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Department of
Environmental, Health &
Safety Affairs (DEHSA)

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February 27, 1995
RAJ-027-95

Attn: TSCA Section 8(e) Coordinator
Document Processing Center (TS-790)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, D.C. 20460



03/07/95
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Subject: 8EHQ-95-13307

Dear Sir or Madam:

As stated in the January 3, 1995 letter on this subject, Hoechst Celanese Corporation is submitting the final reports on the following previously reported studies for 3-(2H-benzotriazole-2-yl)-1,1,1'-tris(4-hydroxyphenyl)ethane:

acute toxicity to rainbow trout
acute toxicity to fathead minnow
acute toxicity to daphnids

Contains No CBI

This submission contains no confidential business information.

If any further information is required, do not hesitate to contact Dr. Richard A. Jourdenais, Manager, Product Stewardship at 908-231-3746.

Sincerely,

Dr. Richard A. Jourdenais
Manager, Product Stewardship



RAJ/mcs

CERTIFIED MAIL/
RETURN RECEIPT REQUESTED

File: Log No. 181

mm
3/28/95

110-1-13

STUDY TITLE

C-1771 (3-Benzotriazoloyl-THPE):
Acute Toxicity To The Rainbow Trout, *Oncorhynchus mykiss*,
Under Static Test Conditions

DATA REQUIREMENT

TSCA Environmental Effects Testing Guideline 797.1400

AUTHORS

Leslie D. Hartman
Jay W. Davis

Contains No CBI

STUDY COMPLETION DATE

February 9, 1995

SPONSOR

Hoechst-Celanese Corporation
Route 202-206, P.O. Box 2500
Somerville, NJ 08876-1258

CONFIDENTIAL
UNCLASSIFIED

PERFORMING LABORATORY

Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

LABORATORY PROJECT ID

J9409010b

STATEMENT OF GLP COMPLIANCE

Test Substance: C-1771 (3-Benzotriazoloyl-THPE)

Title: C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The
Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test
Conditions

This study was conducted in accordance with published Good
Laboratory Practices (GLP) regulations for tests of substances
regulated under the Toxic Substances Control Act (TSCA 40 CFR
Part 792) by the U.S. Environmental Protection Agency.



Jay W. Davis
Study Director

2/9/95

Date

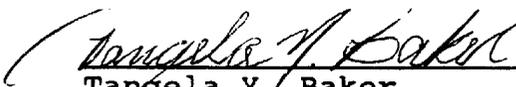
STATEMENT OF QUALITY ASSURANCE

Test Substance: C-1771 (3-Benzotriazoloyl-THPE)

Title: C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The
Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test
Conditions

Test data were reviewed by the Quality Assurance Unit to assure that standard operating procedures and the protocol developed for the study were followed. This report is an accurate reflection of the raw data. The dates of all quality assurance audits are documented below.

<u>TYPE OF AUDIT</u>	<u>DATE OF AUDIT</u>	<u>DATE FINDINGS REPORTED TO THE STUDY DIRECTOR AND TO MANAGEMENT</u>
In-Life Audit: (Test Termination)	12/20/94	12/20/94
Study Data Review:	01/27/95	01/30/95
Draft Report Review:	01/27/95	01/30/95
Final Report Review:	02/09/95	02/09/95



Tangela Y. Baker
Quality Assurance Auditor
Toxikon Environmental Sciences

2/09/95

Date

LIST OF SCIENTIFIC PERSONNEL

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Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test
Conditions

Study Director: Jay W. Davis

Principal Investigator: Leslie D. Hartman

Biologists: Hui (Jeff) Liu
Nicola Kernaghan
John Aufderheide
Andreas E. Daehnick
Flynn Jones

Aquaculturists: Jeff V. Wheat
Keith Ferris

SUMMARY

Sponsor: Hoechst-Celanese Corporation
Route 202-206, P.O. Box 2500
Somerville, NJ 08876-1258

Study Director: Jay W. Davis; (407) 575-2477

Study Location: Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

**Location of Raw Data
and Final Report:** Pathology Associates, Inc.
Frederick, Maryland

Test Substance: C-1771 (3-Benzotriazoloyl-THPE);
Lot No. SN-11311; Purity: 99.2%

Test Species: Juvenile rainbow trout, (*Oncorhynchus
mykiss*); 28.5 ± 1.8 mm average standard
length and 0.25 ± 0.06 g average wet
weight

Source of Organisms: Mt. Lassen Trout Farm
Red Bluff, CA 96080

**Condition at
Study Initiation:** Rainbow trout appeared to be in good
physical condition at study initiation,
mortality was <1% during the 48-hour
period prior to test initiation.

Dilution Water: Reconstituted freshwater with an initial
hardness and alkalinity of 83 and 54 mg/L
as CaCO₃, respectively; test temperature
range of 10.5 to 11.4°C.

**Nominal
Concentrations:** Control, Solvent (acetone) Control, 0.063,
0.13, 0.25, 0.50 and 1.00 mg ai/L

Experimental Dates: December 16 - 20, 1994

Study Length: 96 hours

Results:

The 96-hour LC₅₀ was calculated to be 0.35 mg ai/L (based upon nominal concentrations) with 95% confidence limits of 0.25 to 0.50 mg ai/L. The NOEC was 0.25 mg ai/L based on the lack of mortality and significant sub-lethal effects observed at this and lower test concentrations.

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1.0 INTRODUCTION

A static freshwater toxicity test was conducted at Toxikon Environmental Sciences, Jupiter, Florida, to determine the acute toxicity of C-1771 (3-Benzotriazoloyl-THPE) to rainbow trout, *Oncorhynchus mykiss*. The criterion for effect was death. Results of the test are expressed as a 96-hour median lethal concentration (LC₅₀), the concentration of C-1771 calculated to result in death to 50 percent of the test population at the specified time.

2.0 MATERIALS AND METHODS

2.1 TEST SUBSTANCE

The test substance, C-1771 (3-Benzotriazoloyl-THPE; Lot No. SN-11311) was received at Toxikon Environmental Sciences on October 3, 1994 in a white plastic container labeled "3-Benzotriazoloyl-THPE" from the Hoechst-Celanese Corporation. The test substance was a white powder which was stored in the dark at ambient room temperature. Hoechst-Celanese reported that the purity of the test substance was 99.2% and that the test compound was insoluble in water and highly soluble in acetone.

Nominal test concentrations are reported as milligrams (mg) of C-1771 as active ingredient (ai) per liter (L) of dilution water.

2.2 TEST SPECIES

Juvenile rainbow trout, *Oncorhynchus mykiss*, were received on November 29, 1994 from Mount Lassen Trout Farm, Red Bluff, California. Rainbow trout were maintained in filtered laboratory freshwater at a temperature of 11.2 to 18.9°C during the 14-day period prior to test initiation. Rainbow trout were maintained on a diet of salmon starter and flake food (both obtained from Zeigler Brothers, Inc., Gardners, PA) until test initiation, but were not fed during the test. Rainbow trout appeared to be in good health at test initiation and mortality was <1% during the 48 hours prior to test initiation.

Rainbow trout used for the test ranged from 25.1 to 32.0 millimeters (mm) standard length (mean and standard deviation = 28.5 ± 1.8 mm) and from 0.15 to 0.37 grams (g) wet weight (mean and standard deviation = 0.25 ± 0.06 g) as measured from the

control fish at test termination. Loading was calculated to be 0.50 g of fish tissue per liter of test solution.

2.3 TEST WATER

The dilution water was a prepared moderately hard reconstituted freshwater with an initial hardness of 83 mg/L as calcium carbonate (CaCO_3), alkalinity of 54 mg/L as CaCO_3 , specific conductivity of 283 micromhos per centimeter ($\mu\text{mhos/cm}$), and a total organic carbon content of <1.0 mg/L. The reconstituted water was prepared by the addition of measured quantities of mineral salts (NaHCO_3 , $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, MgSO_4 and KCl) to a base of deionized water and was re-aerated prior to use.

Chemical characterization of a recent representative sample of this base deionized water is presented in Appendix A.

2.4 TEST METHODS

Methods used for the 96-hour static test were those described in Toxikon Environmental Sciences' test protocol entitled: "C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions."

A 96-hour static range-finding test was conducted prior to the definitive test. Initially, three fish were exposed at nominal concentrations of 0.10, 1.00, 10.0, 100 and 1000 mg ai/L. After 96 hours, mortality in concentrations 1.00 and 10.0 mg ai/L was 100 percent. All other treatment concentrations and controls had zero percent mortality. Due to solvent restrictions and the insolubility of the test compound in acetone at 100,000 mg ai/L, the test compound was directly added to treatments 10.0, 100, and 1000 mg ai/L after adding the maximum amount of acetone allowed

(0.1 mL/L). Stock solutions in acetone were used for treatments 0.10 and 1.00 mg ai/L. It was observed during the range-finding test that all treatments ≥ 10.0 mg ai/L had precipitate in the test vessels. This observation of the test substance not being in solution may have explained why no mortality was noted at 100 and 1000 mg ai/L during the range-finding test. Based upon these results, nominal C-1771 concentrations selected for definitive testing were 0.063, 0.13, 0.25, 0.50 and 1.00 mg ai/L.

A primary stock was prepared by adding 0.1126 g of test substance to a 10-mL volumetric flask and bringing to volume with acetone. The acetone was analytical grade J.T.Baker brand solvent; lot number H11297. Additional stocks were prepared through 50:50 serial dilutions of the previously prepared stock solution resulting in a total of five stock solutions. Approximately 0.5 mL of each of these stock solutions was then added to 5-L volumes of dilution water and stirred in order to prepare the nominal test concentrations of 0.063, 0.13, 0.25, 0.50 and 1.00 mg ai/L. A dilution water control and a solvent (acetone) control were maintained concurrently with the test solutions. The acetone concentration in the solvent control and all test solutions was 0.1 mL/L.

Following the monitoring of initial water quality parameters, the 96-hour test was initiated on December 16, 1994 with the impartial addition of rainbow trout, by twos, to all test chambers until 10 trout were distributed to each chamber. All treatments were duplicated resulting in a total of 20 trout per treatment. The test chambers were 10-L glass jars (22-cm diameter x 30-cm height) containing 5.0 L of dilution water and providing a water depth of 13.9 cm. All test chambers were

covered throughout the exposure period to reduce evaporation. The test chambers were positioned in a water bath under fluorescent lighting regulated to a photoperiod of 16 hours light and 8 hours darkness. The light intensity at the surface of the test solutions ranged between 5.3 and 9.2 microEinsteins per square meter per second as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a 2π quantum sensor.

Survival of the rainbow trout was monitored daily and any dead trout removed. Any abnormalities in the behavior or physical appearance of the trout were also noted. Test solutions were not aerated during the conduct of the test. All test solutions were clear and colorless throughout the test indicating that the test substance was in solution.

Water quality (i.e., pH and dissolved oxygen concentrations) were measured in each replicate daily. The temperature was also measured daily in a control replicate. The diurnal range of the water bath temperature was continuously monitored using a minimum/maximum thermometer and recorded daily. Specific conductivity, total alkalinity, and total hardness of the dilution water were measured at test initiation and termination. Total organic carbon of the dilution water was measured at test initiation. Specific conductivity was measured using a Corning Model Checkmate 90 digital conductivity meter. Water hardness and alkalinity were determined by EDTA and potentiometric titration, respectively (APHA et al., 1992). The total organic carbon was determined using EPA Method 9060 (U.S. EPA, 1986). Dissolved oxygen concentrations and chamber temperatures were determined using a YSI Model 58 oxygen meter utilizing a membrane

electrode. The pH was measured with a Fisher Scientific Accumet® 1002 pH meter.

2.5 CHEMICAL SAMPLING AND ANALYSIS

No water samples were collected or analyzed during the test. Nominal concentrations were used during both the range-finding and definitive tests.

2.6 STATISTICAL ANALYSIS

Based on the results of the test, the 24-, 48-, 72- and 96-hour LC₅₀ values and their 95 percent confidence limits were calculated. The LC₅₀ values were estimated by a computer program (Wheat, 1989) using the following statistical methods: moving average angle, probit, logit, and non-linear interpolation. Confidence limits for LC₅₀ values determined by non-linear interpolation were calculated by binomial probability. The method selected for reporting the test results was determined by the characteristics of the data, i.e., the presence or absence of 0-percent and 100-percent mortality and the number of concentrations in which mortalities between 0 and 100 percent occurred (Stephan, 1977).

2.7 ARCHIVES

The final report and all raw data related to this study will be archived for Hoechst Celanese Corporation at Pathology Associates, Inc., Frederick, Maryland.

3.0 RESULTS AND DISCUSSION

Mortality of rainbow trout exposed for 96 hours to C-1771 (3-Benzotriazoloyl-THPE) ranged from 0 percent at test concentrations \leq 0.25 mg ai/L to 100 percent at test concentrations of 0.50 and 1.00 mg ai/L (Table 1). Mortality in the dilution water control and solvent (acetone) control was zero percent. Sublethal effects, specifically lethargy, were noted as a result of exposure to the chemical substance (Table 1). The 96-hour LC_{50} was calculated to be 0.35 mg ai/L (based on nominal concentrations) with 95% confidence limits of 0.25 to 0.50 mg ai/L using the binomial calculation method (Table 2). The no-observed-effect concentration (NOEC) was 0.25 mg ai/L based on the lack of mortality and lack of significant sublethal effects at and below this test concentration.

The test temperature during the 96-hour exposure ranged from 10.5 to 11.4°C (Table 3). The total organic carbon content of the dilution water at test initiation was <1.0 mg/L. The dilution water possessed an initial hardness and alkalinity of 83 and 54 mg/L as calcium carbonate ($CaCO_3$), respectively, and a conductivity of 283 μ mhos/cm. At test termination, the hardness and alkalinity of the dilution water (control) were 70 and 58 mg/L as $CaCO_3$, respectively, and the conductivity was 304 μ mhos/cm (Table 4). The dissolved oxygen concentrations in all control and test solutions at test initiation ranged from 10.4 to 11.2 mg/L (95 to >100 percent of saturation). The dissolved oxygen concentrations of the controls and all test solutions ranged from 7.2 to 9.5 mg/L (65 to 86 percent of saturation) during the remainder of the test (Table 5). The pH values of all controls and all test solutions ranged from 8.1 to 8.2 at test

initiation. The pH values of all controls and all test solutions ranged from 7.3 to 7.6 during the remainder of the test (Table 6).

4.0 PROTOCOL DEVIATIONS

There were no protocol deviations from the test protocol during the conduct of this test.

REFERENCES

- American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 1992. Standard Methods for the Examination of Water and Wastewater, 18th Edition.
- Stephan, C.E. 1977. Methods for calculating an LC_{50} . pp. 65-84
In: F.L. Mayer and J.L. Hamelink, Editors, Aquatic Toxicology and Hazard Evaluation, ASTM STP 534, Philadelphia, PA.
- U.S. EPA. Office of Solid Waste. 1986. Test Methods for Evaluating Solid Waste: Physical/Chemical Methods. EPA SW-846 (Third Edition) 1986.
- Wheat, J.V. 1989. Basic program for computing sets of LC_{50} values adapted from the U.S. Environmental Protection Agency, Duluth, MN, August 1978.

Table 1. Mortality of Rainbow Trout, *Oncorhynchus mykiss*, During a 96-Hour Static Exposure to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	Cumulative Number Dead (Percent Mortality)							
	24 Hour		48 Hour		72 Hour		96 Hour	
Control	0	(0)	0	(0)	0	(0)	0	(0)
Solvent Control	0	(0)	0	(0)	0	(0)	0	(0)
0.063	0	(0)	0	(0)	0	(0)	0	(0)
0.13	0	(0)	0	(0)	0	(0)	0 ^b	(0)
0.25	0	(0)	0	(0)	0	(0)	0	(0)
0.50	19 ^a	(95)	20	(100)	20	(100)	20	(100)
1.00	20	(100)	20	(100)	20	(100)	20	(100)

^a One fish appeared lethargic.

^b One fish appeared dark in coloration.

Table 2. Calculated LC₅₀ Values for Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE), Under Static Test Conditions

Exposure Period (Hour)	LC ₅₀ (mg ai/L)	95-Percent Confidence Limits (mg ai/L)	Statistical Method
24	0.37	0.25 - 0.50	Binomial
48	0.35	0.25 - 0.50	Binomial
72	0.35	0.25 - 0.50	Binomial
96	0.35	0.25 - 0.50	Binomial

Table 3. Temperatures During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	Temperature (°C)				
		0 HR	24 HR	48 HR	72 HR	96 HR
Control	A	11.4	10.7	10.8	10.5	11.0

NOTE: The diurnal temperature of the water bath ranged from 9.7 to 11.7°C as measured by a minimum/maximum thermometer during the test.

Table 4. Hardness, Alkalinity, and Conductivity Measurements During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to C-1771 (3-Benzotriazoloyl-THPE)

Day	Hardness* (mg/L)	Alkalinity* (mg/L)	Conductivity (μ mhos/cm)
0	83	54	283
4	70	58	304

* Hardness and alkalinity measurements expressed as milligrams per liter as CaCO₃.

NOTE: Dilution water for determination of hardness, alkalinity, and conductivity was collected from the control test chamber.

Table 5. Dissolved Oxygen Concentrations During a 96-Hour Static Exposure of the Rainbow Trout, *Oncorhynchus mykiss*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	Dissolved Oxygen Concentration (mg/L)				
		0 HR	24 HR	48 HR	72 HR	96 HR
Control	A	11.0	9.1	9.1	8.7	8.6
	B	11.1	9.4	9.4	8.9	8.8
Solvent Control	A	11.2	9.4	8.5	9.0	8.4
	B	11.2	9.5	8.6	9.0	8.5
0.063	A	11.0	8.6	8.0	8.0	7.3
	B	11.0	8.5	8.1	8.1	7.9
0.13	A	11.0	8.6	8.5	7.8	7.6
	B	11.0	8.5	8.7	7.8	7.3
0.25	A	10.9	9.2	8.5	8.2	7.4
	B	10.9	9.2	8.9	8.1	7.2
0.50	A	10.8	9.3	9.1	--- ^b	--- ^b
	B	10.8	9.1	--- ^b	--- ^b	--- ^b
1.00	A	10.6	9.5	--- ^b	--- ^b	--- ^b
	B	10.4	9.5	--- ^b	--- ^b	--- ^b

O ₂ Saturation ^a		11.0	11.0	11.0	11.0	11.0

^a Dissolved oxygen saturation values based on the temperature in one control chamber (control replicate A) at the specified times.

^b Water quality measurement discontinued due to 100% mortality in this test replicate.

Table 6. The pH Values During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	pH				
		0 HR	24 HR	48 HR	72 HR	96 HR
Control	A	8.1	7.4	7.3	7.5	7.4
	B	8.1	7.4	7.3	7.5	7.4
Solvent Control	A	8.1	7.4	7.3	7.5	7.4
	B	8.1	7.4	7.3	7.5	7.3
0.063	A	8.1	7.4	7.3	7.5	7.3
	B	8.1	7.4	7.3	7.5	7.3
0.13	A	8.1	7.4	7.3	7.5	7.3
	B	8.1	7.4	7.3	7.5	7.3
0.25	A	8.1	7.4	7.3	7.5	7.3
	B	8.2	7.5	7.4	7.5	7.3
0.50	A	8.2	7.5	7.4	---- ^a	---- ^a
	B	8.2	7.5	---- ^a	---- ^a	---- ^a
1.00	A	8.2	7.6	---- ^a	---- ^a	---- ^a
	B	8.2	7.6	---- ^a	---- ^a	---- ^a

^a Water quality measurement discontinued due to 100% mortality in this test replicate.

APPENDIX A
DEIONIZED WATER CHARACTERIZATION

DEIONIZED WATER CHARACTERIZATION*

Parameter	Concentration ^b
Aluminum	<0.030 mg/L
Arsenic	<0.005 mg/L
Boron	0.061 mg/L
Beryllium	<0.005 mg/L
Bromide	<0.10 mg/L
Cadmium	<0.001 mg/L
Calcium	<0.050 mg/L
Chloride	3.00 mg/L
Chromium (hexavalent)	<0.05 mg/L
Chromium (total)	<0.005 mg/L
Cobalt	<0.01 mg/L
Copper	<0.001 mg/L
Fluoride	<0.1 mg/L
Iodide	<0.050 mg/L
Iron	<0.030 mg/L
Lead	<0.003 mg/L
Manganese	<0.005 mg/L
Magnesium	<0.05 mg/L
Mercury	<0.0002 mg/L
Molybdenum	<0.005 mg/L
Nickel	<0.008 mg/L
Potassium	<0.050 mg/L
Selenium	<0.003 mg/L
Silver	<0.0001 mg/L
Sodium	<0.050 mg/L
Tin	<0.010 mg/L
Zinc	<0.005 mg/L
Ammonia (total)	<0.01 mg/L
Cyanide (total)	<0.020 mg/L
Nitrates (total as N)	<0.050 mg/L
Nitrites (total as N)	<0.050 mg/L
Phosphates (total)	<0.01 mg/L
Sulfide (total)	--
Sulfate (total)	<2.0 mg/L
TDS	68 mg/L
TOC	<1.0 mg/L
TSS	<4 mg/L
COD	<5.0 mg/L
Total organophosphorus pesticides	<1.0 µg/L
Total phenoxy herbicides	<1.2 µg/L
Total organochlorine pesticides	<0.01 µg/L
PCBs	<0.10 µg/L

* The characterized deionized water is Jupiter, Florida, town water which has been deionized by a Continental Water ion exchange system and passed through activated carbon.

^b Sample of deionized water collected June 16, 1994.

STUDY TITLE

C-1771 (3-Benzotriazoloyl-THPE):
Acute Toxicity To The Fathead Minnow, *Pimephales promelas*,
Under Static Test Conditions

DATA REQUIREMENT

TSCA Environmental Effects Testing Guideline 797.1400

AUTHORS

Leslie D. Hartman
Jay W. Davis

STUDY COMPLETION DATE

February 9, 1995

SPONSOR

Hoechst-Celanese Corporation
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Somerville, NJ 08876-1258

PERFORMING LABORATORY

Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

LABORATORY PROJECT ID

J9409010c

STATEMENT OF GLP COMPLIANCE

Test Substance: C-1771 (3-Benzotriazoloyl-THPE)

Title: C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The
Fathead Minnow, *Pimephales promelas*, Under Static Test
Conditions

This study was conducted in accordance with published Good
Laboratory Practices (GLP) regulations for tests of substances
regulated under the Toxic Substances Control Act (TSCA 40 CFR
Part 792) by the U.S. Environmental Protection Agency.

Jay W. Davis
Jay W. Davis
Study Director

2/9/95
Date

STATEMENT OF QUALITY ASSURANCE

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Test data were reviewed by the Quality Assurance Unit to assure that standard operating procedures and the protocol developed for the study were followed. This report is an accurate reflection of the raw data. The dates of all quality assurance audits are documented below.

<u>TYPE OF AUDIT</u>	<u>DATE OF AUDIT</u>	<u>DATE FINDINGS REPORTED TO THE STUDY DIRECTOR AND TO MANAGEMENT</u>
In-Life Audit:	12/09/94	12/12/94
Study Data Review:	01/19, 20/95	01/23/95
Draft Report Review:	01/19, 20/95	01/23/95
Final Report Review:	02/09/95	02/09/95



Tangela Y. Baker
Quality Assurance Auditor
Toxikon Environmental Sciences

Date 2/09/95

LIST OF SCIENTIFIC PERSONNEL

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Study Director: Jay W. Davis

Principal Investigator: Leslie D. Hartman

Biologists: Hui (Jeff) Liu
Nicola Kernaghan
John Aufderheide

Aquaculturists: Jeff V. Wheat
Keith Ferris
Richard F. Tamblyn

SUMMARY

Sponsor: Hoechst-Celanese Corporation
Route 202-206, P.O. Box 2500
Somerville, NJ 08876-1258

Study Director: Jay W. Davis; (407) 575-2477

Study Location: Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

Location of Raw Data and Final Report: Pathology Associates, Inc.
Frederick, Maryland

Test Substance: C-1771 (3-Benzotriazoloyl-THPE);
Lot No. SN-11311; Purity: 99.2%

Test Species: Juvenile fathead minnow, (*Pimephales promelas*); 16.1 ± 2.7 mm average standard length and 0.025 ± 0.022 g average wet weight

Source of Organisms: Toxikon Environmental Sciences
Jupiter, Florida

Condition at Study Initiation: Fathead minnows appeared to be in good physical condition at study initiation, mortality was 0% during the 48-hour period prior to test initiation.

Dilution Water: Reconstituted freshwater with an initial hardness and alkalinity of 72 and 54 mg/L as CaCO₃, respectively; test temperature range of 22.0 to 25.4°C.

Nominal Concentrations: Control, Solvent (acetone) Control, 0.063, 0.13, 0.25, 0.50 and 1.00 mg ai/L

Experimental Dates: December 7 - 11, 1994

Study Length: 96 hours

Results: The 96-hour LC₅₀ was calculated to be 0.16 mg ai/L (based upon nominal concentrations) with 95% confidence limits of 0.14 to 0.19 mg ai/L. The NOEC was 0.063 mg ai/L based on the lack of significant mortality and sub-lethal effects observed at this and lower test concentrations.

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1.0 INTRODUCTION

A static freshwater toxicity test was conducted at Toxikon Environmental Sciences, Jupiter, Florida, to determine the acute toxicity of C-1771 (3-Benzotriazoloyl-THPE) to the fathead minnow, *Pimephales promelas*. The criterion for effect was death. Results of the test are expressed as a 96-hour median lethal concentration (LC₅₀), the concentration of C-1771 calculated to result in death to 50 percent of the test population at the specified time.

2.0 MATERIALS AND METHODS

2.1 TEST SUBSTANCE

The test substance, C-1771 (3-Benzotriazoloyl-THPE; Lot No. SN-11311) was received at Toxikon Environmental Sciences on October 3, 1994 in a white plastic container labeled "3-Benzotriazoloyl-THPE" from the Hoechst-Celanese Corporation. The test substance was a white powder which was stored in the dark at ambient room temperature. The purity of the test substance was reported to be 99.2% and it was reported to be insoluble in water and highly soluble in acetone by Hoechst-Celanese Corporation.

Nominal test concentrations are reported as milligrams (mg) of C-1771 as active ingredient (ai) per liter (L) of dilution water.

2.2 TEST SPECIES

Juvenile fathead minnows, *Pimephales promelas*, were hatched from existing cultures at Toxikon Environmental Sciences, Jupiter, FL. Parental fish were received September 2, 1994 from Northeastern Biologists, Inc., Rhinebeck, New York. Fathead minnows were maintained in filtered laboratory freshwater at a temperature of 24.3 to 26.1°C during the 8-day period prior to test initiation. Fathead minnows were maintained on a diet of live brine shrimp (*Artemia salina*) nauplii (hatched from cysts obtained from Aquarium Products, Glen Burnie, MD) until test initiation, but were not fed during the test. Fathead minnows appeared to be in good health at test initiation and mortality was 0% during the 48 hours prior to test initiation.

Fathead minnows used for the test ranged from 13 to 22 millimeters (mm) standard length (mean and standard deviation = 16.1 ± 2.7 mm) and from 0.006 to 0.078 grams (g) wet weight (mean

and standard deviation = 0.0254 ± 0.022 g) as measured from the control fish at test termination. Loading was calculated to be 0.13 g of fish tissue per liter of test solution.

2.3 TEST WATER

The dilution water was a prepared moderately hard reconstituted freshwater with an initial hardness of 72 mg/L as calcium carbonate (CaCO_3), alkalinity of 54 mg/L as CaCO_3 , specific conductivity of 315 micromhos per centimeter ($\mu\text{mhos/cm}$), and a total organic carbon content <1.0 mg/L. The reconstituted water was prepared by the addition of measured quantities of mineral salts (NaHCO_3 , $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, MgSO_4 , and KCl) to a base of deionized water and was re-aerated prior to use.

Chemical characterization of a recent representative sample of this base deionized water is presented in Appendix A.

2.4 TEST METHODS

Methods used for the 96-hour static test were those described in Toxikon Environmental Sciences' test protocol entitled: "C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The Fathead Minnow, *Pimephales promelas*, Under Static Test Conditions."

A 96-hour static range-finding test was conducted prior to the definitive test. Initially, five fish were exposed at nominal concentrations of 0.001, 0.01, 0.10, 1.00 and 10.0 mg ai/L. After 96 hours, mortality in concentrations 1.00 and 10.0 mg ai/L was 100 percent. All other treatment concentrations and controls had zero percent mortality. A second range-finding test was conducted at nominal test concentrations of 0.10, 1.00, and 10.0 mg ai/L. Three fish, approximately twice the standard length of

the fish in the first range-finding test, were exposed at each concentration to determine if differences in the sensitivity of fish size to mortality existed. After 96 hours, mortality was 100 percent at 1.00 and 10.0 mg ai/L and zero percent at 0.10 mg ai/L. Based upon these results, nominal C-1771 concentrations selected for definitive testing were 0.063, 0.13, 0.25, 0.50 and 1.00 mg ai/L.

A primary stock was prepared by adding 0.1126 g of test substance to a 10-mL volumetric stock and bringing to volume with acetone. The acetone, lot number H11297, was J.T. Baker brand and analytical reagent grade. Additional stocks were prepared through 50:50 serial dilution of the previously prepared stock solution resulting in 5 stock solutions. Approximately 0.2 mL of each stock solution was then added to individual 2-L volumes of dilution water and stirred in order to prepare the nominal test concentrations of 0.063, 0.13, 0.25, 0.50, and 1.00 mg ai/L. A dilution water control and a solvent (acetone) control were maintained concurrently with the test solutions. The acetone concentration in the solvent control and all test solutions was 0.1 mL/L.

Following the monitoring of the initial water quality parameters, the 96-hour test was initiated on December 7, 1994 with the impartial addition of fathead minnows, by twos, to all test chambers until 10 fatheads were distributed to each chamber. All treatments were duplicated resulting in a total of 20 fathead minnows per treatment. The test chambers were 3.8-L glass jars (14-cm diameter x 23.5-cm height) containing 2.0 L of dilution water and providing a water depth of 12.4 cm. All test chambers were covered throughout the exposure period to reduce

evaporation. The test chambers were positioned in a water bath under fluorescent lighting regulated to a photoperiod of 16 hours light and 8 hours darkness. The light intensity at the surface of the test solutions ranged between 4.1 and 6.9 microEinsteins per square meter per second ($\mu\text{E}/\text{m}^2/\text{s}$) as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a 2π quantum sensor. Due to a chiller malfunction on day 2, the test chambers were moved to a temperature-controlled environmental chamber. The light intensity in the environmental chamber ranged between 14.7 to 31.6 $\mu\text{E}/\text{m}^2/\text{s}$.

Survival of fathead minnows was monitored daily and any dead fathead minnows removed. Any abnormalities in the behavior or physical appearance of the fathead minnows were also noted. Test solutions were not aerated during the conduct of the test. All test solutions remained clear and colorless throughout the test indicating that the test substance was in solution.

Water quality (i.e., pH and dissolved oxygen concentrations) were measured in each replicate daily. The temperature was also measured daily in a control replicate. The diurnal range of the water bath and/or environmental chamber temperature was continuously monitored using a minimum/maximum thermometer and recorded daily. Specific conductivity, total alkalinity, and total hardness of the dilution water were measured at test initiation and termination. Total organic carbon of the dilution water was measured at test initiation. Specific conductivity was measured using a Corning Model Checkmate 90 digital conductivity meter. Water hardness and alkalinity were determined by EDTA and potentiometric titration, respectively (APHA et al., 1992). The total organic carbon was determined using EPA Method 9060 (U.S.

EPA, 1986). Dissolved oxygen concentrations and chamber temperatures were determined using a YSI Model 58 oxygen meter utilizing a membrane electrode. The pH was measured with a Fisher Scientific Accumet® 1002 pH meter.

2.5 CHEMICAL SAMPLING AND ANALYSIS

No water samples were collected or analyzed during the test. Nominal concentrations were used during both the range-finding and definitive tests.

2.6 STATISTICAL ANALYSIS

Based on the results of the test, the 24-, 48-, 72- and 96-hour LC₅₀ values and the 95 percent confidence limits were calculated. The LC₅₀ values were estimated by a computer program (Wheat, 1989) using the following statistical methods: moving average angle, probit, logit, and non-linear interpolation. Confidence limits for LC₅₀ values determined by non-linear interpolation were calculated by binomial probability. The method selected for reporting the test results was determined by the characteristics of the data, i.e., the presence or absence of 0-percent and 100-percent mortality and the number of concentrations in which mortalities between 0 and 100 percent occurred (Stephan, 1977).

2.7 ARCHIVES

The final report and all raw data related to this study will be archived for Hoechst Celanese Corporation, Somerville, New Jersey at Pathology Associates, Inc., Frederick, Maryland.

3.0 RESULTS AND DISCUSSION

Mortality of the fathead minnow exposed for 96 hours to C-1771 (3-Benzotriazoloyl-THPE) ranged from 0 percent at a test concentration of 0.063 mg ai/L to 100 percent at test concentrations of 0.50 and 1.00 mg ai/L (Table 1). Mortality in the dilution water control and solvent (acetone) control were both zero percent. Several sublethal effects, including a loss of equilibrium, erratic behavior, and dark coloration, were noted as a result of exposure to the test substance (Table 1). The 96-hour LC₅₀ was calculated to be 0.16 mg ai/L (based on nominal concentrations) with 95% confidence limits of 0.14 to 0.19 mg ai/L using the probit calculation method (Table 2). The no-observed-effect concentration (NOEC) was 0.063 mg ai/L based on the lack of mortality and lack of significant sublethal effects at this test concentration.

The test temperature during the 96-hour exposure ranged from 22.0 to 25.4°C (Table 3). Due to a chiller malfunction on day 2, the test chambers were moved to a temperature-controlled environmental chamber. Before moving the test solutions to the environmental chamber, a control replicate had a measured temperature of 25.4°C. Within 1 hour and 45 minutes of the noted malfunction, temperature in the control chamber had returned to the targeted test range of $22 \pm 2^\circ\text{C}$ and remained within range for the duration of the test. The total organic carbon content of the dilution water at test initiation was <1.0 mg/L. The dilution water possessed an initial hardness and alkalinity of 72 and 54 mg/L as calcium carbonate (CaCO₃), respectively, and a conductivity of 315 µmhos/cm. At test termination, the hardness and alkalinity of the dilution water (control) were 80 and 56 mg/L as CaCO₃, respectively, and conductivity was 306 µmhos/cm

(Table 4). The dissolved oxygen concentrations in all control and test solutions at test initiation ranged from 8.6 to 8.7 mg/L (99 to 100 percent of saturation). The dissolved oxygen concentrations of the controls and all test concentrations ranged from 5.1 to 7.8 mg/L (59 to 90 percent of saturation) during the remainder of the test (Table 5). The pH of the controls and test solutions ranged from 8.0 to 8.1 at test initiation and ranged from 7.4 to 7.7 during the remainder of the test (Table 6).

4.0 PROTOCOL DEVIATIONS

One deviation from the test protocol occurred during the conduct of this test.

1. Due to a malfunction in the laboratory chiller unit, temperature in a control tank got as high as 25.4°C on day 2 of the test. This exceeded the stipulated temperature range of $22 \pm 2^\circ\text{C}$. In order to correct the problem, the test chambers were removed from the designated waterbath and relocated to an environmental (temperature-controlled) chamber which is maintained at approximately 24.0°C. Within 1 hour and 45 minutes of the noted malfunction, temperature in the control chamber was back in range at 23.9°C and remained within range for the remainder of the test.

In the scientific judgement of the Study Director, this deviation did not affect the outcome or validity of the test results.

REFERENCES

- American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 1992. Standard Methods for the Examination of Water and Wastewater, 18th Edition.
- Stephan, C.E. 1977. Methods for calculating an LC_{50} . pp. 65-84
In: F.L. Mayer and J.L. Hamelink, Editors, Aquatic Toxicology and Hazard Evaluation, ASTM STP 534, Philadelphia, PA.
- U.S. EPA. Office of Solid Waste. 1986. Test Methods for Evaluating Solid Waste: Physical/Chemical Methods. EPA SW-846 (Third Edition) 1986.
- Wheat, J.V. 1989. Basic program for computing sets of LC_{50} values adapted from the U.S. Environmental Protection Agency, Duluth, MN, August 1978.

Table 1. Mortality of the Fathead Minnow, *Pimephales promelas*, During a 96-Hour Static Exposure to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	Cumulative Number Dead (Percent Mortality)							
	24 Hour		48 Hour		72 Hour		96 Hour	
Control	0	(0)	0	(0)	0	(0)	0	(0)
Solvent Cont.	0	(0)	0	(0)	0	(0)	0	(0)
0.063	0	(0)	0 ^b	(0)	0 ^b	(0)	0 ^b	(0)
0.13	0	(0)	1	(5)	1 ^d	(5)	4	(20)
0.25	1 ^a	(5)	11 ^c	(55)	14 ^c	(70)	19 ^c	(95)
0.50	19 ^b	(95)	20	(100)	20	(100)	20	(100)
1.00	20	(100)	20	(100)	20	(100)	20	(100)

^a One fish exhibited erratic behavior.

^b One fish appeared dark in coloration.

^c Six fish appeared dark in coloration.

^d Two fish exhibited a complete loss of equilibrium.

^e All surviving fish exhibited a complete loss of equilibrium.

Table 2. Calculated LC₅₀ Values for the Fathead Minnow, *Pimephales promelas*, Exposed to C-1771 (3-Benzotriazoloyl-THPE), Under Static Test Conditions

Exposure Period (Hour)	LC ₅₀ (mg ai/L)	95-Percent Confidence Limits (mg ai/L)	Statistical Method
24	0.35	0.30 - 0.41	Probit
48	0.23	0.20 - 0.28	Probit
72	0.21	0.18 - 0.25	Probit
96	0.16	0.14 - 0.19	Probit

Table 3. Temperatures During a 96-Hour Static Exposure of the Fathead Minnow, *Pimephales promelas*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	Temperature (°C)				
		0 HR	24 HR	48 HR	72 HR	96 HR
Control	A	22.0	22.4	23.9 ^a	24.0	23.3

^a Due to a malfunction in the laboratory chilling unit, the temperature in a control tank was measured to be 25.4°C on day 2 of the test. In order to correct the problem, the test chambers were removed from the waterbath and relocated to a temperature-controlled environmental chamber. Within 1 hour and 45 minutes of the noted malfunction, the temperature in the control chamber was measured to be 23.9°C, within the protocol range, and remained within range for the remainder of the test.

NOTE: The diurnal temperature of the water bath ranged from 21.6 to 26.2°C as measured by a minimum/maximum thermometer during the test. The diurnal temperature of the environmental chamber was 19.2 to 23.6°C as monitored by a min/max thermometer.

Table 4. Hardness, Alkalinity, and Conductivity Measurements During a 96-Hour Static Exposure of the Fathead Minnow, *Pimephales promelas*, to C-1771 (3-Benzotriazoloyl-THPE)

Day	Hardness* (mg/L)	Alkalinity* (mg/L)	Conductivity (μ mhos/cm)
0	72	54	315
4	80	56	306

* Hardness and alkalinity measurements expressed as milligrams per liter as CaCO₃.

NOTE: Dilution water for determination of hardness, alkalinity, and conductivity was collected from the control test chamber.

Table 5. Dissolved Oxygen Concentrations During a 96-Hour Static Exposure of the Fathead Minnow, *Pimephales promelas*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	Dissolved Oxygen Concentration (mg/L)				
		0 HR	24 HR	48 HR	72 HR	96 HR
Control	A	8.7	7.4	6.7	5.8	5.7
	B	8.7	7.5	6.2	5.7	6.7
Solvent Control	A	8.7	7.6	7.0	6.6	6.5
	B	8.7	7.6	6.1	5.6	5.1
0.063	A	8.7	7.7	6.5	5.6	5.8
	B	8.7	7.7	6.6	5.9	6.1
0.13	A	8.7	7.8	6.0	5.7	6.0
	B	8.7	7.6	6.2	5.6	5.8
0.25	A	8.7	7.6	6.6	6.4	6.2
	B	8.7	7.6	6.8	6.7	5.8
0.50	A	8.7	6.7	6.2	--- ^b	--- ^b
	B	8.7	6.8	--- ^b	--- ^b	--- ^b
1.00	A	8.6	6.7	--- ^b	--- ^b	--- ^b
	B	8.6	6.0	--- ^b	--- ^b	--- ^b

O ₂ Saturation ^a		8.7	8.7	8.4	8.4	8.6

^a Dissolved oxygen saturation values based on the temperature in one control chamber at the specified times.

^b Water quality measurement discontinued due to 100% mortality in this test replicate.

Table 6. The pH Values During a 96-Hour Static Exposure of the Fathead Minnow, *Pimephales promelas*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	pH				
		0 HR	24 HR	48 HR	72 HR	96 HR
Control	A	8.0	7.5	7.6	7.5	7.5
	B	8.0	7.5	7.6	7.5	7.6
Solvent Control	A	8.0	7.7	7.7	7.6	7.6
	B	8.1	7.6	7.5	7.4	7.4
0.063	A	8.1	7.7	7.6	7.5	7.5
	B	8.1	7.7	7.6	7.5	7.5
0.13	A	8.1	7.7	7.5	7.5	7.5
	B	8.1	7.7	7.5	7.4	7.5
0.25	A	8.1	7.7	7.7	7.6	7.6
	B	8.1	7.7	7.7	7.7	7.5
0.50	A	8.1	7.6	7.5	---- ^a	---- ^a
	B	8.1	7.5	---- ^a	---- ^a	---- ^a
1.00	A	8.1	7.6	---- ^a	---- ^a	---- ^a
	B	8.1	7.5	---- ^a	---- ^a	---- ^a

^a Water quality measurement discontinued due to 100% mortality in this test replicate.

APPENDIX A
DEIONIZED WATER CHARACTERIZATION

DEIONIZED WATER CHARACTERIZATION*

Parameter	Concentration ^b
Aluminum	<0.030 mg/L
Arsenic	<0.005 mg/L
Boron	0.061 mg/L
Beryllium	<0.005 mg/L
Bromide	<0.10 mg/L
Cadmium	<0.001 mg/L
Calcium	<0.050 mg/L
Chloride	3.00 mg/L
Chromium (hexavalent)	<0.05 mg/L
Chromium (total)	<0.005 mg/L
Cobalt	<0.01 mg/L
Copper	<0.001 mg/L
Fluoride	<0.1 mg/L
Iodide	<0.050 mg/L
Iron	<0.030 mg/L
Lead	<0.003 mg/L
Manganese	<0.005 mg/L
Magnesium	<0.05 mg/L
Mercury	<0.0002 mg/L
Molybdenum	<0.005 mg/L
Nickel	<0.008 mg/L
Potassium	<0.050 mg/L
Selenium	<0.003 mg/L
Silver	<0.0001 mg/L
Sodium	<0.050 mg/L
Tin	<0.010 mg/L
Zinc	<0.005 mg/L
Ammonia (total)	<0.01 mg/L
Cyanide (total)	<0.020 mg/L
Nitrates (total as N)	<0.050 mg/L
Nitrites (total as N)	<0.050 mg/L
Phosphates (total)	<0.01 mg/L
Sulfide (total)	—
Sulfate (total)	<2.0 mg/L
TDS	68 mg/L
TOC	<1.0 mg/L
TSS	<4 mg/L
COD	<5.0 mg/L
Total organophosphorus pesticides	<1.0 µg/L
Total phenoxy herbicides	<1.2 µg/L
Total organochlorine pesticides	<0.01 µg/L
PCBs	<0.10 µg/L

* The characterized deionized water is Jupiter, Florida, town water which has been deionized by a Continental Water ion exchange system and passed through activated carbon.

^b Sample of deionized water collected June 16, 1994.

STUDY TITLE

C-1771 (3-Benzotriazoloyl-THPE):
Acute Toxicity To The Water Flea, *Daphnia magna*,
Under Static Conditions

DATA REQUIREMENT

TSCA Environmental Effects Testing Guideline 797.1300

AUTHORS

Hui (Jeff) Liu
Jay W. Davis

STUDY COMPLETION DATE

February 8, 1995

SPONSOR

Hoechst-Celanese Corporation
Route 202-206, P.O. Box 2500
Somerville, NJ 08876-1258

PERFORMING LABORATORY

Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

LABORATORY PROJECT ID

J9409010d

STATEMENT OF GLP COMPLIANCE

Test Substance: C-1771 (3-Benzotriazoloyl-THPE)

Title: C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The
Water Flea, *Daphnia magna*, Under Static Conditions

This study was conducted in accordance with published Good
Laboratory Practices (GLP) regulations for tests of substances
regulated under the Toxic Substances Control Act (TSCA 40 CFR
Part 792) by the U.S. Environmental Protection Agency.

Jay W. Davis
Jay W. Davis
Study Director

2/8/95
Date

STATEMENT OF QUALITY ASSURANCE

Test Substance: C-1771 (3-Benzotriazoloyl-THPE)

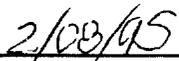
Title: C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The Water Flea, *Daphnia magna*, Under Static Conditions

Test data were reviewed by the Quality Assurance Unit to assure that standard operating procedures and the protocol developed for the study were followed. This report is an accurate reflection of the raw data. The dates of all quality assurance audits are documented below.

<u>TYPE OF AUDIT</u>	<u>AUDIT DATE</u>	<u>DATE FINDINGS REPORTED TO THE STUDY DIRECTOR AND TO MANAGEMENT</u>
In-Life Audit: (Test Initiation)	12/09/94	12/12/94
Study Data Review:	01/24/95	01/27/95
Draft Report Review:	01/24/95	01/27/95
Final Report Review:	02/08/95	02/08/95



Tangela Y. Baker
Quality Assurance Auditor
Toxikon Environmental Sciences



Date

LIST OF SCIENTIFIC PERSONNEL

Test Substance: C-1771 (3-Benzotriazoloyl-THPE)

Title: C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The
Water Flea, *Daphnia magna*, Under Static Conditions

Study Director: Jay W. Davis

Principal Investigator: Hui (Jeff) Liu

Biologists: Leslie D. Hartman
John A. Aufderheide

Aquaculturists: Jeff V. Wheat
Keith Ferris

SUMMARY

Sponsor: Hoechst-Celanese Corporation
Route 202-206, P.O. Box 2500
Somerville, NJ 08876-1258

Study Director: Jay W. Davis; (407) 575-2477

Study Location: Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

Location of Raw Data and Final Report: Pathology Associates, Inc.
Frederick, Maryland

Test Substance: C-1771 (3-Benzotriazoloyl-THPE);
Lot No. SN-11311; Purity: 99.2%

Test Species: *Daphnia magna* (neonates; < 24 hrs. old)

Source of Organisms: Toxikon Environmental Sciences
Jupiter, Florida

Condition at Study Initiation: Daphnids appeared to be in good physical condition at study initiation.

Dilution Water: Reconstituted freshwater with an initial hardness and alkalinity of 64 and 60 mg/L as CaCO₃, respectively; test temperature range of 19.0 to 20.3°C.

Nominal Concentrations: Control, Solvent (acetone) Control, 0.16, 0.31, 0.63, 1.25, 2.50, and 5.00 mg ai/L

Test Dates: December 9 to 11, 1994

Study Length: 48 hours

Results: The 48-hour EC₅₀ was 0.40 mg ai/L (based upon nominal concentrations) with 95% confidence limits of 0.33 to 0.48 mg ai/L. The NOEC was 0.16 mg ai/L based on the lack of mortality and sub-lethal effects observed at this test concentration.

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1.0 INTRODUCTION

A static freshwater toxicity test was conducted at Toxikon Environmental Sciences, Jupiter, Florida, to determine the acute toxicity of C-1771 (3-Benzotriazoloyl-THPE) to the water flea, *Daphnia magna*. The criterion for effect was death or immobilization (i.e., no movement except for minor activity of appendages). Results of the test are expressed as a 48-hour median effect concentration (EC_{50}), the concentration of C-1771 calculated to result in immobilization or death to 50 percent of the test population at the specified time.

2.0 MATERIALS AND METHODS

2.1 TEST SUBSTANCE

The test substance, C-1771 (3-Benzotriazoloyl-THPE, Lot No. SN-11311) was received at Toxikon Environmental Sciences on October 3, 1994 in a white plastic container labeled "3-Benzotriazoloyl-THPE" from Hoechst-Celanese Corporation. The test substance was a white powder which was stored in the dark at ambient room temperature. The purity of the test substance was reported to be 99.2% and it was reported to be insoluble in water and highly soluble in acetone by the Hoechst-Celanese Corporation.

Nominal test concentrations are reported as milligrams (mg) of C-1771 as active ingredient per liter (L) of dilution water.

2.2 TEST SPECIES

Daphnia magna used for testing were obtained from Toxikon Environmental Sciences' cultures which originated from animals received from the U.S. Environmental Protection Agency, Duluth, Minnesota in 1989. A subculture of adults was isolated from these cultures and maintained prior to testing. The cultures were generally fed the green algae, *Selenastrum capricornutum*, and a solution prepared from cereal leaves daily. Less than 24 hours prior to test initiation, the adults were re-isolated in dilution water containing food. Neonates (<24 hours old) were collected in food-free dilution water for test initiation on December 9, 1994. *Daphnia magna* were cultured and isolated in moderately hard freshwater. No ephippia were produced during culture and adults produced, on average, over 3 young per adult per day over the 7-day period prior to the test.

2.3 TEST WATER

The dilution water was a prepared moderately hard reconstituted freshwater with an initial hardness of 64 mg/L as calcium carbonate (CaCO_3), alkalinity of 60 mg/L as CaCO_3 , specific conductivity of 278 micromhos per centimeter ($\mu\text{mhos/cm}$), and a total organic carbon content of <1.0 mg/L. The reconstituted water was prepared by the addition of measured quantities of mineral salts (NaHCO_3 , $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, MgSO_4 , and KCl) to a base of deionized water and was re-aerated prior to use.

Chemical characterization of a recent representative sample of this base deionized water is presented in Appendix A.

2.4 TEST METHODS

Methods used for the 48-hour static test were those described in Toxikon Environmental Sciences' test protocol entitled: "C-1771 (3-Benzotriazoloyl-THPE): Acute Toxicity To The Water Flea, *Daphnia magna*, Under Static Conditions."

A 48-hour static range-finding test was conducted at nominal concentrations of 0.001, 0.01, 0.10, 1.00, and 10.0 mg ai/L prior to performing the definitive test. Ten water fleas were tested at each concentration. After 48 hours of exposure, mortality of water fleas in the range-finding test was 0 percent at test concentrations ≤ 0.10 mg ai/L, 50 percent at 1.00 mg ai/L, and 60 percent at 10 mg ai/L, respectively. Mortality in the control and solvent control was 30 and 0 percent, respectively. Based upon these results, nominal C-1771 concentrations selected for definitive testing were 0.16, 0.31, 0.63, 1.25, 2.50, and 5.00 mg ai/L.

A series of C-1771 stock solutions were prepared and utilized in this test. A stock solution of 50,000 mg ai/L was first prepared by adding 1.2604 g of C-1771 to a 25-mL volumetric flask and bringing to volume with acetone. The acetone, lot number BG810, was Burdick and Jackson brand and analytical reagent grade. Due to the limited solubility of this stock, it was only used to make the 5.00 mg ai/L test solution by adding 0.10 mL to 1 L of dilution water. A 25,000 mg ai/L primary stock solution was prepared by adding 0.6302 g of C-1771 to a 25-mL volumetric flask and bringing to volume with acetone. Secondary stocks (12,503, 6,252, 3,126, and 1,563 mg ai/L) were prepared by adding appropriate amounts of primary stock to individual 10-mL volumetric flasks and bringing them to the volume with acetone. Nominal test concentrations of 5.00, 2.50, 1.25, 0.63, 0.31, and 0.16 mg ai/L were prepared by adding 0.1 mL of the appropriate stock solutions to 1-L volumes of reconstituted freshwater. A dilution water control and solvent (acetone) control were maintained concurrently with the test solutions. The acetone concentration in the solvent control and all test solutions was 0.1 mL acetone/L.

The 48-hour definitive test was initiated on December 9, 1994 with the impartial addition of water fleas, by ones or twos, to all test chambers until 10 water fleas were distributed to each chamber following monitoring of the initial water quality parameters. Test concentrations were duplicated resulting in a total of 20 water fleas per treatment. The test chambers were 300-mL glass crystallizing dishes (10-cm diameter x 5-cm height) containing 250 mL of dilution water and providing a water depth of 4.0 cm. All test chambers were covered throughout the exposure period to reduce evaporation. The test chambers were

positioned in a temperature-controlled chamber under fluorescent lighting regulated to a photoperiod of 16 hours light and 8 hours darkness. The light intensity at the surface of the test solutions ranged between 16.2 and 27.4 microEinsteins per square meter per second as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a 2π quantum sensor.

Survival of *D. magna* was monitored daily and any dead or immobilized water fleas were removed. Any abnormalities in the behavior or physical appearance of the daphnids were also noted. Water fleas were not fed, nor were test solutions aerated during the test. Test solutions ≤ 1.25 mg ai/L (nominal concentration) remained clear and colorless for the duration of the test. A white precipitate was observed at concentrations ≥ 2.50 mg ai/L (nominal concentration) throughout the test indicating that the solubility limit of the test material was exceeded at these test concentrations.

At test initiation, water quality (i.e., pH and dissolved oxygen concentrations) was measured in composites of all test solutions. The temperature was measured daily in a control replicate. At test termination, water quality (i.e., pH and dissolved oxygen concentrations) was measured in each replicate of all test solutions. The diurnal temperature range of the temperature-controlled chamber was continuously monitored using a minimum/maximum thermometer and recorded daily. Specific conductivity, total alkalinity, total hardness, and total organic carbon of the dilution water were measured at test initiation. Specific conductivity was measured using a Corning Model Checkmate 90 digital conductivity meter. Water hardness and alkalinity were determined by EDTA and potentiometric titration,

respectively (APHA et al., 1992). The total organic carbon was determined using EPA Method 9060 (U.S. EPA, 1986). Dissolved oxygen concentrations and chamber temperatures were determined using a YSI Model 58 oxygen meter utilizing a membrane electrode. The pH was measured with a Fisher Scientific Accumet® 1002 pH meter.

2.5 CHEMICAL SAMPLING AND ANALYSIS

No water samples were collected or analyzed during the test. Nominal concentrations were used during both the range-finding and definitive tests.

2.6 STATISTICAL ANALYSIS

Based on results of the test, the 24- and 48-hour EC₅₀ values and their 95 percent confidence limits were calculated. The EC₅₀ values were estimated by a computer program (Wheat, 1989) using the following statistical methods: moving average angle, probit, logit and non-linear interpolation. Confidence limits for EC₅₀ values determined by non-linear interpolation were calculated by binomial probability. The method selected for reporting the test results was determined by the characteristics of the data, i.e., the presence or absence of 0-percent and 100-percent immobilization or mortality and the number of concentrations in which immobilization or mortality between 0 and 100 percent occurred (Stephan, 1977).

2.7 ARCHIVES

The final report and all raw data related to this study will be archived by Hoechst-Celanese Corporation, at Pathology Associates, Inc., Frederick, Maryland.

3.0 RESULTS AND DISCUSSION

Mortality of water fleas exposed for 48 hours to C-1771 (3-Benzotriazoloyl-THPE) ranged from 0 percent at 0.16 mg ai/L to 100 percent at test concentrations ≥ 1.25 , mg ai/L (Table 1). No adverse effects, other than death and lethargy, were noted as a result of exposure to the chemical substance. There was no mortality observed in the solvent (acetone) control and 5 percent mortality in the dilution water control. The 48-hour EC_{50} was calculated to be 0.40 mg ai/L (based on nominal concentrations) with 95 percent confidence limits of 0.33 and 0.48 mg ai/L as calculated using the probit method (Table 2). The no-observed-effect concentration (NOEC) was 0.16 mg ai/L based on the lack of mortality and sublethal effects at this lowest test concentration.

The test temperature during the 48-hour exposure ranged from 19.0 to 20.3°C (Table 3). The initial dilution water conductivity and total organic carbon content were 278 μ mhos/cm and <1.0 mg/L, respectively. The initial dilution water had a hardness of 64 mg/L as calcium carbonate ($CaCO_3$) and an alkalinity of 60 mg/L as $CaCO_3$. The dissolved oxygen concentrations in all control and test solutions at test initiation were 9.5 mg/L (≥ 100 percent of saturation). The dissolved oxygen concentrations of the controls and test solutions ranged from 8.6 to 9.2 (95 to >100 percent of saturation) at test termination (Table 4). Initial pH values of all control and test solutions ranged from 7.9 to 8.0 and pH at test termination ranged from 8.0 to 8.2 in all control and test solutions (Table 5).

4.0 PROTOCOL DEVIATIONS

Two deviations from the test protocol occurred during the conduct of this study.

1. Less than 24 hours prior to test initiation, the gravid adult water fleas were isolated in dilution water containing food. Neonates (<24 hours old) were collected in food-free dilution water for test initiation the following morning after the adult isolation.
2. *Daphnia magna* culture temperature ranged from 20.3 to 23.0°C during the week before test initiation. This range slightly exceeded the targeted range of $20 \pm 2^\circ\text{C}$.

In the scientific opinion of the Study Director, these deviations did not affect the outcome or validity of the test results.

REFERENCES

- American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 1992. Standard Methods for the Examination of Water and Wastewater, 18th Edition.
- Stephan, C.E. 1977. Methods for Calculating an LC50. In: American Society for Testing and Materials (ASTM) Aquatic Toxicology and Hazard Evaluation, pp. 65 - 84, F.L. Mayer and J.L. Hamelink, Editors. ASTM STP 534, Philadelphia, Pennsylvania.
- U.S. EPA, Office of Solid Waste. 1986. Test Methods for Evaluating Solid Waste: Physical/Chemical Methods. EPA SW-846 (Third Edition) 1986.
- Wheat, J.V. 1989. Basic program for computing sets of LC50 values adapted from the U.S. Environmental Protection Agency, Duluth, MN, August, 1978.

Table 1. Mortality of Water Flea, *Daphnia magna*, During a 48-Hour Static Exposure to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	Cumulative Number Dead (Percent Mortality)			
	24 Hour		48 Hour	
Control	0	(0) ^a	1	(5)
Sol. Control	0	(0)	0	(0)
0.16	0	(0)	0	(0)
0.31	0	(0) ^c	5	(25) ^c
0.63	11	(55) ^d	18	(90) ^b
1.25	11	(55) ^d	20	(100)
2.50	17	(85) ^c	20	(100)
5.00	18	(90) ^b	20	(100)

^a One daphnid observed floating on the surface of the water.

^b Two daphnids appeared lethargic.

^c Three daphnids appeared lethargic.

^d Nine daphnids appeared lethargic.

Table 2. Calculated EC₅₀ Values for Water Flea, *Daphnia magna*, Exposed to C-1771 (3-Benzotriazoloyl-THPE), Based on Nominal Concentrations, Under Static Test Conditions

Exposure Period (Hour)	EC ₅₀ (mg ai/L)	95-Percent Confidence Limits (mg ai/L)	Statistical Method
24	1.09	0.82 - 1.50	Moving Average
48	0.40	0.33 - 0.48	Probit

Table 3. Temperatures During a 48-Hour Static Exposure of Water Flea, *Daphnia magna*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	Temperature (°C)		
		0 HR ^a	24 HR	48 HR
Control	A	19.0	20.3	20.0
	B	---	---	---

^a Temperature measured on composite before dividing solutions into duplicates.

NOTE: The diurnal temperature of the temperature-controlled chamber ranged from 19.1 to 21.1°C as measured by a minimum/maximum thermometer during the test.

Table 4. Dissolved Oxygen Concentrations During a 48-Hour Static Exposure of Water Flea, *Daphnia magna*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	Dissolved Oxygen Concentration (mg/L)	
		0 HR ^b	48 HR
Control	A	9.5	8.7
	B	---	8.6
Solvent Control	A	9.5	8.9
	B	---	8.8
0.16	A	9.5	8.9
	B	---	9.0
0.31	A	9.5	8.9
	B	---	8.9
0.63	A	9.5	9.1
	B	---	9.0
1.25	A	9.5	9.1
	B	---	9.1
2.50	A	9.5	9.1
	B	---	9.0
5.00	A	9.5	9.2
	B	---	9.2

Saturation ^a		9.3	9.1

^a Dissolved oxygen saturation values based on the temperature in one control chamber at the specified times.

^b Dissolved oxygen concentrations measured on composite before dividing solutions into duplicates.

Table 5. The pH Values During a 48-Hour Static Exposure of Water Flea, *Daphnia magna*, to C-1771 (3-Benzotriazoloyl-THPE)

Nominal Concentrations (mg ai/L)	R E P	pH	
		0 HR ^a	48 HR
Control	A	7.9	8.0
	B	---	8.0
Solvent Control	A	8.0	8.0
	B	---	8.0
0.16	A	8.0	8.1
	B	---	8.1
0.31	A	8.0	8.1
	B	---	8.1
0.63	A	8.0	8.1
	B	---	8.1
1.25	A	8.0	8.1
	B	---	8.1
2.50	A	8.0	8.1
	B	---	8.2
5.00	A	8.0	8.2
	B	---	8.2

^a pH values measured on composite before dividing solutions into duplicates.

APPENDIX A
DEIONIZED WATER CHARACTERIZATION

DEIONIZED WATER CHARACTERIZATION*

Parameter	Concentration ^b
Aluminum	<0.030 mg/L
Arsenic	<0.005 mg/L
Boron	0.061 mg/L
Beryllium	<0.005 mg/L
Bromide	<0.10 mg/L
Cadmium	<0.001 mg/L
Calcium	<0.050 mg/L
Chloride	3.00 mg/L
Chromium (hexavalent)	<0.05 mg/L
Chromium (total)	<0.005 mg/L
Cobalt	<0.01 mg/L
Copper	<0.001 mg/L
Fluoride	<0.1 mg/L
Iodide	<0.050 mg/L
Iron	<0.030 mg/L
Lead	<0.003 mg/L
Manganese	<0.005 mg/L
Magnesium	<0.05 mg/L
Mercury	<0.0002 mg/L
Molybdenum	<0.005 mg/L
Nickel	<0.008 mg/L
Potassium	<0.050 mg/L
Selenium	<0.003 mg/L
Silver	<0.0001 mg/L
Sodium	<0.050 mg/L
Tin	<0.010 mg/L
Zinc	<0.005 mg/L
Ammonia (total)	<0.01 mg/L
Cyanide (total)	<0.020 mg/L
Nitrates (total as N)	<0.050 mg/L
Nitrites (total as N)	<0.050 mg/L
Phosphates (total)	<0.01 mg/L
Sulfide (total)	---
Sulfate (total)	<2.0 mg/L
TDS	68 mg/L
TOC	<1.0 mg/L
TSS	<4 mg/L
COD	<5.0 mg/L
Total organophosphorus pesticides	<1.0 µg/L
Total phenoxy herbicides	<1.2 µg/L
Total organochlorine pesticides	<0.01 µg/L
PCBs	<0.10 µg/L

* The characterized deionized water is Jupiter, Florida, town water which has been deionized by a Continental Water ion exchange system and passed through activated carbon

^b Sample of deionized water collected June 16, 1994.

Triage of 8(e) Submissions

Date sent to triage: MAR 2 1996

NON-CAP

CAP

Submission number: 13307 B

TSCA Inventory: Y N **D**

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO **AQUATO**

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX
STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

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entire document: 0 1 2 pages _____ pages _____

Notes:

Contractor reviewer: FOR Date: 12/13/95

CHEMICALS TRACKING DATABASE ENTRY FORM

CHEM DATA: Substitution # BEHQ-0395-13307 SEQ. B
 TYPE: INT. SUPP FLWP
 SUBMITTER NAME: Hoechst Celanese Corporation

INFORMATION REQUESTED: FLWP DATE
 0301 NO INFO REQUESTED
 0302 INFO REQUESTED (TECH)
 0303 INFO REQUESTED (VOL. ACTIONS)
 0304 INFO REQUESTED (REPORTING RATIONAL/FI)
 DISPOSITION:
 0305 REFER TO CHEMICAL SCREENING
 0306 CAP NOTICE

OPTIONARY ACTIONS:
 0401 MATERIAL PURIFIED
 0402 STUDY'S PLANNED IN THE FUTURE
 0403 DISTRIBUTION IN WORK IN PROGRESS
 0404 LABEL/ASSAYS (TIAMINIS)
 0405 PROCESS/ANALYSIS (TIAMINIS)
 0406 APPROUSE DISCONTINUED
 0407 PRODUCTION DISCONTINUED
 0408 CONFIDENTIAL

SUB. DATE: 02/27/95 OTR DATE: 03/07/95 CERAD DATE: 03/28/95
 CHEMICAL NAME: Ethbase, 3-(2H-benzotriazole-2-yl)-
1,1,1'-tris(4-hydroxyphenyl)-
 CAS# unknown

INFORMATION TYPE	P.F.C.	INFORMATION TYPE	P.F.C.	INFORMATION TYPE	P.F.C.
0201 ONCO (HUMAN)	01 02 04	0216 EPICUR	01 02 04	0241 BIRLINGO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 BIRLINGO (HUMAN)	01 02 04
0203 CELL TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEMOPHYT PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 BODWATER TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCURENCE/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 BIASER ENCI OF ENV CONTAM	01 02 04	0247 DNA DAMAGE/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQUEST DELAY	01 02 04	0248 PRODUCE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PRODUCE/PROC ID	01 02 04	0251 MEDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	0259 OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX. (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX. (ANIMAL)	01 02 04	0229 METAPHARMACOD (ANIMAL)	01 02 04		
0215 CHRONIC TOX. (ANIMAL)	01 02 04	0230 METAPHARMACOD (HUMAN)	01 02 04		

TRADE NAME: NON-CHEM INVENTORY YES NO (CONTINUE)
 CAS SR: NON-CHEM INVENTORY
 TOXICOLOGICAL CONCERN: LOW
 SPECIES: Fish
Daphnid
 USE: PRODUCTION
 1-250-13 Non-Cap, 55

KDCN: 8895000070

Hoechst Celanese

RECEIVED
03/13/95

Contains No CBI

95 MAR 20 AM 8:44

Department of
Environmental, Health &
Safety Affairs (DEHSA)

Hoechst Celanese Corporation
Route 202-206
PO Box 2500
Springville, NJ 08876-1258
908 231 2000
Tele: 833 449
Fax: 908 231 4554

March 13, 1995
RAJ-031-95

8EHQ-0395-13307

~~B~~ C

Attn: TSCA Section 8(e) Coordinator
Document Processing Center (TS-790)
U.S. Environmental Protection Agency
401 M Street, S.W.
Washington, DC 20460

ORIGINAL

Subject: 8EHQ-95-13307

Dear Sir or Madam:

Additional fish toxicity information has been obtained for 3-(2H-benzotriazole-2-yl)-1',1',1'-tris(4-hydroxyphenyl)ethane.

The preliminary results of a rangefinding study to determine the toxicity levels of this chemical on rainbow trout eggs/embryos indicate that the LC₅₀ is in the range of 0.200 ppm. Summary information is found on the attached table.

The final report will be forwarded to EPA upon completion of the study.

This submission contains no confidential business information.

If any further information is required, do not hesitate to contact Dr. Richard A. Jourdenais, Manager, Product Stewardship at 908-231-3746.

Sincerely,

Susan Engelman
Vice President, Environmental, Health &
Safety Affairs

RAJ/mcs
Attachment

CERTIFIED MAIL/
RETURN RECEIPT REQUESTED

File: Log No. 181



8EHQ-95-13307
SP003 03/20/95

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LEGAL DEPT.



89950000156

4/4/95

Hoechst Celanese

Percent Hatch and Survival of Trout Eggs/Embryos in the Presence of C-1771. Static-Renewal Rangelinding Study.

Group	Nominal conc (ppb)	Measured range	Avg Conc (ppb)	Percent Hatch	Day-14 Survival	Day-15 Survival	
T5	500	180-630	380	90	1/18	0/18	
T4	250		190	90	11/18	7/18	
T3	100		74	80	18/18	18/18	
T2	25		19	100	18/18	18/18	
T1	10		7.5	100	18/18	18/18	
Control	0		0	100	18/18	18/18	
Solvent	0		0	100	18/18	18/18	

Only Group 1 concentration measured. Remainder extrapolated from nominal concentrations and percent loss from Group 1 measurements.

- All static renewal with three renewals total.
- Hatching occurred from day-8 through day-10.