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# RESULTS OF ALGAL TOXICITY TESTING WITH SODIUM PERCHLORATE

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*September 1999*

*EA Project 70003.10*

*Report Number 3188*

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# 1. INTRODUCTION

At the request of Armstrong Laboratory, EA Engineering, Science, and Technology performed 96-hour toxicity testing with sodium perchlorate ($NaClO_4$) using the freshwater algal species *Selenastrum capricornutum*. The objective of the toxicity testing was to assess the chronic toxicity of perchlorate to the algal test species based on cell growth. An initial rangefinding toxicity test was conducted in order to broadly assess the toxicity of perchlorate to the test species. Based on the results of this rangefinding test, an appropriate concentration series was selected for conducting the definitive toxicity test. This report summarizes the results of both the rangefinding and definitive toxicity testing.

# 2. METHODS AND MATERIALS

## 2.1 TEST MATERIAL

The test material was perchlorate ($ClO_4$), administered as sodium perchlorate ($NaClO_4$). Stocks of $ClO_4$ were prepared from reagent grade (99%+ pure) $NaClO_4$ and deionized water, and these stocks were used to dose the test solutions for the algal toxicity tests.

## 2.2 TEST ORGANISMS

*Selenastrum capricornutum* (freshwater green algae) was cultured in-house by EA. The original stock culture was obtained from the Culture Collection of Algae, Department of Biology, University of Texas (Austin, Texas). The laboratory stock cultures were maintained in an environmentally controlled incubator at 25°C with continuous illumination of 400±40 ft candles. New stock cultures were started every 7 days. The algal inoculum for these tests was prepared from stock cultures that were 7 days old.

## 2.3 TOXICITY TEST OPERATIONS AND PERFORMANCE

Toxicity testing was performed in accordance with US EPA (1994) guidance, and methodology followed EA's standard toxicity testing protocols (EA 1996) which are included in Attachment I. A 96-hour rangefinding toxicity test was conducted 30 July-3 August 1999 using the concentration series of 5, 50, 500, and 5,000 mg/L $ClO_4$ with a laboratory dilution media control. Based on the results of the rangefinding test, a graded concentration series of $ClO_4$ was selected for the definitive toxicity test. The definitive test (conducted 6-10 August) consisted of five concentrations of $ClO_4$ (20, 50, 200, 500, and 1,200 mg/L) and a laboratory dilution media control. Test concentrations were prepared by measuring volumes of test material with pipets, adding to a volumetric flask or graduated cylinder, and bringing to final volume with dilution media. Each test solution was mixed and split equally into the replicate test vessels.

The algal toxicity tests were performed in 250-ml Erlenmeyer flasks equipped with breathable

metal caps, and each flask contained 100 ml of test solution. The rangefinding test utilized one replicate per concentration, while the definitive test had three replicates per concentration. Each flask was inoculated with *S. capricornutum* to give an initial target cell concentration of 10,000 cells/ml. The flasks were kept in an environmentally controlled chamber at 25±1°C with 400±40 ft. candles of continuous light. The flasks were randomized inside the environmental chamber at test initiation and then re-randomized daily. The vessels were also swirled twice daily.

At the end of the 96-hour exposure period, cell counts were performed on each replicate using a compound microscope and hemacytometer. The results of the definitive toxicity test were statistically analyzed according to US EPA (1994) guidance using hypothesis and point estimation techniques. The endpoints from the hypothesis statistical analyses are expressed as the No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC), and the Chronic Value (ChV). The definitions of these chronic endpoints are as follows (US EPA 1994):

- The NOEC is the highest concentration of toxicant to which organisms are exposed in a full life-cycle or partial life-cycle test, which causes no statistically significant adverse effect on the observed parameter (usually hatchability, survival, growth, and reproduction).

- The LOEC is the lowest concentration of toxicant to which organisms are exposed in a life-cycle or partial life-cycle test, which causes a statistically significant adverse effect on the observed parameters (usually hatchability, survival, growth, and reproduction).

- The ChV is a value lying between the NOEC and LOEC, derived by calculating the geometric mean of the NOEC and LOEC. The term is sometimes used interchangeably with Maximum Acceptable Toxicant Concentration (MATC).

For the point estimation analysis, a 25 percent Inhibition Concentration (IC25) value was calculated. This endpoint is defined below:

- Inhibition Concentration (ICp) - A point estimate of the toxicant concentration that causes a given percent reduction in a non-quantal biological measurement such as

fecundity or growth. For example, an IC25 is the estimate concentration of toxicant that causes a 25 percent reduction in mean young per female or some other non-quantal biological measurement.

Copies of the statistical analyses are included with copies of the original test data sheets in Attachment II. The Report Quality Assurance Record is presented in Attachment III.

## 2.4 REFERENCE TOXICANT TESTING

In conformance with EA's quality assurance/quality control program, a reference toxicant test was performed with the test species. The reference toxicant test was a 96-hour test and used a graded concentration series of hexavalent chromium ($Cr^{+6}$), administered as potassium dichromate ($K_2Cr_2O_7$). The results of the reference toxicant test were compared to EA's established control chart limits.

## 2.5 ARCHIVES

Original data sheets, records, memoranda, notes, and computer printouts are archived at EA's Baltimore Office in Sparks, Maryland. These data will be retained for a period of 5 years unless a longer period of time is requested by Armstrong Laboratory.

## 3. RESULTS AND DISCUSSION

The results of the *Selenastrum capricornutum* toxicity tests are summarized in Table 1. In the rangefinding toxicity test, there was a 98 percent inhibition of growth in the highest concentration tested (5,000 mg/L $ClO_4$). The lower concentrations (500, 50, and 5 mg/L $ClO_4$) had 23, 15, and 0 percent growth inhibition with respect to the control. The rangefinding toxicity test yielded a sufficient dose response for selection of test concentrations for the definitive test.

In the definitive toxicity test, there was a 40 percent inhibition of cell growth in the highest test concentration (1,200 mg/L $ClO_4$). The next highest test concentration (500 mg/L $ClO_4$) had 17 percent growth inhibition which was similar to the 500 mg/L $ClO_4$ test concentration in the rangefinding test. The three lowest test concentrations in the definitive test (200, 50, and 20 mg/L $ClO_4$) had stimulation of cell growth as compared to the control. The NOEC from the definitive toxicity test was 500 mg/L $ClO_4$, the LOEC was 1,200 mg/L $ClO_4$, and the ChV was 775 mg/L $ClO_4$. The IC25 was 615 mg/L $ClO_4$.

The results of reference toxicant testing conducted on the algal species fell within EA's acceptable control chart limits. The 96-hour EC50 was 0.60 mg/L $Cr^{+6}$ with acceptable control chart limits of 0-0.68 mg/L $Cr^{+6}$.

# 4. REFERENCES

EA. 1996. Quality Control and Standard Operating Procedures Manual for the EA Ecotoxicology Laboratory. Revision No.5. EA Manual ATS-102. Internal document prepared by EA's Ecotoxicology Laboratory, EA Engineering, Science, and Technology, Inc., Sparks, Maryland.

US EPA. 1994. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. Third Edition. EPA/600/4-91/002. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.

TABLE 1 RESULTS OF *Selenastrum capricornutum* TOXICITY TESTING WITH SODIUM PERCHLORATE

| | | |
|---|---|---|
| Test Type: | 96-Hour Rangefinding | 96-Hour Definitive |
| Test Material: | Sodium perchlorate ($NaClO_4$) | Sodium perchlorate ($NaClO_4$) |
| EA Test Number: | TN-99-513 | TN-99-525 |
| Test Initiation: | 1350, 30 July 1999 | 1055, 6 August 1999 |
| Test Termination: | 1530, 3 August 1999 | 1300, 10 August 1999 |

| Nominal Test Concentration (mg/L $ClO_4$) | 96-Hour Mean Cell Count | Nominal Test Concentration (mg/L $ClO_4$) | 96-Hour Mean Cell Count |
|---|---|---|---|
| Control | 1,521,875 | Control | 1,996,875 |
| 5 | 1,528,125 | 20 | 2,092,708 |
| 50 | 1,290,625 | 50 | 2,243,750 |
| 500 | 1,168,750 | 200 | 2,023,958 |
| 5,000 | 34,375 | 500 | 1,659,375 |
| | | 1,200 | 1,195,833[(a)] |

| Chronic Endpoints (as mg/L $ClO_4$) | | |
|---|---|---|
| NOEC: | not calculated | 500 |
| LOEC: | not calculated | 1,200 |
| ChV: | not calculated | 775 |
| 96-Hour IC25: (95 percent confidence limits) | not calculated | 615 (149-1,126) |

(a) Concentration was significantly different from the control based on 96-hour mean cell count.