGE METHOD

The moving average method cannot be used with this data set because no span which produces average angles bracketing 45 degrees also uses two percent dead between 0 and 100 percent.

PROBIT METHOD

No convergence in 25 iterations. Probit method probably cannot be use with this set of data.

Summary of statistical analysis for the *D. ambigua* 48-hour acute test with copper.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 6-16-97 DURATION: 48 hours
SAMPLE: Copper sulfate SPECIES: Daphnia ambigua

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	6.618	5.600	8.450	2.850
MAA	5.545	4.920	6.202	1.282
Probit	6.000	****	****	****
Spearman-Karber	6.538	6.172	6.925	0.753

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 4%
LC50: 6.538
95% Lower confidence: 6.172
95% Upper confidence: 6.925

BINOMIAL METHOD

Concentration (μg/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
1.9	50	2	4	.1133D-09
2.9	50	2	4	.1133D-09
5.6	50	6	12	.1622D-05
8.45	50	49	98	.4530D-11
13.3	50	50	100	.8882D-13
21.8	50	50	100	.8882D-13

The binomial test shows that 5.60 and 8.45 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 100.000 percent. An approximate LC50 for this data set is 6.618.

RESULTS USING MOVING AVERAGE

Span	G	LC50	95% Confidence limit	
5	.0233	5.54	4.92	6.20

RESULTS CALCULATED BY PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT
10	2.2609	23.155	.000

A probability of 0 means less than 0.001

SLOPE = 6.08
95% CONFIDENCE LIMITS: -3.06 AND 15.23

LC50= 6.00
95% CONFIDENCE LIMITS: 0 AND + INFINITY

LC1 = 2.49
95% CONFIDENCE LIMITS: 0 AND 5.04

TEST: Copper sulfate with *C. dubia*
Start Date: 6/14/97

Initial Water Chemistry

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	7.70	8.28	24.8	0.263	60	66
2	7.88	8.28	25.6	0.265		
3.05	7.87	8.28	24.7	0.268		
5.75	7.84	8.23	25.6	0.270		
8.35	7.50	8.21	24.4	0.270		
13.05	8.11	8.16	24.6	0.270		
22.45	8.24	8.16	24.3	0.27	60	70

24-Hour Readings

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.08	8.37	24.1
2	7.74	8.39	25.7
3.05	7.73	8.41	25.2
5.75	7.91	8.42	25.0
8.35	7.96	8.48	24.9
13.05	8.14	8.45	24.9
22.45	7.99	8.42	24.8

48-Hour Readings

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)
Control	7.66	8.51	24.3
2	7.53	8.43	25.2
3.05	7.74	8.46	25.3
5.75	7.72	8.47	25.2
8.35	7.42	8.60	25.3
13.05	8.02	8.55	24.2
22.45	7.91	8.51	24.3

TEST: Copper sulfate with *D. ambigua*
Start Date: 6/14/97

Initial Water Chemistry

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	8.52	8.17	21.1	0.263	60	68
1.9	8.31	8.19	21.1	0.266		
2.9	8.42	8.22	21.9	0.268		
5.6	8.39	8.21	22.0	0.269		
8.45	8.48	8.17	21.9	0.270		
13.3	8.27	8.16	21.8	0.270		
21.8	8.40	8.14	21.9	0.270	60	72

24-Hour Readings

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)
Control	9.05	8.35	20.9
1.9	8.58	8.31	20.5
2.9	8.84	8.32	21.4
5.6	9.04	8.30	21.2
8.45	8.95	8.31	21.3
13.3	8.78	8.30	21.1
21.8	8.90	8.31	21.1

48-Hour Readings

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.84	8.41	21.0
1.9	7.82	8.35	21.2
2.9	8.55	8.37	21.3
5.6	8.74	8.37	21.1
8.45	8.90	8.36	20.7
13.3	8.71	8.32	21.3
21.8	8.79	8.32	21.2

APPENDIX 1 C

ACUTE TOXICITY TEST DATA:

D. ambigua AND *C. dubia* EXPOSED TO SODIUM LAURYL SULFATE

APPENDIX 1 C - ANALYTICAL CHEMISTRY DATA

Nominal and actual concentrations of sodium lauryl sulfate in solutions used for acute toxicity testing. Actual concentrations were measured on initial test solutions and are applicable to the testing for both species. Values were determined by the MBAS method (USEPA, 1983).

Nominal SLS Concentration (mg/L)	Actual SLS Concentration (mg/L)
Background	< detection limit
1	0.953
2	1.92
3	2.9
4	4.08
6	6.15

APPENDIX 1 C

Forty-eight hour mortality observations on *C. dubia* in the sodium lauryl sulfate toxicity test.

Actual SLS Concentration (mg/L)	Replicate										Total	
	1		2		3		4		5			
	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	10	0	10	0	10	0	50
0.953	2	8	4	6	2	8	2	8	3	7	13	37
1.92	10	0	8	2	10	0	6	4	9	1	43	7
2.9	10	0	10	0	10	0	10	0	10	0	50	0
4.08	10	0	10	0	10	0	10	0	10	0	50	0
6.15	10	0	10	0	10	0	10	0	10	0	50	0

48-hour LC_{50} = 1.26 mg/L (95% confidence limits = 1.14 - 1.39 mg/L).

Forty-eight hour mortality observations on *D. ambigua* in the sodium lauryl sulfate toxicity test.

Actual SLS Concentration (mg/L)	Replicate										Total	
	1		2		3		4		5			
	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	10	0	10	0	10	0	50
0.953	0	10	0	10	0	10	0	10	0	10	0	50
1.92	2	8	0	10	4	6	4	6	1	9	11	39
2.9	7	3	7	3	6	4	6	4	7	3	33	17
4.08	9	1	10	0	9	1	9	1	10	0	47	3
6.15	10	0	9	1	10	0	10	0	10	0	49	1

48-hour LC_{50} = 2.44 mg/L (95% confidence limits = 2.23 - 2.67 mg/L).

Summary of statistical analysis for the *C. dubia* 48-hour acute test with sodium lauryl sulfate.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 7-2-97 DURATION: 48 hours
SAMPLE: SLS SPECIES: Ceriodaphnia dubia

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	1.245	0.950	1.920	0.970
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	1.259	1.140	1.389	0.249

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 26%
LC50: 1.259
95% Lower confidence: 1.140
95% Upper confidence: 1.389

BINOMIAL METHOD

Concentration (μg/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.95	50	13	26	.4681D-01
1.92	50	43	86	.1049D-04
2.90	50	50	100	.8882D-13
4.08	50	50	100	.8882D-13
6.15	50	50	100	.8882D-13

The binomial test shows that 0.95 and 1.92 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.9532 percent.

An approximate LC50 for this data set is 1.245.

MOVING AVERAGE METHOD

The moving average method cannot be used with this data set because no span which produces average angles bracketing 45 degrees also uses two percent dead between 0 and 100 percent.

RESULTS CALCULATED BY PROBIT METHOD

No convergence in 25 iterations. Probit method probably can not be use with this set of data.

Summary of statistical analysis for the *D. ambigua* 48-hour acute test with sodium lauryl sulfate.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 7-2-97 DURATION: 48 hours
SAMPLE: sodium lauryl sulfate SPECIES: Daphnia ambigua

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	2.506	1.920	2.900	0.980
MAA	2.376	2.128	2.621	0.493
Probit	2.513	2.300	2.723	0.423
Spearman-Karber	2.436	2.226	2.665	0.440

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 2%
LC50: 2.436
95% Lower confidence: 2.226
95% Upper confidence: 2.665

BINOMIAL METHOD

Concentration (mg/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.95	50	0	0	.8882D-13
1.92	50	11	22	.4511D-02
2.90	50	33	66	.1642D+01
4.08	50	47	94	.1854D-08
6.15	50	49	98	.4530D-11

The binomial test shows that 1.92 and 2.90 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 98.3535 percent. An approximate LC50 for this data set is 2.506.

RESULTS USING MOVING AVERAGE

Span	G	LC50	95% Confidence limit	
4	.0292	2.38	2.13	2.62

RESULTS CALCULATED BY PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT
5	.0540	1.000	.505

SLOPE = 6.50
95% CONFIDENCE LIMITS: 4.99 AND 8.01

LC50= 2.51
95% CONFIDENCE LIMITS: 2.30 AND 2.72

LC1 = 1.10
95% CONFIDENCE LIMITS: .83 AND 1.32

TEST: Sodium lauryl sulfate with *C. dubia*
Start Date: 6/30/97

Initial Water Chemistry

Concentration (mg/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	7.91	8.28	24.5	0.286	65	72
0.953	7.84	8.21	24.2	0.286		
1.92	8.23	8.31	24.3	0.285		
2.9	8.02	8.31	24.4	0.281		
4.08	8.41	8.25	24.2	0.289		
6.15	8.40	8.16	24.6	0.294	60	80

24-Hour Readings

Concentration (mg/L)	DO (mg/L)	pH	Temperature (°C)
Control	9.00	8.66	24.8
0.953	8.28	8.49	24.1
1.92	8.62	8.49	24.0
2.9	7.97	8.46	24.1
4.08	8.45	8.60	24.3
6.15	8.05	8.60	24.3

48-Hour Readings

Concentration (mg/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.65	8.36	24.8
0.953	8.76	8.37	24.8
1.92	8.65	8.33	25.0
2.9	8.65	8.36	25.1
4.08	8.32	8.38	24.9
6.15	8.25	8.31	24.7

TEST: Sodium lauryl sulfate with *D. ambigua*
Start Date: 3/30/97

Initial Water Chemistry

Concentration (mg/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	7.46	8.25	21.2	0.267	64	66
0.953	7.81	8.23	21.3	0.267		
1.92	7.53	8.28	21.4	0.268		
2.9	7.61	8.21	21.4	0.274		
4.08	8.32	8.11	21.4	0.275		
6.15	8.73	8.05	21.4	0.289	60	80

24-Hour Readings

Concentration (mg/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.23	8.34	20.8
0.953	9.14	8.34	20.8
1.92	9.11	8.33	20.9
2.9	9.11	8.32	20.8
4.08	8.87	8.30	21.3
6.15	9.00	8.34	20.9

48-Hour Readings

Concentration (mg/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.75	8.35	21.2
0.953	8.85	8.35	21.3
1.92	8.87	8.34	21.5
2.9	8.64	8.33	21.1
4.08	9.01	8.30	21.1
6.15	8.96	8.30	21.0

APPENDIX 1 D

ACUTE TOXICITY TEST DATA:

D. ambigua AND *C. dubia* EXPOSED TO CHLORPYRIFOS

APPENDIX 1 D - ANALYTICAL CHEMISTRY DATA

Nominal and actual concentrations of chlorpyrifos in solutions used for acute toxicity testing. Values were determined by solid-phase microextraction and GC/MS.

Test Species	Nominal Chlorpyrifos Concentration (µg/L)	Measured Chlorpyrifos Concentration in Initial Test Solutions (µg/L)	Measured Chlorpyrifos Concentration in Mid-point Test Solutions (µg/L)	Measured Chlorpyrifos Concentration in Final Test Solutions (µg/L)	Geometric Mean of Chlorpyrifos Concentrations (µg/L)
C. dubia	0.075	0.0558	0.0202	0.0236	0.030
	0.1	0.1288	0.0333	0.0206	0.044
	0.2	0.2182	0.0222	0.0729	0.071
	0.5	0.5847	0.0433	0.0218	0.082
	0.9	0.8304	0.0843	0.0917	0.185
D. ambigua	0.0125	0.0185	0.0173	0.0116	0.015
	0.025	0.0288	0.0428	0.0312	0.033
	0.05	0.0566	0.0436	0.0176	0.035
	0.075	0.1024	0.0662	0.0355	0.062
	0.1	0.1149	0.0962	0.0462	0.080

Chlorpyrifos concentrations decreased notably in 24 and 48-hour readings, probably due to the volatile nature of the compound, thus the decrease was more evident in the *C. dubia* test which was conducted at a higher temperature than the *D. ambigua* test (25°C vs. 21°C). Therefore, the LC_{50}s for each species were relatively close, even though nominal test concentrations and nominal LC_{50} values were quite different. Actual concentrations were determined by taking the geometric mean of the three analytical values.

APPENDIX 1 D

Forty-eight hour mortality observations on *C. dubia* in the chlorpyrifos toxicity test.

Measured Chlorpyrifos Concentration (µg/L)	Replicate										Total	
	1		2		3		4		5			
	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	10	0	10	0	10	0	50
Solvent Control	0	10	0	10	0	10	0	10	0	10	0	50
0.03	0	10	0	10	0	10	0	10	0	10	0	50
0.04	2	8	0	10	0	10	2	8	0	10	4	46
0.07	9	1	10	0	9	1	9	1	10	0	47	3
0.09	10	0	10	0	10	0	8	2	10	0	48	2
0.19	10	0	10	0	10	0	10	0	10	0	50	0

48-hour LC_{50} = 0.056 µg/L (95% confidence limits = 0.054 - 0.059 µg/L).

Forty-eight hour mortality observations on *D. ambigua* in the chlorpyrifos toxicity test.

Chlorpyrifos Concentration (µg/L)	Replicate										Total	
	1		2		3		4		5			
	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	10	0	10	1	9	1	49
Solvent control	0	10	0	10	0	10	0	10	0	10	0	50
0.02	0	10	0	10	0	10	0	10	0	10	0	50
0.03	2	8	3	7	1	9	1	9	2	8	9	41
0.04	7	3	9	1	7	3	7	3	9	1	39	11
0.06	8	2	10	0	10	0	10	0	9	1	47	3
0.08	10	0	10	0	10	0	10	0	10	0	50	0

48-hour LC_{50} = 0.035 µg/L (95% confidence limits = 0.032 - 0.037 µg/L).

Summary of statistical analysis for the *C. dubia* 48-hour acute test with chlorpyrifos.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 9-6-97 DURATION: 48 hours
SAMPLE: chlorpyrifos SPECIES: Ceriodaphnia dubia

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	0.055	0.044	0.071	0.027
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	0.056	0.054	0.059	0.006

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 0%
LC50: 0.055
95% Lower confidence: 0.054
95% Upper confidence: 0.059

BINOMIAL METHOD

Concentration (μg/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.03	50	0	0	.8882D-13
0.04	50	4	8	.2231D-07
0.07	50	47	94	.1854D-08
0.09	50	48	96	.1133D-09
0.19	50	50	100	.8882D-13

The binomial test shows that 0.04 and 0.07 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 100 percent.

An approximate LC50 for this data set is 0.055.

RESULTS USING MOVING AVERAGE

The moving average method cannot be used with this data set because no span which produces average angles bracketing 45 degrees also uses two percent dead between 0 and 100 percent.

RESULTS CALCULATED BY PROBIT METHOD

No convergence in 25 iterations. Probit method probably can not be use with this set of data.

Summary of statistical analysis for the *D. ambigua* 48-hour acute test with chlorpyrifos.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 9-10-97 DURATION: 48 hours
SAMPLE: chlorpyrifos SPECIES: Daphnia ambigua

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	0.034	0.033	0.035	0.002
MAA	0.035	0.032	0.037	0.005
Probit	0.035	****	****	****
Spearman-Karber	0.035	0.032	0.037	0.005

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 0%
LC50: 0.035
95% Lower confidence: 0.032
95% Upper confidence: 0.037

BINOMIAL METHOD

Concentration (μg/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.02	50	0	0	.8882D-13
0.03	50	9	18	.2807D-03
0.04	50	39	78	.4511D-02
0.06	50	47	94	.1854D-08
0.08	50	50	100	.8882D-13

The binomial test shows that 0.03 and 0.04 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.9952 percent. An approximate LC50 for this data set is 0.034.

RESULTS USING MOVING AVERAGE

SPAN	G	LC50	95% CONFIDENCE LIMIT	
4	.0179	.03	.03	.04

RESULTS CALCULATED BY PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT
5	2.0081	9.865	.000

A PROBABILITY OF 0 MEANS LESS THAN 0.001

SLOPE = 7.54
95% CONFIDENCE LIMITS: -3.14 AND 18.22

LC50= .04
95% CONFIDENCE LIMITS: 0 AND + INFINITY

LC1 = .02
95% CONFIDENCE LIMITS: 0 AND .03

TEST: Chlorpyrifos with *C. dubia*
Start Date: 9/4/97

Initial Water Chemistry

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	7.80	8.47	24.1	0.256	54	56
0.03	8.15	8.18	24.2	0.256		
0.044	7.90	8.18	24.1	0.256		
0.071	7.95	8.21	24.4	0.265		
0.082	7.97	8.21	24.2	0.267		
0.185	8.07	8.20	24.4	0.267	56	58

24-Hour Readings

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)
Control	7.89	8.15	24.4
0.03	7.46	8.26	24.5
0.044	7.66	8.25	24.5
0.071	7.72	8.21	24.5
0.082	7.88	8.18	24.3
0.185	7.95	8.20	24.1

48-Hour Readings

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)
Control	7.66	8.21	24.8
0.03	7.43	8.27	25.0
0.044	7.35	8.22	25.1
0.071	7.65	8.24	25.1
0.082	7.62	8.22	24.8
0.185	7.54	8.18	24.7

TEST: Chlorpyrifos with *D. ambigua*
Start Date: 9/8/97

Initial Water Chemistry

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	7.99	8.39	21.8	0.249	56	62
0.015	8.12	8.26	21.8	0.250		
0.033	8.19	8.22	21.3	0.251		
0.035	8.44	8.22	21.8	0.251		
0.062	8.38	8.22	22.0	0.251		
0.08	8.35	8.23	21.6	0.252	58	62

24-Hour Readings

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.69	8.26	21.4
0.015	8.66	8.28	21.2
0.033	8.66	8.27	21.2
0.035	8.67	8.25	21.3
0.062	8.77	8.26	21.1
0.08	8.74	8.25	21.2

48-Hour Readings

Concentration (ug/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.76	8.27	21.0
0.015	8.69	8.25	21.1
0.033	8.56	8.30	21.3
0.035	8.55	8.25	21.2
0.062	8.55	8.22	21.0
0.08	8.43	8.24	21.1

APPENDIX 2

CHRONIC TOXICITY TEST DATA:

GENERAL SENSITIVITY OF *D. ambigua* VS. *C. dubia* EXPOSED TO

1. SODIUM CHLORIDE

2. COPPER SULFATE

APPENDIX 2 A

CHRONIC TOXICITY TEST DATA:

C. dubia EXPOSED TO SODIUM CHLORIDE

APPENDIX 2 - ANALYTICAL CHEMISTRY DATA - SODIUM CHLORIDE

Nominal and actual concentrations of sodium chloride in solutions used for chronic toxicity testing. Actual concentrations of sodium were measured on three samples taken during the chronic test period. Values were determined by Trace Scan ICP.

Sample Time	Nominal NaCl Concentration (g/L)	Measured Na Concentration (g/L)	Measured Na minus Background Na Concentration[1] (g/L)	Actual NaCl Concentration[2] (g/L)
Initial	0.25	0.108	0.0826	0.210
	0.5	0.202	0.1766	0.450
	1.0	0.365	0.3396	0.865
	1.5	0.536	0.5106	1.30
	2.0	0.714	0.6886	1.75
	2.5	0.885	0.8596	2.19
Mid-point	0.25	0.108	0.0826	0.210
	0.5	0.194	0.1686	0.430
	1.0	0.353	0.3276	0.835
	1.5	0.531	0.5056	1.29
	2.0	0.710	0.6846	1.74
	2.5	0.865	0.8396	2.14
Final	0.25	0.110	0.0846	0.215
	0.5	0.200	0.1746	0.445
	1.0	0.356	0.3306	0.842
	1.5	0.535	0.5096	1.30
	2.0	0.689	0.6636	1.69
	2.5	0.862	0.8366	2.13

[1]Background Na concentration = 0.0254 g/L.
[2]Concentration of NaCl was calculated using the following formula:
[NaCl] = ([Na]/22.9) * 58.35

Geometric Means of the sodium chloride concentrations.

Nominal NaCl Concentration (g/L)	Measured NaCl Concentration in Initial Test Solutions (g/L)	Measured NaCl Concentration in Mid-way Point Solutions (g/L)	Measured NaCl Concentration in Final Test Solutions (g/L)	Geometric Mean of NaCl Concentrations (g/L)
0.25	0.210	0.210	0.215	0.212
0.50	0.450	0.430	0.445	0.441
1.0	0.865	0.835	0.842	0.847
1.5	1.30	1.29	1.30	1.29
2.0	1.75	1.74	1.69	1.73
2.5	2.19	2.14	2.13	2.15

3-BROOD CHRONIC TOXICITY TEST WITH CERIODAPHNIA
Survival and Reproduction Data

Toxicant: sodium chloride
Test Initiation Date: 7/25/1997

Reproduction at the contol concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	0	4	9	14	27
2	0	4	8	10	22
3	4	9	0	12	25
4	0	4	7	11	22
5	0	5	8	11	24
6	0	4	9	11	24
7	0	5	8	12	25
8	0	5	9	12	26
9	0	6	9	12	27
10	4	6	1	7	18
11	4	4	8	10	26
12	0	4	7	12	23
13	0	6	9	13	28
14	0	4	9	5	18
15	0	5	8	12	25
16	0	4	5	10	19
17	0	4	8	11	23
18	0	5	7	10	22
19	0	4	8	13	25
20	0	4	8	10	22
Mean number of young per female					23.55

Reproduction at the 0.21 g/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	5	0	5	11	21
2	4	0	11	12	27
3	5	0	8	9	22
4	4	0	10	13	27
5	4	7	0	13	24
6	3	0	11	0	14
7	5	0	11	10	26
8	4	8	0	11	23
9	3	0	7	12	22
10	4	10	0	11	25
11	5	0	12	12	29
12	4	0	6	11	21
13	4	0	11	0	15
14	4	5	0	12	21
15	4	7	0	12	23
16	4	0	6	11	21
17	4	0	7	9	20
18	1	5	6	11	23
19	4	0	11	0	15
20	4	0	5	11	20
Mean number of young per female					21.95

Reproduction at the 0.44 g/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	4	0	7	12	23
2	3	0	8	9	20
3	4	6	0	13	23
4	0	5	8	13	26
5	4	0	6	13	23
6	5	0	9	13	27
7	0	5	8	0	13
8	3	8	0	13	24
9	4	0	9	13	26
10	3	0	7	13	23
11	4	0	5	8	17
12	3	0	7	6	16
13	4	4	6	0	14
14	4	0	8	9	21
15	4	7	0	13	24
16	4	7	0	13	24
17	5	0	8	12	25
18	5	0	7	12	24
19	4	0	7	12	23
20	4	0	7	9	20
Mean number of young per female					21.8

Reproduction at the 0.85 g/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	4	0	7	11	22
2	3	0	5	8	16
3	0	4	6	1	11
4	0	3	8	0	11
5	4	0	7	12	23
6	3	0	8	10	21
7	0	4	6	0	10
8	4	0	6	5	15
9	2	2	6	0	10
10	3	0	6	0	9
11	0	4	8	0	12
12	0	5	6	0	11
13	3	0	7	10	20
14	2	0	7	8	17
15	3	0	7	9	19
16	3	0	8	9	20
17	2	0	8	0	10
18	0	5	5	0	10
19	2	0	7	9	18
20	0	4	7	0	11
Mean number of young per female					14.8

X denotes death of test organism

3-BROOD CHRONIC TOXICITY TEST WITH CERIODAPHNIA
Survival and Reproduction Data

Toxicant: sodium chloride
Test Initiation Date: 7/25/1997

Reproduction at the 1.3 g/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	3	0	0	10	13
2	2	0	0	10	12
3	2	0	0	8	10
4	2	0	0	8	10
5	0	2	X	X	2
6	0	3	0	0	3
7	3	0	7	7	17
8	3	0	0	7	10
9	4	0	0	7	11
10	4	0	8	9	21
11	1	0	4	8	13
12	3	0	4	9	16
13	3	0	4	9	16
14	0	2	4	11	17
15	0	4	4	10	18
16	3	0	1	11	15
17	3	4	0	6	13
18	2	0	6	9	17
19	2	0	2	9	13
20	0	3	6	0	9
Mean number of young per female					12.8

Reproduction at the 1.7 g/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	0	0	0		0
2	0	0	0		0
3	0	0	2		2
4	0	0	2		2
5	0	0	0		0
6	0	0	2		2
7	0	0	2		2
8	0	0	2		2
9	0	0	0		0
10	0	0	0		0
11	0	0	0		0
12	0	0	0		0
13	0	0	0		0
14	0	0	X	X	0
15	X	X	X	X	0
16	X	X	X	X	0
17	0	0	0		0
18	0	0	0		0
19	0	0	0		0
20	0	0	0		0
Mean number of young per female					0.5

Reproduction at the 2.2 g/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	X	X	X	X	0
2	X	X	X	X	0
3	X	X	X	X	0
4	X	X	X	X	0
5	X	X	X	X	0
6	X	X	X	X	0
7	X	X	X	X	0
8	X	X	X	X	0
9	X	X	X	X	0
10	X	X	X	X	0
11	X	X	X	X	0
12	X	X	X	X	0
13	X	X	X	X	0
14	X	X	X	X	0
15	X	X	X	X	0
16	X	X	X	X	0
17	X	X	X	X	0
18	X	X	X	X	0
19	X	X	X	X	0
20	X	X	X	X	0
Mean number of young per female					0

X denotes death of test organism

APPENDIX 2 A

Summary of statistical data from the chronic *C. dubia* test with sodium chloride

Group	Identification	N	Reproduction					
			Min	Max	Mean	Variance	SD	CV %
1	Control	20	18	28	23.55	8.261	2.874	12.20
2	0.21 ppt	20	14	29	21.95	16.050	4.006	18.25
3	0.44 ppt	20	13	27	21.80	15.853	3.982	18.26
4	0.85 ppt	20	9	23	14.80	23.011	4.797	32.41
5	1.3 ppt	20	2	21	12.80	22.484	4.742	37.04
6	1.7 ppt	20	0	2	0.50	0.789	0.889	177.70
7	2.2 ppt	20	no reproduction due to mortality of all test organisms					

APPENDIX 2 A

Summary of statistical analyses performed on data from the chronic *C. dubia* test with sodium chloride.

SUMMARY OF FISHER'S EXACT TESTS ON MORTALITY DATA

Group	Identification	Number Exposed	Number Dead	Sigificant (p=0.05)
1	Control	20	0	
2	0.21 ppt	20	0	
3	0.44 ppt	20	0	
4	0.85 ppt	20	0	
5	1.3 ppt	20	1	
6	1.7 ppt	20	3	
7	2.2 ppt	20	20	*

APPENDIX 2 A

Summary of statistical analyses performed on data from the chronic *C. dubia* test with sodium chloride.

CHI-SQUARE TEST FOR NORMALITY: ACTUAL AND EXPECTED FREQUENCIES

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	8.040	29.040	45.840	29.040	8.040
OBSERVED	10	35	33	32	10

Calculated Chi-Square goodness of fit test statistic = 6.0771
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE

Calculated B1 statistic = 43.64

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data FAIL B1 homogeneity test at 0.01 level. Try another transformation.

STEEL'S MANY-ONE RANK TEST - Ho:Control<Treatment

Group	Identification	Mean	Rank Sum	Critical Value	df	Significant
1	Control	23.55			20	
2	0.21 ppt	21.95	357.00	327.00	20	
3	0.44 ppt	21.80	362.00	327.00	20	
4	0.85 ppt	14.80	235.50	327.00	20	*
5	1.3 ppt	12.80	214.00	327.00	20	*
6	1.7 ppt	0.50	210.00	327.00	20	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Summary of water quality parameters measured on daily renewal solutions for the *C. dubia* sodium chloride chronic test. Values are expressed as means, ranges are presented in parentheses, N=7.

Concentration (µg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Control	8.38 (7.68-8.69)	8.22 (8.12-8.30)	24.6 (24.1-25.6)	0.255 (0.252-0.258)	57 (54-60)	65 (58-76)
19	8.30 (7.96-8.79)	8.17 (8.05-8.32)	24.5 (24.2-24.7)	0.736 (0.723-0.749)		
27	8.26 (8.06-8.39)	8.15 (8.07-8.20)	24.5 (24.1-24.9)	1.199 (1.118-1.214)		
33	8.24 (7.78-8.56)	8.16 (8.05-8.25)	24.6 (24.1-25.2)	2.169 (2.150-2.190)		
39	8.30 (7.74-8.59)	8.14 (8.08-8.24)	24.5 (24.1-24.9)	3.103 (3.040-3.150)		
49	8.40 (7.99-8.79)	8.12 (8.04-8.25)	24.5 (24.1-25.1)	3.863 (3.520-3.960)		
53	8.44 (7.74-9.27)	8.13 (7.98-8.26)	24.6 (24.1-25.6)	4.764 (4.630-4.870)	58 (54-62)	69 (64-74)

Summary of water quality parameters measured on 24-hour old solutions for the *C. dubia* sodium chloride chronic test. Values are expressed as means, ranges are presented in parentheses, N=7.

Concentration (µg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
Control	7.83 (7.76-7.90)	8.27 (8.17-8.33)	24.9 (24.6-25.1)
19	7.83 (7.64-7.99)	8.27 (8.19-8.35)	24.6 (24.1-25.1)
27	8.01 (7.82-8.28)	8.26 (8.17-8.37)	24.7 (24.3-25.1)
33	7.87 (7.45-8.23)	8.23 (8.14-8.30)	24.6 (24.2-24.9)
39	8.00 (7.73-8.26)	8.21 (8.12-8.28)	24.4 (24.1-24.7)
49	7.94 (7.59-8.32)	8.18 (8.03-8.27)	24.6 (24.2-25.2)
53	7.86 (7.43-8.21)	8.16 (7.98-8.24)	24.7 (24.1-25.3)

APPENDIX 2 B

CHRONIC TOXICITY TEST DATA:

D. ambigua EXPOSED TO SODIUM CHLORIDE

APPENDIX 2 - ANALYTICAL CHEMISTRY DATA - SODIUM CHLORIDE

Nominal and actual concentrations of sodium chloride in solutions used for chronic toxicity testing. Actual concentrations of sodium were measured on three samples taken during the chronic test period. Values were determined by Trace Scan ICP.

Sample Time	Nominal NaCl Concentration (g/L)	Measured Na Concentration (g/L)	Measured Na minus Background Na Concentration[1] (g/L)	Actual NaCl Concentration[2] (g/L)
Initial	0.25	0.108	0.0826	0.210
	0.5	0.202	0.1766	0.450
	1.0	0.365	0.3396	0.865
	1.5	0.536	0.5106	1.30
	2.0	0.714	0.6886	1.75
	2.5	0.885	0.8596	2.19
Mid-point	0.25	0.108	0.0826	0.210
	0.5	0.194	0.1686	0.430
	1.0	0.353	0.3276	0.835
	1.5	0.531	0.5056	1.29
	2.0	0.710	0.6846	1.74
	2.5	0.865	0.8396	2.14
Final	0.25	0.110	0.0846	0.215
	0.5	0.200	0.1746	0.445
	1.0	0.356	0.3306	0.842
	1.5	0.535	0.5096	1.30
	2.0	0.689	0.6636	1.69
	2.5	0.862	0.8366	2.13

[1]Background Na concentration = 0.0254 g/L.
[2]Concentration of NaCl was calculated using the following formula:
[NaCl] = ([Na]/22.9) * 58.35

Geometric Means of the sodium chloride concentrations.

Nominal NaCl Concentration (g/L)	Measured NaCl Concentration in Initial Test Solutions (g/L)	Measured NaCl Concentration in Mid-way Point Solutions (g/L)	Measured NaCl Concentration in Final Test Solutions (g/L)	Geometric Mean of NaCl Concentrations (g/L)
0.25	0.210	0.210	0.215	0.212
0.50	0.450	0.430	0.445	0.441
1.0	0.865	0.835	0.842	0.847
1.5	1.30	1.29	1.30	1.29
2.0	1.75	1.74	1.69	1.73
2.5	2.19	2.14	2.13	2.15

3-BROOD CHRONIC TOXICITY TEST WITH DAPHNIA AMBIGUA
Survival and Reproduction Data

Toxicant: Sodium chloride
Test Initiation Date: 8-8-97

Reproduction at the control concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	3	3	0	0	11	17
2	4	11	0	0	5	20
3	4	3	0	0	13	20
4	4	0	0	0	6	10
5	4	0	10	0	7	21
6	0	0	15	0	12	27
7	4	10	X	X	X	14
8	6	0	4	0	7	17
9	5	0	3	0	3	11
10	4	0	0	0	10	14
11	0	0	0	0	14	14
12	0	0	6	0	10	16
13	3	0	4	0	6	13
14	3	0	4	0	6	13
15	4	0	4	0	4	12
16	5	0	6	0	0	11
17	0	0	3	7	0	10
18	5	0	0	0	12	17
19	4	0	3	3	6	16
20	4	4	X	X	X	8
Mean number of young per female						15.05

Reproduction at the 0.21 g/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	4	0	0	3	0	7
2	4	0	4	0	0	8
3	4	0	6	4	0	14
4	4	0	8	10	15	37
5	0	4	X	X	X	4
6	4	0	0	0	0	4
7	6	0	6	4	X	16
8	3	0	3	0	0	6
9	5	0	9	0	6	20
10	5	5	0	0	0	10
11	5	0	0	6	4	15
12	0	6	0	0	4	10
13	6	0	15	0	9	30
14	4	0	0	17	0	21
15	4	0	13	0	15	32
16	6	0	14	12	0	32
17	5	0	0	1	0	6
18	6	4	0	0	4	14
19	4	0	0	2	0	6
20	5	0	5	X	X	10
Mean number of young per female						15.1

Reproduction at the 0.44 g/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	3	0	9	0	5	17
2	5	0	6	0	9	20
3	3	0	0	0	8	11
4	2	4	0	0	6	12
5	0	4	0	7	4	15
6	2	0	3	0	7	12
7	3	0	0	4	6	13
8	4	0	3	0	5	12
9	0	5	0	0	4	9
10	3	0	8	0	5	16
11	4	0	7	0	4	15
12	2	0	4	0	7	13
13	4	0	4	0	5	13
14	3	0	9	0	7	19
15	3	0	2	X	X	5
16	2	0	3	0	6	11
17	3	0	4	0	8	15
18	3	0	3	0	5	11
19	2	0	3	0	6	11
20	3	0	0	4	5	12
Mean number of young per female						13.1

Reproduction at the 0.85 g/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	2	0	0	0	3	5
4	0	0	1	0	X	1
5	0	0	0	0	2	2
6	0	0	0	0	0	0
7	0	0	0	0	X	0
8	X	X	X	X	X	0
9	0	0	4	0	3	7
10	0	0	0	0	5	5
11	X	X	X	X	X	0
12	0	0	6	0	6	12
13	0	0	0	0	0	0
14	X	X	X	X	X	0
15	0	0	0	0	11	11
16	2	0	X	X	X	2
17	0	0	4	0	6	10
18	0	0	0	0	0	0
19	X	X	X	X	X	0
20	3	0	0	0	4	7
Mean number of young per female						3.1

X denotes death of test organism

3-BROOD CHRONIC TOXICITY TEST WITH DAPHNIA AMBIGUA
Survival and Reproduction Data

Toxicant: Sodium chloride
Test Initiation Date: 8-8-97

Reproduction at the 1.3 g/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	X	X	X	X	X	0
2	0	0	0	2	0	2
3	0	0	0	3	0	3
4	X	X	X	X	X	0
5	0	0	0	0	0	0
6	X	X	X	X	X	0
7	0	0	0	0	0	0
8	0	0	0	0	0	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0
11	X	X	X	X	X	0
12	X	X	X	X	X	0
13	X	X	X	X	X	0
14	X	X	X	X	X	0
15	X	X	X	X	X	0
16	0	0	0	0	0	0
17	X	X	X	X	X	0
18	X	X	X	X	X	0
19	X	X	X	X	X	0
20	X	X	X	X	X	0
Mean number of young per female						0.25

Reproduction at the 1.7 g/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0
11	X	X	X	X	X	0
12	X	X	X	X	X	0
13	X	X	X	X	X	0
14	X	X	X	X	X	0
15	X	X	X	X	X	0
16	X	X	X	X	X	0
17	X	X	X	X	X	0
18	X	X	X	X	X	0
19	X	X	X	X	X	0
20	X	X	X	X	X	0
Mean number of young per female						0

Reproduction at the 2.2 g/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	X	X	X	X	X	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	X	X	X	X	X	0
10	X	X	X	X	X	0
11	X	X	X	X	X	0
12	X	X	X	X	X	0
13	X	X	X	X	X	0
14	X	X	X	X	X	0
15	X	X	X	X	X	0
16	X	X	X	X	X	0
17	X	X	X	X	X	0
18	X	X	X	X	X	0
19	X	X	X	X	X	0
20	X	X	X	X	X	0
Mean number of young per female						0

X denotes death of test organism

APPENDIX 2 B

Summary of statistical data from the chronic *D. ambigua* test with sodium chloride

Group	Identification	N	Reproduction					
			Min	Max	Mean	Variance	SD	CV %
1	Control	20	8	27	15.05	20.787	4.559	30.29
2	0.21 ppt	20	4	37	15.10	106.516	10.321	68.35
3	0.44 ppt	20	5	20	13.10	11.674	3.417	26.08
4	0.85 ppt	20	0	12	3.10	17.358	4.166	134.4
5	1.3 ppt	20	0	3	0.25	0.618	0.786	314.56
6	1.7 ppt	20	no reproduction due to mortality of all test organisms					
7	2.2 ppt	20	no reproduction due to mortality of all test organisms					

APPENDIX 2 B

Summary of statistical analyses performed on data from the chronic *D. ambigua* test with sodium chloride.

SUMMARY OF FISHER'S EXACT TESTS ON MORTALITY DATA

Group	Identification	Number Exposed	Number Dead	Sigificant (p=0.05)
	Control	20	2	
1	0.21 ppt	20	2	
2	0.44 ppt	20	1	
3	0.85 ppt	20	10	*
4	1.3 ppt	20	14	*
5	1.7 ppt	20	20	*
6	2.2 ppt	20	20	*

Note: Those concentrations with significant mortality were dropped from further statistical analysis.

APPENDIX 2 B

Summary of statistical analyses performed on data from the chronic *D. ambigua* test with sodium chloride.

CHI-SQUARE TEST FOR NORMALITY: ACTUAL AND EXPECTED FREQUENCIES

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	2	17	25	10	6

Calculated Chi-Square goodness of fit test statistic = 4.0096
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE

Calculated B1 statistic = 25.01

Table Chi-square value = 9.21 (alpha = 0.01, df = 2)
Table Chi-square value = 5.99 (alpha = 0.05, df = 2)

Data FAIL B1 homogeneity test at 0.01 level.

STEEL'S MANY-ONE RANK TEST - Ho:Control<Treatment

Group	Identification	Mean	Rank Sum	Critical Value	df	Significant
1	Control	15.05			20	
2	0.21 ppt	15.10	374.00	339.00	20	
3	0.44 ppt	13.10	362.50	339.00	20	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Summary of water quality parameters measured on daily renewal solutions for the *D. ambigua* sodium chloride chronic test. Values are expressed as means, ranges are presented in parentheses, N = 10.

Concentration (µg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Control	8.86 (8.46-9.10)	8.17 (8.10-8.22)	20.9 (20.5-21.3)	0.252 (0.248-0.257)	59 (54-64)	70 (64-74)
19	8.86 (8.54-9.11)	8.17 (8.05-8.23)	21.1 (20.8-21.5)	0.738 (0.734-0.741)		
27	8.87 (8.65-9.06)	8.14 (8.07-8.20)	21.1 (20.7-21.5)	1.213 (1.211-1.218)		
33	8.78 (8.39-9.10)	8.16 (8.02-8.21)	21.2 (20.8-21.7)	2.186 (2.160-2.220)		
39	8.87 (8.52-9.08)	8.14 (8.04-8.20)	21.2 (20.8-21.9)	3.131 (3.110-3.160)		
49	8.91 (8.61-9.10)	8.11 (7.98-8.19)	21.0 (20.5-21.6)	3.948 (3.900-3.980)		
53	8.87 (7.99-9.08)	8.14 (7.96-8.21)	21.2 (20.8-21.8)	4.763 (4.630-4.850)	57 (54-60)	70 (68-74)

Summary of water quality parameters measured on 24-hour old solutions for the *D. ambigua* sodium chloride chronic test. Values are expressed as means, ranges are presented in parentheses, N = 10.

Concentration (µg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
Control	8.81 (8.63-9.16)	8.11 (8.00-8.21)	21.2 (20.9-21.6)
19	8.91 (8.79-9.11)	8.12 (7.96-8.19)	21.2 (20.8-21.5)
27	9.01 (8.76-9.24)	8.13 (7.90-8.21)	21.0 (20.1-21.7)
33	9.00 (8.78-9.20)	8.10 (7.87-8.17)	21.0 (20.1-21.7)
39	9.00 (8.78-9.22)	8.11 (7.87-8.20)	21.0 (19.9-21.6)
49	8.94 (8.72-9.13)	8.11 (7.85-8.21)	20.9 (20.0-21.6)
53	8.96 (8.78-9.22)	8.11 (7.83-8.20)	20.9 (20.0-21.5)

APPENDIX 2 C

CHRONIC TOXICITY TEST DATA:

C. dubia EXPOSED TO COPPER SULFATE

APPENDIX 2 - ANALYTICAL CHEMISTRY DATA - COPPER

Nominal and actual concentrations of copper in solutions used for chronic toxicity testing. Values were determined by Trace Scan ICP.

Nominal Cu Concentration (μg/L)	Measured Cu Concentration in Initial Test Solutions[1] (μg/L)	Measured Cu Concentration in Mid-point Test Solutions (μg/L)	Measured Cu Concentration in Final Test Solutions (μg/L)	Geometric Mean of Cu Concentrations (μg/L)
15	19.2	20.0	19.1	19.4
20	27.2	27.0	28.1	27.4
25	33.6	32.6	31.9	32.7
30	40.5	38.3	39.6	39.4
35	48.3	51.4	48.4	49.3
40	53.2	52.7	53.5	53.1

[1]Background Cu concentration was less than 0.005 μg/L.

3-BROOD CHRONIC TOXICITY TEST WITH CERIODAPHNIA
Survival and Reproduction Data

Toxicant: Copper sulfate
Test Initiation Date: 8-20-97

Reproduction at the contol concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	5	0	8	0	13
2	4	0	8	17	29
3	6	0	8	14	28
4	6	0	11	17	34
5	6	0	11	16	33
6	6	0	5	18	29
7	5	0	9	14	28
8	6	0	8	17	31
9	5	0	9	17	31
10	6	0	10	15	31
11	6	0	7	19	32
12	6	9	0	0	15
13	5	0	9	16	30
14	6	0	7	18	31
15	5	0	8	19	32
16	5	0	10	14	29
17	5	0	9	16	30
18	5	0	8	18	31
19	5	0	5	17	27
20	0	0	10	14	24
Mean number of young per female					28.4

Reproduction at the 19 ug/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	6	0	7	5	18
2	6	0	9	15	30
3	6	0	7	12	25
4	5	0	7	17	29
5	5	0	11	15	31
6	5	0	7	15	27
7	6	0	8	15	29
8	4	0	8	14	26
9	4	0	10	10	24
10	5	0	7	9	21
11	6	0	7	20	33
12	5	0	8	12	25
13	6	0	11	3	20
14	6	0	10	17	33
15	3	0	8	12	23
16	6	11	16	0	33
17	4	0	3	17	24
18	6	0	7	15	28
19	5	0	8	X 10	23
20	6	0	2	13	21
Mean number of young per female					26.15

Reproduction at the 27 ug/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	5	0	4	13	22
2	3	0	7	8	18
3	4	0	5	17	26
4	5	0	5	12	22
5	5	0	7	15	27
6	6	0	8	16	30
7	6	0	0	18	24
8	4	0	10	9	23
9	6	0	10	14	30
10	5	0	4	9	18
11	6	0	0	20	26
12	5	0	4	15	24
13	4	7	X	X	11
14	5	0	0	20	25
15	5	0	6	16	27
16	5	8	11	0	24
17	6	0	7	18	31
18	6	0	X	X	6
19	5	0	0	18	23
20	5	0	10	18	33
Mean number of young per female					23.5

Reproduction at the 33 ug/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	5	0	7	17	29
2	6	0	7	16	29
3	5	0	7	15	27
4	5	0	3	13	21
5	4	3	X	X	7
6	0	6	X	X	6
7	6	0	8	11	25
8	4	0	8	14	26
9	4	0	X	X	4
10	4	0	7	0	11
11	4	0	9	7	20
12	5	0	X	X	5
13	6	0	6	16	28
14	6	0	2	8	16
15	0	14	X	X	14
16	5	0	8	12	25
17	0	8	X	X	8
18	5	0	4	16	25
19	4	0	X	X	4
20	0	10	X	X	10
Mean number of young per female					17

X denotes death of test organism

3-BROOD CHRONIC TOXICITY TEST WITH CERIODAPHNIA
Survival and Reproduction Data

Toxicant: Copper sulfate
Test Initiation Date: 8-20-97

Reproduction at the 39 ug/Lconcentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	4	0	8	0	12
2	4	0	6	8	18
3	3	0	X	X	3
4	6	0	4	9	19
5	6	0	2	0	8
6	4	9	X	X	13
7	4	0	5	11	20
8	4	0	X	X	4
9	0	0	X	X	0
10	6	0	8	5	19
11	6	0	X	X	6
12	6	7	X	X	13
13	5	5	X	X	10
14	6	9	X	X	15
15	4	0	6	0	10
16	2	6	X	X	8
17	5	9	11	0	25
18	5	0	8	15	28
19	4	0	X	X	4
20	0	0	0	0	0
Mean number of young per female					11.75

Reproduction at the 49 ug/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	0	0	0	0	0
2	0	0	X	X	0
3	0	0	0	0	0
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	X	X	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	X	X	0
10	0	0	X	X	0
11	X	X	X	X	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	X	X	0
15	0	0	0	0	0
16	0	0	X	X	0
17	0	0	0	0	0
18	0	0	4	0	4
19	0	0	0	0	0
20	0	0	4	0	4
Mean number of young per female					0.4

Reproduction at the 53 ug/L concentration					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	3	0	0	15	18
2	0	0	0	0	0
3	5	0	0	6	11
4	0	0	0	0	0
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	5	0	0	2	7
9	0	0	0	0	0
10	0	0	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	8	8
15	2	0	0	0	2
16	0	0	0	2	2
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
Mean number of young per female					2.4

X denotes death of test organism

APPENDIX 2 C

Summary of statistical data from the chronic *C. dubia* test with copper

Group	Identification	N	Reproduction					
			Min	Max	Mean	Variance	SD	CV %
1	Control	20	13	34	28.40	29.305	5.413	19.06
2	19 ppb	20	18	33	26.15	20.450	4.522	17.29
3	27 ppb	20	6	33	23.50	42.053	6.485	27.59
4	33 ppb	20	4	29	17.00	88.737	9.420	55.41
5	39 ppb	20	0	28	11.75	63.461	7.966	67.80
6	49 ppb	20	0	4	0.40	1.516	1.231	307.79
7	53 ppb	20	0	18	2.40	23.726	4.871	202.96

APPENDIX 2 C

Summary of statistical analyses performed on data from the chronic *C. dubia* test with copper.

SUMMARY OF FISHER'S EXACT TESTS ON MORTALITY DATA

Group	Identification	Number Exposed	Number Dead	Sigificant (p=0.05)
	Control	20	0	
1	19 ppb	20	0	
2	27 ppb	20	2	
3	33 ppb	20	8	*
4	39 ppb	20	10	*
5	49 ppb	20	7	*
6	53 ppb	20	0	

Note: Concentrations with significant mortality were excluded from further statisitcal analysis.

APPENDIX 2 C

Summary of statistical analyses performed on data from the chronic *C. dubia* test with copper.

CHI-SQUARE TEST FOR NORMALITY: ACTUAL AND EXPECTED FREQUENCIES

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	5.360	19.360	30.560	19.360	5.360
OBSERVED	5	8	46	16	5

Calculated Chi-Square goodness of fit test statistic = 15.0981
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. No need to continue analysis.

STEEL'S MANY-ONE RANK TEST - Ho:Control<Treatment

Group	Identification	Mean	Rank Sum	Critical Value	df	Significant
1	Control	28.40				
2	19 ppb	26.15	338.5	333.00	20	
3	27 ppb	23.50	297.5	333.00	20	*
4	53 ppb	2.40	212.0	333.00	20	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Summary of water quality parameters measured on daily renewal solutions for the *C. dubia* copper sulfate chronic test. Values are expressed as means, ranges are presented in parentheses, N=7.

Concentration (µg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Control	8.48 (8.32-8.72)	8.25 (8.20-8.30)	24.6 (24.0-25.1)	0.291 (0.276-0.323)	65 (64-68)	69 (66-72)
19	8.18 (7.69-8.51)	8.29 (8.25-8.35)	24.4 (24.1-25.2)	0.293 (0.277-0.330)		
27	8.23 (7.56-8.45)	8.29 (8.26-8.32)	24.9 (24.0-25.5)	0.300 (0.278-0.332)		
33	8.22 (7.95-8.37)	8.31 (8.24-8.36)	24.5 (24.1-25.3)	0.298 (0.273-0.335)		
39	8.20 (7.95-8.57)	8.31 (8.27-8.36)	24.5 (24.0-25.1)	0.298 (0.276-0.334)		
49	8.18 (7.96-8.49)	8.34 (8.30-8.38)	24.6 (24.2-25.0)	0.295 (0.277-0.354)		
53	8.20 (7.89-8.46)	8.31 (8.28-8.32)	24.5 (24.2-25.0)	0.296 (0.273-0.358)	65 (64-66)	76 (74-78)

Summary of water quality parameters measured on 24-hour old solutions for the *C. dubia* copper sulfate chronic test. Values are expressed as means, ranges are presented in parentheses, N=7.

Concentration (µg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
Control	7.49 (7.35-7.64)	8.38 (8.29-8.45)	25.0 (24.6-25.4)
19	7.71 (7.48-7.95)	8.35 (8.23-8.45)	24.6 (24.3-25.2)
27	7.58 (7.35-7.84)	8.36 (8.28-8.42)	24.6 (24.1-25.1)
33	7.70 (7.36-8.12)	8.36 (8.27-8.43)	24.6 (24.1-25.1)
39	7.68 (7.48-7.91)	8.34 (8.27-8.42)	24.5 (24.1-24.8)
49	7.74 (7.38-8.04)	8.38 (8.29-8.48)	24.5 (24.1-25.1)
53	7.70 (7.35-7.95)	8.41 (8.37-8.46)	24.5 (24.1-25.2)

APPENDIX 2 D

CHRONIC TOXICITY TEST DATA:

D. ambigua EXPOSED TO COPPER SULFATE

APPENDIX 2 - ANALYTICAL CHEMISTRY DATA - COPPER

Nominal and actual concentrations of copper in solutions used for chronic toxicity testing. Values were determined by Trace Scan ICP.

Nominal Cu Concentration (μg/L)	Measured Cu Concentration in Initial Test Solutions[1] (μg/L)	Measured Cu Concentration in Mid-point Test Solutions (μg/L)	Measured Cu Concentration in Final Test Solutions (μg/L)	Geometric Mean of Cu Concentrations (μg/L)
15	19.2	20.0	19.1	19.4
20	27.2	27.0	28.1	27.4
25	33.6	32.6	31.9	32.7
30	40.5	38.3	39.6	39.4
35	48.3	51.4	48.4	49.3
40	53.2	52.7	53.5	53.1

[1]Background Cu concentration was less than 0.005 μg/L.

3-BROOD CHRONIC TOXICITY TEST WITH DAPHNIA AMBIGUA
Survival and Reproduction Data

Toxicant: copper sulfate
Test Initiation Date: 8-20-97

Reproduction at the control concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	0	0	6	0	X	6
2	4	0	10	0	7	21
3	4	0	10	0	11	25
4	4	0	0	10	4	18
5	4	0	14	0	10	28
6	0	0	7	0	15	22
7	3	0	5	5	3	16
8	5	0	10	0	10	25
9	4	0	7	0	0	11
10	4	0	12	0	12	28
11	4	0	0	10	9	23
12	0	0	6	4	10	20
13	3	0	0	13	10	26
14	5	0	7	0	5	17
15	3	0	7	0	7	17
16	3	0	5	0	1	9
17	4	0	0	8	8	20
18	5	0	7	0	10	22
19	0	0	5	8	7	20
20	2	0	4	6	7	19
Mean number of young per female						19.65

Reproduction at the 19 ug/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	4	0	0	0	11	15
2	5	0	0	8	9	22
3	3	0	10	0	3	16
4	4	0	10	0	9	23
5	4	0	10	0	8	22
6	5	0	0	0	3	8
7	5	0	0	0	8	13
8	6	0	0	3	5	14
9	5	12	0	0	10	27
10	5	4	0	6	5	20
11	3	2	0	0	4	9
12	4	1	0	4	7	16
13	6	6	0	0	10	22
14	4	0	3	0	10	17
15	6	0	5	0	8	19
16	0	0	6	0	9	15
17	4	0	5	0	5	14
18	4	0	5	0	0	9
19	5	0	0	0	4	9
20	5	0	6	0	3	14
Mean number of young per female						16.2

Reproduction at the 27 ug/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	4	0	4	5	0	13
2	5	0	8	1	9	23
3	4	0	11	0	7	22
4	3	0	6	0	4	13
5	3	0	0	0	0	3
6	5	0	0	4	0	9
7	0	0	3	X	X	3
8	4	0	0	10	10	24
9	4	0	0	2	0	6
10	0	0	3	0	4	7
11	5	0	9	0	0	14
12	4	0	7	0	5	16
13	3	0	7	0	5	15
14	0	0	6	0	12	18
15	0	0	7	0	12	19
16	5	0	8	0	0	13
17	4	0	7	0	6	17
18	0	0	4	0	9	13
19	4	0	8	0	7	19
20	0	0	9	0	7	16
Mean number of young per female						14.15

Reproduction at the 33 ug/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	0	0	0	0	0	0
2	0	0	0	6	0	6
3	0	0	0	3	0	3
4	0	4	0	0	0	4
5	0	0	0	0	0	0
6	0	5	0	6	0	11
7	4	0	0	1	0	5
8	4	0	0	5	0	9
9	5	11	0	0	5	21
10	4	0	0	6	0	10
11	3	0	0	0	0	3
12	3	0	0	2	0	5
13	4	0	0	0	3	7
14	0	4	0	7	0	11
15	4	4	0	0	0	8
16	3	0	0	8	0	11
17	3	7	0	0	0	10
18	5	0	0	5	0	10
19	0	5	0	9	0	14
20	3	0	0	0	0	3
Mean number of young per female						7.55

X denotes death of test organism

3-BROOD CHRONIC TOXICITY TEST WITH DAPHNIA AMBIGUA
Survival and Reproduction Data

Toxicant: copper sulfate
Test Initiation Date: 8-20-97

Reproduction at the 39 ug/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	X	X	X	X	X	0
2	4	0	6	0	0	10
3	0	0	3	0	0	3
4	0	0	3	7	0	10
5	3	0	0	0	0	3
6	4	0	0	0	0	4
7	X	X	X	X	X	0
8	X	X	X	X	X	0
9	3	0	0	5	0	8
10	X	X	X	X	X	0
11	2	0	4	0	0	6
12	3	0	0	0	0	3
13	0	0	0	0	0	0
14	X	X	X	X	X	0
15	2	0	3	0	0	5
16	4	0	0	0	2	6
17	X	X	X	X	X	0
18	2	0	0	0	5	7
19	X	X	X	X	X	0
20	4	0	0	0	0	4
Mean number of young per female						3.45

Reproduction at the 49 ug/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	0	0	0	0	0	0
2	X	X	X	X	X	0
3	X	X	X	X	X	0
4	X	X	X	X	X	0
5	X	X	X	X	X	0
6	0	0	4	3	0	7
7	X	X	X	X	X	0
8	0	0	0	0	0	0
9	X	X	X	X	X	0
10	0	0	0	4	0	4
11	4	0	7	0	0	11
12	X	X	X	X	X	0
13	0	0	4	0	0	4
14	X	X	X	X	X	0
15	3	0	2	6	0	11
16	0	0	0	0	0	0
17	0	0	0	0	0	0
18	3	0	3	0	0	6
19	X	X	X	X	X	0
20	3	0	6	0	0	9
Mean number of young per female						2.6

Reproduction at the 53 ug/L concentration						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	X	X	X	X	X	0
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	X	X	X	X	X	0
5	0	0	0	0	0	0
6	X	X	X	X	X	0
7	X	X	X	X	X	0
8	0	0	2	0	0	2
9	0	X	X	X	X	0
10	0	X	X	X	X	0
11	X	X	X	X	X	0
12	X	X	X	X	X	0
13	0	0	0	0	0	0
14	X	X	X	X	X	0
15	0	0	0	0	0	0
16	X	X	X	X	X	0
17	X	X	X	X	X	0
18	0	0	0	0	0	0
19	0	0	2	0	0	2
20	0	0	0	0	0	0
Mean number of young per female						0.2

X denotes death of test organism

APPENDIX 2 D

Summary of statistical data from the chronic *D. ambigua* test with copper

Group	Identification	N	Reproduction					
			Min	Max	Mean	Variance	SD	CV %
1	Control	20	6	28	19.65	35.082	5.923	30.14
2	19 ppb	20	8	27	16.20	28.274	5.317	32.82
3	27 ppb	20	3	24	14.15	37.503	6.124	43.28
4	33 ppb	20	0	21	7.55	25.418	5.042	66.78
5	39 ppb	20	0	10	3.45	12.155	3.486	101.06
6	49 ppb	20	0	11	2.60	16.042	4.005	154.05
7	53 ppb	20	0	2	0.20	0.379	0.616	307.79

APPENDIX 2 D

Summary of statistical analyses performed on data from the chronic *D. ambigua* test with copper.

SUMMARY OF FISHER'S EXACT TESTS ON MORTALITY DATA

Group	Identification	Number Exposed	Number Dead	Sigificant (p=0.05)
	Control	20	1	
1	19 ppb	20	0	
2	27 ppb	20	1	
3	33 ppb	20	0	
4	39 ppb	20	7	*
5	49 ppb	20	9	*
6	53 ppb	20	11	*

Note: Concentrations with significant mortality were excluded from further statisitcal analysis.

APPENDIX 2 D

Summary of statistical analyses performed on data from the chronic *D. ambigua* test with copper.

CHI-SQUARE TEST FOR NORMALITY: ACTUAL AND EXPECTED FREQUENCIES

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	5.360	19.360	30.560	19.360	5.360
OBSERVED	5	17	34	21	3

Calculated Chi-Square goodness of fit test statistic = 1.8771
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE

Calculated B1 statistic = 0.92

Table Chi-square value = 11.34 (alpha = 0.01, df = 3)
Table Chi-square value = 7.81 (alpha = 0.05, df = 3)

Data PASS B1 homogeneity test at 0.01 level.

APPENDIX 2 D

Summary of statistical analyses performed on data from the chronic *D. ambigua* test with copper.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	3	1555.738	518.579	16.427
Within (Error)	76	2399.250	31.569	
Total	79	3954.988		

Critical F value = 2.76 (0.05,3,60)
Since F > Critical F REJECT Ho: All equal

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	19.650	19.650		
2	19 ppb	16.200	16.200	1.942	
3	27 ppb	14.150	14.150	3.096	*
4	33 ppb	7.550	7.550	6.810	*

Dunnett table value = 2.10 (1 Tailed Value, P=0.05, df=60,3)

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	20			
2	19 ppb	20	3.731	19.0	3.450
3	27 ppb	20	3.731	19.0	5.500
4	33 ppb	20	3.731	19.0	

Summary of water quality parameters measured on daily renewal solutions for the *D. ambigua* copper sulfate chronic test. Values are expressed as means, ranges are presented in parentheses, N = 10.

Concentration (µg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)
Control	8.97 (8.74-9.36)	8.35 (8.25-8.42)	21.1 (20.5-21.4)	0.289 (0.276-0.323)	65 (64-68)	69 (66-72)
19	8.97 (8.73-9.39)	8.34 (8.23-8.44)	21.0 (20.2-21.5)	0.294 (0.276-0.330)		
27	8.96 (8.64-9.12)	8.31 (8.21-8.45)	21.1 (20.5-21.6)	0.296 (0.277-0.332)		
33	8.90 (8.54-9.14)	8.31 (8.24-8.42)	21.0 (20.1-21.5)	0.300 (0.273-0.335)		
39	8.96 (8.65-9.39)	8.36 (8.25-8.45)	21.0 (20.4-21.4)	0.296 (0.276-0.334)		
49	8.96 (8.65-9.28)	8.35 (8.27-8.42)	21.0 (20.2-21.6)	0.295 (0.277-0.354)		
53	8.87 (8.65-9.00)	8.32 (8.24-8.42)	21.0 (20.3-21.6)	0.298 (0.273-0.358)	65 (64-66)	77 (74-80)

Summary of water quality parameters measured on 24-hour old solutions for the *D. ambigua* copper sulfate chronic test. Values are expressed as means, ranges are presented in parentheses, N = 10.

Concentration (µg/L)	Dissolved Oxygen (mg/L)	pH	Temperature (°C)
Control	9.02 (8.81-9.25)	8.36 (8.27-8.43)	21.0 (20.4-21.6)
19	9.09 (8.85-9.37)	8.34 (8.23-8.45)	20.8 (19.4-21.4)
27	9.14 (8.97-9.45)	8.35 (8.27-8.42)	20.8 (19.6-21.6)
33	9.03 (8.76-9.45)	8.31 (8.22-8.39)	20.7 (19.6-21.4)
39	9.08 (8.79-9.52)	8.32 (8.22-8.41)	20.7 (19.5-21.4)
49	9.07 (8.76-9.52)	8.35 (8.20-8.42)	20.7 (20.1-21.5)
53	8.94 (8.12-9.48)	8.35 (8.20-8.42)	20.7 (20.1-21.4)

APPENDIX 3

STREAM WATER TEST DATA:

D. ambigua AND *C. dubia* EXPOSED TO

1. UNALTERED STREAM WATER

2. STREAM WATER SPIKED WITH SODIUM CHLORIDE

APPENDIX 3 A

STREAM WATER TEST DATA:

C. dubia EXPOSED TO UNALTERED STREAM WATER

3-BROOD CHRONIC TOXICITY TEST WITH CERIODAPHNIA
Survival and Reproduction Data

Toxicant: Unaltered laboratory and stream water

Test Initiation Date: 8-23-97

Reproduction in laboratory reconstituted water					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	7	12	18	0	37
2	6	11	16	0	33
3	6	12	0	18	36
4	3	12	0	17	32
5	5	9	0	13	27
6	7	10	0	17	34
7	6	8	15	0	29
8	6	10	0	17	33
9	6	10	0	17	33
10	6	12	0	17	35
11	5	11	0	16	32
12	6	8	9	0	23
13	6	9	16	0	31
14	7	10	18	0	35
15	6	10	0	16	32
16	5	9	0	17	31
17	6	9	16	0	31
18	5	10	0	16	31
19	6	12	0	16	34
20	6	13	16	0	35
Mean number of young per female					32.2

Reproduction in Pen Branch Water					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	5	8	14	0	27
2	6	10	0	18	34
3	5	13	0	16	34
4	5	13	16	0	34
5	6	10	16	0	32
6	6	10	11	0	27
7	5	11	0	17	33
8	6	10	9	7	32
9	7	12	0	17	36
10	7	9	14	0	30
11	6	12	18	0	36
12	6	11	14	0	31
13	16	1	17	0	34
14	6	9	16	0	31
15	6	12	18	0	36
16	5	12	15	0	32
17	13	3	15	0	31
18	6	10	16	0	32
19	6	11	17	0	34
20	2	11	19	0	32
Mean number of young per female					32.4

Reproduction in Four Mile Branch Water					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	3	0	6	5	14
2	1	0	5	8	14
3	1	0	6	6	13
4	1	0	5	6	12
5	0	2	6	0	8
6	3	0	4	7	14
7	0	2	6	0	8
8	0	2	0	5	7
9	0	4	0	6	10
10	2	6	0	6	14
11	2	0	4	0	6
12	3	4	0	6	13
13	2	0	6	0	8
14	0	0	6	0	6
15	2	0	4	0	6
16	2	0	6	0	8
17	2	0	5	6	13
18	0	2	6	0	8
19	0	3	6	0	9
20	0	2	3	0	5
Mean number of young per female					9.8

Reproduction in Upper Three Runs Water					
Rep.	Day 3/4	Day 5	Day 6	Day 7	Total
1	7	10	0	13	30
2	4	7	0	9	20
3	5	14	0	10	29
4	5	10	0	12	27
5	6	9	11	0	26
6	5	5	X	X	10
7	6	7	10	0	23
8	4	9	0	8	21
9	6	10	11	0	27
10	5	8	0	10	23
11	5	6	0	8	19
12	5	10	0	8	23
13	4	12	0	10	26
14	5	8	0	9	22
15	6	10	0	12	28
16	6	9	X	X	15
17	5	11	12	0	28
18	5	11	16	0	32
19	6	10	16	0	32
20	6	10	0	10	26
Mean number of young per female					24.35

X denotes death of test organism

APPENDIX 3 A

Summary of statistical data from the chronic *C. dubia* test in unaltered stream water

Group	Identification	N	Reproduction					
			Min	Max	Mean	Variance	SD	CV %
1	lab water	20	23	37	32.20	10.379	3.222	10.01
2	Pen Branch	20	27	36	32.40	6.463	2.542	7.85
3	Four Mile	20	5	14	9.80	10.379	3.222	32.87
4	Upper Three Runs	20	10	32	24.35	30.661	5.537	22.74

APPENDIX 3 A

Summary of statistical analyses performed on data from the chronic *C. dubia* test with unaltered stream water.

CHI-SQUARE TEST FOR NORMALITY: ACTUAL AND EXPECTED FREQUENCIES

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	5.360	19.360	30.560	19.360	5.360
OBSERVED	6	18	27	29	0

Calculated Chi-Square goodness of fit test statistic = 10.7467
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE

Calculated B1 statistic = 13.38

Table Chi-square value = 11.34 (alpha = 0.01, df = 3)
Table Chi-square value = 7.81 (alpha = 0.05, df = 3)

Data FAIL B1 homogeneity test at 0.01 level.

STEEL'S MANY-ONE RANK TEST - Ho:Control<Treatment

Group	Identification	Mean	Rank Sum	Critical Value	df	Significant
1	Control	32.20				
2	Pen Branch	32.40	410.50	333.00	20	
3	Four Mile	9.80	210.00	333.00	20	*
4	Upper Three Runs	24.35	244.00	333.00	20	*

Critical values use k = 5, are 1 tailed, and alpha = 0.05

APPENDIX 3 B

STREAM WATER TEST DATA:

D. ambigua EXPOSED TO UNALTERED STREAM WATER

3-BROOD CHRONIC TOXICITY TEST WITH DAPHNIA AMBIGUA
Survival and Reproduction Data

Toxicant: Unaltered laboratory and stream water
Test Initiation Date: 8-23-97

Reproduction in laboratory reconstituted water						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	7	0	0	12	0	19
2	5	0	0	10	8	23
3	4	0	0	10	10	24
4	3	0	0	11	0	14
5	8	0	0	12	5	25
6	6	0	0	9	0	15
7	5	0	0	8	0	13
8	2	0	0	9	0	11
9	2	0	0	10	8	20
10	8	0	0	11	0	19
11	8	0	0	15	0	23
12	8	0	0	9	0	17
13	3	0	0	12	0	15
14	5	0	0	9	8	22
15	4	0	0	10	0	14
16	6	0	0	12	5	23
17	7	0	0	11	0	18
18	5	0	0	11	0	16
19	5	0	0	6	10	21
20	4	0	0	10	0	14
Mean number of young per female						18.3

Reproduction in Pen Branch Water						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	8	11	0	0	11	30
2	7	0	0	10	5	22
3	8	0	0	13	0	21
4	11	0	0	14	15	40
5	6	0	0	10	9	25
6	6	0	0	10	10	26
7	7	0	0	12	13	32
8	10	0	0	13	0	23
9	9	0	0	10	0	19
10	9	0	0	10	0	19
11	10	0	0	13	2	25
12	5	0	0	10	9	24
13	6	0	0	10	10	26
14	6	0	0	13	0	19
15	8	0	0	12	9	29
16	5	0	0	12	0	17
17	7	0	0	11	13	31
18	10	0	0	10	0	20
19	10	0	0	9	10	29
20	9	0	0	10	0	19
Mean number of young per female						24.8

Reproduction in Four Mile Branch Water						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	5	0	0	13	12	30
2	6	0	0	12	11	29
3	5	0	0	12	11	28
4	8	0	0	10	0	18
5	7	0	0	13	12	32
6	7	1	0	12	0	20
7	6	0	0	14	0	20
8	6	0	0	10	0	16
9	9	0	0	15	0	24
10	7	0	0	14	0	21
11	5	0	0	10	12	27
12	6	0	0	12	10	28
13	7	0	0	13	0	20
14	7	0	0	12	10	29
15	8	0	0	10	0	18
16	6	0	0	10	11	27
17	5	0	0	11	0	16
18	7	0	0	11	0	18
19	8	0	0	12	11	31
20	6	0	0	13	0	19
Mean number of young per female						23.55

Reproduction in Upper Three Runs Water						
Rep.	Day 5/6	Day 7	Day 8	Day 9	Day 10	Total
1	9	0	0	10	0	19
2	5	0	0	13	15	33
3	6	0	0	9	10	25
4	6	0	0	10	10	26
5	5	0	0	11	12	28
6	6	0	0	9	8	23
7	5	0	0	8	9	22
8	6	0	0	13	10	29
9	7	0	0	13	10	30
10	9	0	0	12	10	31
11	8	0	0	12	0	20
12	9	0	0	13	8	30
13	6	0	0	13	0	19
14	6	0	0	13	0	19
15	7	0	0	10	10	27
16	7	0	0	12	0	19
17	5	0	0	12	0	17
18	8	0	0	10	0	18
19	9	0	0	10	8	27
20	7	0	0	10	11	28
Mean number of young per female						24.5

X denotes death of test organism

APPENDIX 3 B

Summary of statistical data from the chronic *D. ambigua* test in unaltered stream water

Group	Identification	N	Reproduction					
			Min	Max	Mean	Variance	SD	CV %
1	lab water	20	11	25	18.30	17.589	4.194	22.92
2	Pen Branch	20	17	40	24.80	33.221	5.764	23.24
3	Four Mile	20	16	32	23.55	29.629	5.443	23.11
4	Upper Three Runs	20	17	33	24.50	25.421	5.042	20.58

APPENDIX 3 B

Summary of statistical analyses performed on data from the chronic *D. ambigua* test with unaltered stream water.

CHI-SQUARE TEST FOR NORMALITY: ACTUAL AND EXPECTED FREQUENCIES

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	5.360	19.360	30.560	19.360	5.360
OBSERVED	1	30	20	25	4

Calculated Chi-Square goodness of fit test statistic = 15.0313
Table Chi-Square value (alpha = 0.01) = 13.277

Data FAIL normality test. No need to continue analysis.

BARTLETT'S TEST FOR HOMOGENEITY OF VARIANCE

Calculated B1 statistic = 2.02

Table Chi-square value = 11.34 (alpha = 0.01, df = 3)
Table Chi-square value = 7.81 (alpha = 0.05, df = 3)

Data PASS B1 homogeneity test at 0.01 level.

STEEL'S MANY-ONE RANK TEST - Ho:Control<Treatment

Group	Identification	Mean	Rank Sum	Critical Value	df	Significant
1	Control	18.30				
2	Pen Branch	24.80	537.00	333.00	20	
3	Four Mile	23.55	514.00	333.00	20	
4	Upper Three Runs	24.50	535.00	333.00	20	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

APPENDIX 3 C

STREAM WATER TEST DATA:

D. ambigua AND *C. dubia* EXPOSED TO

STREAM WATER SPIKED WITH SODIUM CHLORIDE

APPENDIX 3 C - ANALYTICAL CHEMISTRY DATA
NaCl IN PEN BRANCH ACUTE

Nominal and actual concentrations of sodium chloride in solutions used for acute toxicity testing. Actual concentrations of sodium were measured in the spiking solution, and final concentrations were calculated based on the amount of stock solutions spiked into the final dilution water. These values are applicable to the testing for both species. Values were determined by Trace Scan ICP.

Nominal NaCl Concentration in the Test Solution (g/L)	NaCl Concentration in the Spiking Solution (g/L)[1]	Amount of Spiking Solution Used (mL)	Final Volume of Test Solution (mL)	Actual Concentration of NaCl in Test Solution (g/L)
0.5	87.9	2.5	500	0.44
1.0	87.9	5.0	500	0.88
1.5	87.9	7.5	500	1.32
2.0	87.9	10.0	500	1.76
2.5	87.9	12.5	500	2.20
3.0	87.9	15.0	500	2.64

[1]The measured sodium concentration for the spiking solution in Pen Branch was 34.5 g/L. The sodium chloride concentration of 87.9 g/L was calculated using the following formula: [NaCl] = ([Na]/22.9) * 58.35.

APPENDIX 3 C

Forty-eight hour mortality observations on *C. dubia* in the Pen Branch sodium chloride toxicity test.

Sodium Chloride Concentration (g/L)	Replicate				Total	
	1		2			
	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	20
0.44	0	10	0	10	0	20
0.88	0	10	0	10	0	20
1.32	0	10	1	9	1	19
1.76	9	1	9	1	18	2
2.20	10	0	10	0	20	0
2.64	10	0	10	0	20	0

48-hour LC_{50} = 1.54 g/L (95% confidence limits = 1.47 - 1.61 g/L).

Forty-eight hour mortality observations on *D. ambigua* in the Pen Branch sodium chloride toxicity test.

Sodium Chloride Concentration (g/L)	Replicate				Total	
	1		2			
	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	20
0.44	0	10	0	10	0	20
0.88	0	10	0	10	0	20
1.32	2	8	3	7	5	15
1.76	10	0	10	0	20	0
2.20	10	0	10	0	20	0
2.64	10	0	10	0	20	0

48-hour LC_{50} = 1.40 g/L (95% confidence limits = 1.31 - 1.50 g/L).

Summary of statistical analysis for the *C. dubia* 48-hour acute test with sodium chloride in water collected from Pen Branch.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 8-29-97 DURATION: 48 hours
SAMPLE: NaCl in Pen Branch SPECIES: Ceriodaphnia dubia

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	1.544	1.320	1.760	0.440
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	1.537	1.465	1.613	0.148

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 0%
LC50: 1.537
95% Lower confidence: 1.465
95% Upper confidence: 1.613

BINOMIAL METHOD

Concentration (g/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.44	20	0	0	.9537D-04
0.88	20	0	0	.9537D-04
1.32	20	1	5	.2003D-02
1.76	20	18	90	.2012D-01
2.20	20	20	100	.9537D-04
2.64	20	20	100	.9537D-04

The binomial test shows that 1.32 and 1.76 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.9779 percent. An approximate LC50 for this data set is 1.544.

RESULTS USING MOVING AVERAGE

The moving average method cannot be used with this data set because no span which produces average angles bracketing 45 degrees also uses two percent dead between 0 and 100 percent.

RESULTS CALCULATED BY PROBIT METHOD

No convergence in 25 iterations. Probit method probably can not be use with this set of data.

Summary of statistical analysis for the *D. ambigua* 48-hour acute test with sodium chloride in water collected from Pen Branch.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 8-29-97 DURATION: 48 hours
SAMPLE:NaCl in Pen Branch SPECIES: Daphnia ambigua

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	1.426	1.320	1.760	0.440
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	1.398	1.307	1.495	0.188

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 0%
LC50: 1.398
95% Lower confidence: 1.307
95% Upper confidence: 1.495

BINOMIAL METHOD

Concentration (g/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.44	20	0	0	.9537D-04
0.88	20	0	0	.9537D-04
1.32	20	5	25	.2069D+01
1.76	20	20	100	.9537D-04
2.20	20	20	100	.9537D-04
2.64	20	20	100	.9537D-04

The binomial test shows that 1.32 and 1.76 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 97.9304 percent. An approximate LC50 for this data set is 1.426.

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

TEST: Sodium chloride test in Pen Branch water for *C. dubia*
Start Date: 8/29/97

Initial Water Chemistry

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	8.39	7.70	24.4	0.050	26	18
0.44	8.45	7.69	24.0	1.000		
0.88	8.63	7.68	24.5	1.930		
1.32	8.39	7.63	24.1	2.790		
1.76	8.54	7.66	24.2	3.680		
2.2	8.24	7.71	24.2	4.750		
2.64	8.36	7.66	24.2	5.540	24	18

24-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.36	7.65	24.0
0.44	8.44	7.71	24.0
0.88	8.45	7.70	24.1
1.32	7.49	7.69	24.5
1.76	8.35	7.68	24.5
2.2	8.39	7.70	24.2
2.64	8.37	7.71	24.3

48-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.44	7.62	24.5
0.44	8.47	7.65	24.4
0.88	8.50	7.69	24.4
1.32	8.22	7.68	24.2
1.76	8.47	7.68	24.4
2.2	8.05	7.67	24.6
2.64	8.33	7.65	24.2

TEST: Sodium chloride test in Pen Branch water for *D. ambigua*
Start Date: 8/29/97

Initial Water Chemistry

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	8.63	7.74	21.2	0.050	26	18
0.44	8.56	7.72	21.1	1.000		
0.88	8.44	7.68	21.4	1.930		
1.32	8.39	7.71	21.5	2.790		
1.76	8.55	7.70	21.1	3.680		
2.2	8.21	7.73	21.2	4.750		
2.64	8.34	7.77	21.3	5.540	24	20

24-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	7.90	7.56	21.7
0.44	8.31	7.58	21.5
0.88	8.13	7.53	21.4
1.32	8.14	7.54	21.5
1.76	8.04	7.53	21.7
2.2	8.33	7.54	21.8
2.64	7.88	7.55	21.7

48-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.77	7.92	20.2
0.44	9.03	7.81	19.7
0.88	9.09	7.82	19.3
1.32	9.45	7.82	19.3
1.76	9.32	7.75	19.5
2.2	9.06	7.76	19.8
2.64	9.19	7.74	19.6

APPENDIX 3 C - ANALYTICAL CHEMISTRY DATA
NaCl IN UPPER THREE RUNS ACUTE

Nominal and actual concentrations of sodium chloride in solutions used for acute toxicity testing. Actual concentrations of sodium were measured in the spiking solution, and final concentrations were calculated based on the amount of stock solutions spiked into the final dilution water. These values are applicable to the testing for both species. Values were determined by Trace Scan ICP.

Nominal NaCl Concentration in the Test Solution (g/L)	NaCl Concentration in the Spiking Solution (g/L)[1]	Amount of Spiking Solution Used (mL)	Final Volume of Test Solution (mL)	Actual Concentration of NaCl in Test Solution (g/L)
0.0625	87.9	0.312	500	0.05
0.125	87.9	0.625	500	0.11
0.25	87.9	1.25	500	0.22
0.5	87.9	2.5	500	0.44
1.0	87.9	5.0	500	0.88
1.5	87.9	7.5	500	1.32

[1]The measured sodium concentration for the spiking solution in Upper Three Runs was 34.5 g/L. The sodium chloride concentration of 87.9 g/L was calculated using the following formula: [NaCl] = ([Na]/22.9) * 58.35.

APPENDIX 3 C

Forty-eight hour mortality observations on *C. dubia* in the Upper Three Runs sodium chloride toxicity test.

Sodium Chloride Concentration (g/L)	Replicate				Total	
	1		2			
	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	20
0.05	0	10	0	10	0	20
0.11	1	9	2	8	3	17
0.22	10	0	8	2	18	2
0.44	10	0	10	0	20	0
0.88	10	0	10	0	20	0
1.32	10	0	10	0	20	0

48-hour LC_{50} = 0.15 g/L (95% confidence limits = 0.13 - 0.17 g/L).

Forty-eight hour mortality observations on *D. ambigua* in the Upper Three Runs sodium chloride toxicity test.

Sodium Chloride Concentration (g/L)	Replicate				Total	
	1		2			
	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	20
0.05	0	10	0	10	0	20
0.11	0	10	0	10	0	20
0.22	0	10	0	10	0	20
0.44	0	10	2	8	2	18
0.88	10	0	10	0	20	0
1.32	10	0	10	0	20	0

48-hour LC_{50} = 0.58 g/L (95% confidence limits = 0.53 - 0.64 g/L).

Summary of statistical analysis for the *C. dubia* 48-hour acute test with sodium chloride in water collected from Upper Three Runs.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 9-1-97 DURATION: 48 hours
SAMPLE: NaCl in Upper Three Runs SPECIES: Ceriodaphnia dubia

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	0.151	0.110	0.220	0.110
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	0.149	0.128	0.173	0.045

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 0%
LC50: 0.149
95% Lower confidence: 0.128
95% Upper confidence: 0.173

BINOMIAL METHOD

Concentration (g/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.05	20	0	0	.9537D-04
0.11	20	3	15	.1288D-00
0.22	20	18	90	.2012D-01
0.44	20	20	100	.9537D-04
0.88	20	20	100	.9537D-04
1.32	20	20	100	.9537D-04

The binomial test shows that 0.11 and 0.22 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.8510 percent. An approximate LC50 for this data set is 0.151

RESULTS USING MOVING AVERAGE

The moving average method cannot be used with this data set because no span which produces average angles bracketing 45 degrees also uses two percent dead between 0 and 100 percent.

RESULTS CALCULATED BY PROBIT METHOD

No convergence in 25 iterations. Probit method probably can not be use with this set of data.

Summary of statistical analysis for the *D. ambigua* 48-hour acute test with sodium chloride in water collected from Upper Three Runs.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 9-1-97 DURATION: 48 hours
SAMPLE: NaCl in Upper Three Runs SPECIES: Daphnia ambigua

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	0.577	0.440	0.880	0.440
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	0.581	0.529	0.637	0.108

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 0%
LC50: 0.581
95% Lower confidence: 0.529
95% Upper confidence: 0.637

BINOMIAL METHOD

Concentration (g/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.05	20	0	0	.9537D-04
0.11	20	0	0	.9537D-04
0.22	20	0	0	.9537D-04
0.44	20	2	10	.2012D-01
0.88	20	20	100	.9537D-04
1.32	20	20	100	.9537D-04

The binomial test shows that 0.44 and 0.88 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.9798 percent. An approximate LC50 for this data set is 0.577.

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

TEST: Sodium chloride test in Upper Three Runs water for *C. dubia*
Start Date: 8/29/97

Initial Water Chemistry

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	8.35	6.55	25.0	0.014	4	10
0.05	8.42	6.56	25.1	0.135		
0.11	8.46	6.58	25.2	0.254		
0.22	8.52	6.49	25.3	0.515		
0.44	8.36	6.53	25.2	1.000		
0.88	8.25	6.58	25.2	1.940		
1.32	8.35	6.47	25.3	2.880	4	8

24-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.15	6.47	24.5
0.05	8.25	6.50	24.6
0.11	8.32	6.52	24.5
0.22	8.16	6.49	24.4
0.44	8.19	6.53	24.7
0.88	8.27	6.52	24.4
1.32	8.35	5.46	24.5

48-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	7.97	6.75	24.1
0.05	8.12	6.62	24.4
0.11	8.03	6.57	24.0
0.22	8.16	6.52	24.0
0.44	7.95	6.45	24.3
0.88	8.34	6.33	24.2
1.32	8.21	6.29	24.0

TEST: Sodium chloride test in Upper Three Runs water for *D. ambigua*
Start Date: 8/29/97

Initial Water Chemistry

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	8.89	6.45	20.3	0.014	4	8
0.05	9.01	6.43	20.1	0.135		
0.11	9.03	6.46	20.1	0.254		
0.22	8.96	6.47	20.1	0.515		
0.44	8.88	6.41	20.0	1.000		
0.88	8.99	6.46	20.0	1.940		
1.32	8.91	6.46	19.9	2.880	4	10

24-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	9.10	6.52	20.6
0.05	9.11	6.52	20.5
0.11	8.96	6.53	20.6
0.22	8.87	6.51	20.7
0.44	9.13	6.49	21.0
0.88	8.86	6.51	21.1
1.32	8.95	6.56	20.9

48-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.95	6.68	20.3
0.05	8.92	6.56	20.4
0.11	9.02	6.34	19.8
0.22	8.76	6.50	20.0
0.44	9.13	6.33	19.9
0.88	9.05	6.23	19.8
1.32	9.27	6.20	19.9

APPENDIX 3 C - ANALYTICAL CHEMISTRY DATA
NaCl IN FOUR MILE BRANCH ACUTE

Nominal and actual concentrations of sodium chloride in solutions used for acute toxicity testing. Actual concentrations of sodium were measured in the spiking solution, and final concentrations were calculated based on the amount of stock solutions spiked into the final dilution water. These values are applicable to the testing for both species. Values were determined by Trace Scan ICP.

Nominal NaCl Concentration in the Test Solution (g/L)	NaCl Concentration in the Spiking Solution (g/L)[1]	Amount of Spiking Solution Used (mL)	Final Volume of Test Solution (mL)	Actual Concentration of NaCl in Test Solution (g/L)
0.125	89.4	0.625	500	0.11
0.25	89.4	1.25	500	0.22
0.5	89.4	2.5	500	0.45
1.0	89.4	5.0	500	0.89
1.5	89.4	7.5	500	1.34
2.0	89.4	12.0	600	1.79

[1]The measured sodium concentration for the spiking solution in Four Mile Branch was 35.1 g/L. The sodium chloride concentration of 89.4 g/L was calculated using the following formula: [NaCl] = ([Na]/22.9) * 58.35.

APPENDIX 3 C

Forty-eight hour mortality observations on *C. dubia* in the Four Mile Branch sodium chloride toxicity test.

Sodium Chloride Concentration (g/L)	Replicate				Total	
	1		2			
	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	20
0.11	0	10	0	10	0	20
0.22	0	10	0	10	0	20
0.45	7	3	9	1	16	4
0.89	9	1	10	0	19	1
1.34	10	0	10	0	20	0
1.79	10	0	10	0	20	0

48-hour LC_{50} = 0.37 g/L (95% confidence limits = 0.33 - 0.43 g/L).

Forty-eight hour mortality observations on *D. ambigua* in the Four Mile Branch sodium chloride toxicity test.

Sodium Chloride Concentration (g/L)	Replicate				Total	
	1		2			
	dead	alive	dead	alive	dead	alive
Control	0	10	0	10	0	20
0.11	0	10	0	10	0	20
0.22	0	10	0	10	0	20
0.45	0	10	0	10	0	20
0.89	10	0	10	0	20	0
1.34	10	0	10	0	20	0
1.79	10	0	10	0	20	0

48-hour LC_{50} = 0.63 g/L (95% confidence limits = 0.45 - 0.89 g/L).

Summary of statistical analysis for the *C. dubia* 48-hour acute test with sodium chloride in water collected from Four Mile Branch.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 9-1-97 DURATION: 48 hours
SAMPLE: NaCl in Four Mile SPECIES: Ceriodaphnia dubia

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	0.360	0.220	0.450	0.230
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	0.372	0.325	0.426	0.101

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 0%
LC50: 0.372
95% Lower confidence: 0.325
95% Upper confidence: 0.426

BINOMIAL METHOD

Concentration (g/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.11	20	0	0	.9537D-04
0.22	20	0	0	.9537D-04
0.45	20	16	80	.5909D+00
0.89	20	19	95	.2003D-02
1.34	20	20	100	.9537D-04
1.79	20	20	100	.9537D-04

The binomial test shows that 0.22 and 0.45 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.4090 percent. An approximate LC50 for this data set is 0.360.

RESULTS USING MOVING AVERAGE

The moving average method cannot be used with this data set because no span which produces average angles bracketing 45 degrees also uses two percent dead between 0 and 100 percent.

RESULTS CALCULATED BY PROBIT METHOD

No convergence in 25 iterations. Probit method probably can not be use with this set of data.

Summary of statistical analysis for the *D. ambigua* 48-hour acute test with sodium chloride in water collected from Four Mile Branch.

CT-TOX MULTI-METHOD PROGRAM
Binomial, Moving average, Probit, and Spearman-Karber Methods

TEST SUMMARY

DATE: 9-1-97 DURATION: 48 hours
SAMPLE: NaCl in Four Mile Branch SPECIES: Daphnia ambigua

METHOD	LC50	CONFIDENCE LIMITS		
		LOWER	UPPER	SPAN
Binomial	0.633	0.450	0.890	0.440
MAA	****	****	****	****
Probit	****	****	****	****
Spearman-Karber	0.633	****	****	****

**** = Limit does not exist.

TRIMMED SPEARMAN-KARBER SUMMARY

Trim: 0%
LC50: 0.633
95% confidence limits are unreliable

BINOMIAL METHOD

Concentration (g/L)	Number Exposed	Number Dead	Percent Dead	Binomial Probability (%)
0.11	20	0	0	.9537D-04
0.22	20	0	0	.9537D-04
0.45	20	0	0	.9537D-04
0.89	20	20	100	.9537D-04
1.34	20	20	100	.9537D-04
1.79	20	20	100	.9537D-04

The binomial test shows that 0.45 and 0.89 can be statistically sound conservative 95 percent confidence limits since the actual confidence level associated with these limits is 99.9998 percent. An approximate LC50 for this data set is 0.633.

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

TEST: Sodium chloride test in Four Mile Branch water with *C. dubia*
Start Date: 9/1/97

Initial Water Chemistry

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	8.11	6.40	24.1	0.026	6	8
0.11	8.26	6.45	24.5	0.260		
0.22	8.35	6.35	24.3	0.476		
0.45	8.64	6.44	24.4	0.847		
0.89	8.44	6.47	24.3	1.850		
1.34	8.15	6.40	24.1	2.830		
1.79	8.36	6.39	24.0	2.740	6	8

24-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	7.95	6.37	24.2
0.11	7.89	6.43	24.1
0.22	7.82	6.42	24.2
0.45	8.12	6.39	24.4
0.89	8.06	6.32	24.5
1.34	8.11	6.32	24.1
1.79	7.96	6.40	24.3

48-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.14	7.03	24.8
0.11	8.08	6.88	24.1
0.22	8.27	6.70	24.1
0.45	8.02	6.63	24.4
0.89	8.04	6.51	24.0
1.34	7.86	6.42	24.2
1.79	8.21	6.39	24.4

TEST: Sodium chloride test in Four Mile Branch water with *D. ambigua*
Start Date: 9/1/97

Initial Water Chemistry

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)	Conductivity (ms/cm)	Alkalinity (mg/L as $CaCO_3$)	Hardness (mg/L as $CaCO_3$)
Control	8.87	6.35	21.1	0.026	6	8
0.11	8.89	6.35	21.0	0.260		
0.22	8.71	6.39	21.4	0.476		
0.45	8.77	6.42	21.3	0.847		
0.89	8.65	6.40	21.1	1.850		
1.34	8.66	6.37	21.1	2.830		
1.79	8.76	6.38	21.1	2.740	6	8

24-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.54	6.45	21.0
0.11	8.56	6.46	20.9
0.22	8.49	6.42	20.8
0.45	8.46	6.48	20.8
0.89	8.48	6.42	21.0
1.34	8.44	6.42	21.0
1.79	8.44	6.44	20.8

48-Hour Readings

Concentration (g/L)	DO (mg/L)	pH	Temperature (°C)
Control	8.84	6.65	19.7
0.11	9.11	6.56	19.8
0.22	8.78	6.46	19.9
0.45	9.03	6.42	19.5
0.89	9.00	6.38	19.7
1.34	9.01	6.30	19.9
1.79	8.91	6.28	19.8