CHRONIC TOXICITY OF EFFLUENT FROM VISTA CHEMICAL COMPANY, VCM PLANT TO THE FATHEAD MINNOW (*Pimephales promelas*) IN A STATIC RENEWAL BIOASSAY

EN89305

Performed for:

Vista Chemical Company
Attn: Paul Fetzer
VCM Plant Road
Westlake, LA 70669

on

September 25 - October 2, 1989

Performed by:

ENSR Consulting and Engineering
(formerly ERT)
Environmental Laboratory
2400 West Loop South
Suite 300
Houston, TX 77027

ENSR

Formerly ERT

ENSR Consulting
and Engineering
2400 West Loop South
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Houston, TX 77027
(713) 960-8557

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REPORT FORMAT
Version 3.2
July, 1989

1. EXECUTIVE SUMMARY

1.1 OBJECTIVE: The objective of this study was to determine the chronic toxicity to *Pimephales promelas* of an effluent submitted by Vista Chemical Company.

1.2 SAMPLE DATES: The composite samples were collected by Vista Chemical Company personnel and received at ENSR Consulting and Engineering Environmental Toxicology Lab on 9/25/89.

1.3 TEST DATES: A 7-day static renewal test was conducted September 25 - October 2, 1989.

1.4 TEST PROTOCOL: The test was conducted according to methods specified by the U. S. Environmental Protection Agency (1985b, 1989), Method 1000.0.

1.5 TEST CONDITIONS: The test was conducted at a temperature of 25±1°C and a light intensity of 50 to 100 foot candles with a photoperiod of 16-hr light and 8-hr dark. 7 dilutions were tested: 26%, 52%, 59%, 67%, 76%, 86% and 100%.

76% is the critical concentration for pass/fail determination.

1.6 STANDARD TOXICANT: 24-Hour LC_{50} = 9.2 parts per thousand (ppt) Sodium Chloride (NaCl).
95% Confidence Limits = 8-11 ppt.

1.7 DILUTION WATER: Receiving Water (Bayou Verdine)

1.8 DATA ANALYSIS: This set of data could not be analyzed becausee of 100% control mortality.

1.9 TEST RESULTS-LC_{50}: An LC_{50} could not be calculated from this set of data

1.10 TEST ACCEPTABILITY: Control 1 survival, 0%, does not meet minimum 80% survival.

1.11 CONCLUSIONS AND COMMENTS: Laboratory water control survival (88%) and growth (0.18 mg/fish) demonstrated the fish used in this test were suitable for toxicity testing.

2. INTRODUCTION

The objective of this study was to determine by a 7-day static renewal test the chronic toxicity to fathead minnow (*Pimephales promelas*) larval survival and growth of an effluent. Samples were collected by the permittee and shipped to ENSR Consulting and Engineering Environmental Toxicology Lab - Houston, Texas. The 7-day chronic toxicity test was performed September 25 - October 2, 1989 in the Environmental Toxicology Laboratory at the ENSR Environmental Lab. This report follows the recommended outline by EPA (1985b, 1989) for chronic toxicity reports. All data, laboratory notebooks, and associated documentation are archived at ENSR Environmental Toxicology Lab for future reference.

3. FACILITIES AND STAFF

All toxicity testing at the ENSR Environmental Lab was performed in the Environmental Toxicology Laboratory (approximately 3,000 sq. ft.). Test organisms were cultured or acclimated to test conditions in the Aquatic Culture Facility (1,000 sq ft) separate from the Environmental Toxicology Laboratory. Both labs are located in the basement of the ENSR Consulting and Engineering building, constructed of 8" thick concrete walls and designed to minimize the effects of changing ambient climatic conditions. All chemical tests were performed in the Chemistry Laboratory (500 sq ft) located on the fourth floor of the same building or at ENSR's Chemistry Laboratory (2925 Richmond Avenue, Houston, TX 77098). All areas of the ENSR Environmental Toxicology Lab have separate, dedicated environmental control systems to avoid any possible air or water cross-contamination between facilities. A Sample Receiving Center (SRC) is located adjacent to the building's loading dock. It includes numerous laboratory refrigerators and freezers for sample storage at 4±1°C. All samples were assigned a unique, coded number upon receipt and the progress of the sample was tracked on a computer sample tracking system (MASS-11) designed for ENSR Environmental Lab.

The Environmental Laboratory is supplied with fresh water for toxicity testing from a 1,000 gallon freshwater system. Fresh water is aerated and conditioned prior to use and is filtered through a series of physical (5 micron), chemical, and biological filters, then oxygenated. Water is chemically analyzed quarterly for various inorganic and organic parameters. Two high capacity Millipore Deionized water systems provide high quality makeup water. A Millipore Q Distilled Water system provides distilled water of even higher quality water for phytoplankton cultures or chemical analyses.

All biological testing was performed in a Nor-Lake Scientific (2'x6.5') or two Bally self-contained Environmental Chambers (12'x18' and 8'x10') with dedicated microprocessor control systems capable of maintaining

temperatures within ±1.0°C in a temperature range of 5-30°C. Temperature is displayed digitally and recorded on 7-day Partlow recording thermometers for documentation. A hi/low temperature alarm system is built in. Lighting is provided by cool-white vaporproof fluorescent lights at an intensity of 70-foot candles. Light and dark photoperiods are maintained by Dayton automatic timers. Air is supplied by an oil-less Rotron Air Blower. An emergency backup power generator maintains the supply of air during power shutdowns.

Test species raised in the culture facility have been taxonomically verified and voucher specimens are maintained. Where possible, the original source of the test organisms was from EPA stocks. Genetically characterized strains are maintained for various species. All culture stocks are tested monthly and quarterly with a standard reference toxicant for the development of a cumulative database through successive generations.

Culturing of test organisms and the performance of all toxicity testing is carried out by a staff of degreed biologists. Biological and chemical analyses are performed using appropriate EPA protocols. Quality control and quality assurance audits are performed by the QA/QC Officer of the Environmental Laboratory.

4. METHODS AND MATERIALS

The type of plant, operating schedule, final products, schematic of waste-treatment, discharge information, flows, etc. have been previously submitted to the EPA by the permittee in EPA Form 2C.

Composite samples of the effluent were collected by the permittee, immediately placed on ice in a cooler and transported to Houston (Appendix A). Chain-of-custody procedures were observed and the completed documents are attached (Appendix B). Effluent samples were stored at 4°C.

Dilution water was receiving water. Appendix D describes the chemical characteristics of the effluent and receiving water.

Testing was carried out according to procedures specified in permittee's permit and Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA, 1985b, 1989).

Test organisms were fathead minnow (*Pimephales promelas*) larvae less than 24-48 hrs old, as per the EPA freshwater chronic manual (1989). The larvae were exposed in a static renewal test for 7 days to 7 different effluent concentrations 26%, 52%, 59%, 67%, 76%, 86% and 100% and 2 controls. Control 1 (dilution water) was receiving water (Bayou Verdine) and Control 2 was moderately hard lab water.

Renewal solutions (effluent concentrations) were prepared the day that each composite sample arrived at ENSR Environmental Toxicology Lab. A sufficient quantity of each test solution was prepared for three days of testing (i.e., three consecutive renewal periods). The reservoirs of test solution were stored at 4°C.

All glassware was cleaned according to procedures specified in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA, 1985b, 1989) and described in detail in "ENSR Consulting and Engineering, ENSR Environmental Laboratory Standard Operating Procedures Manual, (September 1988; revised May, 1989).

The test chambers were Nitex cylinders placed inside 1-liter beakers. The Nitex cylinders were constructed by attaching a 7-cm high collar of 363-micrometer mesh Nitex screen to a 15x60 mm glass Petri dish with silicone sealant. Larvae were placed into the cylinders in random order until 10 larvae were in each cylinder.

The test was initiated by randomly placing a Nitex cylinder containing 10 larvae into the test chamber. A total of 40 larvae was tested for each concentration (4 replicates per concentration).

The light quality and intensity were at ambient laboratory levels of 50 to 100 foot candles, with a photoperiod of 16-hour light and 8-hour dark (16L:8D). The water temperature in each test chamber was maintained at 25±1°C. The larvae in each test chamber were fed newly hatched (<24-hr old) brine shrimp nauplii three times daily: at the beginning, midway and at the end of each day. The nauplii were soaked in fatty acid, vitamin and trace element solution to enhance their nutritional value. The nauplii were rinsed with moderately hard lab water just prior to feeding.

The number of live larvae in each test chamber were recorded daily and the dead larvae discarded. Just prior to the daily renewal of the test solutions, uneaten and dead brine shrimp and other debris were carefully removed from the bottom of each Nitex cylinder with a new polyethylene dropper.

Test solutions were renewed by gently lifting the Nitex cylinders out of the test chambers and placing them into new test chambers (1-liter beakers) containing new test solutions (250 mL). During the transfer, the larvae remained in the solution filled petri dishes at the bottom of the Nitex cylinders.

This procedure for the daily renewal of the test solutions was observed to be far less stressful to the larvae than the recommended siphon technique (Method 1000.0; EPA, 1985b) which often leads to the inadvertent removal of larvae (i.e. caught up in the siphon).

The test solution volume was also reduced from the recommended 500 mL (EPA 1000.0) to 250 mL per replicate to reduce the volume of sample required for the test. This alternative is consistent with the testing protocol used by EPA Region VI Toxicity Testing Laboratory, Houston, Texas and the second edition of the EPA manual (1989).

Dissolved oxygen was measured at the beginning and end of each 24-hr exposure period at all test concentrations and in the control. Temperature, pH, and conductivity were measured at the beginning of each 24-hr exposure period at all test concentrations and in the controls. This information is contained in Appendix D.

The test was terminated after seven days of exposure. At termination, the live larvae in each test chamber were counted and preserved as a group in 4% formalin or 70% ethanol. For the dry weight analysis, the preserved larvae from each test chamber were rinsed in deionized water, transferred to tared weighing boats and dried at 100°C for a minimum of 2-hrs. Immediately upon removal from the drying oven, the weighing boats were placed in a dessicator. Weights were measured to the nearest 0.10 mg.

All data were analyzed according to the statistical flow chart outlined in the second edition of the EPA Freshwater Chronic Methods Manual (1989). Figure 1 is a generic flow chart of the statistical approach used in this report. Statistical analyses are included in Appendix E.

Survival and growth data from the fathead minnow larval survival and growth test are analyzed using hypothesis testing techniques. TOXSTAT, TOXDAT and a proprietary computer program are used for all calculations. Survival data are arc-sine transformed and both data sets are tested for normality by Shapiro-Wilk's test. Normal data are then tested for homogeneity of variances by Bartlett's test. Data passing both tests are analyzed by Dunnett's test (Dunnett, 1955). Dunnett's test is a two stage test and consists of an analysis-of-variance (ANOVA) to determine if treatment means are different from each other. If treatment means are significantly different, then the second test, a post-hoc multiple-range comparison is made between the treatment means and the control mean. If treatment means are equal, the post-hoc comparison is not performed (Snedecor and Cochran, 1980).

Data that do not meet the normality assumptions are analyzed by a nonparametric test, Steel's Many-One Rank test. If an unequal number of replicates exist, Wilcoxon Rank Sum test with Bonferroni's Adjustment is used alternatively.

A modified version of the TOXDAT program is used to calculate an LC_{50} if mortality occurred in the higher test concentrations. The LC_{50} is calculated by three different statistical models (binomial, moving-average, and probit) and the model fitting the data best is used. LC_{50}'s are corrected for control mortality by a maximum likelihood estimation method. The LC_{50} is reported as an unadjusted value.

5. RESULTS - Effluent

The results derived from the 7-day static renewal test with the fathead minnow (*Pimephales promelas*) larvae and effluent are shown in Table 1. Table 2 lists the percent survival in replicate chambers and the mean percent survival at 24-hr (Day 1), 48-hr (Day 2) and 7-days. The test results were not acceptable with survival in the controls less than 80% (EPA, 1985b, 1989). Table 3 is a summary of the growth data.

Fathead Minnow

Table 1. Survivorship during the 7-day static renewal test.

Conc.(%)		0	Test Day 1	2	3	4	5	6	7	Proportion Surviving
0 %	A	10	0	0	0	0	0	0	0	0.00
CONTROL 1	B	10	0	0	0	0	0	0	0	0.00
	C	10	0	0	0	0	0	0	0	0.00
	D	10	0	0	0	0	0	0	0	0.00
26 %	A	10	0	0	0	0	0	0	0	0.00
	B	10	0	0	0	0	0	0	0	0.00
	C	10	0	0	0	0	0	0	0	0.00
	D	10	0	0	0	0	0	0	0	0.00
52 %	A	10	0	0	0	0	0	0	0	0.00
	B	10	0	0	0	0	0	0	0	0.00
	C	10	0	0	0	0	0	0	0	0.00
	D	10	0	0	0	0	0	0	0	0.00
59 %	A	10	0	0	0	0	0	0	0	0.00
	B	10	0	0	0	0	0	0	0	0.00
	C	10	0	0	0	0	0	0	0	0.00
	D	10	0	0	0	0	0	0	0	0.00
67 %	A	10	0	0	0	0	0	0	0	0.00
	B	10	0	0	0	0	0	0	0	0.00
	C	10	0	0	0	0	0	0	0	0.00
	D	10	0	0	0	0	0	0	0	0.00
76 %	A	10	0	0	0	0	0	0	0	0.00
	B	10	0	0	0	0	0	0	0	0.00
	C	10	0	0	0	0	0	0	0	0.00
	D	10	0	0	0	0	0	0	0	0.00
86 %	A	10	0	0	0	0	0	0	0	0.00
	B	10	0	0	0	0	0	0	0	0.00
	C	10	0	0	0	0	0	0	0	0.00
	D	10	0	0	0	0	0	0	0	0.00
100 %	A	10	0	0	0	0	0	0	0	0.00
	B	10	0	0	0	0	0	0	0	0.00
	C	10	0	0	0	0	0	0	0	0.00
	D	10	0	0	0	0	0	0	0	0.00
CONTROL 2	A	10	10	10	10	10	9	9	8	0.80
	B	10	10	10	10	9	9	9	9	0.90
	C	10	10	9	9	9	8	8	8	0.80
	D	10	10	10	10	10	10	10	10	1.00

FATHEAD MINNOW

Table 2. Summary of survival data of fathead minnow larvae exposed for 7 days to the effluent.

Conc.(%)	Replicate A	B	C	D	Time 24 H	48 H	7 D	CV*
0 %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
26 %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
52 %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
59 %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
67 %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
76 %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
86 %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
100 %	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
CONTROL 2	0.80	0.90	0.80	1.00	1.00	0.98	0.88	9

* CV = StDev*100/Mean

Table 3. Summary of growth data of fathead minnow larvae exposed for 7 days to the effluent.

Conc.(%)	Replicate A	B	C	D	Statistics Mean	StDev	CV%
CONTROL 2	0.19	0.19	0.20	0.15	0.18	0.02	10

The survival data were analyzed with the EPA TOXDAT Program. TOXDAT could not calculate an LC_{50} due to 100% control mortality. Survival and growth data were not analyzed because of dilution water (receiving water) toxicity. The dilution water control showed 100% mortality in 24 hours.

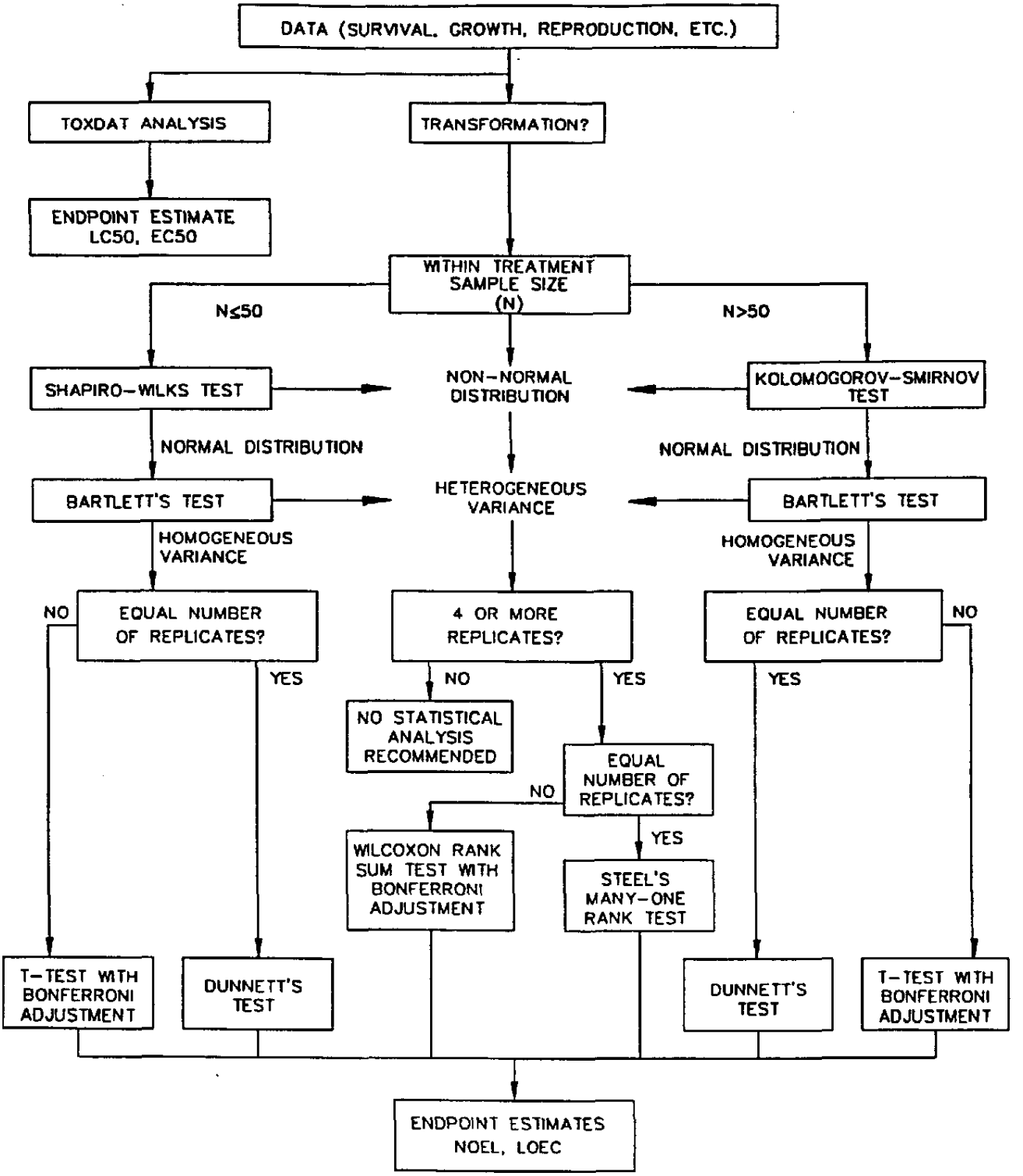

FIGURE 1
FLOW CHART FOR STATISTICAL ANALYSIS OF CHRONIC TOXICITY TEST DATA
MODIFIED FROM EPA (1988, 1989)

8615001

ENSR™

VVV 000004080

6. DISCUSSION

The response of a subsample of the fathead minnow larvae population used in this toxicity test to a reference (standard) toxicant (NaCl), as well as survivorship in the laboratory water control showed that the test organisms were in suitable condition for use in toxicity testing. ENSR Environmental Toxicology Lab routinely runs bioassays with a reference toxicant to determine the sensitivity of test organisms. When compared with historical standard toxicant results, these data indicate the similarity in sensitivities of test organism populations at different times and thus the extent to which tests performed with the same species at different times may be considered comparable. All requirements of the prescribed protocol (EPA, 1985a) were adhered to and conditions were maintained within the required limits throughout the test.

1. NOEL (or NOEC). No observed Effect Level (or Concentration) - the highest concentration of toxicant (effluent) to which the organisms are exposed in a full life-cycle or partial life-cycle test, which causes no statistically significant adverse effect on the observed parameters (EPA, 1985b)

7. LITERATURE CITED

Dunnett, C.W. 1955. Multiple comparison procedure for comparing several treatments with a control. J. Amer. Stat. Assoc. 50:1096-1112.

EPA, 1985a. Methods for measuring the acute toxicity of effluents to freshwater and marine organisms. EPA/600/4-85/013. Environmental Monitoring and Support Lab. Cincinnati, Ohio. Third Edition. 216 pp.

EPA, 1985b. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. EPA/600/4-85/014. Environmental Monitoring and Support Lab. Cincinnati, Ohio. 162 pp.

EPA, 1988. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. Environmental Monitoring and Support Lab. Cincinnati, OH. EPA 600/4-88-028. 417 pp.

EPA, 1989. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms. Environmental Monitoring and Support Laboratory. Cincinnati, OH. EPA 600/4-89-001. February, 1989. 250 pp.

Snedecor, G.W., and W.G. Cochran. 1980. Statistical Methods. Seventh Edition. Iowa State University Press. Ames. 593 pp.

8. QUALITY ASSURANCE/QUALITY CONTROL CHECKLIST AND CERTIFICATION

ENSR CONSULTING AND ENGINEERING
ENVIRONMENTAL LABORATORY - HOUSTON

QUALITY ASSURANCE / QUALITY CONTROL PROCEDURES CHECKLIST
FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST
METHOD 1000 (EPA-600/4-85/014, FEB 1989)
(Section numbers in parentheses refer to method section(s) where requirement is discussed.)

	Yes	No
A. Sample Characteristics		
1. Sample quality maintained according to protocol requirements (8.0)?	✓	
2. Sample tested within time period required by protocol (8.3.1)?	✓	
3. Salinity measured to determine if freshwater (5 ppt) test required?	✓	
B. Testing Conditions		
1. Standard toxicant LC_{50} is reasonable?	✓	
2. <24-48 hr old larvae acclimated (if necessary)	✓	
3. Test temperature maintained at 25±1°C?	✓	
4. Dissolved oxygen concentration ≥40% saturation?	✓	
5. Aeration required?		✓
6. Protocol followed without deviation?	✓	
C. Test Acceptability Criteria		
1. Laboratory water controls		
a. Average survival ≥80%?	✓	
b. Average dry weight of preserved larvae ≥0.25 mg/fish?	✓	
2. Receiving water controls		
a. Average survival ≥80%?		✓
b. Average dry weight of preserved larvae ≥0.25 mg/fish?		NA
3. Coefficient of variation between replicates ≤40% for survival?	✓	
4. Coefficient of variation between replicates ≤40% for growth?	✓	
D. Report		
1. Report pages are numbered in sequence?	✓	
2. Each page contains the sample number?	✓	
3. Page numbers listed in the table of contents correspond to pages in the body of the report?	✓	

4. Headings listed in the Table of Contents correspond to headings in the body of the report?	✓	—
5. Titles of tables listed in the Table of Contents correspond to tables in the body of the report?	✓	—
6. Titles of figures listed in the Table of Contents correspond to figures in the body of the report?	✓	—
7. Values appearing more than once in the text agree?	✓	—
8. Tables and figures appear in the order in which they are referenced in the report?	✓	—
9. Horizontal tables and figures are bound above the top edge?	✓	—
10. Sample conditions upon receipt adequately reported?	✓	—
11. Explanations are provided for all footnoted items?	✓	—
12. Units are provided for all columns and rows of data?	✓	—
13. Appendices attached to report?		
A. Sample Receiving Forms	✓	—
B. Chain-of-Custody	✓	—
C. Chemical analysis of tap water, deionized water and saltwater used in ENSR Environmental Laboratory - Houston	✓	—
D. Initial characterization of effluent and receiving water	✓	—
E. Water Quality Parameters Lab Notebook	✓	—
F. Statistical Analyses of Data	—	✓

E. COMMENTS

TABLE OF CONTENTS

CERTIFICATION

Data presented in this report were derived by methods and materials identified in the section of the report entitled "Methods and Materials," in accordance with the protocol specified by the U.S. Environmental Protection Agency (1985b, 1989). All laboratory practices and procedures were in accordance with ENSR Environmental Toxicology Lab Standard Operating Procedure and the ENSR Environmental Toxicology Lab Standard Operating Procedures Manual, (September 1988, May 1989).

A project notebook was maintained during the testing program, and all data transcribed from the notebook to the report were checked for accuracy by two people.

Hugh Johnson, Section Manager
Freshwater Biomonitoring Section
ENSR Environmental Toxicology Lab

QA/QC Officer,
ENSR Environmental Toxicology Lab

HJ/mr

23-OCT-89

APPENDIX A

Sample Receiving Form

SAMPLE CHECK-IN FORM

Vista Set #1

PROJECT #: 8615-MZ-039 EFF

EN # : 89305.01

DATE : 9/25

SALINITY : 6 ✓

DO : 9.5 [✓] YES [ ] NO

pH : 7.6 [✓] YES [ ] NO

TEMP °C : 13.0 [✓] YES [ ] NO

CONDUCTIVITY : 6,000 [✓] YES [ ] NO

BLACK SPOTS : [ ] YES [✓] NO

OUL ODOR : [ ] YES [✓] NO

SAMPLE CONTAINER [ ] POLY [ ] 1 gallon Letica pail [✓] OTHER 2.5 Gallon Cube

[ ] GLASS [ ] 500 ml [ ] 1000 ml [ ] OTHER

ENSR KIT: [✓] yes [ ] no

If not, describe: [ ] Igloo Cooler [ ] styrofoam cooler [ ] _____

IGLOO ICE: [ ] frozen [✓] cool [ ] warm

[ ] no Igloo ice

RETORT % (vol/vol): [ ] yes [✓] no

_____ % water _____ % oil _____ % solids

NITROGEN: [ ] yes [✓] no

Signed

9/25 Date

VVV 000004088

SAMPLE CHECK-IN FORM

Vista Set #1

PROJECT #: 8615-002-039 RW

EN # : 89305.02

DATE : 9/25

SALINITY : 5 -V

DO : 2.8 [4] YES [] NO

pH : 7.4 [4] YES [] NO

TEMP °C : 12.2 [4] YES [] NO

CONDUCTIVITY : 5,500 [4] YES [] NO

BLACK SPOTS : [] YES [4] NO

OIL ODOR : [] YES [4] NO

SAMPLE CONTAINER [] POLY [] 1 gallon Letica pail [4] OTHER 15 Gallon Cube

[] GLASS [] 500 ml [] 1000 ml [] OTHER

ENSR KIT: [4] yes [] no

If not, describe: [] Igloo Cooler [] styrofoam cooler [] _____

IGLOO ICE: [] frozen [4] cool [] warm

[] no Igloo ice

RETORT % (vol/vol): [] yes [4] no

_____ % water _____ % oil _____ % solids

NITROGEN: [] yes [] no

Signed

9/25/89 Date

VVV 000004089

APPENDIX B

Chain-of-Custody

Vista Chemical Company

☐ Lake Charles Chemical Plant ☒ Lake Charles VCM Plant ☐ Lake Charles LAB Plant

TST

R/A Control No. _____

Chain-of-Custody Record

C/C Control No. 01460

PROJECT NAME/NUMBER Chronic Biomonitoring

LAB DESTINATION ENSR

SAMPLE TEAM MEMBERS Fetzer - Corkran -

CARRIER/WAYBILL NO. _____

Sample Number	Sample Location and Description	Date and Time Collected	Sample Type	Container Type	Condition on Receipt (Name and Date)	Disposal Destination
1	001 Outfall					
2	001 Outfall					
3	001 Outfall					
4	001 Outfall					
~~5~~	~~001 OUTFALL~~					

Special Instructions: _____

Possible Sample Hazards: _____

SIGNATURES: (Name, Company, Date and Time)

1. Relinquished By: Doug Whaley 9-24-89 1250

Received By: Pat Fetzer ✓ ✓

2. Relinquished By: David G. Goss 9-24-89 ~~1745~~ 1645

Received By: George M. Ben 9-24-89 1645

3. Relinquished By: David G. Goss 2125 9-24-89

Received By: George M. Ben 9-24-89 2125

4. Relinquished By: Ralph Lucian 9-25-89 02:

Received By: Doris Smith 9-25-89 02:

00000091 VV

V8-65-X3

The white copy is to be returned to the Environmental Coordinator at the plant of origination after the analysis is completed.

White - To Accompany Samples
Canary - Laboratory Copy
Pink - Sample Team Copy

☐ Lake Charles Chemical Plant ☐ Lak ırles VCM Plant ☐ Lake Charles LAB Plant

R/A Control No. _____

Chain-of-Custody Record

C/C Control No. 01461

PROJECT NAME/NUMBER Chronic Biomonitoring (Specie Report LAB DESTINATION EWSR

SAMPLE TEAM MEMBERS _____ CARRIER/WAYBILL NO. _____

Sample Number	Sample Location and Description	Date and Time Collected	Sample Type	Container Type	Condition on Receipt (Name and Date)	Disposal Destination
5	001 OUTFALL					
LCVCM-8-24-89-195	001 Outfall	9-24-89 TO 9-25-89 0700 0700				
	Composite mode of sampler	Left on C/C	No 01460			

Special Instructions: _____

Possible Sample Hazards: _____

SIGNATURES: (Name, Company, Date and Time)

1. Relinquished By: CHRIS MATTE 9-25-89
   Received By: Paul Fenter Vista 9-25-89 0700
2. Relinquished By: Paul Fenter Vista 9-25-89 0900
   Received By: [signature] 9-25-89 0900
3. Relinquished By: [signature] 9/25/89 11:42am
   Received By: [signature] EWSR 9/25 11:42
4. Relinquished By: _____
   Received By: _____

VW 00004092

VB-55 X2

The white copy is to be returned to the Environmental Coordinator at the plant of origination after the analysis is completed.

White - To Accompany Samples
Canary - Laboratory Copy
Pink - Sample Team Copy

Vista Chemical Company

☐ Lake Charles Chemical Plant ☒ Lake Charles VCM Plant ☐ Lake Charles LAB Plant

TSI

R/A Control No. _____

C/C Control No. 01463

Chain-of-Custody Record

PROJECT NAME/NUMBER Chronic Biomonitoring LAB DESTINATION ENSR

SAMPLE TEAM MEMBERS _____ CARRIER/WAYBILL NO. _____

Sample Number	Sample Location and Description	Date and Time Collected	Sample Type	Container Type	Condition on Receipt (Name and Date)	Disposal Destination
1	Bayou Water	9/25/89 - 0800	GRAB	plastic		ENSR

Special Instructions: _____

Possible Sample Hazards: _____

SIGNATURES: (Name, Company, Date and Time)

1. Relinquished By: Sandra V. Carlson 9-25-89 0800

   Received By: Paul Fetzer Vista 0800 9-25-89

2. Relinquished By: Paul Fetzer Vista 0900 9-25-89

   Received By: [illegible] 0900 9-25-89

3. Relinquished By: [illegible] 9/25/89 11:42 am

   Received By: [illegible] 9/25/89 1150 AM

4. Relinquished By: _____

   Received By: _____

VV 00000403

White - To Accompany Samples
Canary - Laboratory Copy
Pink - Sample Team Copy

The white copy is to be returned to the Environmental Coordinator at the plant of origination after the analysis is completed.

APPENDIX C

Chemical Analysis of Tap Water, Deionized Water, and Saltwater Used in ENSR Environmental Toxicology Lab - Houston

Chemical analysis of tap water, deionized water, and saltwater used in ENSR Environmental Laboratory. Specific analytical references, detection limits and QA/QC results are available upon request. N.D. = Not Detected and N.M. = Not Measured.

PARAMETER	Units	Tap Water	Deionized Water	Saltwater (25 °/oo)
Inorganic Parameters				
Alkalinity	mg/L	210	4	62
Hardness	mg/L	100	N.D.	4000
TDS	mg/L	280	N.D.	20,000
TSS	mg/L	N.D.	N.D.	4
Ammonia	mg/L	1.1	N.M.	N.M.
ICP Metals/Elements				
Aluminum	µg/L	N.D.	N.M.	N.D.
Cadmium	µg/L	N.D.	N.M.	N.D.
Hexavalent Chromium	mg/L	N.D.	N.M.	N.D.
Copper	µg/L	.04	N.D.	.03
Iron	µg/L	0.4	0.08	0.06
Lead	µg/L	N.D.	N.M.	53.4
Manganese	µg/L	4.4	N.M.	N.D.
Mercury	µg/L	N.D.	N.M.	N.D.
Selenium	µg/L	N.D.	N.M.	N.D.
Zinc	µg/L	N.D.	0.3	N.D.
ORGANOCHLORINE, Pesticides				
ALPHA-BHC	µg/L	N.D	N.M.	N.M.
BETA-BHC	µg/L	N.D	N.M.	N.M.
DELTA-BHC	µg/L	N.D.	N.M.	N.M.
GAMMA-BHC (LINDANE)	µg/L	N.D.	N.M.	N.M.
HEPTACHLOR	µg/L	N.D.	N.M.	N.M.
ALDRIN	µg/L	N.D	N.M.	N.M.
HEPTACHLOR EPOXIDE	µg/L	N.D	N.M.	N.M.
ENDOSULFAN I	µg/L	N.D	N.M.	N.M.
DIELDRIN	µg/L	N.D.	N.M.	N.M.
4,4'-DDE	µg/L	N.D.	N.M.	N.M.
ENDRIN	µg/L	N.D.	N.M.	N.M.
ENDOSULFAN II	µg/L	N.D	N.M.	N.M.
4,4.'-DDD	µg/L	N.D.	N.M.	N.M.
ENDRIN ALDEHYDE	µg/L	N.D.	N.M.	N.M.
ENDOSULFAN SULFATE	µg/L	N.D.	N.M.	N.M.
4,4'-DDT	µg/L	N.D.	N.M.	N.M.
ENDRIN KETONE	µg/L	N.D.	N.M.	N.M.
METHOXYCHLOR	µg/L	N.D.	N.M.	N.M.
TOXAPHENE	µg/L	N.D.	N.M.	N.M.
AROCLOR-1016	µg/L	N.D.	N.M.	N.M.
AROCLOR-1221	µg/L	N.D.	N.M.	N.M.
AROCLOR-1232	µg/L	N.D.	N.M.	N.M.
AROCLOR-1242	µg/L	N.D.	N.M.	N.M.
AROCLOR-1248	µg/L	N.D.	N.M.	N.M.
AROCLOR-1254	µg/L	N.D.	N.M.	N.M.
AROCLOR-1260	µg/L	N.D.	N.M.	N.M.

PARAMETER	Units	Tap Water	Deionized Water	Saltwater (25 °/oo)
Organic Parameters, Volatiles				
Chloromethane	µg/L	N.D.	N.D.	N.M.
Bromomethane	µg/L	N.D.	N.D.	N.M.
Vinyl Chloride	µg/L	N.D.	N.D.	N.M.
Chloroethane *	µg/L	*	N.D.	N.M.
Methylene Chloride	µg/L	N.D.	N.D.	N.M.
1,1-Dichloroethane	µg/L	N.D.	N.D.	N.M.
1,1-Dichloroethane	µg/L	N.D.	N.D.	N.M.
1,2-Dichloroethene (total)	µg/L	N.D.	N.D.	N.M.
Chloroform	µg/L	N.D.	N.D.	N.M.
1,2-Dichloroethane	µg/L	N.D.	N.D.	N.M.
1,1,1-Trichloroethane	µg/L	N.D.	N.D.	N.M.
Carbon Tetrachloride	µg/L	N.D.	N.D.	N.M.
Bromodichloromethane	µg/L	N.D.	N.D.	N.M.
1,2-Dichloropropane	µg/L	N.D.	N.D.	N.M.
Cis-1,3-Dichloropropene	µg/L	N.D.	N.D.	N.M.
Trichloroethene	µg/L	N.D.	N.D.	N.M.
Dibromochloromethane	µg/L	N.D.	N.D.	N.M.
1,1,2-Trichloroethane	µg/L	N.D.	N.D.	N.M.
Benzene	µg/L	N.D.	N.D.	N.M.
Trans-1,3,-Dichloropropene	µg/L	N.D.	N.D.	N.M.
2-Chloroethylvinylether	µg/L	N.D.	N.D.	N.M.
Bromoform	µg/L	N.D.	N.D.	N.M.
Tetrachloroethene	µg/L	N.D.	N.D.	N.M.
1,1,2,2-Tetrachloroethane	µg/L	N.D.	N.D.	N.M.
Tolvene	µg/L	N.D.	N.D.	N.M.
Chlorobenzene	µg/L	N.D.	N.D.	N.M.
Ethylbenzene	µg/L	N.D.	N.D.	N.M.
Trichlorofluoromethane	µg/L	N.D.	N.D.	N.M.
Organic Parameters: Pthalate Esters				
Dimethyl Phthalate	µg/L	N.M.	N.M.	N.D.
Diethlyl Phthalate	µg/L	N.M.	N.M.	N.D.
DI-N-Butyl Phthalate	µg/L	N.M.	N.M.	N.D.
Butyl Benzyl Phthalate	µg/L	N.M.	N.M.	N.D.
Bis(2-Ethylhexyl)Phthalate	µg/L	N.M.	N.M.	N.D.
DI-N-Octyl Phthalate	µg/L	N.M.	N.M.	N.D.
Organic Parameters, Semivolatiles (Method 625)				
Benzo(k)fluoranthene	µg/L	N.D.	N.D.	N.D.
bis(2-Chloroethoxy)methane	µg/L	N.D.	N.D.	N.D.
bis(2-Chloroethyl)ether	µg/L	N.D.	N.D.	N.D.
bis(2-Chloroisopropyl)ether	µg/L	N.D.	N.D.	N.D.
bis(2-Ethylhexyl)phthalate	µg/L	N.D.	N.D.	N.D.
4-Bromophenyl phenyl ether	µg/L	N.D.	N.D.	N.D.
Butyl benzyl phthalate	µg/L	N.D.	N.D.	N.D.
2-Chloronaphthalene	µg/L	N.D.	N.D.	N.D.
4-Chlorophenyl phenyl ether	µg/L	N.D.	N.D.	N.D.
Chrysene	µg/L	N.D.	N.D.	N.D.

*Compound is present, but below the detection limit (10 µg/L).

PARAMETER	Units	Tap Water	Deionized Water	Saltwater (25 °/oo)
Dibenzo(a,h)anthracene	µg/L	N.D.	N.D.	N.D.
1,2-Dichlorobenzene	µg/L	N.D.	N.D.	N.D.
1,3-Dichlorobenzene	µg/L	N.D.	N.D.	N.D.
1,4-Dichlorobenzene	µg/L	N.D.	N.D.	N.D.
3,3'-Dichlorobenzidine	µg/L	N.D.	N.D.	N.D.
Diethyl phthalate	µg/L	N.D.	N.D.	N.D.
Dimethyl phthalate	µg/L	N.D.	N.D.	N.D.
Di-n-butyl phthalate	µg/L	N.D.	N.D.	N.D.
2,4-Dinitrotoluene	µg/L	N.D.	N.D.	N.D.
2,6-Dinitrotoluene	µg/L	N.D.	N.D.	N.D.
Di-n-octyl phthalate	µg/L	N.D.	N.D.	N.D.
1,2-Diphenylhydrazine	µg/L	N.D.	N.D.	N.D.
Fluoranthene	µg/L	N.D.	N.D.	N.D.
Fluorene	µg/L	N.D.	N.D.	N.D.
Hexachlorobenzene	µg/L	N.D.	N.D.	N.D.
Hexachlorobutadiene	µg/L	N.D.	N.D.	N.D.
Hexachlorocyclopentadiene	µg/L	N.D.	N.D.	N.D.
Hexachloroethane	µg/L	N.D.	N.D.	N.D.
Indeno(1,2,3-c,d)pyrene	µg/L	N.D.	N.D.	N.D.
Isophorone	µg/L	N.D.	N.D.	N.D.
Naphthalene	µg/L	N.D.	N.D.	N.D.
Nitrobenzene	µg/L	N.D.	N.D.	N.D.
N-Nitroso-dipropylamine	µg/L	N.D.	N.D.	N.D.
N-Nitrosodiphenylamine	µg/L	N.D.	N.D.	N.D.
Phenanthrene	µg/L	N.D.	N.D.	N.D.
Pyrene	µg/L	N.D.	N.D.	N.D.
1,2,4-Trichlorobenzene	µg/L	N.D.	N.D.	N.D.
2-Chlorophenol	µg/L	N.D.	N.D.	N.D.
2,4-Dichlorophenol	µg/L	N.D.	N.D.	N.D.
2,4-Dimethylphenol	µg/L	N.D.	N.D.	N.D.
4,6-Dinitro-2-methylphenol	µg/L	N.D.	N.D.	N.D.
2,4-Dinitrophenol	µg/L	N.D.	N.D.	N.D.
2-Nitrophenol	µg/L	N.D.	N.D.	N.D.
4-Nitrophenol	µg/L	N.D.	N.D.	N.D.
4-Chloro-3-methylphenol	µg/L	N.D.	N.D.	N.D.
Pentachlorophenol	µg/L	N.D.	N.D.	N.D.
Phenol	µg/L	N.D.	N.D.	N.D.
2,4,6-Trichlorophenol	µg/L	N.D.	N.D.	N.D.

APPENDIX D

Initial Characterization of Effluent and Receiving Water

Initial characterization of effluent and receiving water upon receipt and prior to mixing test concentrations.

Sample (Date Rec'd)	Alkalinity (mg/L as $CaCO_3$)	Hardness	Conductivity (μmhos/cm)	Temp. (°C)	pH (units)	D.O. (mg/L)	NH_3 (mg/L)
Effluent[1]							
EN89305.01 (9/25/89)	161	2785	6000	13.0	7.6	9.5	0.04
Receiving Water[2]							
EN89305.02 (9/25/89)	162	2573	5500	12.2	7.4	2.8	0.04

[1]Effluent is Vista Chemical Company, VCM Plant
[2]Receiving water is Bayou Verdine.

APPENDIX E

Water Quality Parameters
Lab Notebook