



RESULTS OF ACUTE TOXICITY TESTS
WITH *Daphnia magna* AND *Pimephales promelas*
ON SAMPLES FROM CALGON CORPORATION

Prepared for:

Calgon Corporation

Prepared by:

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

October 1995

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30 October 1995

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1. INTRODUCTION

At the request of Calgon Corporation, EA Engineering, Science, and Technology (EA) performed acute toxicity screening tests with *Daphnia magna* (water flea) and *Pimephales promelas* (fathead minnow) on samples from Perry Nuclear Power Plant, North Perry, Ohio. Each species was exposed to undiluted (100 percent) (1) influent, (2) effluent grab sample collected prior to chemical treatment for zebra mussels and (3) effluent composite sample collected during chemical treatment.

Test results, test organism source and age, reference toxicant data, and water quality ranges measured during the tests are summarized on pages 3-6. Sample collection and initial water quality data are reported in Table 1. Test protocols, copies of raw data sheets, and the Report Quality Assurance Record are included as Attachments I, II, and III, respectively.

RESULTS

The results of the *D. magna* acute toxicity screening tests performed on the samples collected from Perry Nuclear Power Plant, North Perry, Ohio are presented on page 4. At 48 hours, there was 100 percent survival of organisms exposed to the influent sample, the effluent grab sample collected prior to zebra mussel treatment and the composite effluent sample collected during treatment. Survival in the laboratory control was also 100 percent.

The results of the *Pimephales promelas* tests are summarized on page 6. The three undiluted samples from Perry Nuclear Power Plant had 100 percent survival after 96 hours of exposure. The laboratory control had only 85 percent survival, which is less than the minimum of 90 percent for acceptable control survival based EA quality control criteria. The results of the most recent reference toxicant test performed on the in-house cultured stock of fathead minnows was within an acceptable range, based on EA's Aquatic Toxicology Control Chart. In our best professional judgment, the poor laboratory control survival does not affect the integrity of the results of the tests performed on the three Perry Nuclear Power Plant samples.

SUMMARY OF SAMPLE/TEST INFORMATION

Test: 48-hour *Daphnia magna* Static Acute Toxicity Test

Client Name: **Calgon Corporation**

Sample Site: **Perry Nuclear Power Plant**

Test Procedure: EA Protocol ATS-SAI-DS-04
Acute 48-hour assay with *Daphnia*

Sample Description: **Influent; Effluent grab prior to treatment; Effluent composite during treatment**

EA Accession Number: **AT5-1088, AT5-1089, AT5-1090**

EA QC Test Number: **TN-95-678**

Test initiation time and date: 1330, 6 October 1995

Test completion time and date: 1313, 8 October 1995

Control Water: Dechlorinated tap water

Organism Lot Information

Lot Number: Not Applicable

Source: EA's Culture Facility, Sparks, Maryland

Age: <24 hours

Reference Toxicant (SDS) Test Information

Reference Toxicant: Sodium dodecyl sulfate

EA QC Test Number: RT-09-26-95-162

Dilution Water: Dechlorinated tap water

Results:

48-hour LC50: 17.1 mg/L SDS

Laboratory control chart acceptability range for 48-hour LC50:
8.7 - 19.4 mg/L SDS

SUMMARY OF SAMPLE/TEST INFORMATION (Cont.)

Test Organism: *D. magna*
EA QC Test Number: TN-95-678

<u>Test Material</u> <u>(100 percent concentration)</u>	<u>Percent Survival at 48 Hours</u>
Laboratory Control	100
Influent (AT5-1088)	100
Effluent prior to treatment (AT5-1089)	100
Effluent during treatment (AT5-1090)	100

<u>Selected Water Quality</u>	<u>Range</u>
Temperature (°C)	19.9 - 20.8
pH	7.9 - 8.6
Dissolved oxygen (mg/L)	8.0 - 8.8
Conductivity (μS/cm)	258 - 374

SUMMARY OF SAMPLE/TEST INFORMATION

Test: 96-hour *Pimephales promelas* Static Acute Toxicity Test

Client Name: Calgon Corporation

Sample Site: Perry Nuclear Power Plant

Test Procedure: EA Protocol ATS-SAF-FM-04
Acute assay with fathead minnows (*Pimephales promelas*)

Sample Description: **Influent; Effluent grab prior to treatment; Effluent composite during treatment**

EA Accession Number: AT5-1088, AT5-1089, AT5-1090

EA QC Test Number: TN-95-679

Test initiation time and date: 1500, 6 October 1995

Test completion time and date: 1530, 10 October 1995

Control Water: Dechlorinated tap water

Organism Lot Information

Lot Number: FH 5-10/3

Source: EA's Culture Facility, Sparks, Maryland

Age: 3 days (hatched within a 24-hour window)

Reference Toxicant (CdCl₂) Test Information

Reference Toxicant: Cadmium chloride

EA QC Test Number: RT-09-16-95-158

Dilution Water: Dechlorinated tap water

Results:

48-hour LC50: 20.8 $\mu\text{g/L}$ Cd

Laboratory control chart acceptability range for 48-hour LC50:
15.2 - 55.2 $\mu\text{g/L}$ Cd

SUMMARY OF SAMPLE/TEST INFORMATION (Cont.)

Test Organism: *P. promelas*
 EA QC Test Number: TN-95-579

<u>Test Material</u> (100 percent concentration)	<u>Percent Survival at 96 Hours</u>
Laboratory Control	85 ^(a)
Influent (AT5-1088)	100
Effluent prior to treatment (AT5-1089)	100
Effluent during treatment (AT5-1090)	100
<u>Selected Water Quality</u>	<u>Range</u>
Temperature (°C)	19.6 - 20.8
pH	7.0 - 8.2
Dissolved oxygen (mg/L)	8.5 - 9.1
Conductivity (μS/cm)	261 - 374

(a) See page 2 for discussion.

TABLE 1 SAMPLE COLLECTION AND INITIAL WATER QUALITY DATA FOR SAMPLES FOR CALGON CORPORATION

<u>Sample Description</u>	<u>EA Accession Number</u>	<u>Type of Sample</u>	<u>Sample Collection</u>	<u>Sample Receipt</u>	<u>Alkalinity (mg/L CaCO₃)</u>	<u>Hardness (mg/L CaCO₃)</u>
Influent	AT5-1088	Grab	0940, 4 OCT 95	0930, 6 OCT 95	80	108
Effluent prior to treatment	AT5-1089	Grab	0850, 4 OCT 95	0930, 6 OCT 95	112	132
Effluent during treatment	AT5-1090	Composite	0954, 4 OCT 95 to 2120, 4 OCT 95	0930, 6 OCT 95	100	132

ATTACHMENT I

PROTOCOLS

ACUTE ASSAY WITH *Daphnia* sp.

1. TEST OBJECTIVE

To assess the toxicity of a test material to *Daphnia* and determine the LC50 or EC50 using mortality or immobilization, respectively, as the test end points.

2. TEST ARTICLE

2.1 Description/Identification

Unless otherwise specified, the test material is supplied by the client. Adequate chemical specifications with special reference to hazardous properties and storage conditions is also supplied by the client.

2.2 Methods of Synthesis

In most cases the test article is an effluent sample. Information on the methods of synthesis, stability, and composition or other characteristics which define the test article are on file with the client.

3. EXPERIMENTAL DESIGN

3.1 Test Organisms

A species of *Daphnia* (water fleas), as determined by project needs, is the test organism.

3.2 Source

Daphnia used for acute toxicity tests are obtained from stock cultures maintained in EA's Aquatic Toxicology Culturing Facility.

3.3 Culturing and Holding Conditions

Daphnia cultures are maintained at 20 ± 2 C and a 16-hour light, 8-hour dark photoperiod cycle in an environmentally controlled laboratory. Cultures are maintained in 18.9-L all glass aquaria or other appropriate container and are fed algae (*Selenastrum capricornutum*) and a trout chow/yeast/cereal leaves suspension in the manner described in Weber 1991. New cultures are initiated on a routine basis to ensure healthy, productive populations. Organisms from cultures producing ephippia are not used for toxicity tests. Certain regulatory or project specific objectives may require organism acclimation to the dilution water when it is different from the holding/culture water.

3.4 Age of Test Organisms at Test Initiation

Neonates of known age (i.e., less than 24-hours old) are obtained for testing by segregating adult females from the mass cultures on the day before a test is initiated. On the day of the test, neonates are segregated from the parent organisms.

3.5 Dilution Water

The source of dilution water is the City of Baltimore Municipal Water System. Upon entry to the laboratory, the water is passed through a high-capacity, activated-carbon filtration system to remove chlorine and other possible contaminants such as detergents, and hexane-soluble organic materials. This water source has proven safe for aquatic organism toxicity testing as evidenced by maintenance of the multigeneration *Daphnia* and *Pimephales promelas* cultures, with no evident loss of fecundity. If requested, reconstituted fresh water or an appropriate receiving water can be used as the dilution water.

3.6 Test Concentration Series

The test concentration series consists of a minimum of five dilutions and may be determined from a prior screening of the test material (e.g., 1, 3, 10, 30, and 100 percent effluent; or 6.25, 12.5, 25, 50, and 100 percent effluent plus a control).

3.7 Test Concentration Preparation

Test concentrations are prepared with Class A glassware.

3.8 Test Vessels and Test Volume

Test vessels are 400-ml glass beakers or 200-ml culture bowls; the final test volume of each replicate is 200 ml. Depending on the study requirements, other test vessels and test volumes may be used.

3.9 Test Organism Number

Tests are conducted using two replicates per concentration, with ten organisms per container. Neonates are randomly assigned to each replicate test container. More replicates can be added, if appropriate.

3.10 Test Environment

The test vessels are maintained at 20 ± 1 C or 25 ± 1 C in an environmentally controlled laboratory with a 16-hour light, 8-hour dark photoperiod.

3.11 Analysis of Test Concentrations for Test Article

If required, test solutions may be analyzed for verification of chemical concentrations. The analytical method and number of analyses are determined after consultation with the client. When chemical analyses are necessary, both nominal and actual measured test solution concentrations are reported.

3.12 Test Observations

Each test day, test organisms are observed to record the number of surviving organisms. The study terminates after completion of the 2-day observation period. The study may be extended, however, at the request of the client.

Each sample received is analyzed for temperature, conductivity, alkalinity, and hardness. Measurements of water quality taken daily include dissolved oxygen, pH, temperature and conductivity from a minimum of one replicate of every concentration. Aliquots of effluent and receiving water may be gently aerated (100 bubbles/min) if dissolved oxygen is less than 4 mg/L or greater than 105% saturation. After test initiation, if the dissolved oxygen in any test chamber is less than 4 mg/L, all test chambers are gently aerated or other corrective action is taken. Analytical determinations are conducted according to APHA et al. (1992) and EPA (1979).

3.13 Solution Renewal (When Applicable)

When static-renewal testing is required, the test solutions are renewed at 24-hours. New test solutions are prepared on the day of renewal and dispensed according to Section 3.7. After the new solutions have reached test temperature, the test organisms are transferred from the old test vessels to the new test vessels using a wide bore pipet or glass tube. The number of live organisms is recorded. Caution is given not to stress the test organisms during transfer. After water quality measurements (temperature, pH, dissolved oxygen, and conductivity) are completed, the old solution is discarded.

3.14 Data Analysis

The LC50 or EC50 values and associated statistics are calculated using the probit, moving average, and binomial methods as described by Stephan (1977). Depending on the nature of the data, other methods may be used, including the Trimmed Spearman-Kärber method, the probit approximation method of Litchfield and Wilcoxon (1949), SAS probit analysis (SAS Institute 1985) or graphical interpolation using the log concentration vs. percent mortality as described by APHA et al. (1989). The methods used are specified in the final report.

3.15 Test Acceptability

An individual test may be conditionally acceptable if temperature, DO, and other specified conditions fall outside specifications, depending on the degree of the departure and the objectives of the tests.

4. FINAL REPORT

The final report is prepared to contain at a minimum the following information:

- . Objectives and procedures stated in the approved protocol, including any changes made to the original protocol
- . Identity of the test article(s) by name or code number and the strength (i.e., quality/purity and a description of any pretreatment)
- . Source of the dilution water, its chemical characteristics, and a description of any pretreatment
- . Test concentration series used and duration of the assay
- . Water quality characteristics of dilution water and selected test concentrations during testing (pH, dissolved oxygen, temperature, etc.)
- . Any unforeseen circumstances that may have affected the quality or integrity of the study
- . Signature of the project manager, senior technical reviewer, and quality control officer, authorizing release of the report
- . Location of all archived data and the original copy of the final report at EA

Items of data to be included in the report consist of experimental design and test performance; effects on general appearance of test organisms (if applicable); morbidity and mortality; presentation of water quality characteristics; and survival data.

5. QUALITY ASSURANCE

5.1 Amendments to Protocol

Amendments to the authorized protocol established by EA or by the client are made only after proper authorization. Such authorization is achieved by completion of the Amendment to Protocol Form by EA after consultation with the client.

5.2 Standard Operating Procedures

Unless otherwise specified, all procedures specified in the protocol are subject to detailed Standard Operating Procedures (SOPs) which are contained in the SOP manuals of the participating departments. These SOPs and protocols generally follow the types of requirements as outlined in the U.S. EPA's Good Laboratory Practice Standards (GLPs) (EPA 1989).

5.3 Reference Toxicant

A reference toxicant test, utilizing sodium dodecyl sulfate (SDS), cadmium chloride, or another appropriate chemical is used as an internal quality check of the sensitivity of the test organisms. Testing is conducted at least once monthly on organisms which are cultured in-house. The results of each test are compared with historical, species-specific toxicological information from reference toxicant tests performed at EA, to determine if the results are within acceptable limits. Limits are established using the control charts outlined in Weber (1991).

5.4 Quality Assurance Evaluation

Studies conducted under this protocol may be subject to internal audit by EA's Quality Assurance Unit. A quality control officer is responsible for monitoring each study to assure the client that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with EA's QC program and, if applicable, EPA's GLPs.

5.5 Inspection by Regulatory Authorities

In the event of an inspection of EA by an outside authority during the course of the study, the client whose study is being inspected will be consulted before examiners are permitted access to any of the project records or the experimental areas.

5.6 Archives

Copies of project-specific records shall be transferred to the client promptly after the project is completed or as negotiated and budgeted. Original primary data are retained at EA for 5 years. Primary data include chain-of-custody records, laboratory data sheets, records, memoranda, notes, photographs, microfilm, and computer printouts that are a result of the original observations and activities of the study and which are necessary for the reconstruction and evaluation of the study report.

5.7 Location

Studies are conducted at the Aquatic Toxicology Laboratory of EA Engineering, Science, and Technology, Inc. at the Loveton Office in Sparks, Maryland.

6. SPECIFICATIONS OF THE *Daphnia* ACUTE TOXICITY TEST

6.1 Basic References

American Public Health Association (APHA), American Waterworks Association, Water Environment Federation. 1992. Standard Methods for Examination of Water and Wastewater, 18th edition or most recent version. APHA, Washington, D.C.

EA Manual ATS-102. 1992. Aquatic Toxicology Studies, Quality Control and Standard Operating Procedures Manual. Fourth Revision. Internal document prepared by Aquatic Toxicology Services, EA Engineering, Science, and Technology, Inc.

Litchfield, J.T., Jr. and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharm. Exp. Ther. 96:99-113.

- SAS Institute Inc. 1985. SAS® User's Guide: Basics, Version 5 Edition. Cary, NC: SAS Institute Inc. 1290 pp.
- Stephan, C.E. 1977. Methods for calculating an LC50, in Aquatic Toxicology and Hazard Evaluation (F.L. Mayer and J.L. Hamelink, eds.), pp. 65-84. ASTM STP 634. American Society for Testing and Materials, Philadelphia.
- U.S. Environmental Protection Agency. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020. U.S. EPA, Washington.
- Weber, C.I. 1991. Editor. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. EPA/600/4-90/027. U.S. Environmental Protection Agency. Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.
- U.S. EPA. 1989. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards. Title 40 CFR Part 792. Fed. Regist. 54(158):34034-34074.

6.2 Test Specifications

Test organism:	<i>Daphnia magna</i> or <i>D. pulex</i> ; species to be specified in the study plan and final report
Age:	Less than 24 hours old
Temperature:	20±1 C or 25±1 C
Light quality:	Wide-spectrum fluorescent light
Light intensity:	50-100 f.c.
Photoperiod:	16-hour light, 8-hour dark
Aeration:	None, unless dissolved oxygen falls below 4 mg/L

Dilution water:	Dechlorinated municipal water, reconstituted water, or appropriate receiving water
Test container:	400-ml beaker or 200 ml culture bowl
Test volume:	200 ml per replicate
No. of concentrations:	Minimum of five test concentrations and a control
No. of replicates:	Two
No. of animals per replicate:	Ten
Feeding regime:	Animals will not be fed during test
Test type and duration:	Rangefinding--24 to 48 hours Definitive--48 hours
Endpoints:	Mortality or immobilization Immobilization--defined as cessation of movement except for minor activity of appendages Mortality--defined as cessation of all movement for a period of at least 5 seconds even when the test container is tapped or rotated, or the organism is gently prodded with glass rod
Test acceptability:	90% or greater survival in the control solution.

ACUTE ASSAY WITH FATHEAD MINNOWS
(*Pimephales promelas*)

1. TEST OBJECTIVE

To assess the toxicity of a test material to *Pimephales promelas* and determine the LC50 or EC50 using mortality and/or immobilization, respectively, as the test endpoint(s).

2. TEST ARTICLE

2.1 Description/Identification

Unless otherwise specified, the test material is supplied by the client. Adequate chemical specifications with special reference to hazardous properties and storage conditions are also supplied by the client.

2.2 Methods of Synthesis

In most cases the test article is an effluent sample. Information on the methods of synthesis, stability, and composition or other characteristics which define the test article are on file with the client.

3. EXPERIMENTAL DESIGN

3.1 Test Organisms

The test species is the fathead minnow, *Pimephales promelas*.

3.2 Source

P. promelas used for toxicity tests are usually obtained from stock cultures maintained at EA's Aquatic Toxicology Culturing Facility. However, organisms may be obtained from a scientific organism vendor, if necessary.

3.3 Culturing and Holding Conditions

P. promelas stock are maintained at 20 ± 2 C and a 16-hour light, 8-hour dark photoperiod cycle in an environmentally controlled laboratory. Test organisms are maintained in 18.9-L or 30-L all glass aquaria in a recirculating system of dechlorinated municipal tap water. If organisms are obtained from a scientific vendor, the fish, upon receipt, are transferred to static recirculating holding tanks of an appropriate size containing dechlorinated tap water at the temperature (± 2 C) of the water in which the organisms were shipped. All fish stocks are examined regularly. Dead fish, or those displaying abnormal swimming behavior, discoloration, or pronounced lethargy are removed as observed, and recorded on appropriate log sheets. Fish stocks are fed a commercial fish food or *Artemia* nauplii a minimum of once daily. Certain regulatory or project specific objectives may require organism acclimation to the dilution water when it is different from the holding/culture water.

3.4 Age of Test Organisms at Test Initiation

The age of the test organisms is dependent on the objectives of the study and the specific guidelines being followed, but in general, fathead minnows used for acute toxicity testing are 1-14 days old (with a 24-hour range in age) upon test initiation.

3.5 Dilution Water

The source of dilution water is the City of Baltimore Municipal Water System. Upon entry to the laboratory, the water is passed through a high-capacity, activated-carbon filtration system to remove chlorine and other possible contaminants such as detergents and hexane-soluble organic materials. This water source has proven safe for aquatic organism toxicity testing, as evidenced by maintenance of the multigeneration *Daphnia* and fathead minnow cultures, with no evident loss of fecundity. If requested, reconstituted fresh water or other dilution water may be used.

3.6 Test Concentration Series

The test concentration series consists of a minimum of five dilutions and may be determined from a prior screening of the test material (e.g., 1, 3, 10, 30, and 100 percent effluent; or 6.25, 12.5, 25, 50, and 100 percent effluent plus a control).

3.7 Test Concentration Preparation

Test concentrations are prepared with Class A glassware.

3.8 Test Vessels and Test Volume

Test vessels are 2 L glass culture bowls; the final test volume is 1 L. Other test vessels may be used depending on the study's requirements.

3.9 Test Organism Number

Tests are conducted using 2 replicates per concentration, with 10 organisms per container. Fish are randomly assigned to each replicate test container. More replicates can be added, if appropriate.

3.10 Test Environment

The test vessels are maintained at 20 ± 1 C or 25 ± 1 C (unless a different project-specific temperature is required) in an environmentally controlled laboratory with a 16-hour light, 8-hour dark photoperiod. Temperature within the environmental room is monitored continuously using temperature recorders.

3.11 Analysis of Test Concentrations for Test Article

If required, test solutions may be analyzed for verification of chemical concentrations. The analytical method and number of analyses are determined after consultation with the client.

When chemical analyses are necessary, both nominal and actual measured test concentrations are reported.

3.12 Test Observations

Each test day, test organisms are observed to record the number of surviving organisms. Dead organisms are removed when observed. The study terminates after completion of the observation period (24 to 96 hours). The study may be extended, however, at the request of the client.

Each sample received is analyzed for temperature, conductivity, alkalinity, and hardness. Measurements of water quality taken daily include dissolved oxygen, pH, temperature, and conductivity from a minimum of one replicate of every concentration. Aliquots of effluent and receiving water may be gently aerated (100 bubbles/min) if dissolved oxygen is less than 4 mg/L or greater than 105% saturation. After test initiation, if the dissolved oxygen in any test chamber is less than 4 mg/L, all test chambers are gently aerated or other corrective action is taken. Analytical determinations are conducted according to APHA et al. (1992) and EPA (1979).

At the end of the test period, a minimum of 30 surviving fish are removed from the test vessels, placed in labeled containers, and frozen for later measurements. The fish are subsequently thawed, measured to the nearest mm (standard length), patted dry, and weighed to the nearest 0.01 g. The average length and weight of the test fish are reported, along with their respective ranges and standard deviations. For fish between 1-14 days old and within a 24-hour age range, weights and lengths usually will not be measured.

3.13 Solution Renewal (When Applicable)

When static-renewal testing is required, the test solution is renewed daily. New solutions are prepared on the day of renewal and dispensed according to Section 3.8. After the new solutions have reached test temperature and water quality measurements (temperature, pH, dissolved oxygen, and conductivity) are completed, the solution renewal may be performed by transferring the organisms from one test chamber to another or by replacing the test

solution. If the test solution replacement method is used, caution must be given not to stress the test organisms while the test chamber is siphoned. Usually 75 percent of the old solution is removed and replaced; however, the amount is dependent on the size of the test organisms.

3.14 Data Analysis

The LC50 and/or EC50 values and associated statistics are calculated using the probit, moving average, and binomial methods as described by Stephan (1977). Depending on the nature of the data, other methods may be used including the Trimmed Spearman-Kärber Method, the probit approximation method of Litchfield and Wilcoxon (1949), SAS probit analysis (SAS Institute 1985), or graphical interpolation using the log concentration vs. percent mortality and/or percent affected as described by APHA et al. (1992). The methods used are specified in the final report.

3.15 Test Applicability

An individual test may be conditionally acceptable if temperature, DO, and other specified conditions fall outside specifications, depending on the degree of the departure and the objectives of the tests.

4. FINAL REPORT

The final report is prepared to contain, at a minimum, the following information:

- Objectives and procedures stated in the approved protocol, including any changes made to the original protocol
- Identity of the test article(s) by name or code number and their strength (i.e., quality/purity), and a description of any pretreatment
- Source of the dilution water, its chemical characteristics, and a description of any pretreatment

- Test concentration series used and duration of the assay
- Mean standard lengths and wet weights of test fish, the respective standard deviations, and approximate biomass loadings (g fish/L of test solution), if applicable
- Water quality characteristics (pH, dissolved oxygen, temperature, etc.) of dilution water and selected test concentrations during testing
- Any unforeseen circumstances that may have affected the quality or integrity of the study
- Signature of the project manager, senior technical reviewer, and quality control officer authorizing release of the report
- Location of all archived data and the original copy of the final report at EA

Items of data to be included in the report consist of experimental design and test performance; effects on general appearance of test organisms (if applicable); morbidity and mortality; presentation of water quality characteristics, and survival data.

5. QUALITY ASSURANCE

5.1 Amendments to Protocol

Amendments to the authorized protocol established by EA or by the client are made only after proper authorization. Such authorization is achieved by completion of the Amendment to Protocol Form by EA after consultation with the client.

5.2 Standard Operating Procedures

Unless otherwise specified, all procedures given in the protocol are subject to detailed Standard Operating Procedures (SOPs) which are contained in the SOP manuals of the participating departments. These SOPs and protocols generally follow the types of requirements outlined in the U.S. EPA's Good Laboratory Practice Standards (GLPs) (EPA 1989).

5.3 Reference Toxicant

A reference toxicant test, utilizing sodium dodecyl sulfate (SDS), cadmium chloride, or another appropriate chemical is used as an internal quality check of the sensitivity of the test organisms. Testing is conducted at least once monthly on organisms that are cultured in-house. The results of each test are compared with historical, species-specific toxicological information from reference toxicant tests performed at EA, to determine if the results are within acceptable limits. Limits are established using the control charts outlined in Weber (1991).

5.4 Quality Assurance Evaluation

Studies conducted under this protocol may be subject to internal audit by EA's Quality Assurance Unit. A quality control officer is responsible for monitoring each study to assure the client that the facilities, equipment, personnel, methods, practices, records, and controls are in conformance with EA's QC program and, if applicable, EPA's GLPs.

5.5 Inspection by Regulatory Authorities

In the event of an inspection of EA by an outside authority during the course of the study, the client whose study is being inspected will be consulted before examiners are permitted access to any of the project records or the experimental areas.

5.6 Archives

Copies of project-specific records shall be transferred to the client promptly after the project is completed or as negotiated and budgeted. Original primary data are retained at EA for 5 years. Primary data include chain-of-custody records, laboratory data sheets, records, memoranda, notes, photographs, microfilm, and computer printouts that are a result of the original observations and activities of the study and which are necessary for the reconstruction and evaluation of the study report.

5.7 Location

Studies are conducted at the Aquatic Toxicology Laboratory of EA Engineering, Science, and Technology, Inc. at the Loveton Office in Sparks, Maryland.

6. SPECIFICATIONS OF THE FATHEAD MINNOW ACUTE TOXICITY TEST

6.1 Basic References

American Public Health Association (APHA) American Water Works Association, Water Environment Federation. 1992. Standard Methods for Examination of Water and Wastewater, 18th or most recent version. APHA, Washington, D.C.

American Society for Testing and Materials (ASTM). 1991. Standard Practice for Conducting Acute Tests with Fishes, Macroinvertebrates, and Amphibians. ASTM Designation: E729-80, Philadelphia, Pa.

EA Manual ATS-102. 1992. Aquatic Toxicology Studies, Quality Control and Standard Operating Procedures Manual. Fourth Revision. Internal document prepared by Aquatic Toxicology Services, EA Engineering, Science, and Technology, Inc.

Litchfield, J.T., Jr. and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. *J. Pharm. Exp. Ther.* 96:99-113.

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EA Engineering, Science,
and Technology, Inc.
Effective May 1981
Revision 4, April 1992

SAS Institute Inc. 1985. SAS® User's Guide: Basics, Version 5 Edition. Cary, NC: SAS Institute Inc. 1290 pp.

Stephan, C.E. 1977. Methods for calculating an LC50, in Aquatic Toxicology and Hazard Evaluation (F.L. Mayer and J.L. Hamelink, eds.), pp. 65-84. ASTM STP 634. American Society for Testing and Materials, Philadelphia.

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Weber, C.I. 1991. Editor. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. Fourth Edition. EPA/600/4-90/027. U.S. Environmental Protection Agency. Environmental Monitoring Systems Laboratory, Cincinnati, Ohio.

6.2 Test Specifications

Test organism:	Fathead minnow (<i>Pimephales promelas</i>)
Temperature:	20±1 C or 25±1 C
Fish age:	Dependent on test guidelines being followed, but in general, 1-14 days with a 24-hour range in age
Fish length:	The largest fish is not more than 50 percent longer than the smallest fish
Loading ratio:	In general, test containers may not contain greater than 0.65 g/L biomass

PROTOCOL ATS-SAF-FM-04

Aeration:	None, unless dissolved oxygen falls below 4 mg/L
Light quality:	Wide-spectrum fluorescent light
Light intensity:	50-100 f.c.
Photoperiod:	16-hour light, 8-hour dark
Dilution water:	Dechlorinated municipal tap water, reconstituted fresh water, or appropriate receiving water
Test containers:	2 L glass culture bowl
Test volume:	1 L/replicate
No. of concentrations:	Minimum of five test concentrations and a control
No. of replicates:	2
Test animals per container:	10 (if loading is exceeded, more replicates or larger test containers may be used)
Feeding regime:	Animals are not fed during tests unless fish are too small to survive 96 hours without feeding.
Test type and duration:	Rangefinding--24-96 hours Definitive--24-96 hours

Endpoints:

Mortality and/or Immobilization

Immobilization--defined as cessation of movement except for minor activity of appendages

Mortality--defined as cessation of opercular movement and inability to respond to external stimuli (gentle prodding with glass rod)

Test acceptability:

90 percent or greater survival in the control solution

ATTACHMENT II

DATA SHEETS



AQUATIC ORGANISMS ACUTE TOXICITY TEST DATA SHEET

Project No.: 7000310

TEST: Static, Flow-through, Renewal

TEST ORGANISM:

Beginning Date: 10/6/95 Time: 1330

Client: Calgon

Test Container: 4" bowl

Common Name: waterflea

Ending Date: 10-8-95 Time: 1313

OC Test No.: TN-678

Test Volume: 200 ml

Scientific Name: D. magna

Adults Isolated (Time, Date): 10-5-95 1600

Test Article: effluent, influent, composite

Test Duration: 48 hrs

Lot No.: NA

Organisms Pulled (Time, Date): 10-6-95 1320

Sample No.: ATS-1088, 1089, 1090

Source: lab

Acclimation: NA or Age 24 hrs

Diluent: Dechlor

TARGET VALUES

Culture Water T/S. @ Transfer (± 2) 20°C

Dilution No.: NA

Temp: 20 \pm 1 DO: 7.4 mg/L

pH: 6-9 Salinity: NA

Conc. or %	Rep. No	Number of Live Organisms					ALL TESTS																								
							Temperature (°C)					pH					Dissolved Oxygen (mg/L)					Conductivity (μ S/cm) Salinity (ppt)					Other				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96					
Control	A	10	10	10			19.9	20.2				8.1	8.2				8.8	8.5				277	258								
	B	10	10	10				20.0					8.3					8.0					383								
ATS-1088	A	10	10	10			20.6	20.4				7.9	8.3				8.5	8.5				308	336								
	B	10	10	10				20.4					8.5					8.3					338								
ATS-1089	A	10	10	10			20.7	20.6				8.1	8.3				8.7	8.5				367	340								
	B	10	10	10				20.4					8.5					8.5					348								
ATS-1090	A	10	10	10			20.5	20.6				8.1	8.4				8.6	8.6				374	343								
	B	10	10	10				20.4					8.6					8.4					347								
Instrument Number						73/13	73/13	73/13			73/13	73/13	73/13			56	56	56			73/13	73/13	73/13								
Correction Factor						1.5	1.7	1.5			1.10	1.0	1.10			8.8	8.9	8.9			1.10	1.13	1.13								
Time		1345	1335	1313			1212	1230	1315			142	1230	1315			1217	1230	1315			1215	1230	1316							
Investigator		CB	CB	CB			CB	CB	CB			CB	CB	CB			CB	CB	CB			CB	CB	CB							



AQUATIC TOXICOLOGY BENCH SHEET
SET UP (SMALL VOLUMES)

Project No.: 70003-10
 Client: Calgon
 QC Test No.: IN-678

Date/Time	Activity	Investigator
10-6-95	Dilutions Made	KK
	Test Vessels Filled	
	Transferred Organisms	KK
	Head Counts	CMH

1200
1330
1345

↓

Sample No.: ATS-1088, 1089, 1090
 Dilution No.: Dechlor

Test Concentration	Volume Test Material (ml)	Final Volume
Control	0 ml	400 ml
ATS-1088) 100%	400 ml	↓
ATS-1089) 100%	400 ml	
ATS-1090) 100%	400 ml	
	400 ml	



AQUATIC TOXICOLOGY BENCH SHEET

Project No: 70003.10
Client: Calgon
QC Test No.: TV-678

Date/Time Activity Investigator

10-6-95 1000	<u>Doi</u>	<u>DoF</u>	<u>time</u>	
ATS-1088	8.7	8.5	60min	1d/c
ATS-1089	9.3	8.7	↓	
ATS-1090	8.8	8.6		



AQUATIC ORGANISMS ACUTE TOXICITY TEST DATA SHEET

Project No.: 70003.10

TEST: Static, Flow-through, Renewal

TEST ORGANISM:

Beginning Date: 10/6/95 Time: 1500

Client: Calgon

Test Container: 1-L Beaker

Common Name: fatheads

Ending Date: 10/10/95 Time: 1530

QC Test No.: TN-679

Test Volume: 250ml

Scientific Name: P. promelas

Adults Isolated (Time, Date): NA

Test Article: efitri info comp

Test Duration: 96 hrs

Lot No.: S-10/3 FH

Organisms Pulled (Time, Date): NA

Sample No.: ATS-1088, 1089, 1090

Source: Lab

Acclimation: - or Age 3 days

Diluent: Dechlor

TARGET VALUES

Culture Water T/S. @ Transfer (± 2) 2.7

Dilution No.: NA

Temp: 20.1 DO: 7.4 mg/L

pH: 6.9 Salinity: NA

Conc. or %	Rep. No	Number of Live Organisms					ALL TESTS																				Other					
							Temperature (°C)					pH					Dissolved Oxygen (mg/L)					Conductivity (µS/cm) - Salinity (ppt)										
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96						
Control	A	10	9	9	9	9	19.9	19.9	19.9	20.3	20.4	8.1	7.8	7.9	8.2	8.2	8.8	8.9	8.6	8.6	8.5	277	261	278	278	275						
	B	10	8	8	8	8	19.6					CH																				
115-1088)	100%	A	10	10	10	10	10	20.6	19.8	19.8	20.1	20.4	7.9	7.1	8.1	8.0	8.2	8.5	9.0	8.8	9.0	9.1	308	305	303	299	292					
	B	10	10	10	10	10	19.6																									
115-1089)	100%	A	10	9	10	10	10	20.7	19.8	19.9	20.7	20.4	8.1	7.3	8.2	8.0	8.2	8.7	8.9	8.5	8.6	8.7	367	340	358	349	320					
	B	10	10	10	10	10																										
15-1090)	100%	A	10	10	10	10	10	20.8	19.8	20.0	20.7	20.1	8.1	7.5	8.2	8.1	8.2	8.6	8.8	8.6	8.5	8.7	374	344	363	356	363					
	B	10	10	10	10	10																										
Instrument Number						73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92							
Correction Factor						1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5							
Time		1505	1520	1530	1445	1530	1212	1426	1505	1446	1535	1720	1526	1505	1446	1535	1417	1522	1510	1447	1535	1215	1426	1505	1448	1535						
Investigator		CH	CH	WL	V	CB	WL	CH	WL	V	CB	WL	CH	WL	V	CB	WL	CH	WL	V	CB	WL	CH	WL	V	CB						

(u) checked by DBS



AQUATIC TOXICOLOGY BENCH SHEET
SET UP (SMALL VOLUMES)

Project No.: 70003.10
 Client: Calgon
 QC Test No.: TN-679

Date/Time	Activity	Investigator
10-6-95	1200 Dilutions Made Test Vessels Filled	KK
↓	1500 Transferred Organisms	CMH
↓	1505 Head Counts	CH

Sample No.: ATS-1088, 1089, 1090
 Dilution No.: Archler

Test Concentration	Volume Test Material (ml)	Final Volume
Control	0ml	500ml
(ATS-1088) 100%	500ml	↓
(ATS-1089) 100%	↓	↓
(ATS-1090) 100%	↓	↓



EA Engineering, Science, and Technology

EA Aquatic Toxicology Laboratories
 15 Loveton Circle
 Sparks (Baltimore), Maryland 21152
 Telephone: (410) 771-4950
 Fax: (410) 771-4204



EA Aquatic Toxicology Laboratories

FOR OFFICE USE ONLY
 Species to be tested:

- D. magna
- D. pulex
- C. dubia
- P. promelas
- Other
- Menidia sp.
- P. pugio
- C. variegatus
- M. bahia
- Other

A = Acute C = Chronic B = Bioaccumulation

Client: Perry Nuclear Power plant Project No.: _____

NPDES Number: _____ Client Purchase Order Number: _____

State/City/County Collected: OH / W. Perry / Lake

PLEASE READ SAMPLING INSTRUCTIONS ON BACK OF FORM

Accession Number (office use only)	Grab	Composite	Collection		Sample Description (including Site, Station Number, and Outfall Number)	Number/Volume of Container
			Start Date/Time	End Date/Time		
ATS-1088	✓		10/4/95 0940		Influent Prior to treatment	1/GAL
ATS-1089	✓		10/4/95 0850		Effluent " " "	1/GAL
ATS-1090		✓	10/4/95 0854	10/4/95 2120	Composite Effluent	1/GAL

Sampled By: <u>Perry Nuclear</u>	Date/Time <u>10/4/95 0800</u>	Received By:	Date/Time
Sampler's Printed Name:	Title:	Relinquished By:	Date/Time
Relinquished By: <u>[Signature]</u>	Date/Time <u>10/5/95 1815</u>	Received By Laboratory: <u>[Signature]</u>	Date/Time <u>10/6/95 0930</u>

Was Sample Chilled During Collection? Yes/No

Comments:
 Cooler was open for most of the
 water was gone upon receipt [Signature]

Sample Shipped By: (circle)

Fed. Ex. Puro. UPS Airborne

Other: _____

Air Bill Number: _____

Sample Temperature at Receipt: H C

ATTACHMENT III
REPORT QUALITY ASSURANCE RECORD



REPORT QUALITY ASSURANCE RECORD

Client: Calgon Corporation
 Project Number: 70003-10
 Author: Virginia A. Sohn

EA Report No.: 2141
 Type Analysis: Acute
 Test Organisms: D. magna & P. promelas

REPORT CHECKLIST

QA/QC ITEM	REVIEWER	DATE
1. Samples collected, transported, and received according to study plan requirements.	<u>Virginia A. Sohn</u>	<u>10/25/95</u>
2. Samples prepared and processed according to study plan requirements.	<u>Virginia A. Sohn</u>	<u>10/25/95</u>
3. Data collected using calibrated equipment.	<u>Virginia A. Sohn</u>	<u>10/25/95</u>
4. Calculations checked:		
- Hand calculations checked	<u>Not applicable</u>	<u> </u>
- Documented and verified statistical procedure used.	<u>Not applicable</u>	<u> </u>
5. Data input/statistical analyses complete and correct.	<u>Richard A. Connelly</u>	<u>10/30/95</u>
6. Reported results and facts checked against original sources.	<u>Richard A. Connelly</u>	<u>10/30/95</u>
7. Data presented in figures and tables correct and in agreement with text.	<u>Richard A. Connelly</u>	<u>10/30/95</u>
8. Results reviewed for compliance with study plan requirements.	<u>Virginia A. Sohn</u>	<u>10/25/95</u>

	AUTHOR	DATE
9. Commentary reviewed and resolved.	<u>Virginia A. Sohn</u>	<u>10/30/95</u>
10. All study plan and quality assurance/control requirements have been met and the report is approved:		
	<u>Virginia A. Sohn</u>	<u>10/30/95</u>
	PROJECT MANAGER	DATE
	<u>Richard A. Connelly</u>	<u>10/30/95</u>
	QUALITY CONTROL OFFICER	DATE
	<u>Wayne McIlwade</u>	<u>10/30/95</u>
	SENIOR TECHNICAL REVIEWER	DATE