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COMPARATIVE TOXICITY OF SRC-I WASTEWATER
TO AQUATIC ORGANISMS

Final Report

By
H. C. Bailey

January 1984

Work Performed Under Contract No. AC05-78OR03054

International Coal Refining Company
Allentown, Pennsylvania

Technical Information Center
Office of Scientific and Technical Information
United States Department of Energy

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ABSTRACT

SRI International performed a series of acute and chronic toxicity studies on SRC-I wastewaters using fish, zooplankton, and algae as test organisms. The tests were designed to determine the toxicity of SRC-I wastewaters to aquatic organisms and, based on differences in toxicity of the various water samples, to evaluate the efficacy of various wastewater treatment methods. Survival data from acute and chronic daphnid studies indicate that phenol recovery markedly reduced wastewater toxicity. In treatment processes that did not include phenol recovery, powdered activated carbon reduced toxicity more effectively than granulated activated carbon. All treated water supported algal growth in excess of that in controls, particularly those waters subjected to phenol recovery. The toxicity of each SRC-I wastewater sample was compared with that of a corresponding synthetic salt solution to determine whether the salt load was the toxic element. The wastewaters typically exhibited higher toxicity than their associated salt solutions. The effect was greatest in the daphnid chronic studies.

The aquatic ecotoxicity tests were performed as part of ICRC's post-Baseline environmental R&D program. One objective of the program was to evaluate the impact of phenol recovery on effluent quality. Another objective was to assess the potential impact of wastewater discharge on aquatic organisms. The results of this study have been integrated with results from the rest of the R&D program, and are documented in ICRC's Integration Report for SRC-I Post-Baseline Environmental R&D (6).

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INTRODUCTION

At the request of the International Coal Refining Company (ICRC), SRI International performed a series of acute and chronic toxicity studies on SRC-1 wastewaters using fish (fathead minnows), zooplankton (Daphnia magna) and algae (Selenastrum capricornutum) as test organisms. The purpose of these tests was to determine the toxicity of SRC-1 wastewaters to aquatic organisms and, based on differences in toxicity of the various water samples, to evaluate the efficacy of various treatments proposed to reduce the toxicity of the wastewaters. The treatment and testing approaches are outlined in Figure 1. Not all studies were performed on each water sample because the volume of some of the samples supplied by ICRC was limited. All samples were screened using 48-hour acute studies with daphnids. Only the samples from the final treatment in each treatment train were used for daphnid chronic and 14-day algal studies, and only the samples from the final treatment of the first two treatment trains were used for 96-hour fathead minnow assays. All studies on SRC-I waters were conducted simultaneously with assays on salt solutions formulated to match the Na^+ , Ca^{++} , $\text{SO}_4^{=}$, and Cl^- balance of the SRC-I water under test. These comparisons between the toxicity of each SRC-I water and its corresponding synthetic salt solution were designed to determine whether the observed toxicity was due to the salt load or to other components of the wastewaters.

Tests described herein were initiated on May 22, 1983, and completed on 23 September, 1983. This final report and the raw data notebooks associated with the study will be held in Room C-3, Building 253 for one year from the date of the final report. Thereafter, these materials will be stored in the Records Retention Center at SRI.*

*Footnote added by ICRC: The aquatic ecotoxicity tests were performed as part of ICRC's post-Baseline environmental R&D program. One objective of the program was to evaluate the impact of phenol recovery on effluent quality. Another objective was to assess the potential impact of wastewater discharge on aquatic organisms. The results of this study have been integrated with results from the rest of the R&D program, and are documented in ICRC's Integration Report for SRC-I Post-Baseline Environmental R&D (6).

MATERIALS AND METHODS

Test Chemicals

Samples of SRC-I wastewaters were supplied by ICRC* in one-gallon bottles or larger drums. All containers were labelled by ICRC as to which wastewater they contained. SRC-1 waters were stored at $4 \pm 2^\circ\text{C}$ at SRI until used in experiments, at which time appropriate aliquots were brought up to the desired test temperatures. Before the samples were shipped to SRI, they were analyzed by ICRC for a number of parameters associated with water quality. The results of these analyses are shown in Table 1. To verify the sodium, calcium, sulfate, and chloride analyses performed by ICRC and to provide additional quantification of these ions for purposes of formulating the salt solutions, each wastewater sample was analyzed again by a local laboratory (Sequoia Laboratories, Redwood City, California). The results of these analyses and a comparison with ICRC results are shown in Table 2. In general, agreement between the values obtained by ICRC and Sequoia Laboratories was good although some results deviated by as much as 30 to 50%. Whether these deviations were due to analytical error or to changes in the samples during shipping or storage cannot be determined without further study.

Synthetic salt solutions were prepared on the basis of analytical results obtained by Sequoia Laboratories using reagent grade chemicals. For solutions corresponding to SRC-1 waters 7499, 7500, 7501, 7502, 7503, and 7504, we used NaCl, NaOH, H_2SO_4 , Ca_2SO_4 , and CaO to obtain the desired ionic balance. Because the pH of these solutions was difficult to maintain at a level corresponding to the appropriate SRC-1 waters, it appeared that we were omitting important components from the salt solutions. Consequently, solutions corresponding to SRC-I samples 7493, 7494, 9319, 7488, 7490, 7491, and 7492 incorporated a carbonate buffering system to aid in maintaining pH. These solutions were prepared with NaCl, CaCl, Na_2SO_4 , and NaHCO_3 . After preparation, samples of the solutions were analyzed by Sequoia Laboratories to provide verification of the actual ionic balance. These data are shown in Table 3.**

Diluent Water

Water used to prepare dilutions and synthetic salt solutions for the tests with daphnid and fathead minnows was obtained from Crystal Springs Reservoir and transported to SRI. This water is high-quality, soft water and is identical to the water used at SRI to rear aquatic organisms except that it has not been subjected to chlorination and dechlorination. Water from Crystal Springs was not used for dilutions, preparation of salt solutions or preparation of media associated with the algal tests; instead glass-distilled water was used to avoid potential problems with nutrient addition or contamination.

*See note on page 16.

**See note on page 19.

Test Organisms

Daphnia magna were reared under flow-through conditions at SRI. Daphnid cultures were fed algae (Selenastrum capricornutum) and a vitamin supplement daily. Daphnids used to initiate tests were less than 24 hour old; offspring from the first brood produced by the adult daphnids were not used in any tests. Cultures were maintained at a temperature of $20 \pm 1^\circ\text{C}$, with a photoperiod of 16 hours light:8 hours dark. Water flow into the culture aquaria was set to provide one to two tank volumes per day.

Fathead minnows (Pimephales promelas) were also reared under continuous-flow conditions at SRI. Juvenile minnows were used in all tests. They were maintained at $25 \pm 1^\circ\text{C}$, with a photoperiod of 16 hours light:8 hours dark, and fed Clark's trout diet and frozen adult brine shrimp. Minnows were not fed for 24 hours prior to testing.

Algae (Selenastrum capricornutum) were reared in algal assay media on shaker tables at $24 \pm 1^\circ\text{C}$. Continuous lighting at an intensity of 400 ft candles was provided. All algae used in the assays were taken from cultures in the log growth phase.

Test Procedures¹

Daphnia Acute Studies

Daphnids were exposed in 250-ml beakers containing 200 ml of test solution. Five daphnids were exposed in each beaker. The test duration was 48 hours, the nominal temperature was $17 \pm 1^\circ\text{C}$, and a photoperiod of 16 hours light and 8 hours dark was used.

For preliminary range-finding tests, five daphnids were used for each of five treatment levels to determine the range of concentrations to be used in the definitive tests. Concentrations used in the preliminary tests were 100, 10, 1, 0.1, and 0.01% wastewater. The control group consisted of five daphnids maintained in untreated water.

For the definitive acute studies, twenty organisms were divided between four replicates at each of six treatment levels, including the controls. Daphnids were distributed to the test beakers by stratified random assortment.

For the preliminary and definitive tests, dilutions of the test material were made using measuring devices of appropriate sizes--pipet, graduated cylinder, etc. Definitive test concentrations were on a logarithmic scale that spanned the effect and no-effect levels determined in the preliminary tests.

In the definitive tests, dissolved oxygen and pH were monitored daily in each of the replicate tests in the controls and the high, medium, and low concentrations. The specific conductance, hardness, and alkalinity were measured at the beginning of each test. Temperature was recorded hourly in the controls and daily in the high, medium, and low concentrations. Test beakers were examined daily for dead daphnids; any found were

removed. Disabled or immobilized organisms were also noted. The mortality data were used to calculate LC50 estimates and associated 95% confidence limits. Means, ranges, and standard deviations associated with the water quality parameters were also calculated.

Fathead Minnow Acute Studies

For preliminary range-finding tests, two minnows were exposed for 96 hours at each of five treatment levels to determine the range of concentrations to be used in the definitive tests. Concentrations used in the preliminary tests were 100, 10, 1, 0.1, and 0.01% wastewater. A control group was also used. These tests were conducted in 5-liter (L) animal jars containing two minnows in 2 L of test solution.

For the definitive tests, ten organisms were used in each of three replicates at each of six treatment levels, including the controls. Minnows were distributed to the test containers by stratified random assortment. These assays were performed in 19-L glass pickle jars containing 10 fish in 10 L of test solution. The test duration was 96 hours, the nominal test temperature was $22 \pm 1^{\circ}\text{C}$, and a photoperiod of 16 hours light and 8 hours dark was used.

All dilutions of the test material were made using measuring devices of appropriate size—pipets, graduated cylinders, etc. Definitive test concentrations were on a logarithmic scale that spanned the effect and no-effect levels determined in the preliminary tests.

In the definitive tests, dissolved oxygen and pH were monitored daily in each of the replicate tests in the controls and the high, medium, and low concentrations. The specific conductance, hardness, and alkalinity were measured in the same concentrations at the beginning of each test. Temperature was recorded hourly in the controls and daily in the high, medium, and low concentrations.

Test chambers were examined daily for dead fish; any found were removed. Control fish were measured and weighed at the conclusion of the test. Based on the mortality data, LC50 estimates and associated 95% confidence limits were calculated for 24- and 96-hour time intervals. Means, ranges, and standard deviations associated with the water quality parameters and fish size were also calculated.

14-Day Algal Assay

Algae were exposed for 14 days in 500-ml, foam-stoppered flasks containing 100 ml of test medium.² The test temperature was $24 \pm 1^{\circ}\text{C}$. Continuous illumination (4000 lux) was provided to the flasks. The initial inoculum of algae per flask was 200×10^4 cells contained in 50 ml of media for the preliminary tests or contained in 1 or 2 ml of media for the definitive tests.

Concentrations of wastewater used in the preliminary tests were 10, 1, 0.1, 0.01, and 0.001%. A control group was also used. For the

definitive tests, six treatment levels were tested in triplicate. Treatment flasks were placed randomly on the shaker table.

Serial dilutions of the test material were made using measuring devices of appropriate sizes—pipets, graduated cylinders, etc. Definitive test concentrations were in a descending geometric series beginning at a concentration of 100% wastewater.

Because the wastewater was a significant component of the total 100 ml/flask, some of the effect on growth could have been due to dilution of the nutrient salts. Therefore, concentrations of 10% and greater were accompanied by controls in which the nutrient medium was appropriately diluted with distilled water. This enabled us to differentiate between the effects of the wastewater and the effects of reduced concentrations of media components. An additional control series was also used in which the salt concentrations were adjusted to match those in the test concentrations of wastewater.

In the definitive studies, pH was monitored in each of the test solutions and adjusted to 7.7 ± 0.3 with either HCl or NaOH before the algal inoculum was added. Temperature was monitored every 24 hours. Cell counts were made with an electronic particle counter at 4, 6, 8, 11, and 14 days. Based on cell counts, growth curves from the pooled replicates were plotted on semilogarithmic paper. Percent inhibition of the treatment levels compared with the controls was calculated using analysis of variance (ANOVA), and no-effect levels were determined. EC50 values for 14 days were estimated using graphical interpolation.

28-Day Daphnid Chronic Studies

Ten daphnids were used in each of three replicate treatment groups at each of six treatment levels, including the controls. The daphnids were distributed to the test dishes by stratified random assortment. The test containers were 1000-ml Carolina culture dishes containing 500 ml of test solution. The test duration was 28 days, the temperature was maintained at $20 \pm 1^\circ\text{C}$, and a photoperiod of 16 hours light and 8 hours dark was used. For food, each dish was supplied daily with a mixture of algae (*S. capricornutum*) and vitamins at a rate of approximately 7.5 mg/L (wet weight; approximately 15,000 cells/ml).

Serial dilutions of the wastewaters were made using appropriate measuring devices—pipets, graduated cylinders, etc. A geometric series of concentrations with an interval of about $\sqrt{10}$ was used, starting with the 48-hour LC50 as the highest concentration and ending at about 1/100 of the 48-hour LC50 as the lowest concentration. The test solutions were renewed on Monday, Wednesday, and Friday. Dissolved oxygen and pH were monitored on Monday, Wednesday, and Friday in one of the replicate dishes in each concentration before and after each renewal. The specific conductance, hardness, and alkalinity in the diluent water were measured weekly. Temperature in one of the control dishes was recorded hourly.

Test dishes were examined on Monday, Wednesday, and Friday for dead organisms and young; any found were removed at each observation period.

The following parameters were used to determine effect and no-effect concentrations: mortality at 7, 14, 21, and 28 days; the time at which the first young were produced; and the number of young produced per female at 7, 14, 21, and 28 days. Means, ranges, and standard deviations associated with the water quality data were also calculated.

Water Quality Analyses

Dissolved oxygen and conductivity measurements were made with a Yellow Springs Instrument Co. probes, pH was determined with an Orion Ionalyzer, and temperature in individual containers was measured with a glass, mercury-filled thermometer. Hourly temperature recordings in the control containers were made with a Honeywell recording thermograph. Hardness, alkalinity, and acidity determinations on the diluent water were obtained with Hach chemical titrants.

Statistical Methods

Estimation of the LC50³

To estimate the median lethal concentration (LC50) we used a computerized program developed at SRI and composed of several statistical methods for estimating LC50s and EC50s. For this project, we chose estimates derived only from the log-probit or binomial methods. The specific method that we selected depended on the number and pattern of partial responses (i.e., > 0%, < 100%).

We used the binomial method when there were no partial responses or when only an incongruous response or nonresponse occurred. A single response (e.g., death) at a concentration was considered to be incongruous when no responses occurred at the next higher concentration. Also, a single nonresponse (e.g., non-death) at a concentration was considered to be incongruous when all of the organisms responded at the next lower concentration. We also used the binomial method when other incongruous response patterns occurred (e.g., 0, 0, 100, 90, 100, and 100% mortality in a series of six increasingly higher test concentrations).

The binomial method is valid regardless of the form of the underlying tolerance distribution and therefore gives statistically valid, but conservative, confidence intervals in all cases. It is the only appropriate method when a data set contains no partial responses. The method is a two-step process. In the first step, at each concentration level with an observed mortality of 50% or more, a significance level is computed for the hypothesis that the true mortality at that concentration is 50% or less, using only the observations at that concentration. In the second step, at each concentration level with an observed mortality of less than 50 percent, a significance level is computed for the hypothesis that the true mortality at that concentration is 50 percent or more. An estimate of the LC50 is also provided as the geometric average of the adjacent concentrations with 0 and 100% mortality. The 95% confidence interval for the LC50 is the shortest interval (with end-points at the concentrations or at plus or minus infinity) such that at the upper end-point and all higher

concentrations, 50% or more of the animals have died and the significance level is 0.025 or less, and at the lower end-point and all lower concentrations, less than 50% of the animals have died and the significance level is 0.025 or less.

The probit method is a parametric technique that depends on the assumption that the tolerance of the organisms to the test material follows a normal distribution. The computer routine performs the probit analysis twice—once for the concentration levels expressed in linear units and once for the concentration levels expressed in logarithmic units. In either case, Berkson's adjustment (one-half of a response at the highest concentration with no response and one-half of a nonresponse at the lowest concentration with all 100 percent response) is used when there is only one partial response.

The LC50 estimate is the maximum likelihood estimate for the mean of the tolerance distribution. The "unadjusted" confidence interval for the LC50 is derived by inverting the likelihood ratio test for determining whether any specified concentration is the LC50. A Chi Square test is computed to determine how well the estimated tolerance distribution fits the data (which are also plotted). In computing this test, adjacent concentration levels are collapsed until the expected responses (mortality and nonmortality) are everywhere greater than 2.0. Finally, if the probability of poor fit is 0.75 or greater, a heterogeneity factor is derived from the Chi Square test and the confidence interval is adjusted outward, using the heterogeneity factor.

Chronic Tests

These tests were designed to detect statistically significant differences between control and treatment groups. It was assumed that any treatment effect would be detrimental to the organism, so that the tests were all one-tailed in the direction of greater mortality or reduced reproduction. One of two types of test was used, depending on the type of data analyzed:

1. Proportional data, such as survival, were analyzed in an untransformed state using Fisher's Exact Test for analysis of 2 x 2 contingency tables.⁴ Probability levels of less than 0.01 for each comparison were flagged as statistically significant, yielding experiment-wise alpha levels of approximately 0.05.
2. Nonproportional data, including length and young production, were first subjected to an analysis of variance (ANOVA), using concentration as the independent variable.⁵ The mean square error from the ANOVA was then used to perform Dunnett's test of control-treatment differences.

Transformations

In cases where homogeneity of variance assumptions were unwarranted, a variance-stabilizing transformation was applied before the statistical tests were performed. This transformation was a square root

transformation, $Y = \sqrt{X}$, which was used for the fertility measures, which were assumed to be Poisson-distributed.

We analyzed data from the algal assays by applying the SAS Statistical Package for the ANOVA package.⁵ Whenever the F test was significant at the 5% level, control group contrasts were examined to identify the toxicant-exposed groups that differed significantly from the controls. Because the tests for homogeneity of variance (Cochran's C and Bartlett-Box's F tests) typically indicated that the variances were unequal, we computed the contrasts using separate variance estimates.

RESULTS AND DISCUSSION

The results of the 48-hour daphnid acute studies are presented in Table 4. In samples without phenol recovery, it appears that granulated activated carbon (GAC) reduces the toxicity of the wastewater but that subsequent ozonation increases it markedly. This observation suggests that the GAC treatment is only partially effective at removing organics and that those remaining are oxidized into more toxic forms by the ozonation treatment. In samples in which powdered activated carbon (PAC) was added to the bioreactor, the LC50 values are similar to each other and to those for the corresponding synthetic salt solutions. This suggests that PAC treatment removes most of the toxic materials present and that further treatment with GAC does not improve the wastewater. In contrast to the results obtained from wastewater treated with GAC, ozonation of PAC-treated wastewater did not significantly increase its toxicity, thus providing further indication of the efficacy of PAC treatment.

A dramatic reduction in the acute toxicity of SRC-1 wastewaters to daphnids occurred when they were subjected to phenol recovery. Daphnids exposed to wastewaters subjected to phenol recovery exhibited no marked mortality at concentrations as high as 100%. Unfortunately, the lack of significant acute toxicity precludes determining the effects of further treatment (e.g., PAC, GAC, and ozonation) on the quality of the wastewater following phenol recovery.

Water quality associated with the daphnid acute studies is summarized in Table 5.

Results of the acute toxicity studies on fathead minnows are summarized in Table 6. Only GAC- and PAC-treated wastewaters not subjected to phenol recovery were tested on fathead minnows. The comparatively high toxicity of the former water again suggests that PAC treatment markedly reduces the toxicity of SRC-1 wastewater. For example, the 96-hr LC50 for fathead minnows exposed to 9319 was 61.1%. In contrast, minnows exposed to 7491 and the synthetic salt solutions showed no appreciable mortality at concentrations as high as 100%. Because waters resulting from intermediate steps in these pathways were not tested on minnows, we cannot assess whether ozonation or other treatment steps would have affected the acute toxicity of the wastewater in a manner similar to that observed with the daphnids. Water quality data associated with these tests are summarized in Table 7.

Survival of daphnids during a 28-day chronic exposure to SRC-1 wastewater 9319 and the corresponding synthetic salt solution is shown in Table 8. Survival in the wastewater appeared unaffected at concentrations of 2.4% and below and did not appear to be affected in the synthetic salt solution at the highest concentration tested--24%. The number of young produced per daphnid did not seem to be affected in any of the concentrations of wastewater (0.2 to 7.6%) in which at least some adults survived (Table 9). The affect of the synthetic salt solution on

production of young is more difficult to interpret because this parameter was reduced in concentrations of 0.2 to 2.4 and 24.0% but not in 7.6%. The fact that the number of young was higher in 7.6% than in the controls suggests that the effects seen on reproduction at 0.2 to 2.4% were not toxicant-related. Neither the wastewater nor the salt solution appeared to retard the onset of young production. Furthermore, the wastewater did not appear to affect the number of young produced per reproductive day (Table 10). However, the salt solution appeared to reduce the number of young produced per day at concentrations of 0.8, 2.4, and 24.0%. As stated previously, the lack of effect of 7.6% suggests that the effects seen at 0.8 and 2.4% salt solution were not toxicant-related. On the basis of these data, it appears that 9319 contained materials that induced toxicity greater than would be expected from just the salt content of the water alone. The toxic effect was limited primarily to survival and the no-effect level was between 2.4 and 7.6%.

Survival of daphnids exposed for 28 days to SRC-1 wastewater 7491 and the corresponding synthetic salt solution is shown in Table 11. Survival was reduced significantly at concentrations of 13 and 42% wastewater, but the only significant effect in the salt solution occurred at 13%. Because of the lack of effect at 42%, the effect seen at 13% was probably an artifact and not toxicant-related. The survival data are confounded by the mortality between Days 21 and 28. Nearly all of the deaths occurred on Day 26 and did not appear to be related to the toxicants as they occurred in all concentrations (including 0%) in both the wastewater and synthetic salt assays as well as in the test on 9319, which was conducted at the same time. The water quality data (Table 21) do not indicate a problem that would cause the deaths. The reproductive data are shown in Tables 12 and 13. Neither the wastewater nor the salt solution had any effect on the onset of reproduction. The salt solution did not appear to have any effect on the total young per female or the number of young per day at any of the concentrations tested (0.4 to 42.0%). In contrast, both of these parameters were significantly reduced in daphnids exposed to SRC-1 wastewater at concentrations of 4.2 to 42.0% and were appreciably, although not statistically significantly so, reduced at a concentration of 1.3%. From these data, it appears that SRC-1 wastewater 7491 is more toxic than the salt solution alone. In contrast to the results obtained from 9319, the effect of 7491 was primarily on reproduction. Based on the statistical analysis, the no-effect level was between 1.3 and 4.2%. This level should be treated with caution because the data may suggest an effect at 1.3% as well.

The effect of SRC-1 wastewater 7501 and its corresponding synthetic salt solution on survival of daphnids during a 28-day exposure is shown in Table 14. Survival in the wastewater was reduced at concentrations of 32 and 100% but was not affected by the salt solution at concentrations of 1.0 to 100.0%. The effects of the wastewater and salt solution on daphnid reproduction are shown in Tables 15 and 16. Neither the salt solution (1.0 to 100%) nor the wastewater (1.0 to 32.0%) appeared to affect the time to first spawn. It is interesting that the controls for the salt solution did not commence spawning until Day 17, compared with 11 to 13 days for the other treatment groups. The number of young per day was significantly reduced at 32% for the wastewater but not in the salt solutions at concentrations up to and including 100%. The most marked effect of the

wastewater occurred on total young produced, which was significantly reduced at concentrations of 3.2% and higher. In contrast, reproduction appeared unaffected by the salt solution at concentrations of 1.0-100.0%. These data again indicate that the wastewater 7501 was more toxic than the corresponding salt solution under conditions of chronic exposure. Judging from the reproductive effects, it appears that the no-effect level is between 1.0 and 3.2%.

The effect of SRC-1 wastewater 7504 and its corresponding salt solution on survival of daphnids during 28-day exposures is summarized in Table 17. Survival was significantly reduced in the wastewater at 32 and 100% and in the salt solution at 100%. The effect of the wastewater and salt solution on reproductive parameters are shown in Tables 18 and 19. Neither the wastewater (1.0 to 32.0%) nor the salt solution (1.0 to 100.0%) appeared to have any effect on time to first reproduction. The number of young produced per day and the total young produced per female were significantly reduced by the wastewater at a concentration of 32.0%. A reduction was also apparent at 10% but was not statistically significant. There were no statistically significant effects on reproduction at any of the concentrations (1.0 to 100.0%) of the salt solution. These data indicate that wastewater 7504 is more toxic than its corresponding salt solution. In terms of statistically significant effects, both survival and reproduction were affected at 32.0% and higher concentrations. However, the reproductive data strongly suggest that a toxicant-related effect also occurred at 10% wastewater. Thus, depending on how conservative one wishes to be, the no-effect level of wastewater 7504 could be estimated to lie between 3.2 and 10% or between 10 and 32.0%.

It is tempting to speculate about differences in chronic toxicity between the wastewaters and thus arrive at some conclusions about the efficacy of the different treatment processes. With this in mind, Table 20 shows the concentrations surrounding the no-effect level for each of the wastewaters tested in daphnid chronic studies.*

The mortality data are generally consistent with the expectation of improvement in survival coincident with improved treatment processes; the effect appears most marked in the samples subjected to phenol recovery (7501 and 7504). In contrast, the reproductive data vary markedly without any apparent relationship to the treatment processes. Whereas the survival data were generally consistent between tests (e.g., generally excellent survival among controls and unaffected treatment groups at least through 21 days), the reproductive data varied considerably in areas such as time to first spawn and total young produced per adult daphnid. This variability also showed up within tests and in many cases precluded assigning statistical significance to what were probably toxicant-related decreases in reproduction. For example, a decrease of 23% in average young per adult was found significant in the test on 7501, but decreases of 37 and 32% were not found significant in tests on 7491 and 7504, respectively. Unfortunately, the experimental approach was designed to differentiate between the effects of the wastewaters and their salt solutions and not between the wastewaters. To determine the latter differences, it would be necessary to conduct the assays simultaneously on all four wastewaters, using identical concentrations.

*See the footnote on page 39.

Water quality data associated with chronic studies on SRC-1 wastewaters and their associated salt solutions are summarized in Table 21.

The effects of SRC-1 wastewaters 9319, 7491, 7501, and 7504, their corresponding salt solutions, and dilute media on the growth of S. capricornutum after 4 and 14 days of exposure are summarized in Table 22. In addition, the growth of the algae over the 14-day exposure periods is shown in Figures 2 through 10. The growth of algae exposed to 9319 was not markedly inhibited except at 100% wastewater. The growth of algae exposed to the 100% salt solution (9319-S) was also significantly reduced, which suggests that the effect seen in 100% wastewater was due largely to the salt concentration. All other concentrations of the wastewater supported somewhat higher growth than that in the controls. A similar response occurred in the salt solution, thus suggesting that the growth increases were due to increased salt concentrations.

The growth of algae exposed to 7491 and its corresponding salt solution was significantly decreased compared with the controls at 100%. Algal growth in other concentrations of the salt solution closely paralleled the growth of the controls. Growth at 3.2 and 10.0% wastewater was markedly increased over the controls at the end of the 14-day period. Because a similar response did not occur in algae exposed to the salt solution alone, the observed response suggests that this wastewater contains additional materials that can support algal growth.

Sample 7501 was the only wastewater that supported algal growth at 100% at a level comparable with that in the controls. Although this response lagged behind that of the other concentration groups, it reached the control level by Day 11. This initial lag suggests that photolysis of some toxic component may have occurred. At 10% concentration, the wastewater also clearly supported algal growth above that observed in the controls and salt solutions. Growth in the salt solution at 100% did not approach that in the controls.

Growth in 100% wastewater 7504 appeared to respond similarly, although not as dramatically, to the response observed in 7501. Again, an initial lag in growth was followed by a continual increase in number of cells, although the levels had not reached those in the controls by the end of the 14-day exposure period. For 7504, concentrations of 3.2, 10, and 32% supported growth in excess of that in the controls or the corresponding salt solutions.

When one attempts to interpret data from algal tests, there are two areas of concern: determining what concentrations are toxic and determining whether the material under test is likely to stimulate algal growth—a process that in the field might result in nuisance "algal blooms." For all of the wastewaters, a concentration of 100% resulted in a marked lag or reduction in growth compared with controls. Because a somewhat similar response occurred in the salt solutions, probably at least part of the observed effect on the algae was due to the salt load of the wastewaters. This effect was least in sample 7501 and was not apparent in any of the wastewaters or salt solutions at 32%. Consequently, the 14-day EC50 for the wastewaters would be estimated at 56%.

All of the wastewaters increased algal growth above the control or the corresponding salt solution groups. This effect was most apparent at concentrations of 3.2, 10.0, and 32.0% for 7501 and 7504 and at 3.2 and 10% for 9319 and 7491. The effect was not trivial; for example, the number of cells per milliliter in a 10% solution of 7491 was twice the number in the controls or corresponding salt solution after a 14-day exposure. On the basis of these data, it appears that phenol recovery enhances the wastewater as a nutrient source for the algae. This conclusion is based on the increased growth that was observed in a 32% concentration of 7501 and 7504 but not of 9319 or 7491.

Further support for the hypothesis that the wastewaters support algal growth comes from data on algae grown in diluted algal assay media (Table 22 and Figure 10). In contrast to algae grown in the wastewaters whose cell counts frequently exceeded those of the controls, algae grown in diluted media had cell counts equal to or less than the controls. Thus it appears that components of the wastewaters are a satisfactory substitute for nutrients found in the algal assay medium when that medium is diluted by addition of wastewater.

CONCLUSIONS *

- Survival data from acute and chronic daphnid studies indicate that phenol recovery markedly reduces the toxicity of the wastewater.
- In the treatment processes that did not include phenol recovery, powdered activated carbon was more effective than granulated activated carbon in reducing toxicity.
- Water from all treatment processes supported algal growth in excess of that in the controls. This effect was most apparent in the waters subjected to phenol recovery.
- The wastewaters typically exhibited higher toxicity than did their associated salt solutions. However, the effect generally was not large in the acute studies, ranging between factors of 0 and 3. In the daphnid chronic studies, survival was affected at concentrations five or six times less than in the salt solutions. Daphnid reproduction was the most sensitive parameter with effects being seen at concentrations 20 to 50 times less than in the salt solutions.

*Footnote added by ICRC: See ICRC's Integration Report⁶ for further discussion of the impact of these test results.

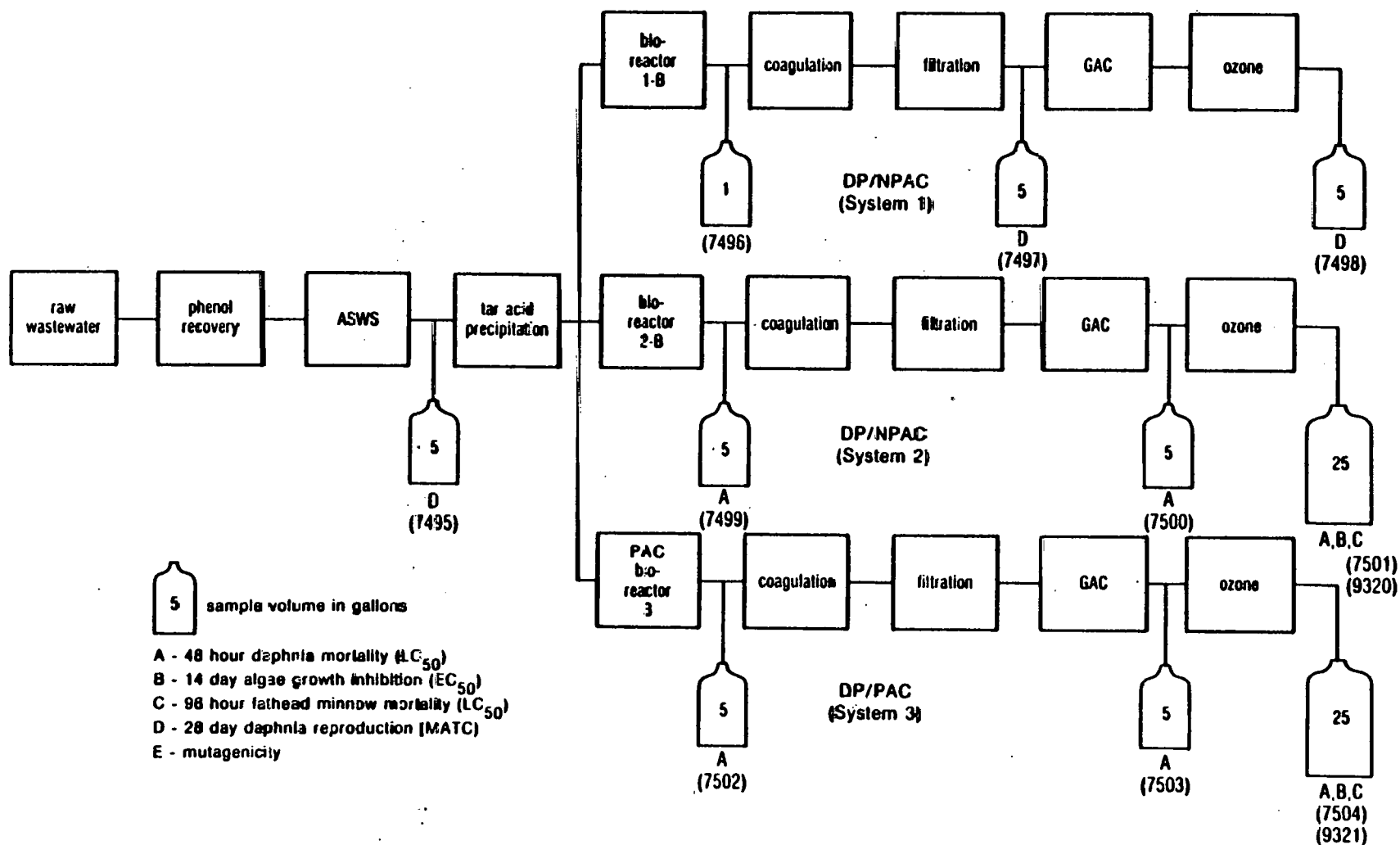
RECOMMENDATIONS *

- Perform fathead minnow acute studies on the treatment processes subjected to phenol recovery.
- Perform fathead minnow 30-day embryolarval studies on the end product of all four treatment processes. If the studies cannot be conducted simultaneously, paired comparisons (9319-7491 and 7501-7504) should be performed simultaneously.
- Depending on the relative costs associated with the different treatment processes, consider repeating the daphnid chronic studies in order to reduce ambiguities associated with selecting between the treatments on the basis of toxicity. These studies should be performed simultaneously, incorporating a different experimental design to allow better differentiation of the effects on reproduction. Suggested modifications include exposure of isolated daphnids using 10 replicates and identical concentrations.

*Footnote added by ICRC: Please see ICRC's Integration Report⁶ for alternative recommendation for further aquatic ecotoxicity testing.

Figure 1

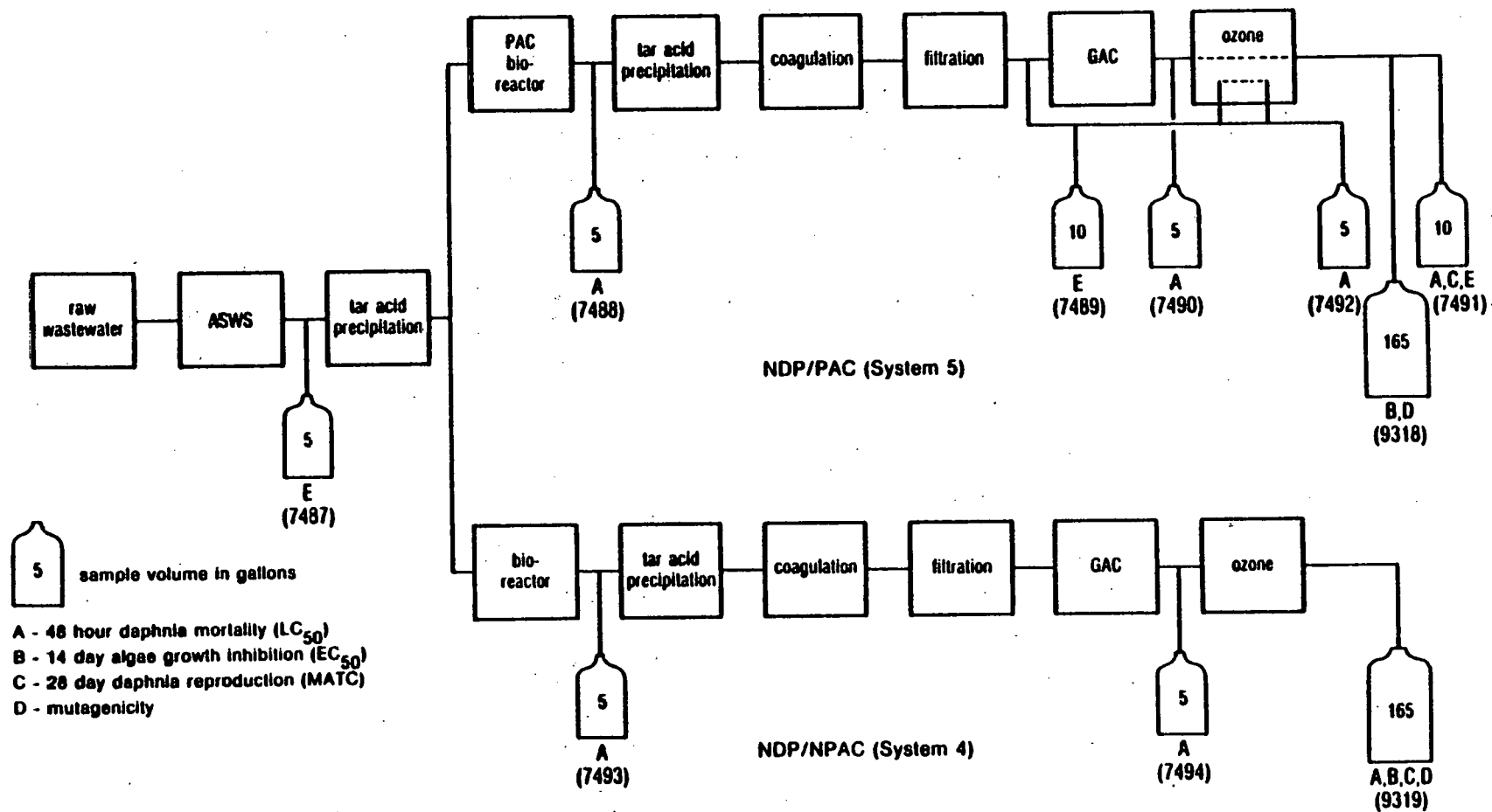
**Toxicology Study Sampling Points for
Aquatic Bioassay and Mutagenicity Tests: Systems 1, 2, and 3**



*Footnote added by ICRC: Note that raw wastewater was obtained from the Ft. Lewis, Washington SRC pilot plant. See reference 7 for more details on sample history.

Figure 1 (cont'd)

**Toxicology Study Sampling Points for
Aquatic Bioassay and Mutagenicity Tests: Systems 4 and 5**



*Footnote added by ICRC: Note that raw wastewater was obtained from the Ft. Lewis, Washington SRC pilot plant. See reference 7 for more details on sample history.

Table 1

CHARACTERIZATION OF WATER QUALITY OF SRC-1 WASTEWATERS

Parameter	Treatment Path I			Treatment Path II				Treatment Path III			Treatment Path IV		
	7493	7494	9319	7488	7490	7491*	7492	7499	7500	7501	7502	7503	7504
Alk-pH 4.3 as mg/L													
CaCO ₃	103	122	0	538	285	268	173	87	131	143	304	186	173
BOD ₅ (mg/L)	5	4	0	24	ND(1)	ND(1)	11	46	ND(1)	ND(2)	35	1	ND(2)
COD (mg/L)	1603	424	114	810	274	143	314	233	74	46	282	115	47
TOC (mg/L)	400	ND(1)*	12	210	ND(1)	ND(1)	10	52	ND(1)	ND(1)	61	ND(1)	ND(1)
TIC (mg/L)	20	9	0	140	53	46	37	90	13	12	93	27	22
TDS (mg/L)	10860	10150	9410	11090	10480	10510	10330	4740	4970	4970	4730	4650	4720
TSS (mg/L)	420	16	18	300	6	8	16	145	10	2	220	8	2
pH	6.5	8.8	0	7.3	7.7	7.8	6.7	7.1	8.2	7.6	7.7	8.2	7.6
Conductivity (u/mho)	14600	13900	0	14400	14400	14400	14700	6340	7330	7290	6220	6717	6750
Color Pt-Co Units	7000	ND(5)	ND(5)	3000	25	ND(5)	ND(5)	1500	ND(5)	10	1000	ND(5)	ND(5)
Turbidity-NTU	130	1.8	0	120	3.6	.45	.62	45	.25	1.7	85	.25	.75
Chloride (mg/L)	2240	2400	2580	2300	2500	2470	2525	360	650	580	380	615	650
Cyanide (mg/L)	.243	ND(.004)	ND(.004)	ND(.004)	ND(.069)	ND(.074)	ND(.004)	.688	ND(.005)	ND(.004)	.957	ND(.004)	ND(.004)
Thiocyanate (mg/L)	12.5	ND(.2)		2.3	.3	ND(.2)	ND(.2)	2.8	ND(.2)	ND(.2)	2	ND(.2)	ND(.2)
Fluoride (mg/L)	.92	.74	.713	1.1	.81	.81	.91	.41	.41	.37	.37	.43	.35
Ammonia-N (mg/L)	30	27	25	4	4	2	ND(.3)	25	ND(.3)	ND(.3)	52	ND(.3)	ND(.3)
Organic-N (mg/L)	49	9	0	36	9	10	3	9	3	2	21	ND(.3)	ND(.3)
Nitrite-N (mg/L)	ND(.25)	.59	0	1.1	.43	.33	ND(.25)	15	14	ND(.25)	103	81.7	ND(.25)
Nitrate-N (mg/L)	131	101	123	67.3	67	54.4	45.1	135	121	71.9	36	28.3	80.5
Phenolics (u/L)	.093	.001	ND(.025)	.026	.004	.063	ND(.001)	.035	.007	.005	.032	.01	.013
Sulfide, total (mg/L)	ND(.001)	ND(.001)	0	ND(.001)	.006	.004	ND(.001)	ND(.001)	.01	.004	.25	.006	ND(.001)
T-Phosphate ^{3P} (mg/L)	13.1	.04	.03	15.8	.21	.16	.12	4.3	.08	.095	10.6	.09	.09
Silica (mg/L)	34	17	22.5	403	18.3	8.7	17	20.3	6.6	7.5	21.4	9.2	8.5
Sulfate (mg/L)	3580	3500	3000	3500	3400	3400	3300	1900	1800	2000	2000	1800	1800
Aluminum (mg/L)	1.41	.13	ND(.4)	2.26	.16	.24	ND(.09)	1.22	.17	.17	2.01	.1	ND(.09)
Calcium (mg/L)	754	558	0	839	656	627	593	375	170	170	562	280	273
Iron (mg/L)	.39	.05	ND(.05)	1.22	.72	.18	.35	.94	ND(.04)	ND(.04)	1.36	ND(.04)	ND(.04)
Magnesium (mg/L)	12.1	14.8		12.6	14	14.4	14.4	14.1	18.6	19.8	14.2	17.6	18.6
Manganese (mg/L)	.012	.224		.057	.239	.101	.108	.008	.202	.19	.02	.139	.114
Antimony (mg/L)	ND(.2)	ND(.2)		ND(.2)	ND(.2)	ND(.2)	ND(.2)	ND(.2)	ND(.2)	ND(.2)	ND(.2)	ND(.2)	ND(.2)
Arsenic (mg/L)	ND(.015)	ND(.015)		.1	.04	.07	ND(.015)	ND(.015)	ND(.015)	ND(.015)	.06	.09	.35
Barium (mg/L)	.6	.8		1.5	ND(.5)	.7	.6	ND(.5)	ND(.5)	ND(.5)	ND(.5)	ND(.5)	ND(.5)
Beryllium (mg/L)	ND(.003)	ND(.003)		ND(.003)	ND(.003)	ND(.003)	ND(.003)	ND(.003)	ND(.003)	ND(.003)	ND(.003)	ND(.003)	ND(.003)
Boron (mg/L)	84	62		76	67	65	91	56	44	59	70	52	45
Cadmium (mg/L)	ND(.01)	.01		ND(.01)	.094	.09	.116	ND(.01)	ND(.01)	ND(.01)	ND(.01)	ND(.01)	ND(.01)
Chromium (mg/L)	ND(.03)	ND(.03)		.03	ND(.03)	ND(.03)	ND(.03)	ND(.03)	ND(.03)	ND(.03)	ND(.03)	ND(.03)	ND(.03)
Copper (mg/L)	.05	.04		.06	.04	.04	.06	.07	ND(.02)	.02	.07	ND(.02)	ND(.02)
Lead (mg/L)	ND(.11)	ND(.11)		ND(.11)	ND(.11)	ND(.11)	ND(.11)	ND(.11)	ND(.11)	ND(.11)	ND(.11)	ND(.11)	ND(.11)
Mercury (mg/L)	.0008	.0005		.0004	.0018	ND(.0002)	.0004	ND(.0004)	ND(.0002)	ND(.0002)	ND(.0004)	ND(.0002)	ND(.0002)
Nickel (mg/L)	ND(.06)	ND(.06)		ND(.06)	ND(.06)	ND(.06)	ND(.06)	ND(.06)	ND(.06)	ND(.06)	ND(.06)	ND(.06)	ND(.06)
Thallium (mg/L)	ND(.08)	ND(.08)		ND(.08)	ND(.08)	ND(.08)	ND(.08)	ND(.08)	ND(.08)	ND(.08)	ND(.08)	ND(.08)	ND(.08)
Selenium (mg/L)	.3	ND(.015)		ND(.015)	ND(.015)	ND(.03)	ND(.015)	.085	ND(.015)	ND(.025)	ND(.04)	ND(.026)	ND(.026)
Silver (mg/L)	.058	.033		.073	.055	.044	.039	ND(.006)	ND(.006)	ND(.006)	.022	.014	.014
Sodium (mg/L)	1900	1700		2240	2000	1900	1900	810	920	960	790	770	790
Titanium (mg/L)	ND(.3)	ND(.3)		ND(.3)	ND(.3)	ND(.3)	ND(.3)	ND(.3)	ND(.3)	ND(.3)	ND(.3)	ND(.3)	ND(.3)
Vanadium (mg/L)	ND(.15)	ND(.15)		ND(.15)	ND(.15)	ND(.15)	ND(.15)	ND(.15)	ND(.15)	ND(.15)	ND(.15)	ND(.15)	ND(.15)
Zinc (mg/L)	1.569	ND(.01)		.394	.083	.059	.365	.066	.01	.01	.07	.01	.01

*The composition of sample No. 9318 (Figure 1) closely resembles that of No. 7491.

Table 2

SODIUM, CALCIUM, CHLORIDE, AND SULFATE CONCENTRATIONS (mg/L)
IN SRC-1 WASTEWATERS *

	Sodium		Calcium		Sulfate		Chloride	
	ICRC	Seq. Labs	ICRC	Seq. Labs	ICRC	Seq. Labs	ICRC	Seq. Labs
7493	1900	2000	754	540	3500	3600	2240	2300
7494	1700	2200	558	840	3500	3000	2400	2400
9319	--	2200	--	800	3000	3000	2580	2600
7488	2240	2200	839	580	3500	3900	2300	2400
7490	2000	2200	656	800	3400	3600	2500	2700
7491	1900	2300	627	770	3400	3600	2470	2700
7492	1900	2200	593	800	3300	3500	2525	2700
7499	810	900	375	420	1900	1900	360	400
7500	920	1200	170	190	1800	1900	650	770
7501	960	1400	170	130	2000	2300	580	640
7502	790	650	562	770	2000	2100	380	400
7503	770	1200	280	330	1800	1600	615	820
7504	790	1000	273	420	1800	2100	650	570

*Footnote added by ICRC: The concentrations of the ions in the synthetic salt solutions prepared by SRI, which are shown in Table 3, are close to those shown in this table.

Table 3

SODIUM, CALCIUM, CHLORIDE, AND SULFATE CONCENTRATIONS (mg/L)
IN SALT SOLUTIONS CORRESPONDING TO SRC-1 WASTEWATERS

	Parameter (mg/L)			
	<u>Sodium</u>	<u>Calcium</u>	<u>Sulfate</u>	<u>Chloride</u>
7493-S				
Daphnid acute	2000	420	2800	2100
7494-S				
Daphnid acute	1900	650	2800	2000
9319-S				
Daphnid acute	2200	480	3000	2000
Daphnid chronic	2100	590	3100	1900
Algal assay	2400	540	3500	2000
7488-S				
Daphnid acute	2800	260	3900	2300
7490-S				
Daphnid acute	2500	600	3600	2000
7491-S				
Daphnid acute	2200	800	3500	2700
Daphnid chronic	2700	790	4000	2000
Algal assay	2800	330	3800	2400
7492-S				
Daphnid acute	2600	560	3300	2200
7499-S				
Daphnid acute	840	340	2000	410
7500-S				
Daphnid acute	1200	200	2000	860
7501-S				
Daphnid acute	1200	160	2500	720
Daphnid chronic	1300	80	1800	570
Algal assay	1200	40	1700	630
7502-S				
Daphnid acute	1000	660	3100	410
7503-S				
Daphnid acute	1200	330	2200	880
7504-S				
Daphnid acute	1300	360	2400	770
Daphnid chronic	1100	80	1700	630
Algal assay	1100	40	1500	530

Table 4

MORTALITY OF DAPHNIA MAGNA* DURING 48-HOUR EXPOSURE TO SRC-1 WASTEWATERS
AND CORRESPONDING SYNTHETIC SALT SOLUTIONS

	Number Dead at Concentration (%) of						LC50 (o/o)	95% Confidence Limits
	0	10	18	32	56	100		
7493	0	0	0	2	8	20	58.3	50.9-67.6
7493-S	1	0	0	0	0	1	>100	
7494	0	0	0	0	0	20	74.8	66.4-84.3
7494-S	0	0	0	0	0	6	90.8	56-100
9319	0	0	0	20	20	20	24.0	18-32.0
9319-S	0	0	0	0	0	10	74.8	56-100
7488	0	0	0	0	3	20	70.5	62.1-79.9
7488-S	0	0	1	1	0	10	74.8	56-100
7490	0	0	0	0	0	20	74.8	66.4-84.3
7490-S	0	0	0	0	0	10	74.8	56-100
7492	0	0	0	0	7	20	60.6	53.0-69.6
7492-S	0	0	0	0	0	10	74.8	56-100
7491	0	0	0	0	9	20	57.6	50.3-66.2
7491-S	0	0	0	0	1	10	73.1	61.0-87.9
7499	0	0	0	0	0	4	>100	
7499-S	--	--	--	--	--	0	>100	
7500	0	0	0	0	0	3	>100	
7500-S	--	--	--	--	--	0	>100	
7501	0	0	0	0	0	0	>100	
7501-S	--	--	--	--	--	0	>100	
7502	0	0	0	0	0	6	>100	
7502-S	--	--	--	--	--	0	>100	
7503	0	0	0	0	0	0	>100	
7503-S	--	--	--	--	--	0	>100	
7504	0	0	0	0	0	0	>100	
7504-S	--	--	--	--	--	0	>100	

* n per group = 20 for SRC-1 wastewater, 10 for synthetic salt solution.

Table 5

WATER QUALITY ASSOCIATED WITH DAPHNID ACUTE STUDIES ON SRC-1 WASTEWATERS AND THEIR ASSOCIATED SALT SOLUTIONS

Water	Replicate	Concentration (%)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity μ hos	Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃
			x	SD	Range	n	x	SD	Range	n	x	SD	Range	n			
7493		0	8.9	0.36	8.5-9.2	3	7.1	0.31	6.8-7.4	3	19.5	0.71	19.0-20.0	2	195	90	78
		10	8.8	0.25	8.6-9.1	3	7.4	0.32	7.0-7.6	3	19.5	0.71	19.0-20.0	2	1400	400	140
		32	8.9	0.17	8.8-9.1	3	7.4	0.29	7.1-7.6	3	19.5	0.71	19.0-20.0	2	3900	910	140
		100	9.1	0.30	8.8-9.4	3	7.3	0.29	7.0-7.5	3	19.0	1.41	18.0-20.0	2	10000	2000	280
7493-S		0	8.9	0.46	8.4-9.2	3	7.0	0.32	6.6-7.2	3	19.5	0.71	19.0-20.0	2	195	83	77
		10	8.9	0.26	8.6-9.1	3	7.2	0.29	6.9-7.4	3	19.5	0.71	19.0-20.0	2	1500	250	120
		32	9.0	0.15	8.8-9.1	3	7.4	0.35	7.0-7.6	3	19.5	0.71	19.0-20.0	2	3000	400	140
		100	9.2	0.06	9.1-9.2	3	7.6	0.49	7.0-7.9	3	19.0	1.41	18.0-20.0	2	9500	1060	140
7494		0	8.9	0.31	8.6-9.2	3	7.0	0	—	3	20.7	2.31	18.0-22.0	3	245	72	74
		10	8.9	0.42	8.6-9.4	3	7.5	0.17	7.4-7.7	3	20.7	2.31	18.0-22.0	3	1500	370	140
		32	9.0	0.35	8.8-9.4	3	7.9	0.10	7.8-8.0	3	20.7	2.31	18.0-22.0	3	3900	720	150
		100	8.9	0.31	8.6-9.2	3	8.1	0.06	8.0-8.1	3	20.7	2.31	18.0-22.0	3	10000	1970	170
7494-S		0	9.0	0.22	8.8-9.2	3	7.0	0	—	3	20.7	2.31	18.0-22.0	3	230	72	80
		10	9.1	0.31	8.8-9.4	3	7.5	0.17	7.3-7.6	3	20.7	2.31	18.0-22.0	3	1500	300	150
		32	8.9	0.42	8.6-9.4	3	7.8	0.21	7.6-8.0	3	20.7	2.31	18.0-22.0	3	3550	600	190
		100	9.0	0.44	8.7-9.5	3	7.9	0.20	7.7-8.1	3	20.7	2.31	18.0-22.0	3	10000	1500	240
9319		0	8.7	0.81	7.8-9.2	3	7.1	0.06	7.1-7.2	3	18.7	0.58	18.0-19.0	3	190	82	73
		10	8.9	0.58	8.2-9.2	3	7.4	0.06	7.4-7.5	3	18.7	0.58	18.0-19.0	3	1000	220	120
		32	8.8	0.53	8.2-9.2	3	7.7	0.17	7.6-7.9	3	18.7	0.58	18.0-19.0	3	3400	800	180
		100	8.9	0.42	8.4-9.2	3	7.6	0.06	7.6-7.7	3	18.7	0.58	18.0-19.0	3	10000	2200	220
9319-S		0	8.7	0.92	7.6-9.2	3	7.2	0.15	7.0-7.3	3	18.7	0.58	18.0-19.0	3	190	87	102
		10	8.7	0.76	7.8-9.2	3	7.3	0.21	7.1-7.5	3	18.7	0.58	18.0-19.0	3	1000	220	150
		32	8.6	0.69	7.8-9.0	3	7.8	0.20	7.6-8.0	3	18.7	0.58	18.0-19.0	3	3300	640	220
		100	8.6	0.69	7.8-9.0	3	8.0	0.06	7.9-8.0	3	18.7	0.58	18.0-19.0	3	9000	1660	240
7488		0	8.9	0.42	8.4-9.2	3	7.2	0.38	6.8-7.5	3	19.3	0.58	19.0-20.0	3	195	80	120
		10	8.9	0.40	8.4-9.1	3	7.6	0.26	7.3-7.8	3	19.3	0.58	19.0-20.0	3	1500	340	170
		32	8.7	0.42	8.2-9.0	3	7.9	0.32	7.5-8.1	3	19.3	0.58	19.0-20.0	3	3500	950	290
		100	7.9	0.17	5.9-9.0	3	7.9	0.35	7.5-8.1	3	19.0	1.00	18.0-20.0	3	8500	2460	600
7488-S		0	8.9	0.49	8.3-9.2	3	7.2	0.35	6.8-7.4	3	19.3	0.58	19.0-20.0	3	190	79	70
		10	8.8	0.40	8.6-9.1	3	7.6	0.29	7.2-7.7	3	19.3	0.58	19.0-20.0	3	1400	200	160
		32	8.9	0.15	8.9-9.1	3	8.0	0.32	7.6-8.1	3	19.3	0.58	19.0-20.0	3	3600	430	220
		100	9.1	1.00	8.8-9.4	3	8.1	0.15	7.9-8.2	3	19.0	1.00	18.0-22.0	3	9500	800	200
7490		0	8.7	0.12	8.6-8.8	3	7.1	0.10	7.0-7.2	3	20.5	2.12	19.0-22.0	2	240	88	88
		10	8.7	0.12	8.6-8.8	3	7.4	0.10	7.3-7.5	3	20.5	2.12	19.0-22.0	2	1700	420	210
		32	8.7	0.10	8.7-8.9	3	7.6	0.15	7.5-7.8	3	20.5	2.12	19.0-22.0	2	4500	930	250
		100	8.7	0.12	8.6-8.8	3	7.8	0.10	7.7-7.8	3	20.0	2.83	18.0-22.0	2	11500	2350	290

Table 5 (continued)

Water	Replicate	Concentration (%)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity μmhos	Hardness mg/L CaCO_3	Alkalinity mg/L CaCO_3
			x	SD	Range	n	x	SD	Range	n	x	SD	Range	n			
7490-S		0	8.7	0.12	8.6-8.8	3	7.1	0.06	7.0-7.1	3	20.5	2.12	19.0-22.0	2	225	90	80
		10	8.8	0.06	8.7-8.8	3	7.4	0.10	7.3-7.5	3	20.5	2.12	19.0-22.0	2	1400	350	200
		32	8.9	0.12	8.8-9.0	3	7.6	0	—	3	20.5	2.12	19.0-22.0	2	4100	710	260
		100	8.8	0.15	8.7-9.0	3	7.8	0	—	3	20.0	2.83	18.0-22.0	2	11000	1630	220
7492		0	8.7	0.12	8.6-8.8	3	7.1	0.06	7.0-7.1	3	20.5	2.12	19.0-22.0	2	240	91	78
		10	8.8	0	—	3	7.2	0.06	7.2-7.3	3	20.5	2.12	19.0-22.0	2	2000	390	200
		32	8.8	0.20	8.6-9.0	3	7.5	0.06	7.4-7.5	3	20.5	2.12	19.0-22.0	2	4800	830	210
		100	8.6	0.21	8.4-8.8	3	7.6	0.06	7.5-7.6	3	20.0	2.83	18.0-22.0	2	14000	2180	280
7492-S		0	8.7	0.12	8.6-8.8	3	7.1	0.10	7.0-7.1	3	20.5	2.12	19.0-22.0	2	235	128	86
		10	8.8	0	—	3	7.1	0.10	7.0-7.2	3	20.5	2.12	19.0-22.0	2	2350	410	200
		32	8.8	0.15	8.7-9.0	3	7.4	0.17	7.2-7.5	3	20.5	2.12	19.0-22.0	2	5000	820	250
		100	8.9	0.31	8.6-9.2	3	7.4	0.35	7.0-7.6	3	20.0	2.83	18.0-22.0	2	19000	2300	350
7491		0	7.6	—	—	1	7.1	0.10	7.0-7.2	3	17.5	2.12	16.0-19.0	2	200	88	82
		10	7.4	—	—	1	7.5	0.06	7.5-7.6	3	17.5	2.12	16.0-19.0	2	1650	400	140
		32	7.6	—	—	1	8.1	0.06	8.1-8.2	3	17.5	2.12	16.0-19.0	2	25500	1500	200
		100	7.4	—	—	1	8.2	0.06	8.1-8.2	3	17.5	2.12	16.0-19.0	2	9500	2460	360
7499		0	10	0.19	9.8-10.2	3	7.5	0.6	7.4-7.5	3	16.0	0	—	3	150	100	
		10	9.4	0.35	9.2-9.8	3	7.6	0.39	7.2-7.9	3	16.0	0	—	3	800	230	
		32	8.4	0.32	8.2-8.8	3	7.5	0.15	7.3-7.6	3	16.0	0	—	3	1700	443	
		100	5.8	1.07	4.6-6.5	3	7.3	0.10	7.2-7.4	3	16.0	0	—	3	4000	1550	
7499-S		100	9.4	0.57	8.8-9.8	3	7.8	0	—	3	16.0	0	—	3	3700	1120	
7491-S		0	7.8	—	—	1	7.1	0.12	7.0-7.2	3	18.0	0.14	17.0-19.0	2	200	88	73
		10	8.2	—	—	1	7.3	0.26	7.0-7.5	3	17.5	2.12	16.0-19.0	2	1900	400	200
		32	8.1	—	—	1	7.9	0.15	7.7-8.0	3	17.5	2.12	16.0-19.0	2	3200	540	200
		100	8.4	—	—	1	8.2	0.15	8.1-8.4	3	17.5	2.12	16.0-19.0	2	10000	1080	320
7500		0	9.6	0.35	9.4-10.0	3	7.4	0.06	7.4-7.5	3	16.0	0	—	3	120	87	74
		10	9.3	0.70	8.6-10.0	3	7.9	0.06	7.9-8.0	3	16.0	0	—	3	700	170	120
		32	9.2	0.60	8.6-9.8	3	8.2	0.06	8.1-8.2	3	16.0	0	—	3	1800	290	160
		100	9.2	0.53	8.8-9.8	3	8.3	0.06	8.2-8.3	3	16.0	0	—	3	4500	730	200
7500-S		100	9.5	0.45	9.2-10.0	3	7.6	0.09	7.5-7.7	3	16.0	0	—	3	4500	710	100
7501		0	9.7	0.31	9.4-10.0	3	7.5	0.09	7.4-7.6	3	16.0	0	—	3	150	95	74
		10	9.3	0.42	8.8-9.6	3	8.1	0.12	8.0-8.2	3	16.0	0	—	3	750	190	120
		32	9.1	0.50	8.6-9.6	3	8.2	0.10	8.1-8.3	3	16.0	0	—	3	1750	350	180
		100	9.2	0.38	8.8-9.5	3	8.3	0.15	8.2-8.5	3	16.0	0	—	3	4700	640	230
7501-S		100	9.4	0.32	9.2-9.8	3	8.1	0.29	7.8-8.3	3	16.0	0	—	3	4500	400	10

Table 5 (concluded)

Water	Replicate	Concentration (%)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Conductivity	Hardness	Alkalinity
			x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	µmhos	mg/L CaCO ₃	mg/L CaCO ₃
7502		0	9.4	0.32	9.2-9.8	3	7.6	0	--	3	16.0	0	--	3	150	84	74
		10	9.4	0.67	8.6-9.8	3	7.7	0.06	7.7-7.8	3	16.0	0	--	3	700	340	140
		32	8.5	0.23	8.4-8.8	3	7.8	0.06	7.8-7.9	3	16.0	0	--	3	1700	740	180
		100	8.0	1.07	6.8-8.7	3	7.9	0.12	7.8-8.0	3	16.0	0	--	3	4200	1860	300
7502-S		100	9.9	0.46	9.4-10.2	3	7.4	0.15	7.3-7.6	3	16.0	0	--	3	4200	1610	100
7503		0	9.5	0.21	9.4-9.8	3	7.6	0	--	3	16.0	0	--	3	150	90	74
		10	9.8	0.06	9.7-9.8	3	8.0	0.21	7.8-8.2	3	16.0	0	--	3	700	210	150
		32	9.6	0.35	9.2-9.8	3	8.2	0.16	8.1-8.3	3	16.0	0	--	3	1700	380	170
		100	9.7	0.12	9.6-9.8	3	8.3	0.10	8.2-8.4	3	16.0	0	--	3	4400	990	290
7503-S		100	9.6	0	--	3	8.1	0.15	7.9-8.2	3	16.0	0	--	3	4500	1000	120
7504		0	9.6	0.20	9.4-9.8	3	7.6	0.06	7.6-7.7	3	16.0	0	--	3	150	86	74
		10	9.8	0	--	3	7.8	0.10	7.7-7.9	3	16.0	0	--	3	700	200	120
		32	9.7	0.06	9.7-9.8	3	8.1	0.10	8.0-8.2	3	16.0	0	--	3	1700	370	150
		100	9.8	0	--	3	8.2	0.12	8.1-8.3	3	16.0	0	--	3	4400	920	180
7504-S		100	9.8	0.06	9.7-9.8	3	8.1	0.10	8.0-8.2	3	16.0	0	--	3	4600	1000	150

Table 6

MORTALITY OF FATHEAD MINNOWS DURING 96-HOUR ACUTE EXPOSURE TO SRC-1
WASTEWATERS AND CORRESPONDING SYNTHETIC SALT SOLUTIONS

Concentration (%)	Number Dead (original n = 30)							
	9319		9319-S		7491		7491-S	
	24 Hr	96 Hr	24 Hr	96 Hr	24 Hr	96 Hr	24 Hr	96 Hr
0	0	0	—	—	0	6	—	—
10	0	0	—	—	0	0	—	—
18	0	0	—	—	0	1	—	—
32	0	0	—	—	0	0	—	—
56	0	10	—	—	0	0	—	—
100	15	30	0	0	0	4	0	0
LC50	100	61.1	>100	>100	>100	>100	>100	>100

95% Confidence(56-B) (55.1-68.1)
Interval

Table 7

WATER QUALITY ASSOCIATED WITH FATHEAD MINNOW ACUTE STUDIES ON SRC-1 WASTEWATERS

Water	Replicate	Concentration (X)	Dissolved Oxygen (mg/L)				pH				Temperature (°C)				Hardness mg/L CaCO ₃	Alkalinity mg/L CaCO ₃	Conductivity µmhos
			x	SD	Range	n	x	SD	Range	n	x	SD	Range	n			
9319	A	0	7.52	0.53	6.9-8.2	5	7.1	0.26	6.7-7.4	5	21.7	0.58	21.0-22.0	3	65	67	200
		10	7.88	0.47	7.3-8.4	5	7.4	0.14	7.2-7.6	5	21.7	0.58	21.0-22.0	3	400	180	1500
		32	8.5	1.39	6.7-10.5	5	7.5	0.11	7.4-7.7	5	21.7	0.58	21.0-22.0	3	700	180	3900
		100	10.9	5.38	6.1-20.0	5	7.6	0.11	7.5-7.8	5	21.7	0.58	21.0-22.0	3	1870	160	8500
	B	0	7.7	0.49	7.1-8.4	5	7.04	0.27	6.7-7.4	5	21.7	0.58	21.0-22.0	3	--	--	200
		10	8.2	0.38	7.6-8.5	5	7.5	0.18	7.2-7.7	5	21.7	0.58	21.0-22.0	3	--	--	1450
		32	8.66	1.18	7.1-10.4	5	7.6	0.15	7.4-7.8	5	21.7	0.58	21.0-22.0	3	--	--	3900
		100	10.96	5.41	6.0-20.0	5	7.6	0.12	7.5-7.8	5	21.7	0.58	21.0-22.0	3	--	--	8500
	C	0	7.9	0.34	7.5-8.4	5	7.1	0.27	6.8-7.4	5	21.7	0.58	21.0-22.0	3	--	--	200
		10	8.04	0.40	7.4-8.4	5	7.5	0.15	7.3-7.7	5	21.7	0.58	21.0-22.0	3	--	--	1500
		32	8.74	1.29	7.3-10.3	5	7.6	0.15	7.4-7.8	5	21.7	0.58	21.0-22.0	3	--	--	3900
		100	10.9	5.35	6.3-20.0	5	7.6	0.11	7.5-7.8	5	21.7	0.58	21.0-22.0	3	--	--	8500
9319-S	A	100	7.63	1.00	6.5-8.9	4	8.0	0.17	7.7-8.2	5	21.7	0.58	21.0-22.0	3	1530	370	7000
	B	100	7.47	1.09	6.2-8.8	4	8.0	.19	7.7-8.2	5	21.7	0.58	21.0-22.0	3	--	--	7000
	C	100	7.55	0.87	6.6-8.7	4	8.0	.19	7.7-8.2	5	21.7	0.58	21.0-22.0	3	--	--	7000
26 7491	A	0	6.36	0.46	5.9-6.9	5	6.5	0.30	6.2-6.9	4	21.5	1.0	21.0-23.0	4	94	70	200
		10	6.48	0.64	6.1-7.6	5	6.9	0.29	6.5-7.1	4	21.5	1.0	21.0-23.0	4	400	180	1600
		32	7.02	1.54	6.4-9.7	5	7.3	0.39	6.7-7.6	4	21.5	1.0	21.0-23.0	4	840	240	4200
		100	9.54	5.05	5.5-18.0	5	7.5	0.43	6.9-7.9	4	21.5	1.0	21.0-23.0	4	2360	240	11000
	B	0	6.36	0.36	6.0-6.8	5	6.6	0.31	6.2-6.9	4	21.4	0.55	21.0-22.0	5	--	--	200
		10	6.28	0.92	5.4-7.8	5	7.0	0.34	6.5-7.3	4	21.4	0.55	21.0-22.0	5	--	--	1600
		32	6.96	1.60	5.6-9.7	5	7.2	0.39	6.7-7.6	4	21.4	0.55	21.0-22.0	5	--	--	4200
		100	8.64	5.75	4.2-18.4	5	7.5	0.43	6.9-7.9	4	21.4	0.55	21.0-22.0	5	--	--	11500
	C	0	6.5	0.24	6.2-6.8	5	6.7	.26	6.3-6.9	4	21.4	0.55	21.0-22.0	5	--	--	200
		10	6.9	0.47	6.5-7.6	5	7.0	.31	6.5-7.2	4	21.4	0.55	21.0-22.0	5	--	--	1600
		32	6.9	1.47	6.3-9.5	5	7.3	.39	6.7-7.4	4	21.4	0.55	21.0-22.0	5	--	--	4300
		100	9.4	5.52	4.8-18.6	5	7.5	.43	6.9-7.9	4	21.4	0.55	21.0-22.0	5	--	--	11500
7491-S	A	100	5.24	1.74	4.2-8.3	5	7.9	.14	7.8-8.0	2	21.6	0.55	21.0-22.0	5	940	360	11000
	B	100	5.6	1.54	5.0-8.3	5	7.9	.14	7.8-8.0	2	21.6	0.55	21.0-22.0	5	--	--	11000
	C	100	5.88	1.38	5.0-8.3	5	7.9	.14	7.8-8.0	2	21.6	0.55	21.0-22.0	5	--	--	11000

Table 8

SURVIVAL OF DAPHNIDS EXPOSED TO SRC-1 WASTEWATER
9319 AND ITS CORRESPONDING SYNTHETIC SALT SOLUTION

Concentration (%)	Number Surviving on Specified Day							
	SRC-I Wastewater (n = 30)				Synthetic Salt Solution (n = 20)			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0	30	28	28	13	20	19	19	6
0.2	30	26	26	11	19	18	17	4
0.8	30	28	27	19	20	19	19	4
2.4	29	28	27	19	20	18	16	13
7.6	23*	23	21	2*	16	15	13	5
24.0	2*	0*	0*	0*	20	17	17	15

*Statistically significant, $p < 0.05$.

Table 9

YOUNG PRODUCED PER DAPHNID EXPOSED TO
SRC-1 WASTEWATER 9319 AND ITS
CORRESPONDING SYNTHETIC SALT SOLUTION

Concentration (%)	Young per Female							
	SRC-1 Wastewater				Synthetic Salt Solution			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0	0.4	24.3	40.7	62.9	0.0	28.6	42.4	68.9
0.2	0.2	26.4	45.2	69.8	0.0	20.9	36.8	53.4*
0.8	0.7	24.6	38.8	56.8	0.3	22.8	33.4	52.4*
2.4	0.2	22.2	39.1	57.7	0.0	16.2*	30.4*	45.0*
7.6	0.0	18.0	45.6	73.9	0.0	19.6	42.3	75.4
24.0	0.0	0.0*	0.0*	0.0*	0.1	11.2*	24.6*	34.6*

Statistically significant; $p < 0.05$.

Table 10

TIME TO FIRST SPAWN AND NUMBER OF YOUNG PRODUCED PER SPAWNING
 DAY FOR DAPHNIDS EXPOSED TO SRC-1 WASTEWATER 9319
 AND ITS CORRESPONDING SYNTHETIC SALT SOLUTION FOR 28 DAYS

Concentration (%)	Time to First Spawn (Days)		No. of Young per Day per Daphnid	
	<u>SRI-1 Wastewater</u>	<u>Salt Solution</u>	<u>SRC-1 Wastewater</u>	<u>Salt Solution</u>
0.0	8	10	3.0	3.9
0.2	9	10	3.5	2.8
0.8	7	8.5	2.6	2.6*
2.4	9	10	2.9	2.4*
7.6	10	10	3.9	4.0
24.0	—*	8.5	0*	1.7*

*Statistically significant, $p < 0.05$.

Table 11

SURVIVAL OF DAPHNIDS EXPOSED TO SRC-1 WASTEWATER
7491 AND ITS CORRESPONDING SYNTHETIC SALT SOLUTION

Concentration (%)	Number Surviving on Specified Day							
	SRC-1 Wastewater (n = 30)				Synthetic Salt Solution (n = 20)			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0	30	30	27	12	20	20	19	11
0.4	30	29	28	12	20	20	19	8
1.3	30	30	27	8	20	20	20	6
4.2	30	28	24	6	20	18	18	4
13.0	30	21*	5*	5	20	19	19	3*
42.0	22*	11*	2*	0*	20	20	20	12

*Statistically significant, $p < 0.05$.

Table 12

YOUNG PRODUCED PER DAPHNID EXPOSED TO
SRC-1 WASTEWATER 7491 AND ITS
CORRESPONDING SYNTHETIC SALT SOLUTION

Concentration (%)	Young per Female							
	SRC-1 Wastewater				Synthetic Salt Solution			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0.0	0	17.6	42.6	63.0	0	19.3	39.1	57.3
0.4	0	18.6	36.1	58.7	0	19.2	35.2	45.2
1.3	0	15.2	28.2	39.8	0	17.4	37.0	55.1
4.2	0	13.0	18.5*	24.0*	0	20.8	38.4	61.2
13.0	0	3.8*	5.5*	9.2*	0	17.2	35.0	58.6
42.0	0	0.0*	0.0*	0.0*	0	14.2	27.2	42.6

*Statistically significant, $p < 0.05$.

Table 13

TIME TO FIRST SPAWN AND NUMBER OF YOUNG PRODUCED PER SPAWNING
 DAY FOR DAPHNIDS EXPOSED TO SRC-1 WASTEWATER 7491
 AND ITS CORRESPONDING SYNTHETIC SALT SOLUTION FOR 28 DAYS

Concentration (%)	Time to First Spawn (Days)		No. of Young per Day per Daphnid	
	<u>SRI-1 Wastewater</u>	<u>Salt Solution</u>	<u>SRC-1 Wastewater</u>	<u>Salt Solution</u>
0.0	10.0	10.0	3.3	3.0
0.4	10.0	10.0	3.1	2.4
1.3	10.0	10.0	2.1	2.9
4.2	10.0	10.0	1.3*	3.2
13.0	10.7	10.0	0.5*	3.0
42.0	—*	10.0	0.0*	2.2

*Statistically significant, $p < 0.05$.

Table 14

SURVIVAL OF DAPHNIDS EXPOSED TO SRC-1 WASTEWATER
7501 AND ITS CORRESPONDING SYNTHETIC SALT SOLUTION

Concentration (%)	Number Surviving on Specified Day							
	SRC-1 Wastewater (n = 30)				Synthetic Salt Solution (n = 20)			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0	30	27	27	27	20	14	13	9
1.0	29	28	26	24	20	19	15	11
3.2	28	27	27	24	20	19	17	17
10.0	30	26	26	23	18	15	15	15
32.0	28	26	18*	14*	18	13	13	13
100.0	11*	0*	0*	0*	18	18	16	15

* Statistically significant, $p < 0.05$.

Table 15

YOUNG PRODUCED PER DAPHNID EXPOSED TO
SRC-1 WASTEWATER 7501 AND ITS
CORRESPONDING SYNTHETIC SALT SOLUTION

Concentration (%)	Young per Female							
	SRC-1 Wastewater				Synthetic Salt Solution			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0	—	7.6	19.6	35.7	—	0	7.4	25.8
1.0	—	5.9	18.6	32.0	—	4.8	13.1	24.2
3.2	—	3.2*	12.6*	27.5*	—	6.8	15.8	28.2
10.0	—	4.9	11.7*	29.4	—	6.8	18.8	32.8
32.0	—	1.4*	4.0*	10.0*	—	4.6	18.8	38.8
100.0	—	0*	0*	0*	—	6.1	14.4	23.4

*Statistically significant, $p < 0.05$.

Table 16

TIME TO FIRST SPAWN AND NUMBER OF YOUNG PRODUCED PER SPAWNING
 DAY FOR DAPHNIDS EXPOSED TO SRC-1 WASTEWATER 7501
 AND ITS CORRESPONDING SYNTHETIC SALT SOLUTION FOR 28 DAYS

Concentration (%)	Time to First Spawn (Days)		No. of Young per Day per Daphnid	
	SRI-1 Wastewater	Salt Solution	SRC-1 Wastewater	Salt Solution
0	12.0	17.0	2.1	2.2
1.0	10.6	13.0	1.8	1.5
3.2	13.3	13.0	1.7	1.8
10.0	12.0	12.0	1.7	1.9
32.0	12.0	11.0	0.6*	2.1
100.0	—*	12.0	0*	1.4

*Statistically significant, $p < 0.05$.

Table 17

**SURVIVAL OF DAPHNIDS EXPOSED TO SRC-1 WASTEWATER
7504 AND ITS CORRESPONDING SYNTHETIC SALT SOLUTION**

Concentration (%)	Number Surviving on Specified Day							
	SRC-1 Wastewater (n = 30)				Synthetic Salt Solution (n = 20)			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0	30	30	30	30	20	20	20	20
1.0	30	30	30	30	20	20	19	19
3.2	30	30	29	29	20	20	20	18
10.0	30	30	29	29	20	20	18	18
32.0	30	28	24	23*	20	20	20	19
100.0	6*	0*	0*	0*	15	14*	14*	14*

*Statistically significant, $p < 0.05$.

Table 18

YOUNG PRODUCED PER DAPHNID EXPOSED TO
SRC-1 WASTEWATER 7504 AND ITS
CORRESPONDING SYNTHETIC SALT SOLUTION

Concentration (%)	Young per Female							
	SRC-1 Wastewater				Synthetic Salt Solution			
	Day 7	Day 14	Day 21	Day 28	Day 7	Day 14	Day 21	Day 28
0.0	0	1.3	5.3	15.4	0	1.0	2.7	16.8
1.0	0	2.1	6.0	16.4	0	1.2	5.4	17.4
3.2	0	1.0	4.9	17.8	0	1.0	6.4	20.1
10.0	0	1.5	2.9	10.4	0	1.3	4.6	22.6
32.0	0	0.2*	1.9*	4.5*	0	0.9	3.8	18.5
100.0	0	0*	0*	0*	0	0.8	2.0	12.6

*Statistically significant, $p < 0.05$.

Table 19

TIME TO FIRST SPAWN AND NUMBER OF YOUNG PRODUCED PER SPAWNING
 DAY FOR DAPHNIDS EXPOSED TO SRC-1 WASTEWATER 7504
 AND ITS CORRESPONDING SYNTHETIC SALT SOLUTION FOR 28 DAYS

Concentration (%)	Time to First Spawn (Days)		No. of Young per Day per Daphnid	
	SRI-1 Wastewater	Salt Solution	SRC-1 Wastewater	Salt Solution
0.0	13.0	12.5	1.0	1.0
1.0	14.0	14.0	1.0	1.2
3.2	13.0	14.0	1.1	1.4
10.0	13.0	12.5	0.7	1.4
32.0	15.3	14.0	0.4*	1.2
100.0	--*	14.0	0.0*	0.8

*Statistically significant, $p < 0.05$.

Table 20 ***

EFFECT NO-EFFECT CONCENTRATIONS OBTAINED FROM
CHRONIC DAPHNID STUDIES ON SRC-1 WASTEWATERS

<u>SRC-1 Wastewater</u>	<u>No-Effect Limits (%)</u>	
	<u>Survival</u>	<u>Reproduction</u>
9319	2.4 > x > 7.2	7.6 > x > 24.0
7491	4.2 > x > 13.0	1.3 > x > 4.2*
7501	10.0 > x > 32.0	1.0 > x > 3.2
7504	10.0 > x > 32.0	10.0 > x > 32.**

* Nonsignificant but probable effect at 1.3%.

**Nonsignificant but probable effect at 10.0%.

***Footnote added by ICRC: In two of the four Daphnia magna chronic bioassays for which data are given (Tables 8 and 11), more than 50% of the control animals died during the tests. Although this does not completely negate the tests, it does call into question the resulting LC₅₀ values for these wastewaters and precludes comparisons among effluents.

Table 21

WATER QUALITY ASSOCIATED WITH DAP-IND CHRONIC STUDIES ON SRC-1 WASTEWATERS AND ASSOCIATED SALT SOLUTIONS

Water	Concentration (%)	Dissolved Oxygen (mg/L)								pH								Temperature (°C)			
		New Solution				Old Solution				New Solution				Old Solution				x	SD	Range	n
		x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n				
9319	0	8.5	0.4	7.9-9.0	13	8.6	0.2	8.2-9.0	13	6.9	0.4	6.7-7.6	12	7.1	0.2	6.7-7.6	12	21.4	0.73	20.0-22.0	9
	0.2	8.4	0.4	7.8-9.0	13	8.6	0.2	8.2-8.9	13	7.1	0.1	6.9-7.4	12	7.2	0.2	6.9-7.6					
	0.8	8.4	0.3	7.8-8.9	13	8.6	0.2	8.2-9.0	13	7.2	0.1	6.9-7.4	12	7.3	0.2	6.9-7.6	12				
	2.4	8.4	0.3	7.8-9.0	13	8.6	0.2	8.2-9.0	13	7.2	0.2	6.7-7.5	12	7.4	0.2	7.0-7.6	12				
	7.6	8.4	0.3	7.8-9.0	13	8.6	0.3	8.2-9.0	13	7.2	0.2	6.7-7.4	12	7.6	0.2	7.4-7.9	12				
	24.0	8.4	0.6	7.8-9.0	4	8.6	0.3	8.2-9.0	5	7.2	0.3	6.8-7.5	4	7.6	0.3	7.4-7.9	3				
9319-S	0	8.3	0.3	7.8-8.8	12	8.7	0.2	8.4-9.0	12	7.0	0.2	6.8-7.5	12	7.2	0.3	6.8-7.7	12	21.4	0.73	20.0-22.0	9
	0.2	8.4	0.3	7.8-8.8	12	8.6	0.2	8.2-9.0	12	7.1	0.2	6.8-7.5	11	7.2	0.2	6.8-7.7	12				
	0.8	8.3	0.3	7.8-8.8	12	8.6	0.2	8.2-9.0	12	7.1	0.2	6.7-7.4	11	7.4	0.2	6.9-7.7	12				
	2.4	8.4	0.4	7.8-8.8	12	8.6	0.2	8.2-9.0	12	7.2	0.2	6.7-7.5	11	7.4	0.2	7.0-7.6	12				
	7.6	8.4	0.3	7.8-8.8	12	8.6	0.2	8.2-9.0	12	7.3	0.2	6.9-7.6	11	7.6	0.2	7.3-7.9	12				
	24.0	8.4	0.3	7.9-9.0	12	8.6	0.2	8.2-9.0	12	7.5	0.2	7.0-7.7	11	7.8	0.1	7.5-8.0	12				
7491	0	8.5	0.2	8.2-8.8	10	8.6	0.2	8.3-9.0	11	7.1	0.2	6.8-7.6	12	7.0	0.3	6.5-7.5	11	21.4	0.73	20.0-22.0	9
	0.4	8.5	0.4	8.2-9.4	10	8.6	0.2	8.2-9.0	11	7.1	0.2	6.8-7.4	12	7.1	0.3	6.5-7.5	11				
	1.3	8.5	0.3	8.2-9.2	10	8.6	0.2	8.2-9.0	11	7.1	0.2	6.5-7.3	12	7.2	0.2	6.9-7.5					
	4.2	8.5	0.3	8.0-8.8	10	8.7	0.2	8.2-9.0	11	7.2	0.3	6.7-7.5	12	7.4	0.2	7.0-7.6	11				
	13.0	8.4	0.2	8.2-8.6	10	8.8	0.2	8.6-9.0	11	7.4	0.4	6.7-7.8	11	7.8	0.2	7.4-8.0	11				
	42.0	8.8	0.6	8.0-10.2	10	8.8	0.2	8.4-9.0	11	7.8	0.5	6.8-8.1	11	8.0	0.2	7.4-8.2	11				
7491-S	0	8.5	0.4	8.1-9.4	9	8.7	0.3	8.4-9.4	10	7.2	0.3	6.8-7.6	11	7.1	0.2	6.7-7.6	12	20.9	1.17	19.0-22.0	9
	0.4	8.5	0.2	8.2-8.8	9	8.6	0.2	8.4-9.2	10	7.2	0.3	6.8-7.8	11	7.2	0.2	7.0-7.6	12				
	1.3	8.5	0.2	8.2-8.8	9	8.6	0.2	8.4-9.2	10	7.3	0.3	6.8-7.8	11	7.4	0.1	7.2-7.6	12				
	4.2	8.5	0.3	8.1-8.8	9	8.6	0.2	8.4-9.2	10	7.4	0.3	6.7-7.8	11	7.5	0.1	7.3-7.8	12				
	13.0	8.5	0.2	8.2-8.8	9	8.7	0.2	8.4-9.0	10	7.5	0.3	7.0-7.8	11	7.7	0.1	7.4-7.9	12				
	42.0	8.5	0.2	8.2-8.9	9	8.7	0.2	8.4-9.0	10	7.9	0.3	7.3-8.4	11	7.9	0.3	7.4-8.2	12				
7501	0	8.4	0.6	7.5-9.1	12	8.9	0.7	7.6-10.0	12	7.0	0.1	6.8-7.3	12	7.2	0.3	7.0-7.9	12	20.9	1.17	19.0-22.0	9
	1.0	8.3	0.6	7.4-9.0	12	8.9	0.7	7.9-10.1	12	7.1	0.2	6.8-7.3	12	7.4	0.2	7.1-8.0	12				
	3.2	8.3	0.5	7.5-8.8	12	8.9	0.6	8.0-10.1	12	7.1	0.2	6.9-7.4	12	7.5	0.2	7.3-8.1	12				
	10.0	8.3	0.4	7.5-8.8	12	8.9	0.6	8.0-10.2	12	7.4	0.2	7.1-7.7	12	7.6	0.2	7.4-8.1	12				
	32.0	8.4	0.5	7.2-9.4	12	8.9	0.6	8.2-9.8	12	7.8	0.1	7.6-8.0	12	7.9	0.1	7.7-8.1	12				
	100.0	9.9	1.5	8.2-12.2	5	9.6	0.3	9.1-9.8	5	8.2	0.1	8.1-8.3	5	8.2	0.1	8.0-8.3	5				
7501-S	0	8.3	0.7	6.5-9.2	12	8.9	0.5	8.4-9.7	12	7.0	0.1	6.8-7.2	12	7.3	0.2	7.0-7.8	12	20.9	1.17	19.0-22.0	9
	1.0	8.3	0.6	6.7-8.9	12	8.9	0.5	8.3-9.7	12	7.0	0.2	6.7-7.3	12	7.5	0.1	7.3-7.8	12				
	3.2	8.2	0.6	6.6-8.8	12	8.9	0.6	8.3-9.8	12	7.1	0.2	6.8-7.3	12	7.5	0.2	7.3-8.0	12				
	10.0	8.2	0.6	6.7-8.9	12	8.8	0.5	7.7-9.7	12	7.2	0.2	6.9-7.5	12	7.7	0.1	7.5-8.0	12				
	32.0	8.3	0.7	6.7-9.0	12	8.9	0.5	8.0-9.6	12	7.6	0.2	7.3-7.9	12	8.2	0.1	7.9-8.4	12				
	100.0	8.2	0.8	6.4-9.0	12	8.8	0.6	8.1-9.9	12	8.0	0.1	7.8-8.2	12	8.3	0.1	8.1-8.4	12				

Table 21 (concluded)

Water	Concentration (%)	Dissolved Oxygen (mg/L)								pH								Temperature (°C)			
		New Solution				Old Solution				New Solution				Old Solution							
		x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n	x	SD	Range	n
7504	0	8.9	0.2	8.4-9.2	12	9.2	0.2	8.8-9.4	12	7.0	0.1	6.9-7.4	12	7.3	0.2	6.9-7.6	12	18.2	1.77	17.0-22.0	13
	1.0	8.9	0.3	8.3-9.4	12	9.1	0.2	8.8-9.4	12	7.2	0.1	6.9-7.4	12	7.4	0.2	6.9-7.7	12				
	3.2	8.8	0.3	8.3-9.4	12	9.1	0.3	8.8-9.5	12	7.3	0.2	7.0-7.5	12	7.5	0.2	7.0-7.8	12				
	10.0	8.9	0.3	8.4-9.4	12	9.1	0.3	8.6-9.5	12	7.6	0.2	7.2-7.9	12	7.7	0.2	7.2-7.9	12				
	32.0	9.0	0.3	8.4-9.4	12	9.1	0.3	8.6-9.4	12	8.0	0.1	7.7-8.2	12	8.0	0.1	7.8-8.2	12				
	100.0	9.4	0.6	9.0-10.4	5	10.3	1.6	9.4-12.2	3	8.3	0.2	8.0-8.4	5	8.4	0.1	8.3-8.4	4				
7504-S	0	9.0	0.2	8.6-9.2	12	9.2	0.3	8.8-9.6	12	7.0	0.2	6.8-7.4	12	7.3	0.3	6.9-7.8	12				
	1.0	8.9	0.2	8.6-9.2	12	9.1	0.3	8.6-9.4	12	7.1	0.2	6.9-7.5	12	7.5	0.2	7.0-7.8	12				
	3.2	8.9	0.3	8.4-9.4	12	9.1	0.2	8.6-9.2	12	7.2	0.2	7.0-7.7	12	7.6	0.1	7.4-7.8	12				
	10.0	8.9	0.3	8.4-9.4	12	9.1	0.2	8.6-9.3	12	7.4	0.2	7.0-7.9	12	7.8	0.3	7.3-8.1	12				
	32.0	9.0	0.3	8.6-9.6	12	9.1	0.2	8.6-9.4	12	7.6	0.2	7.3-8.0	12	8.0	0.2	7.6-8.3	12				
	100.0	9.0	0.3	8.6-9.6	12	9.1	0.3	8.6-9.4	12	7.8	0.2	7.5-8.1	12	8.1	0.2	7.8-8.5	12				

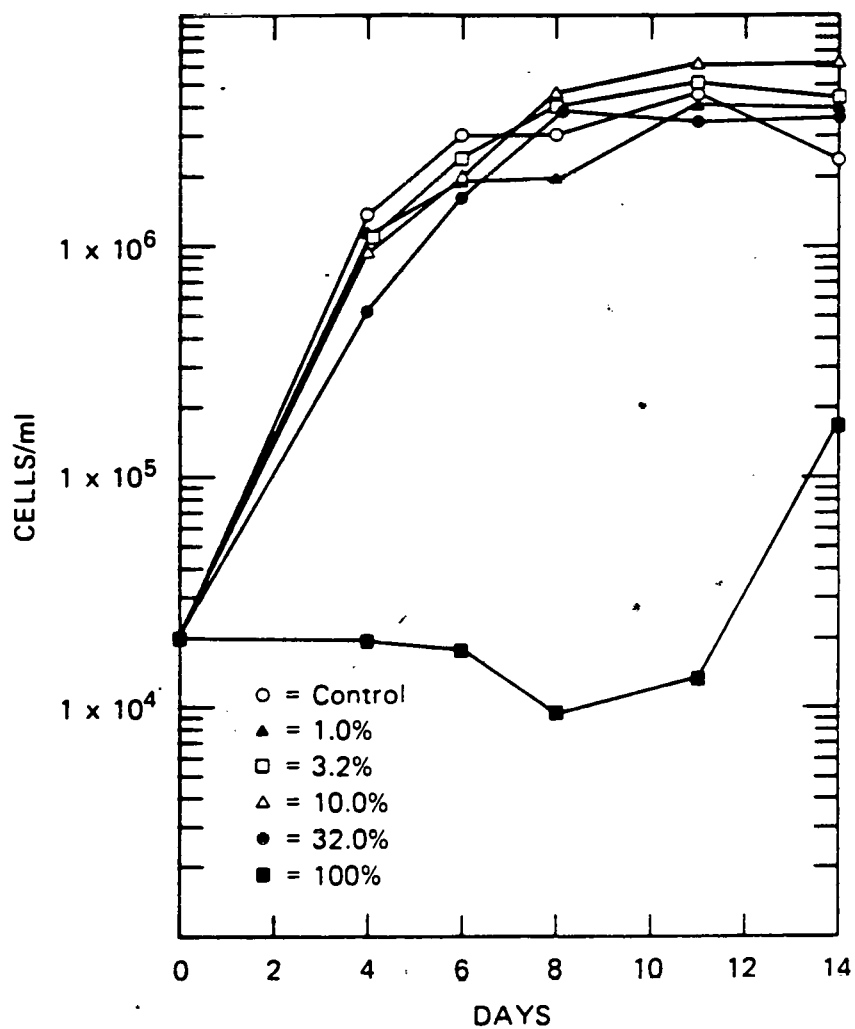
Table 22

MEAN NUMBER OF CELLS OF *S. CAPRICORNUTUM* EXPOSED TO SRC-1 WATERS,
THE CORRESPONDING SYNTHETIC SALT SOLUTIONS, AND DISTILLED WATER
AFTER 4 AND 14 DAYS OF EXPOSURE

	Concentration (%)					
	0	1.0	3.2	10.0	32.0	100.0
9319						
Day 4	1,360,573	1,126,362	1,107,751	927,991	523,858	19,547*
Day 14	2,329,887	3,934,507**	4,180,844**	6,228,035*	3,455,208	168,124*
9319-S						
Day 4	1,360,573	151,360*	670,183*	807,453*	392,000*	121,260*
Day 14	2,329,887	3,866,120**	2,963,520	2,585,493	4,415,333*	56,160*
7491						
Day 4	992,920	1,094,506	683,233	365,240	810,640	32,942*
Day 14	3,580,713	3,640,413	5,999,430*	7,115,467*	3,003,564	217,057*
7491-S						
Day 4	992,920	681,293	1,340,613	1,272,427	1,516,387	217,057*
Day 14	3,580,713	2,714,240	3,062,240	3,028,400	3,342,027	15,630
7501						
Day 4	4,265,500	3,911,351	3,921,680	3,162,196	3,103,458	1,057,733*
Day 14	2,271,233	2,271,227	2,858,880	5,801,333*	3,218,276	2,468,800
7501-S						
Day 4	4,265,500	3,356,947	2,875,707	2,775,640	4,228,520	248,430*
Day 14	2,271,233	3,029,480	2,561,333	2,176,573	1,818,760*	149,813*
7504						
Day 4	663,460	1,216,649	249,502	326,587	845,556	27,528*
Day 14	2,249,173	1,852,498	5,382,436**	5,611,02**	7,337,902*	528,533*
7504-S						
Day 4	663,460	1,389,280**	1,057,200	1,172,213	519,400	90,226*
Day 14	2,249,173	2,631,386	2,655,613	2,368,693	3,996,987	162,480*
Distilled water						
Day 4	1,178,053	--	--	976,951	839,831	53,236*
Day 14	2,070,000	--	--	2,180,000	1,390,000*	111,787*

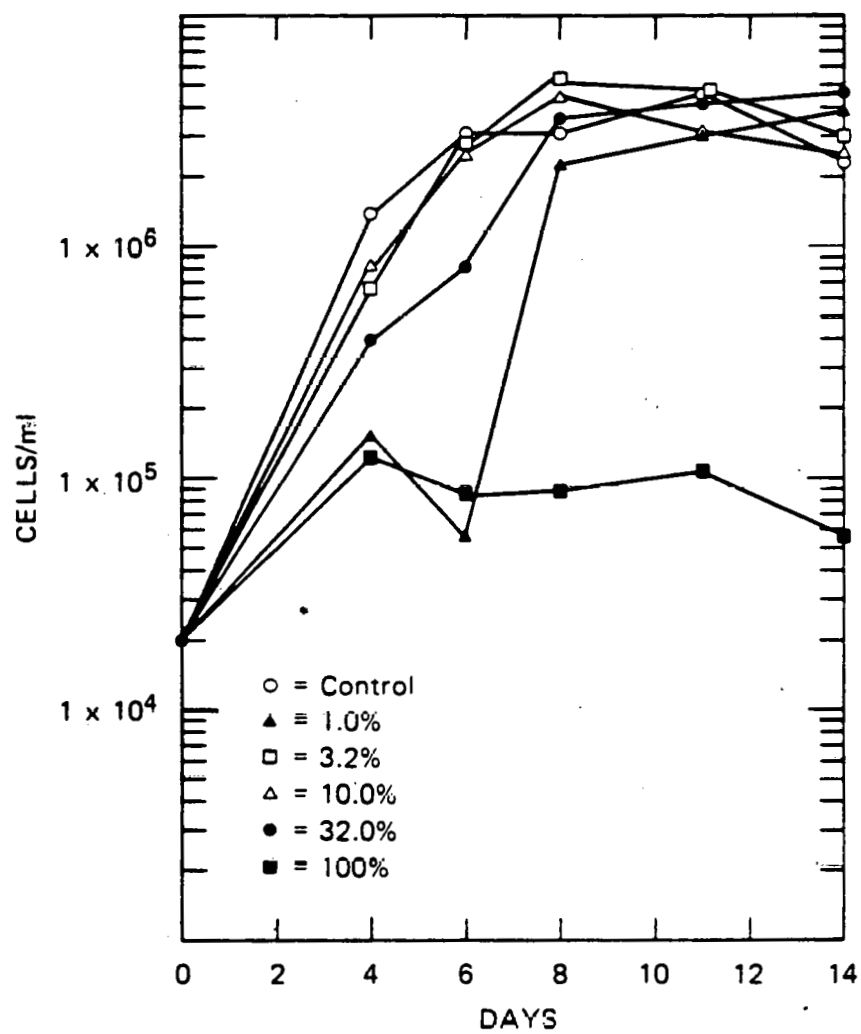
* Statistically significant, $p < 0.05$.

**Statistically significant, $p < 0.10$.



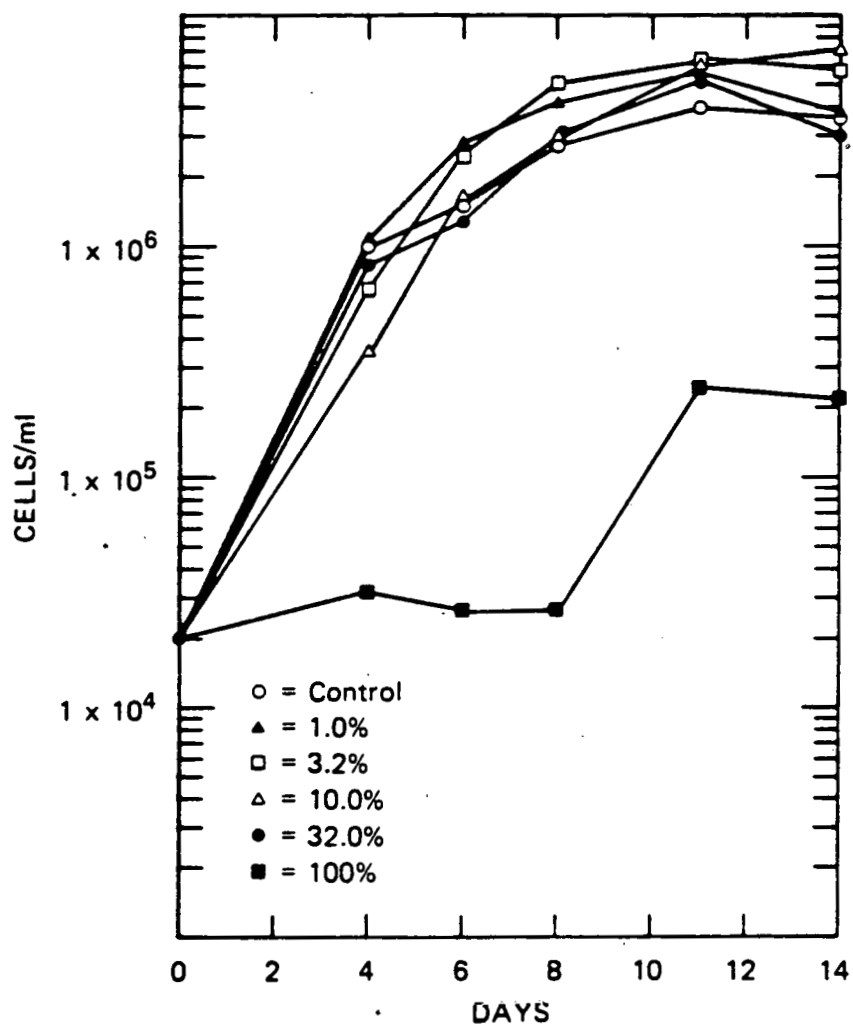
LA-4570-85

FIGURE 2 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO SRC-1 WASTEWATER 9319 FOR 14 DAYS



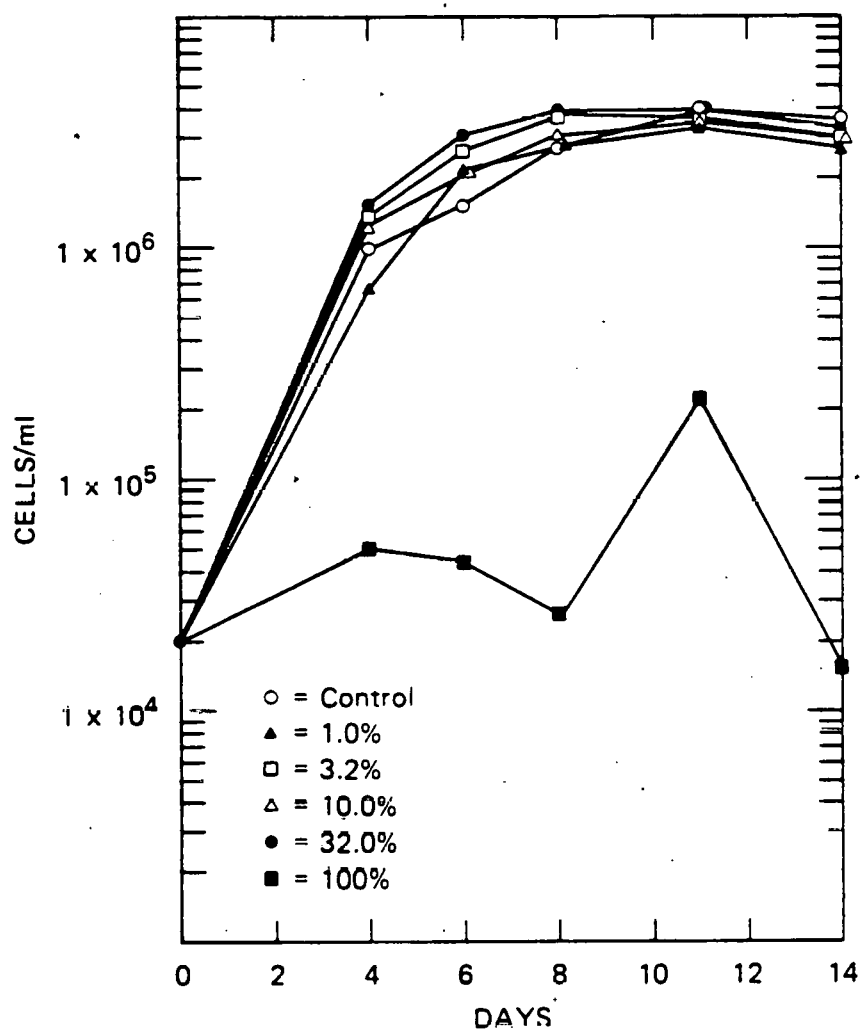
LA-4570-R6

FIGURE 3 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO SALT SOLUTION 9319-S FOR 14 DAYS



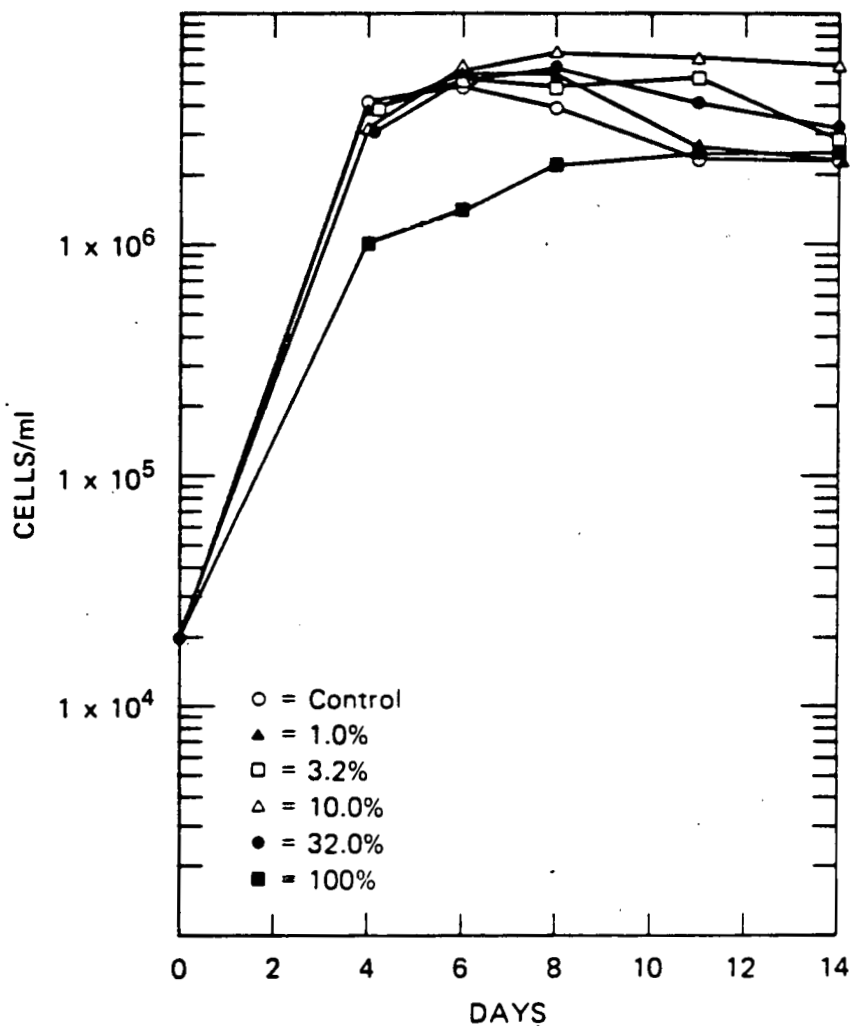
LA-4570-87

FIGURE 4 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO SRC-1 WASTEWATER 7491 FOR 14 DAYS



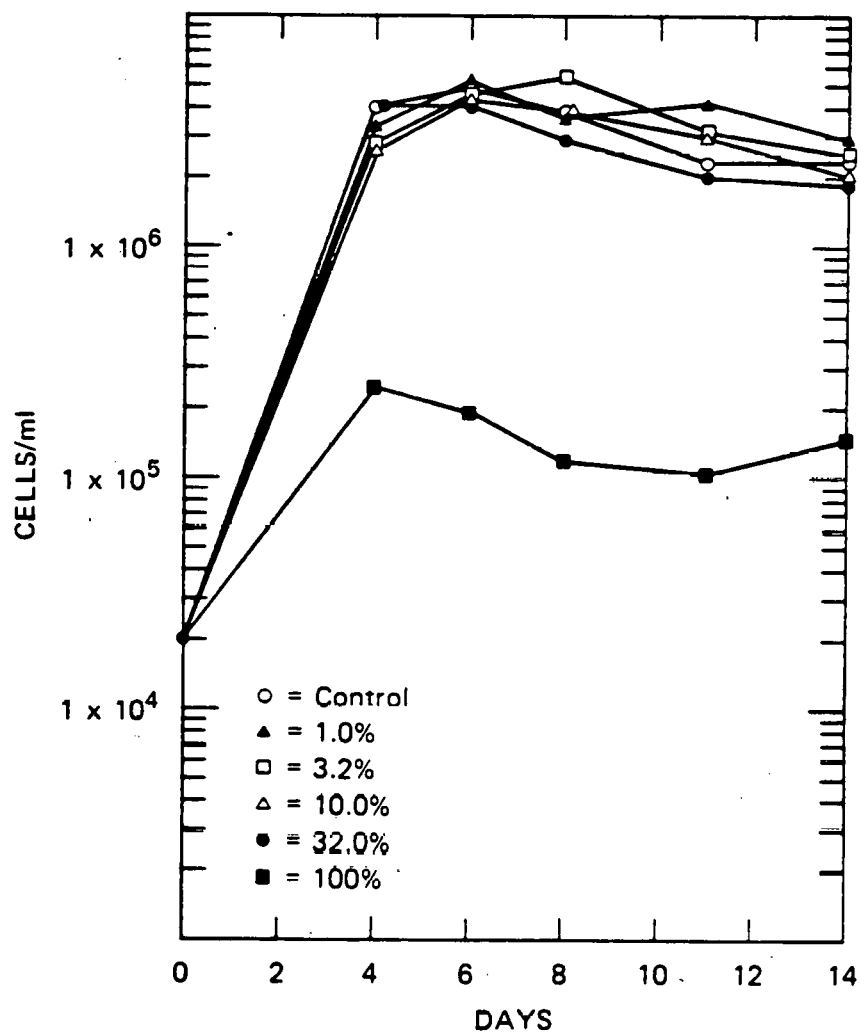
LA-4570-88

FIGURE 5 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO SALT SOLUTION 7491-S FOR 14 DAYS



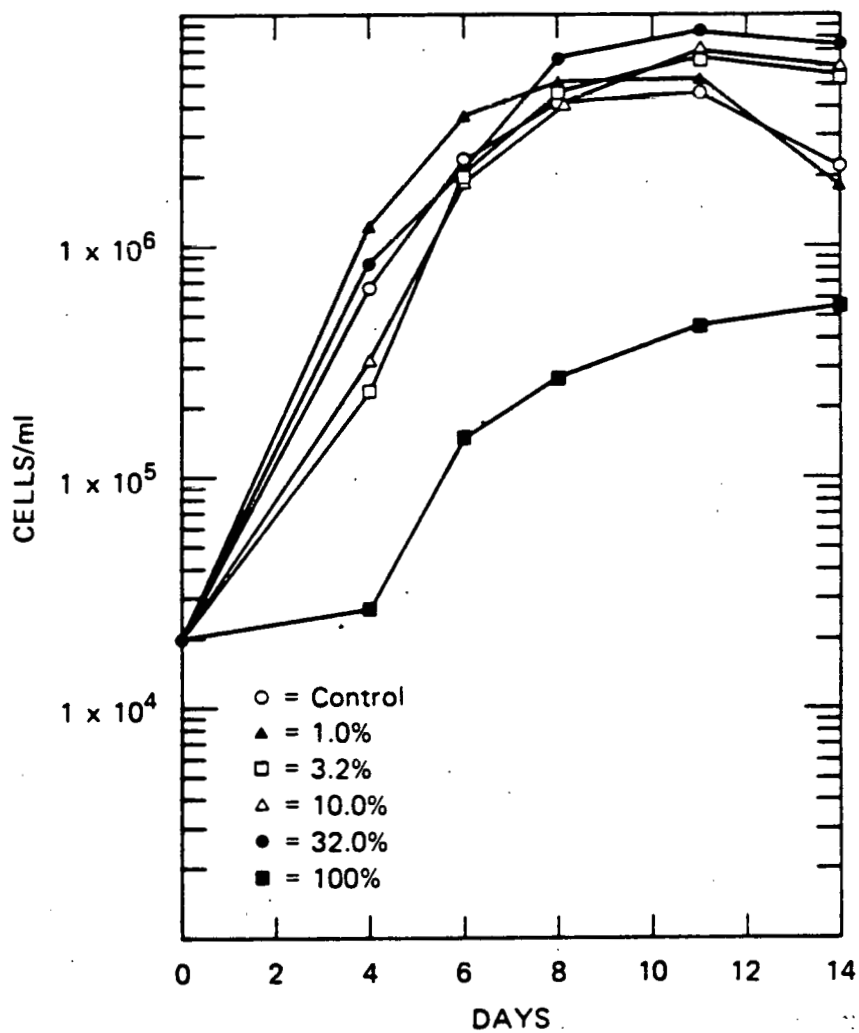
LA-4570-89

FIGURE 6 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO SRC-1 WASTEWATER 7501 FOR 14 DAYS



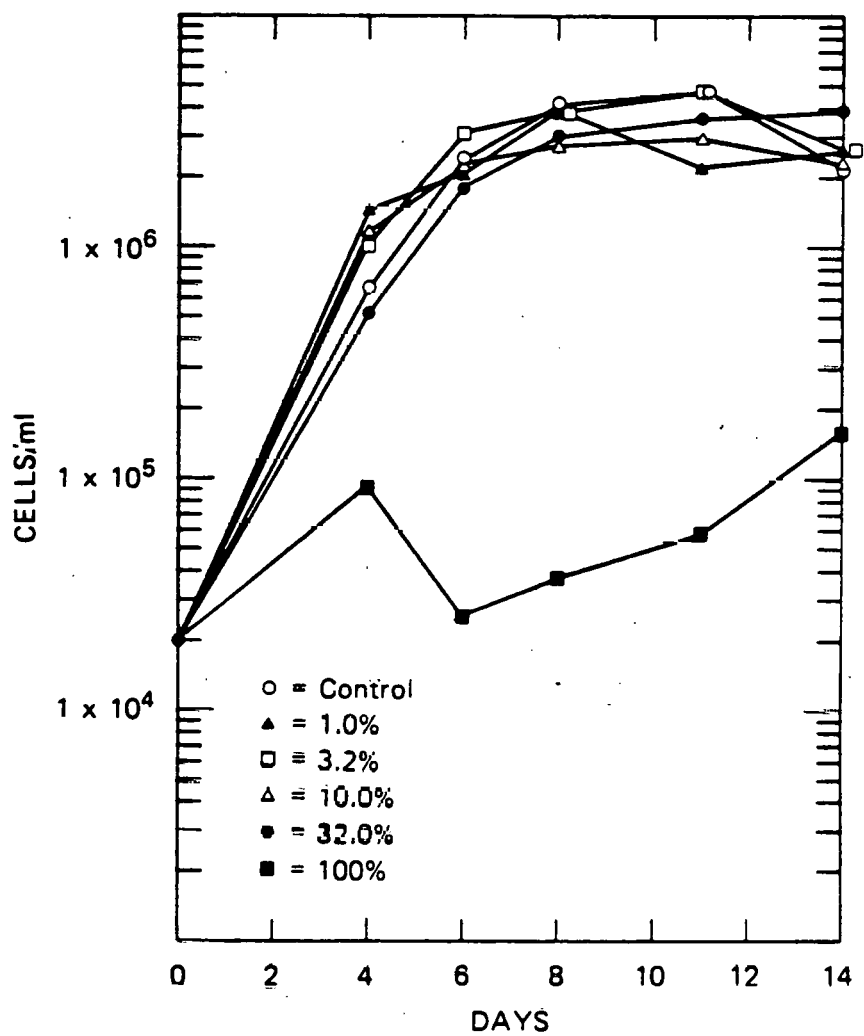
LA-4570-90

FIGURE 7 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO SALT SOLUTION 7501 FOR 14 DAYS



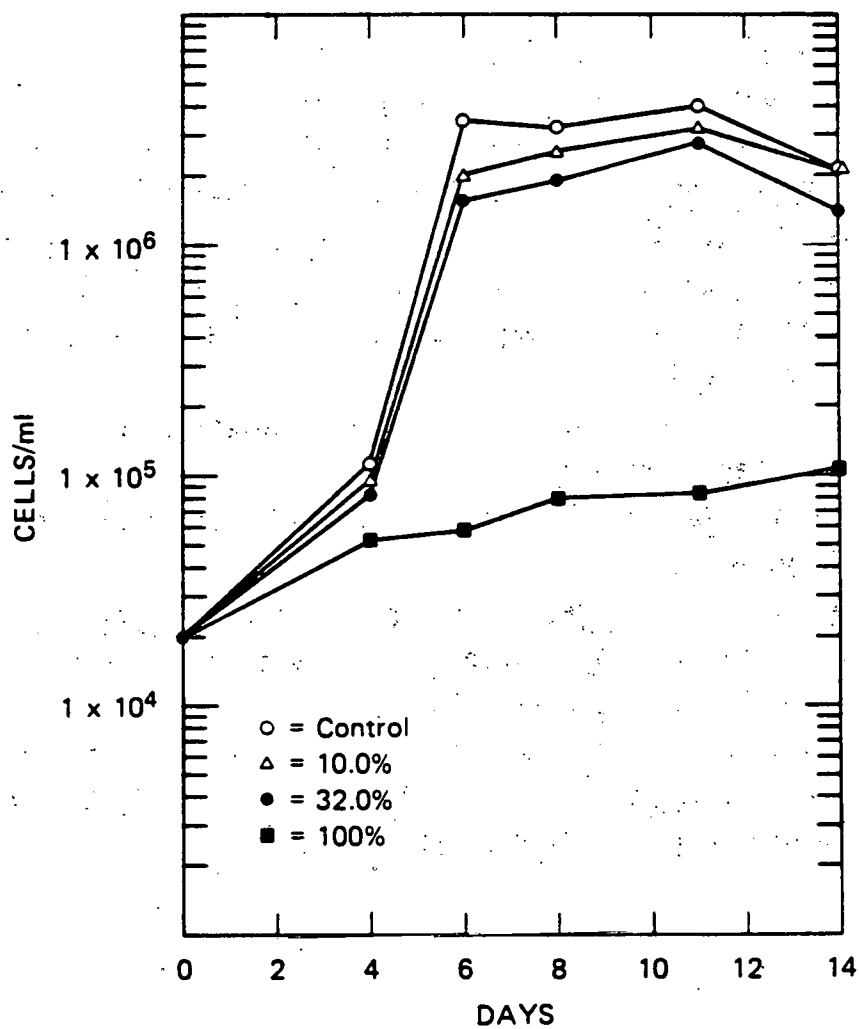
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FIGURE 8 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO SRC-1 WASTEWATER 7504 FOR 14 DAYS



LA-4570-92

FIGURE 9 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO SALT SOLUTION 7504-S FOR 14 DAYS



LA-4570-93

FIGURE 10 GROWTH OF *S. CAPRICORNUTUM* EXPOSED TO DISTILLED WATER FOR 14 DAYS

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