

ATTACHMENT 2



AQUA SURVEY, INC.

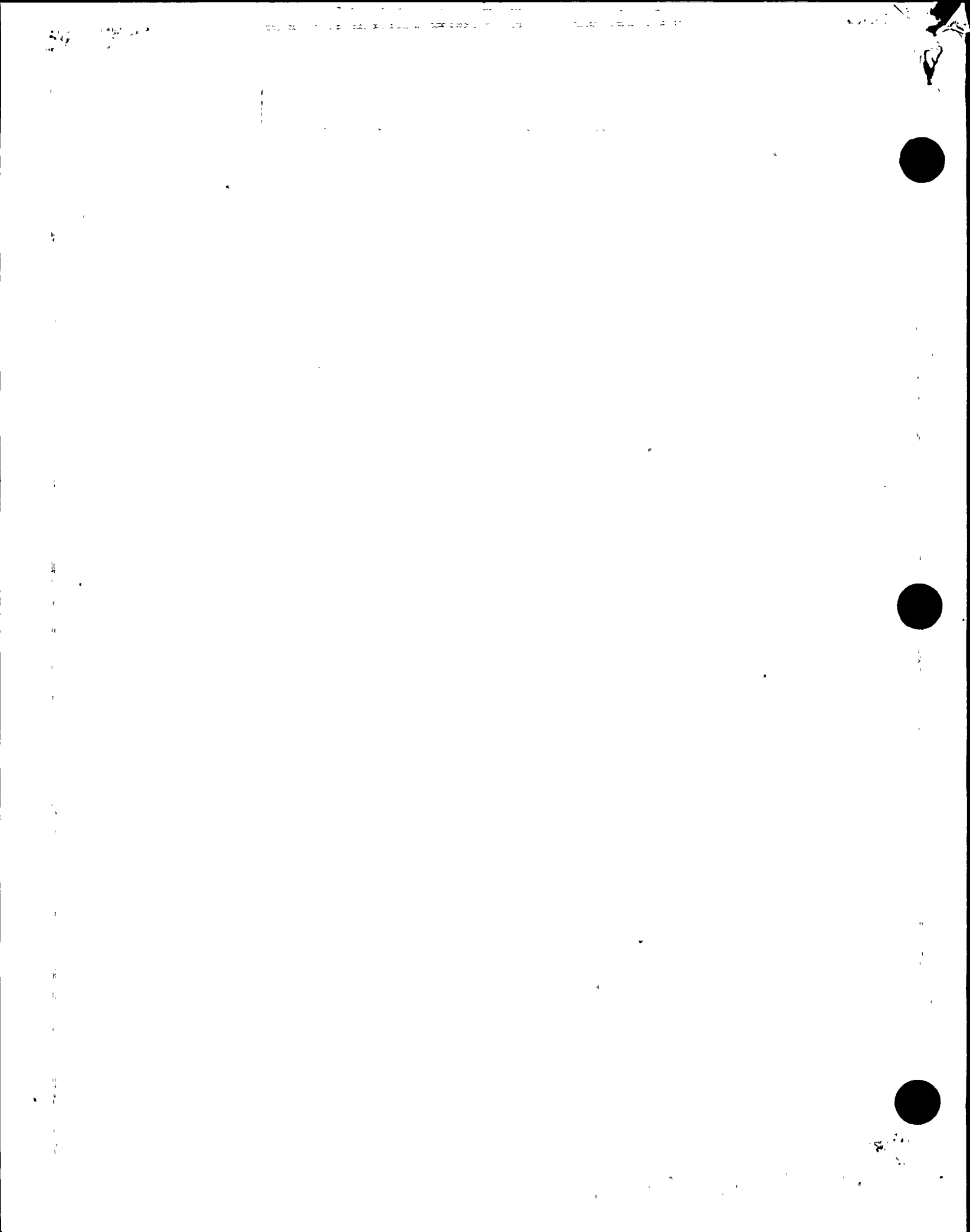
FINAL REPORT

THE ACUTE TOXICITY
OF BETZ CLAM-TROL® CT-1
IN MENIDIA MENIDIA

JULY 24, 1991

BR91-1513E

JOB #91-089E



STUDY TITLE

Clam-Trol® CT-1 Acute Bioassay with
Menidia menidia

Data Requirement

Author

York Terrell

Study Completed on
July 12, 1991

Performing Laboratory
Aqua Survey, Inc
499 Point Breeze Road
Flemington, New Jersey 08822

Laboratory Project I.D.
91-089E

STATEMENT OF NO CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10 (D) (1) (A), (B) or (C).

BETZ LABORATORIES, INC.
4636 Somerton Road
Trevose, PA 19047

Mr. Larry Lyons
Company Agent

Date

Aquatic Toxicologist Laboratory Manager
Title

Signature

ASI Project Number: 91-089E

THE ACUTE TOXICITY BIOASSAY OF CLAM-TROL®, CT-1
ON MENIDIA MENIDIA

This study does not meet the requirements for 40 CFR Part 160,

Submitter: Betz laboratories, Inc.
4636 Somerton Road
Trevose, PA 19047

Signature: _____

Date

Sponsor: Betz Laboratories, Inc.
4636 Somerton Road
Trevose, PA 19047

Signature: _____

Date

Study Director: York Terrell

York Terrell

7/24/91
Date

QUALITY ASSURANCE UNIT INSPECTION
STATEMENT FOR THE ACUTE TOXICITY OF CT-1
TO MENIDIA MENIDIA

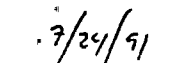
In accordance with the regulations set forth in Good Laboratory Practice for Nonclinical Laboratory Studies, 40 CFR 792, Federal Register, vol. 48, November 29, 1983, part 4, The phase of study inspection, along with the dates(s) of inspection are shown below:

<u>Phase of Study</u>	<u>Date(s) of Inspection</u>
Preparation and set-up	7/8/91
Exposure period and daily observations	7/10-11/91
Notebook entries	7/11/91
Concentration validation	7/8,9/91
Correlation of notebook entries to final report	7/23/91

Comments:

At the time of this inspection, all work had been or was being performed in a manner consistent with the Good Laboratory Practices.


Karl Cerasoli
Quality Assurance Officer


Date

Summary

A sample of Betz Clam-Trol®, CT-1, received from Betz Laboratories, Inc., Somerton Road, Trevose, PA 19047 on March 21, 1991, was tested for potential acute toxicity to the saltwater finfish Menidia menidia. The test was conducted under static conditions with daily renewal of the test solutions at nominal concentrations of 0.625, 1.25, 2.5, 5.0 and 10.0 ppm. Concentration validation made before renewals at T0 and T73 revealed a mean concentration of 0.563, 1.14, 2.46, 4.30 and 10.31 ppm while concentration validation made after renewals at T23 and T96 revealed a mean concentration of 0.39, 0.85, 1.97, 4.11 and 7.73 ppm.

After 96 hours mortality ranged from 25 percent at the 5.0 ppm concentration to 100 percent at the 10.0 ppm concentration.

The nominal 96 hour LC50 was 6.02 ppm calculated by the Binomial method.

I. Objective

The objective of this study was to determine the acute toxic effects of Betz CT-1 in a marine/estuarine aquatic environment with regard to finfish. The measure of potency is that concentration, in water, which produces a significant toxic response in the exposed animals after 96 hours (4 days).

II. Test material

Source: Betz Laboratories, Inc.
Somerton Road
Trevose, PA 19047

Name: Clam-Trol®, (CT-1)

Density:

% Solubility
in Water: 100

Date
Received: March 21, 1991

Amount
Received: 1 pint

III. Materials and Methods

A. Method

The method employed was a modification of the method outlined in Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms EPA/600/4-85/013 March 1985. (See Appendix A for the Protocol).

B. Test Organisms

Species

The test species, representing a typical temperate saltwater finfish, is Menidia menidia.

Size/Age/Physical Condition

Animals used for this test were juvenile field collected Menidia menidia. Animals appeared to be healthy and in good physical condition.

Source/Acclimation

All animals utilized in this test were field collected from Sandy Hook, New Jersey on May 29, 1991. The animals were acclimated to filtered (0.45 microns) Manasquan Inlet Water (natural seawater) for 23.25 hours at a moderate changeover rate.

C. Test System

Source of Dilution Water

Water utilized for this test was filtered (0.45 microns) natural seawater obtained from Manasquan River Inlet, Manasquan, New Jersey.

Temperature

The test temperature was 20 +/- 2° C

Test vessels

The test vessels were 20 liter, 20 x 25 x 40 cm tanks containing 6,000 ml of test solution at a depth of 9.0 cm.

Photoperiod

A 14-hour light/10-hour dark photoperiod with a 30 minute transitional period was utilized.

Loading

Each test chamber contained ten organisms with an average weight of 0.171 gram each in 6,000 mls of test solution, resulting in a loading factor of 0.285 gram per liter.

D. Test Design

Test Levels

A range finding test was conducted separately. Three concentrations of the test material were evaluated using 5 organisms per treatment with no replication. The definitive test was conducted using 10 organisms in each of 2 replicates at each of five treatment levels.

Control

A control treatment was run concurrently with the test substance treatments using the same dilution water and the same number of organisms as per the test concentrations.

Test Initiation

Testing was conducted under static conditions with daily renewal of the test solutions. An initial test material stock solution of 1,000 ppm was prepared with distilled water. Test solutions were prepared from the stock solution and then dispensed into the test chambers. The test was initiated when, within 30 minutes of test solution preparation, the test organisms were transferred to the test chambers.

The range-finding test spanned 24 hours and was conducted using 10 organisms at each of three treatment levels: 0.1, 1.0 and 10 ppm. Test results indicated a toxic response between 1 and 10.0 ppm. Exposure at the lower concentration level resulted in no mortality after 24 hours, while exposure at the higher level resulted in 80% mortality after 24 hours.

The definitive test was of 96 hours (4 days) duration and was conducted using two (2) replicates of ten organisms each at each of five treatment levels, 0.625, 1.25, 2.5, 5.0 and 10.0 ppm. Definitive test concentration levels were determined based upon 24-hour Rangefinding Test results.

Test vessels were examined at each 24 hour interval from test initiation through test termination. Survival/mortality determinations were recorded for each chamber. Dead animals were removed and discarded.

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Chemical Analyses

Concentrations were validated at T0, T23, T73, and T96 using Clam-Trol®, CT-1 methyl orange method (supplied by Betz Lab) in the range of 0.2 - 3.0 mg/l. A Bausch & Lomb Spectronic 21 was used for the absorbance readings (see Appendix, page B-1).

Physical/Chemical Parameters

The temperature, pH, dissolved oxygen (DO) concentration and salinity were measured and recorded for each treatment level at the beginning and end of each 24 hour test interval.

Weighing

At termination of the test, a random sample of test organisms was measured and weighed.

IV. Results

The definitive test resulted in mortality ranging from 25% at the 5.0 ppm concentration to 100% mortality at the 10.0 ppm concentration (See Table I).

The test solution temperature was maintained between 18.0 - 21.5° C (See Table II).

Dissolved oxygen was maintained between 5.1 and 7.4 mg/l (See Table III).

The pH ranges during the test were between 7.8 and 8.1 (See Table IV).

The salinity ranges during the test were between 29.0 and 30.0 ppt (See Table V).

The mean concentration validation before each renewal period at T0 and T73, and at the end of each renewal period at T23 and T96 can be seen in Table VI.

V. Source of Documentation

All original data documentation is being maintained at Aqua Survey, Inc., 499 Point Breeze Road, Flemington, NJ 08822, in Betz Laboratories Notebook 002, pages 93-107.

Table I:

Mortality Data (% Dead)

Exposure Time (hrs.)	<u>Control</u>		<u>0.625</u>		<u>1.25</u>		<u>2.50</u>		<u>5.0</u>		<u>10.0</u>	
	A	B	A	B	A	B	A	B	A	B	A	B
0.0	0	0	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0	100	100
48	0	0	0	0	0	0	0	0	0	0	100	100
72	0	0	0	0	0	0	0	0	20	0	100	100
96	0	0	0	0	0	0	0	0	40	10	100	100

Table II

Temperature in Degrees C.

Exposure Time (hrs.)	<u>Control</u>		<u>0.625</u>		<u>1.25</u>		<u>2.50</u>		<u>5.0</u>		<u>10.0</u>	
	A	B	A	B	A	B	A	B	A	B	A	B
0.0	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5	21.5
24	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5
48	19.5	19.5	19.5	19.5	19.0	19.5	19.5	19.5	19.5	19.5	19.5	19.5
72	19.0	19.0	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5	19.5
96	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.5	18.5

Table III

Dissolved Oxygen mg/l

Exposure Time (hrs.)	<u>Control</u>		<u>0.625</u>		<u>1.25</u>		<u>2.50</u>		<u>5.0</u>		<u>10.0</u>	
	A	B	A	B	A	B	A	B	A	B	A	B
0.0	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
24	7.2	7.2	7.2	7.2	7.3	7.3	7.3	7.3	7.4	7.4	7.4	7.4
48	6.5	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
72	6.5	6.5	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
96	5.4	5.2	5.2	5.3	5.3	5.2	5.2	5.1	5.2	5.1	5.1	5.1

Table IV

pH

Exposure Time (hrs.)	<u>Control</u>		<u>0.625</u>		<u>1.25</u>		<u>2.5</u>		<u>5.0</u>		<u>10.0</u>	
	A	B	A	B	A	B	A	B	A	B	A	B
0.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.1	8.1	8.1	8.1
24	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
48	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
72	7.9	7.9	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
96	7.8	7.8	7.8	7.8	7.8	7.8	7.9	7.8	7.9	7.8	7.8	7.8

Table V

Salinity pptExposure
Time
(hrs.)Control
A B0.625
A B1.25
A B2.50
A B5.0
A B10.0
A B

0.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0
24	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0	29.0
48	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
72	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5
96	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5	29.5

Table VI

Concentration Validation (Before Renewal)

Conc. ppm	<u>T0</u>	<u>T73</u>	Mean mg/l
0.625	0.54	0.59	0.56
1.25	1.09	1.19	1.14
2.5	2.37	2.54	2.46
5.0	4.26	4.34	4.30
10.0	10.31		10.31

Concentration Validation (After Renewal)

Conc. ppm	<u>T23</u>	<u>T96</u>	Mean mg/l
0.625	0.34	0.44	0.39
1.25	0.71	0.98	0.85
2.5	1.92	2.02	1.97
5.0	3.88	4.34	4.11
10.0	7.73		7.73

96 hrs LC50 M. meridia 91-054E (CT-1)

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (%)
0	20	20	100	9.536743E-05
	20	5	25	2.069473
2.5	20	0	0	9.536743E-05
1.25	20	0	0	9.536743E-05
.625	20	0	0	9.536743E-05

THE BINOMIAL TEST SHOWS THAT 5 AND 10 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS SINCE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS 97.93044 PERCENT. AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 6.02491

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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PROTOCOL
ACUTE TOXICITY BIOASSAY FOR
SILVERSIDE
MENIDIA MENIDIA

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PURPOSE:

To determine the acute toxicity of the test material to the Silverside and to establish the median lethal dosage (LC50) of the test material under static renewal conditions for 96 hours.

TEST ARTICLE:

The test article is CT-1 which was received from Betz Labs, Inc. on 3/21/91; the test article is clear liquid (description). The sample is labeled with an ASI number 91-089. Any test article remaining after the test is completed will be disposed of according to instructions from the sponsor or returned to the sponsor.

TEST ARTICLE CHARACTERIZATION:

Data concerning stability, uniformity, composition and additional chemical/physical characteristics of the test article will be provided by the sponsor on the Test Substance Form (supplied by ASI). Chemical analyses will be the responsibility of the sponsor unless prior arrangements have been made for ASI to assume that responsibility.

CONTROL ARTICLE:

None required.

TEST SYSTEM:

Testing is performed with the Silverside, Menidia menidia, representing an estuarine/marine finfish.

JUSTIFICATION:

Silverside are considered the appropriate test system as they are a characteristic system in which the response to toxicological agents can be evaluated.

DILUTION WATER:

Whenever possible, reconstituted sea water will be used. Reconstituted water will be aged one or two weeks and intensely aerated prior to use. If use of reconstituted water is not possible, an alternative dilution water, uncontaminated and of constant quality, will be used.



SOLVENTS:

Whenever possible, the toxicant will be introduced into the test solution without the use of solvents other than water. If alternative solvents are necessary, they will be used sparingly, not to exceed 0.5 ml/l in a solution.

TEST MATERIAL:

The technical grade, end-use product or both will be used in this study. The brand name or chemical names and a lot number are used to define a test material. Mixtures are defined by ingredient and percent of each ingredient, where possible.

TEST ORGANISMS:

The Silverside, Menidia menidia, is the organism specified for this test. Juveniles up to 90 days old, with all organisms within a ± 3 day age class, will be used. The standard length of the longest fish will be no more than twice that of the shortest. The age of the fish will be ascertained from the supplier along with any disease treatment schedule.

EXPOSURE CHAMBER:

Nineteen (19) liter all glass aquarium or other appropriate size glass chambers will be used.

RANGE-FINDING TEST:

If the toxicity of the test substance is not already known, a range finding test will be performed to determine the range of concentrations to be used in the definitive test.

DEFINITIVE TEST:

The purpose of the definitive test is to determine the concentration - response curves and the 48 and 96 hour LC50 values.

A minimum of 20 fish per concentration will be exposed to five or more concentrations of the chemical. Each designated treatment group will be exposed to a concentration of toxicant that is at least 50% of the next highest concentration.

Test organisms will be divided into two chambers per treatment group (10 fish each). All organisms will be randomly assigned to test vessels.

TEST PROCEDURES:

In preparation for the test, the chambers are filled with appropriate volumes of dilution water. The test substance is introduced into each chamber. The test is started by randomly introducing fish acclimated in accordance with the test design into the test chambers within 30 minutes after the addition of the test substance. Fish in the test chambers are observed periodically during the test, the dead fish removed and the findings recorded. Dissolved oxygen concentration, Ph, temperature, the concentration of test substance, and other water quality characteristics are measured at specified intervals in test chambers. The test solution will be renewed daily.

ACCLIMATION:

Organisms will be acclimated to the dilution water as long as possible. The fish will receive standard commercial chow and newly hatched brine shrimp, Artemia salina, daily until two days prior to testing, at which time feeding will be discontinued. Fish will not be fed during test. Test organisms will be observed prior to testing for signs of disease, stress, physical damage and mortality. Injured, dead and abnormal individuals will be discarded.

If organisms show any of these signs, remedial action will be taken during the acclimatization period and not during the test phase.

Organisms that have been treated for disease are acceptable for testing provided they do not exhibit signs of disease during the 48 hours of pretest. Organisms will not be used if they appear to be diseased or stressed or if more than 3% die during the 48 hours immediately prior to testing.

TEST PARAMETERS:

CONCENTRATION MEASUREMENT:

Analytical measurements of the test substance will be made for each concentration at T_0 , T_{23} , T_{73} , and T_{96} of the test by a method validated before beginning the test by appropriate laboratory practices.

TEMPERATURE:

Silverside will be tested at $20 \pm 2^\circ\text{C}$. Temperature will be measured continuously in at least one test vessel during the entire study.

pH:

The pH will be measured at the beginning of the test and daily thereafter at each toxicant concentration as long as animals are surviving at that level.

SALINITY:

The salinity will be measured at the beginning of the test and daily thereafter at each toxicant concentration as long as animals are surviving at that level.

DISSOLVED OXYGEN:

Dissolved oxygen (DO) will be measured at the beginning of the test and daily thereafter. Measurements will be taken from each concentration as long as animals are surviving at that concentration. The DO concentration during the first 48 hours shall be between 60 and 100% saturation and must be between 40% and 100% saturation after 48 hours.

PHOTOPERIOD:

A 14 hour light and 10 hour dark photoperiod with a 15 to 30 minute transition period will be employed.

LOADING:

The size of the test vessels will be such that the loading factor is no greater than 0.8 g/l.

OBSERVATIONS:

The fish will be observed at 24, 48, 72, and 96 hours with particular attention being given to general behavior, partial or total loss of equilibrium, fin erection and mortality.

A study is not acceptable if more than 10% of the control organisms die during a test.

WEIGHT AND LENGTH:

After all 96 hour evaluations have been performed, the test is terminated. A random sample of 30 exposed surviving fish are sacrificed for weight and length determinations.

REPORT:

The following are included in the final report:

1. **Test Material** - If technical grade product is tested, its source, batch and exact purity are reported. When end-use product is tested, the exact percent of active ingredient and the type of formulation (i.e. granular, wettable powder) are reported.
2. **Dilution Water** - Dilution water source, its chemical/physical characteristics, and pretreatment are reported.
3. **Exposure Chambers** - The volume, diluent depth, and container construction material are reported.
4. **Test Organisms** - Source, quarantine, holding and acclimatization, inclusive of feeding schedules and disease treatment procedures, are reported. The age and scientific names are reported.
5. **Range Finding Test** - Procedures and results are reported for the range finding study. Included at a minimum are sample size, concentrations tested and mortality data.
6. **Definitive Test** - Procedures used to prepare toxicant stock solutions are described. The method of dosing is reported. The criteria for determining effects are defined. At 6, 24, and 48 hours, raw data or percentage of deaths/effects at each treatment level along with the number of organisms exposed at each level is reported. Toxic symptoms, both physical and behavioral, observed during the test are reported.
7. **Calculated LC50 Value** - LC50 values (24 and 48 hours if possible) with the corresponding 95% confidence intervals and slopes of the concentration response lines and method of calculation will be reported.
8. **Temperature/DO/pH/salinity** - Temperature, dissolved oxygen, pH and salinity measurements are reported along with the range and mean of temperature.

9. **Chemical Analyses** - When chemical analyses are made to validate toxicant concentrations, the analytical method is reported as well as the results. Residue observed in exposure chambers is described in the report.
10. **Testing Protocols** - This protocol is referenced in the report.
11. **Deviations** - Deviations from protocol or standard operating procedures are reported.
12. **Certification** - The Study Director and the QA Manager certify the final report.

RECORDS:

All raw data, data manipulations, draft reports and final reports will be retained by Aqua Survey, Inc. for a period of five years from the date of completion of testing. Records will be surrendered to the sponsor upon their written request.

QUALITY ASSURANCE:

This study will be conducted in accordance with the EPA Good Laboratory Practice Regulations (Federal Register, Volume 48, November 29, 1983) and Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms EPA/600/4-85/013 March 1985.



analytical data

CLAM-TROL® CT-1 METHYL ORANGE METHOD

APPARATUS REQUIRED

Beaker, glass, 50 mL (2 required)	Code	**
Cylinder, graduated, 25 mL	2622	
Funnel Rack, separatory	936	
Funnel, separatory, with a Teflon stopcock, 250 mL (2 required)	**	
Glass Rod	114	
Optical Cell, (2 required)	**	
Spectrophotometer	**	

GENERAL APPARATUS *

Cylinder, graduated, 100 mL	Code	121
Cylinder, graduated, 250 mL	917	
Flask, volumetric, 1 L, glass (4 required)	935	
Pipet, glass, graduated, 1 mL	140	
Pipet, glass, volumetric, 1 mL	866	
Pipet, glass, volumetric, 3 mL	**	
Pipet, glass, volumetric, 5 mL	124	
Pipet, glass, volumetric, 10 mL	123	
Pipet, glass, volumetric, 15 mL	861	
Pipet, glass, volumetric, 20 mL	**	
Pipet, glass, volumetric, 25 mL	117	
Pipet, glass, volumetric, 30 mL	**	

* The general apparatus required for the test is determined by the specific test procedure used.

** Apparatus not available through Betz Lab Supply should be obtained through a local supplier.

CHEMICALS REQUIRED

1, 2 - Dichloroethane (reagent grade or equivalent)	Code	1666
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CT-1 Buffer Reagent	1591
Methanol (reagent grade or equivalent)	322
Drying Reagent, with a plastic dipper	1271

SUMMARY OF METHOD

In this procedure the dye in the CT-1 Buffer Reagent complexes with the active ingredients in Clam-Trol CT-1. This complex is extracted into 1, 2 - dichloroethane. The organic layer containing the complex is separated from the aqueous layer and dried with a drying reagent containing anhydrous sodium sulfate. The color intensity of the 1, 2 dichloroethane layer is then measured in a spectrophotometer at 415 nm.

This method must be customized to each specific application. Vary the volumes of sample, CT-1 Buffer Reagent, and 1, 2 - dichloroethane according to the test range (see Table 1). If a higher absorbance is needed, increase the volume of sample or decrease the volume of 1, 2 - dichloroethane. When increasing the sample volume it may be necessary to increase the volume of CT-1 Buffer Reagent used. For samples < 150 mL use 10 mL of CT-1 Buffer Reagent; for samples between 150 and 300 mL use 15 mL of CT-1 Buffer Reagent. Make sure that enough 1, 2 - dichloroethane is used to leave a small plug of solvent in the separatory funnel when the bottom layer of solvent is removed and to fill the optical cell properly.

GENERAL PROCEDURE

Use a well-ventilated or hooded area to run the test. Always use a safety bulb when pipetting liquids.

1,2 - Dichloroethane (also known as Ethylene Dichloride) is a priority pollutant and a specifically-listed RCRA-regulated material subject to specific disposal restrictions and/or prohibitions. For this reason, all used 1,2 - dichloroethane should be segregated from other waste streams. Dispose of waste 1,2 - dichloroethane in an approved manner (e.g., labpacking or incineration).

Table 1. Suggested Volumes for Various Ranges of CT-1 .

Range CT-1 (mg/L)	Volume CT-1 Buffer (mL)	Volume Dichloroethane (mL)	Volume Sample (mL)	Optical Cell Size
0.2 - 3.0	15	10	250	1.0 cm *
1.0 - 25.0	10	30	50	2.5 cm **
0.2 - 1.0	15	20	200	5.0 cm ***

* The 1.0-cm cell (Code 1312) can be used with Hach spectrophotometers using a 1-cm cell adapter (Code 2776C).

** The 2.5-cm cell is the standard Hach 1-in. cell (Betz Code 2601).

*** Five centimeter cells are not available for use with the Hach photometers. Many laboratory spectrophotometers require an adapter to accommodate 5-cm cells. Check with the instrument manufacturer.

1. Transfer an aliquot of the water sample to a separatory funnel (the sample). Transfer the same volume of distilled (or deionized) water to a second separatory funnel (the blank). Run the blank once for each set of samples tested (see Notes 1, 2, and 3).
2. Add CT-1 Buffer Reagent to both the sample and the blank.
3. Using a pipet, add 1, 2 - dichloroethane to both separatory funnels.
4. Insert the stoppers in each of the separatory funnels. Invert and briefly open the stopcock to vent the funnels (see Notes 4 and 5). When venting the funnels, point the tip of the funnel away from yourself and others.
5. Shake the funnels moderately for 30 sec, vent the funnels, then allow them to stand for 10 min (but no longer than 15 min).
6. Collect the lower layer (1, 2 - dichloroethane) from each funnel in 50-mL beakers leaving about 1-2 mL in the funnel. This will prevent significant removal of water.
7. Using the plastic dipper, add 2 scoops of Drying Reagent to each beaker and stir with a glass rod for 15 sec (but no longer than 30 sec).
8. Wait approximately 1 to 2 min (but not more than 5 min). Then carefully decant the extract off of the drying reagent into an optical cell.
9. Set the spectrophotometer at 415 nm and zero with 1, 2-dichloroethane. Measure and record the absorbance of the blank and the sample (see Note 6).
10. The sample absorbance minus the blank absorbance is used to determine the concentration of CT-1 in the sample. From a prepared calibration curve, determine the CT-1 concentration in the sample (see Calibration Curve Preparation).
11. Clean the cells after each measurement (see Note 7).

CALIBRATION CURVE PREPARATION

1. Prepare a 1000 mg/L CT-1 stock solution by accurately weighing 1.00 g of CT-1 into 1 L of distilled (or deionized) water.
2. Pipet designated volumes of the stock solution into 1-L volumetric flasks. These are the standard solutions used in preparing a calibration curve. Use Table 2 to make appropriate dilutions of the stock solution for each specific application.
3. Follow the General Procedure using the specific solution volumes that have been determined for the application and prepare a calibration curve. Determine the absorbance of a blank solution using distilled (or deionized) water. This blank can be subtracted from the sample absorbance or used to zero the spectrophotometer so that the calibration curve goes through the origin. The calibration curve should be linear over the indicated ranges.

NOTES

1. For maximum accuracy the calibration curve should be checked by every operator using this test and should be verified a minimum of twice per month using a freshly prepared CT-1 standard.
2. A blank measurement must be recorded for each set of samples. The blank reading may vary slightly; however, the absolute difference between the sample and the blank remains relatively constant.
3. Chlorine causes a negative interference in the test. This can be eliminated by adding 0.1 N Sodium Thiosulfate (Code 235) to the water sample before running the test. The amount added be is based on the concentration of chlorine in the system. For a 100-mL water sample containing 0.3 mg/L chlorine, add 10 drops of 0.1 N Sodium Thiosulfate to remove the interference.

Table 2. Dilutions for Calibration Curve Preparation Based on a Final Solution Volume of 1 L.

Concentration CT-1 Desired (mg/L)	CT-1 Stock Solution Added to Make 1 L (mL)
0.2	0.2
0.4	0.4
0.6	0.6
0.8	0.8
1.0	1.0
5.0	5.0
10.0	10.0
15.0	15.0
20.0	20.0
25.0	25.0

4. A slight emulsion may form when using natural water samples. When this happens, vary step 5 of the

procedure. Shake the funnel for 30 sec, vent it, then allow it to stand for 5 min. Gently invert the funnel once then allow the funnel to stand for 5 min.

5. It is important to vent the separatory funnel both before and after shaking it. Otherwise, a pressure will build up in the funnel that can cause the stopper to be forced out of the top of the funnel.
6. Use caution when inserting or removing the sample cell in the photometer. The 1, 2 - dichloroethane can damage the cell compartment.
7. It is imperative that the sample cells are kept clean during the running of the test. It is recommended that the cells are cleaned after each measurement using the following procedure:
 - a) Rinse the cell three times with distilled (or deionized) water.
 - b) Rinse the cell three times with methanol.
 - c) Rinse the cell three times with 1, 2 - dichloroethane to remove methanol from the cell.
8. This method is based upon Wang, L. K.; Langly, D. F. *Ind. Eng. Chem., Prod. Res. Dev.*, 1975, 14, 3, 210-212.



TOTAL ANALYTICAL SERVICES FOR A SAFE ENVIRONMENT

nytest environmental inc

Project No.: 9117891
Log in No. : 7861
P.O. No. : Pending
Date : April 30, 1991

ANALYTICAL DATA REPORT
PACKAGE FOR

Aqua Survey Inc.

499 Point Breeze Rd.

Flemington, NY 08822

ATTN: Jim Todd
REF: Lab Water

SAMPLE
IDENTIFICATION

LABORATORY
NUMBER

SAMPLE
MATRIX

SEE NEXT PAGE

WE CERTIFY THAT THIS REPORT IS A
TRUE REPORT OF RESULTS OBTAINED
FROM OUR TESTS OF THIS MATERIAL.

PARAG K. SHAH, Ph. D.
ORGANIC LAB. MANAGER

kb

RESPECTFULLY SUBMITTED,
NYTEST ENVIRONMENTAL INC.

[Signature]
DOUGLAS SHEELEY
LABORATORY DIRECTOR
NJ Cert# 73469

Report on sample(s) furnished by client applies to sample(s). Report on sample(s) obtained by us applies only to lot sampled. Information contained herein is not to be used for reproduction except by special permission. Sample(s) will be retained for thirty days maximum after date of report unless specifically requested otherwise by client. In the event that there are portions or parts of sample(s) remaining after Nytest has completed the required tests, Nytest shall have the option of returning such sample(s) to the client at the client's expense.

NYTEST ENVIRONMENTAL Inc.

LABORATORY
NUMBER

SAMPLE
IDENTIFICATION

TYPE OF
SAMPLE

7861001
7861002
7861003

LG SALT
LG FRESH
LP FRESH

Water
Water
Water

DATA REPORTING QUALIFIERS

- U Indicates compound was analyzed for but not detected. Report the minimum detection limit for the sample with the U (e.g. 10 U) based on necessary concentration dilution actions. (This is not necessarily the instrument detection limit.) The footnote should read U-Compound was analyzed for but not detected. The number is the minimum attainable detected limit for the sample.
- J Indicates an estimated value. This flag is used either when estimating a concentration for tentatively identified compounds where a 1:1 response is assumed or when the mass spectral data indicates the presence of a compound that meets the identification criteria but the result is less than the specified detection limit but greater than zero (e.g.: if limit of detection is 10 ug/l and a concentration of 3 ug/l is calculated, report as 3J.)
- B This flag is used when the analyte is found in the blank as well as a sample. It indicates possible/probable blank contamination and warns the data user to take appropriate action.
- T This flag identifies all targeted compounds that were found above the method detection limits.
- NA This flag indicates that the compounds are not applicable.
- A Aldol condensation product.
Note: Data on soil samples expressed on a dry weight basis.
- All non-water samples are reported on soil forms. This includes samples whose matrix is listed as miscellaneous.
- The initial and continuing calibration dates and times for the volatile fraction are listed on the BFB summary forms. The initial and continuing calibration dates and times for the semi-volatile fractions are listed on the DFTPP summary forms.

Report of Tests
Water Suitability Test

We find as follows:

The sample was analyzed according to Standard Methods for Examination of Water and Wastewater, 14th Ed., page 888.

Growth Ratio

Sample ID: LP FRESH

1.08

Lab ID No.: 7861003

Remarks:

The sample is of acceptable suitability for the use in bacteriological testing. A ratio greater than 3.0 would indicate growth promoting substances present in the water. A ratio less than 0.8 indicates the presence of growth inhibiting substances.

nytest environmental inc.

REPORT OF ANALYSIS

Log In No.: 7861

We find as follows:

Parameter(s)	Sample Identification			
	LG SALT (7861001)	LG FRESH (7861002)	LP FRESH (7861003)	Method Blank
pH	7.66	--	--	NA
Salinity, ppt	29.8	--	--	<1
Results in mg/l:				
Ammonia Nitrogen	0.130	0.22	--	<0.05
Bromide	40.8	--	--	<1.0
Fluoride	0.576	--	--	<0.02
Iodine	<1	--	--	<1
Nitrate	<0.04	--	--	<0.04
Phosphate	<0.04	--	--	<0.04
Residual Chlorine	<0.1	<0.1	<0.1	<0.1
Sulfide	<0.10	--	--	<0.10
Sulfate	1910	--	--	<1
Total Cyanide	<0.01	--	--	<0.01
Total Suspended Solids	3	5	--	<1

ppt = parts per thousand

REPORT OF ANALYSIS

Log In No.:7861

We find as follows:

Results in mg/l:

Parameter(s)	Sample Identification			
-----	LG SALT	LG FRESH	LP FRESH	Method
	(7861001)	(7861002)	(7861003)	Blank
	-----	-----	-----	-----
Aluminum	<0.2	--	--	<0.2
Arsenic	<0.010	--	<0.010	<0.010
Beryllium	<0.005	--	--	<0.005
Boron	3.3	--	--	<0.1
Cadmium	<0.005	--	<0.005	<0.005
Calcium	271	--	--	<1.0
Chromium	<0.010	--	--	<0.010
Cobalt	<0.05	--	--	<0.05
Copper	<0.025	--	<0.025	<0.025
Iron	<0.050	--	--	<0.050
Lead	<0.050	--	<0.050	<0.050
Magnesium	932	--	--	<1.0
Manganese	<0.015	--	--	<0.015
Mercury	<0.0002	--	<0.0002	<0.0002
Molybdenum	0.16	--	--	<0.010
Nickel	<0.040	--	<0.040	<0.040
Potassium	348	--	--	<1.0
Selenium	<0.010	--	--	<0.010
Silver	<0.010	--	--	<0.010
Zinc	<0.020	--	<0.020	<0.020
Total Organic Carbon	20.5	11.3	--	<1.0

10-T

NYTEST ENVIRONMENTAL INC.

TCL PESTICIDE/PCB ORGANICS ANALYSIS DATA SHEET

SAMPLE MATRIX: WATER
 CONC. LEVEL: LOW
 EXTRACTION DATE: 4/9/91
 ANALYSIS DATE: 4/18/91

SAMPLE ID: LG SALT
 LAB SAMPLE ID: 7861001
 OIL FACTOR: 1.00
 % MOISTURE: NA

UG/L

CHPD # CAS Number PESTICIDE/PCB COMPOUND

1	319-84-6	Alpha-BHC	0.050 U.
2	319-85-7	Beta-BHC	0.050 U.
3	319-86-8	Delta-BHC	0.050 U.
4	58-89-9	Gamma-BHC(Lindane)	0.050 U.
5	76-44-8	Heptachlor	0.050 U.
6	309-00-2	Aldrin	0.050 U.
7	1024-57-3	Heptachlor Epoxide	0.050 U.
8	959-98-8	Endosulfan I	0.050 U.
9	60-57-1	Dieldrin	0.100 U.
10	72-55-9	4,4'-DDE	0.100 U.
11	70-20-8	Endrin	0.100 U.
12	33213-65-9	Endosulfan II	0.100 U.
13	72-54-8	4,4'-DDD	0.100 U.
14	1031-07-8	Endosulfan Sulfate	0.100 U.
15	50-29-3	4,4'-DDT	0.100 U.
16	53494-70-5	Endrin Ketone	0.100 U.
17	72-43-5	Methoxychlor	0.500 U.
18	57-74-9	Chlordane	0.500 U.
19	8001-35-2	Toxaphene	1.000 U.
20	12674-11-2	Aroclor-1016	0.500 U.
21	11104-28-2	Aroclor-1221	0.500 U.
22	11141-16-5	Aroclor-1232	0.500 U.
23	53469-21-9	Aroclor-1242	0.500 U.
24	12672-29-6	Aroclor-1248	0.500 U.
25	11097-69-1	Aroclor-1254	1.000 U.
26	11096-82-5	Aroclor-1260	1.000 U.

ORGANOPHOSPHOROUS PESTICIDES

Log in No.: 7861
Sample ID No.: LG SALT
Lab ID No.: 7861001

Parameters (ug/l)	Detection Limit (ug/l)	Found
Vapona (Dichlorvos)	0.2	ND
Phorate	0.2	ND
Diazinon	0.6	ND
Naled (Dibron)	0.2	ND
Dementon - S	0.2	ND
Disulfoton	0.2	ND
Ronnel	0.3	ND
Chlorpyrifos (Dursban)	0.3	ND
Bolstar (Sulprofos)	0.2	ND
Stirophos	2.0	ND
Fensulfothion	5.0	ND
Azinphos methyl (Guthion)	1.5	ND
Coumaphos	1.5	ND
Malathion	0.3	ND
Parathion	0.3	ND

ND = Not Detected

10-T

HYTEST ENVIRONMENTAL INC.

TCL PESTICIDE/PCB ORGANICS ANALYSIS DATA SHEET

SAMPLE MATRIX: WATER
 CONC. LEVEL: LOW
 EXTRACTION DATE: 4/9/91
 ANALYSIS DATE: 4/19/91

SAMPLE ID: PBLK1
 LAB SAMPLE ID: A04G005
 OIL FACTOR: 1.00
 % MOISTURE: NA

UG/L

CMPO #	CAS Number	PESTICIDE/PCB COMPOUND	UG/L
1	319-84-6	Alpha-BHC	0.050 U.
2	319-85-7	Beta-BHC	0.050 U.
3	319-86-8	Delta-BHC	0.050 U.
4	58-89-9	Gamma-BHC(Lindane)	0.050 U.
5	76-44-8	Heptachlor	0.050 U.
6	309-00-2	Aldrin	0.050 U.
7	1024-57-3	Heptachlor Epoxide	0.050 U.
8	959-98-8	Endosulfan I	0.050 U.
9	60-57-1	Dieldrin	0.100 U.
10	72-55-9	4,4'-DDE	0.100 U.
11	70-20-8	Endrin	0.100 U.
12	33213-65-9	Endosulfan II	0.100 U.
13	72-54-8	4,4'-DDD	0.100 U.
14	1031-07-8	Endosulfan Sulfate	0.100 U.
15	50-29-3	4,4'-DDT	0.100 U.
16	53494-70-5	Endrin Ketone	0.100 U.
17	72-43-5	Methoxychlor	0.500 U.
18	57-74-9	Chlordane	0.500 U.
19	8001-35-2	Toxapene	1.000 U.
20	12674-11-2	Aroclor-1016	0.500 U.
21	11104-28-2	Aroclor-1221	0.500 U.
22	11141-16-5	Aroclor-1232	0.500 U.
23	53469-21-9	Aroclor-1242	0.500 U.
24	12672-29-6	Aroclor-1248	0.500 U.
25	11097-69-1	Aroclor-1254	1.000 U.
26	11096-82-5	Aroclor-1260	1.000 U.