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RESULTS OF CHRONIC TOXICITY TESTING WITH SODIUM PERCHLORATE USING *Hyalella azteca* AND *Pimephales promelas*

Prepared for:

Armstrong Laboratory
AFIER/RSRE
2513 Kennedy Circle
Brooks AFB, Texas 78235-5114

Prepared by:

EA Engineering, Science, and Technology, Inc.
15 Loveton Circle
Sparks, Maryland 21152

October 2000 *EA Project 70003.10*

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Wayne McCulloch
Project Manager

Date

William L. Goodfellow, Jr.
Senior Scientist

Date

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1. INTRODUCTION

At the request of Armstrong Laboratory, EA Engineering, Science, and Technology performed chronic toxicity tests with sodium perchlorate ($NaClO_4$) using selected freshwater test species. The chronic toxicity tests included the 28-day survival, growth and fecundity test with the freshwater amphipod *Hyalella azteca*, and the 35-day early life stage embryo-larval survival and growth test with the fathead minnow *Pimephales promelas*. The objectives of the chronic toxicity testing were to assess the chronic lethality of perchlorate to the test species and to determine the inhibition of fecundity (*H. azteca*) and growth (*P. promelas* and *H. azteca*) of the perchlorate exposed test organisms as compared to the control

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 chronic toxicity tests.

2.2 TEST ORGANISMS

Hyalella azteca (freshwater amphipod) were obtained from EA's culture facility in Sparks, Maryland. The amphipods were cultured at 20°C in 10-gallon glass aquaria with a substrate of hardwood (maple) leaves and overlying water of dechlorinated municipal tap water. Prior to introduction into the aquaria, the leaves were pre-soaked or boiled to remove tannins. The cultures were fed Tetramin-B flake food weekly in addition to the hardwood leaves. For use in testing, 7-14 day old organisms (2-3 mm) were collected from the cultures and were gradually acclimated to the proper test temperature (23°C).

Pimephales promelas (fathead minnow) embryos were also obtained from EA's Culture Facility. Brood organisms were maintained in recirculating dechlorinated tap water at 25°C in 10-gallon aquaria. Fertilized eggs produced from the brood system were acclimated to testing temperature of 25°C, and were used for testing as lot #FH0-6/19-20.

2.3 DILUTION/OVERLYING WATER

Dechlorinated tap water was used a dilution water for the *P. promelas* embryo-larval toxicity test and as the overlying water for the toxicity test with *H. azteca*. The source of the tap water was the City of Baltimore municipal water system. Upon entering the laboratory, the water was passed through a high-capacity, activated-carbon filtration system to remove chlorine and any other possible trace organic contaminants. This water source has proven safe

for aquatic organism toxicity testing at EA as evidenced by maintenance of multigeneration *Daphnia* sp., *H. azteca*, and fathead minnow cultures with no evident loss of fecundity.

The sediment used in the *H. azteca* toxicity test was collected from the Magothy River, Maryland. Sediment collected from this location has historically been non-toxic and is routinely utilized as a control sediment in EA's toxicity tests.

2.4 TOXICITY TEST OPERATIONS AND PERFORMANCE

Toxicity testing was performed in accordance with US EPA (1993, 1994 and 2000) and ASTM (1992) guidance, and methodology followed EA's standard toxicity testing protocols (EA 1996) which are included in Attachment I. The concentration series used in the 35-day *P. promelas* chronic toxicity test was based on the results of a previous 7-day chronic *P. promelas* test which was included in EA Report #2900. The concentration series used for the 28-day *H. azteca* test was based on results of perchlorate toxicity testing previously performed with other test species, in conjunction with the relative sensitivity of *H. azteca* compared to other species. Each toxicity test included a series of five ClO_4 concentrations and a dilution water control. Prior to preparation of test solutions, aliquots of dilution water were brought to the proper test temperature using a water bath. Aqueous test concentrations were prepared by measuring volumes of test stock solution with pipets or small graduated cylinders, adding to a volumetric flask or larger graduated cylinder, and bringing to final volume with dilution water. Each test solution was mixed and split equally into the replicate test vessels.

Copies of original data sheets for the *H. azteca* and *P. promelas* tests, which include all mortality observations and water quality measurements, and copies of the statistical analyses, are presented in Attachments II and III, respectively. The Report Quality Assurance Record is presented in Attachment IV.

2.4.1 *Hyalella azteca* Chronic Toxicity Test

The 28-day *H. azteca* sediment toxicity test was initiated on 22 June and was completed on 20 July 2000. Test chambers were 250 ml glass beakers each containing 50 ml of sediment and 150 ml of overlying water. The tests were performed with eight replicates per test concentration and control. The sediment and overlying water were added to the chambers 24 hours prior to

~~introduction of the test organisms. The beakers were left undisturbed overnight to allow any~~ suspended sediment particles in the water column to settle. At test initiation, ten organisms were randomly introduced into each replicate beaker. The test chambers were placed in a water bath and maintained at 23 ±1°C with a 16-hour light/8-hour dark photoperiod. The *H. azteca* were fed daily with 1 ml/replicate of YCT (a suspension of yeast, ground cereal leaves, and trout chow) on days 0–14, and 1.5 ml/replicate on days
14-28).

The overlying water in the exposure chambers was renewed three times per week. Test solutions renewals were performed by siphoning 80 percent of the old test solution from each test chamber, and then slowly siphoning new test solution into the chamber, taking care not to disturb the sediment. Temperature, pH, conductivity and dissolved oxygen measurements were recorded daily on the overlying water in one replicate of each test concentration and control. A summary of the water quality measurements recorded during testing is provided in Table 1.

At the end of the 28-day exposure period, the surviving organisms from each replicate were retrieved by screening through a 250 µm sieve, and the number of surviving *H. azteca* from each replicate was recorded. Surviving *H. azteca* were observed microscopically to determine sex and the presence or absence of eggs in the females. The surviving organisms from each replicate were placed in a dried, pre-weighed tin and placed in a drying oven at 100°C for at least 24 hours. At the end of the drying period, the tins were then removed, placed in a desiccator to cool, and each tin was weighed to the nearest 0.01 mg. The dry weight of the

surviving organisms in each replicate was determined by subtracting the weight of the empty tin from the weight of the tin plus dried organisms.

Following US EPA (1994) guidance, statistical analyses were performed on the survival, dry weight, biomass, and fecundity data to determine if any test concentration was significantly different from the control (p=0.05). The chronic toxicity test endpoints reported are expressed as the No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC), and the Chronic Value (ChV). Additionally, a 25 percent Inhibition Concentration (IC25) was calculated for each test. The definitions of these chronic endpoints follow US EPA

(1994) and are as follows:

- 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).

- 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 biomass or some other non-quantal biological measurement.

2.4.2 *Pimephales promelas* 35-Day Early Life Stage Embryo Larval Chronic Toxicity Test

The 35-day *P. promelas* test was conducted in 10-L glass aquaria containing 5 L of test solution. The test was conducted as a flow-through test, with a minimum of 3.5 volume replacements per day. Each test concentration and control had 2 replicates. Each replicate contained two incubation cups with 15 embryos per cup. The incubation cups were suspended in the test chambers so that the embryos were submerged and the test solution could flow through the cups without disturbing the embryos. Each test chamber was equipped with a self-starting siphon with a vertical travel distance of 35-40 mm to ensure adequate flow in and out of the incubation cups. The embryos were observed daily to determine the number of hatched embryos and surviving larvae. The larvae were fed 2-3 drops of a 0.05 g/ml suspension of newly hatched brine shrimp nauplii (*Artemia* sp., less than 24 hours old) once daily. After 96 hours of exposure, 20 larvae from each replicate were transferred from the combined incubation cups into the aquaria, and the incubation cups were removed. In several replicates, less than 20 larvae were transferred because the remaining surviving larvae from those replicates were premature or deformed. Beginning on Day 5, and for the remainder of the 35 days, the *P. promelas* larvae were fed 3 -10

drops (0.2 – 0.5 ml) of the 0.05 g/ml *Artemia* suspension, three times daily.

Observations of mortality were recorded daily. Temperature, pH, dissolved oxygen, and conductivity measurements were recorded daily on one replicate of each test concentration. Alkalinity, hardness, and conductivity was measured on the test solutions at initiation, termination, and on Days 7, 21, and 28. At the end of the 35-day exposure period, the surviving larvae were placed in pre-weighed, oven-dried aluminum pans (one pan per replicate) and dried at 100°C for a minimum of 22 hours. The mean dry weight per surviving organisms, and per originally exposed organisms was calculated for each replicate. The results were statistically analyzed according to EPA (1994) guidance to determine if any test concentration was significantly different from the control with respect to survival, mean dry weight, or biomass (p=0.05). The results of the chronic toxicity test were reported as the NOEC, the LOEC, the ChV, and the IC25, as described in section 2.4.1.

2.5 REFERENCE TOXICANT TESTS

In conformance with EA's quality assurance/quality control program, reference toxicant tests were performed on the in-house cultures of *P. promelas* and *H. azteca*. The *P. promelas* were exposed to the reference toxicant cadmium chloride ($CdCl_2$) to determine the 7-day NOEC. The *H. azteca* were exposed to a graded concentration series of copper sulfate ($CuSO_4$) to determine the 96-hour medium lethal concentration (LC50). The results of the reference toxicant tests were compared to control chart limits established by EA according to US EPA (1993) methodology.

2.6 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

A summary of the water quality parameters (temperature, pH, dissolved oxygen and conductivity) measured during the testing is presented in Table 1. The results of the 28-day amphipod, *Hyalella azteca* chronic toxicity test with sodium perchlorate are summarized in Table 2. The test concentrations are expressed as nominal mg/L (ppm) ClO_4 and range from 30 to 1,000 mg/L ClO_4. After 28 days of exposure, survival in the perchlorate test concentrations ranged from 68 to 85 percent. None of the test concentrations were significantly different (p=0.05) than the control, which had 84 percent survival at test termination. The percent of females with eggs was greater in the perchlorate test concentrations (79 to 88 percent fecundity) than in the control (77 percent). Similarly, there was no statistically adverse effect on growth, based on mean dry weight per surviving organisms or biomass per exposed organisms. The mean dry weight was 0.368 mg per surviving organism in the control, and 0.383–0.432 mg per surviving organism in the perchlorate test solutions. Biomass ranged from 0.259 – 0.340 mg per exposed organism in the perchlorate test concentrations compared to 0.305 mg per exposed organism in the dilution water control. The NOEC for survival and growth was 1000 mg/L ClO_4. The LOEC and ChV were >1000 mg/L ClO_4. The IC25, based on biomass, was also >1000 mg/L ClO_4.

The *P. promelas* 35-day early life stage definitive chronic toxicity test evaluated perchlorate concentrations ranging from 28 to 490 mg/L. During the initial 96-hour exposure period, 78 percent of the embryos exposed to the control water hatched. Between 73 and 83 percent of the embryos exposed to the perchlorate test concentrations hatched during this 96-hour period. Larval fish survival at day 35 ranged from 78 to 95 percent in the perchlorate concentrations, compared to 73 percent survival in the control. None of the perchlorate concentrations were significantly different from the control (p=0.05) when analyzed for either 96-hour embryo hatch rate, or 35-day larval survival. Similarly, there was no statistically significant adverse effect on larval growth or biomass caused by exposure to the perchlorate test concentrations. Mean dry weight ranged from 5.299 to 7.048 mg per surviving larvae in the perchlorate concentrations, compared to 6.386 mg per surviving larvae in the control. Larval biomass ranged from 4.719 to

5.330 mg per exposed larvae in the perchlorate test concentrations compared to 4.633 mg per larvae exposed to the control. However, exposure to the perchlorate concentrations produced an observable effect on the *P. promelas* larvae. All larvae exposed to the five perchlorate concentrations exhibited redness and swelling, which was not observed in the larvae exposed to the control water. The no effect level for the observed internal redness was <28 mg/L ClO_4. The perchlorate NOEC based on larval survival and growth was 490 mg/L ClO_4. The LOEC, ChV and the IC25 (based on biomass) were all >490 mg/L ClO_4. These results are presented in Table 3.

The results of reference toxicant testing conducted on the in-house cultured *H. azteca* and *P. promelas* are presented in Table 4. The values for these species were all within the acceptable control chart limits.

4. REFERENCES

American Society for Testing and Materials (ASTM). 1992. Standard Guide for Conducting Early Life Stage Toxicity Tests with Fishes: E 1241-92. Philadelphia, Pennsylvania.

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. 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. EPA/600/4-90/027F. U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.

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

US EPA. 2000. Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates. Second Edition. EPA/600/R-99/064. U.S. Environmental Protection Agency, Office of Research and Development, Duluth, Minnesota.

TABLE 1 SUMMARY OF WATER QUALITY RANGES MEASURED DURING TOXICITY TESTING FOR ARMSTRONG LABORATORY

EA Test Number	Test Species	Temperature (°C) Range	Temperature (°C) Mean (±S.D.)	pH	Dissolved Oxygen (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)	Conductivity (µS/cm)
TN-00-463	*P. promelas*	23.8 – 28.6	25.3 (± 0.72)	7.1 – 8.3	7.4 – 8.6	52 - 58	92 - 116	261 – 913
TN-00-464	*H. azteca*	22.0 – 24.1	23.0 (± 0.44)	7.2 – 8.2	2.2 – 8.8	N/A	N/A	266 – 2,900

N/A Not analyzed

TABLE 2 RESULTS OF *Hyalella azteca* 28-DAY CHRONIC TOXICITY TESTING WITH SODIUM PERCHLORATE

Test Species:	*Hyalella azteca*
Test Type:	28-day chronic toxicity test
Test Material:	Sodium perchlorate ($NaClO_4$)
Overlying Water:	Dechlorinated tap water
Sediment	Magothy River sediment
EA Test Number:	TN-00-464
Test Initiation:	1000, 22 June 2000
Test Termination:	1100, 20 July 2000

Nominal Test Concentration (mg/L ClO_4)	28-Day Percent Survival	Percent Fecundity	Mean dry weight (per surviving organism ±S.D.)	Mean Biomass (per original organism ±S.D.)
Control	84	77	0.368 (±0.038)	0.305 (±0.038)
30	73	81	0.432 (±0.044)	0.312 (±0.076)
75	71	79	0.416 (±0.054)	0.290 (±0.077)
180	85	86	0.401 (±0.051)	0.340 (±0.066)
420	83	88	0.383 (±0.038)	0.310 (±0.066)
1,000	68	81	0.399 (±0.085)	0.259 (± 0.034)

Endpoints (as mg/L ClO_4)

NOEC (28-day survival):	1,000
NOEC (mean dry weight per surviving organism):	1,000
LOEC (mean dry weight per surviving organism):	>1,000
ChV (mean dry weight per surviving organism):	>1,000
IC25 (biomass per original organism):	>1,000

TABLE 3 RESULTS OF *Pimephales promelas* 35-DAY EARLY LIFE STAGE TESTING WITH SODIUM PERCHLORATE

Test Species:	*Pimephales promelas* (fathead minnow)
Test Type:	35-day early life stage chronic toxicity test
Test Material:	Sodium perchlorate ($NaClO_4$)
Dilution Water:	Dechlorinated tap water
EA Test Number:	TN-00-463
Test Initiation:	1615. 21 June 2000
Test Termination:	1430, 26 July 2000

Nominal Test Concentration (mg/L ClO_4)	96-Hour Percent Embryo Hatch	35-Day Percent Larval Survival	Mean Dry Weight (mg per surviving larvae ±S.D.)	Mean Biomass (mg per original larvae ±S.D.)
Control	78	73	6.386 (±0.055)	4.633 (±0.717)
28	73	85 (a)	5.674 (±0.704)	4.756 (±0.180)
56	83	88 (a)	5.603 (±0.078)	4.904 (±0.266)
115	78	78 (a)	6.113 (±0.355)	4.719 (±0.373)
280	80	95 (a)	5.299 (±0.019)	5.033 (±0.356)
490	75	78 (a)	7.048 (±2.321)	5.330 (±0.655)

Endpoints (as mg/L ClO_4)

96-Hour LC50 (percent hatched embryos):	>490
NOEC (35-day, larval survival):	490
NOEC (35-day, adversely affected larvae):	<28
NOEC (mean dry weight per surviving organism):	490
LOEC (mean dry weight per surviving organism):	>490
ChV (mean dry weight per surviving organism):	>490
IC25 (biomass per original organism):	>490

(a) Survival, dry weight and biomass were not significantly different from the control. Organisms in all perchlorate concentrations exhibited internal redness.

TABLE 4 SUMMARY OF REFERENCE TOXICANT TESTING FOR EA-CULTURED TEST ORGANISMS

Test Species	Reference Toxicant Test Number	Reference Toxicant	Endpoint		Acceptable Control Chart Limits
Hyalella azteca	RT-00-087	Copper sulfate ($CuSO_4$)	96-Hour LC50:	133 µg/L Cu	89 – 450 µg/L Cu
Pimephales promelas	RT-00-074	Cadmium chloride ($CdCl_2$)	7-Day NOEC:	42 µg/L Cd	7.5 – 42 µg/L Cu

TABLE 4 SUMMARY OF REFERENCE TOXICANT TESTING FOR EA-CULTURED TEST ORGANISMS

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Pimephales promelas	RT-00-074	Cadmium chloride ($CdCl_2$)	7-Day NOEC:	42 µg/L Cd	7.5 – 42 µg/L Cu