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December 8, 1995
RAJ-136-95

Attn: TSCA Section 8(e) Coordinator
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U.S. Environmental Protection Agency
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Washington, DC 20460



Subject: 8EHQ-95-13307

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Dear Sir or Madam:

With this submission, Hoechst Celanese Corporation is providing additional aquatic toxicity information for 3-(2H-benzotriazole-2-yl)-1',1',1'-tris(4-hydroxyphenyl)ethane:

- chronic life-cycle toxicity to the water flea, *Daphnia magna*, under flow-through test conditions; NOEC=5.83ppb
- toxicity to embryos and larvae of the rainbow trout, *Oncorhynchus mykiss*, under flow-through test conditions; NOEC=15.2ppb.

Draft reports of both studies are attached.

This submission contains no confidential business information.

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

Sincerely,

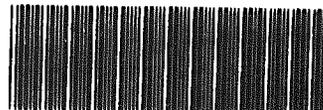
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STUDY TITLE

C-1771 (3-Benzotriazoloyl-THPE):
Chronic Life-Cycle Toxicity To The Water Flea, *Daphnia magna*,
Under Flow-Through Test Conditions

DATA REQUIREMENT

U.S. EPA TSCA Environmental Effects Testing Guideline 797.1330

AUTHORS

Hui (Jeff) Liu
Jay W. Davis

STUDY INITIATION DATE

December 29, 1994

STUDY COMPLETION DATE

1995

SPONSOR

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PERFORMING LABORATORY

Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

LABORATORY PROJECT ID

J9409010e

Contains No CBI

STATEMENT OF GLP COMPLIANCE

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

**Title: C-1771 (3-Benzotriazoloyl-THPE): Chronic Life-Cycle
Toxicity To The Water Flea, *Daphnia magna*, Under Flow-
Through 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 792) by the U.S. Environmental Protection Agency.

Jay W. Davis
Study Director
Toxikon Environmental Sciences

Date

STATEMENT OF QUALITY ASSURANCE

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

Title: C-1771 (3-Benzotriazoloyl-THPE): Chronic Life-Cycle
Toxicity To The Water Flea, *Daphnia magna*, Under Flow-
Through 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:		
Study Data Review:		
Draft Report Review:		
Final Report Review:		

Kelly Eyler
Quality Assurance Manager
Toxikon Environmental Sciences

Date

LIST OF SCIENTIFIC PERSONNEL

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

**Title: C-1771 (3-Benzotriazoloyl-THPE): Chronic Life-Cycle
Toxicity To The Water Flea, *Daphnia magna*, Under Flow-
Through Test Conditions**

Study Director: Jay W. Davis

Principal Investigator: Hui (Jeff) Liu

**Biologists: Leslie D. Hartman
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Michael B. Malone**

**Chemists: Timothy Z. Kendall
Matthew Pike
Kim C. Friesen
Carla Rollins**

**Aquaculturist: Keith Ferris
Jonathan Spalding**

SUMMARY

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

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

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

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

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

Test Species: *Daphnia magna* <24 hours old at test
initiation

Source of Organisms: Toxikon Environmental Sciences,
Jupiter, FL

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

Dilution Water: Freshwater with a hardness range of 66
to 92 mg/L as CaCO₃ and a mean
temperature of 19.6 ± 0.3°C.

**Nominal
Concentrations:** Control, Solvent (DMF) Control, 3.75,
7.50, 15.0, 30.0, 60.0, and 120 µg ai/L.

**Measured
Concentrations:** Control (<1.52), Solvent (DMF) Control
(<1.52), 2.74, 5.83, 9.85, 21.2, 36.2,
and 78.1 µg ai/L

Test Dates: July 6 to 27, 1995

Study Length: 21 days

Results:

The Maximum Allowable Toxicant
Concentration (MATC) was $>5.83 < 9.85 \mu\text{g}$
ai/L with a geometric mean of $7.58 \mu\text{g}$
ai/L. The No-Observed-Effect
Concentration (NOEC) was $5.83 \mu\text{g}$ ai/L.

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1.0 INTRODUCTION

A flow-through freshwater toxicity test was conducted at Toxikon Environmental Sciences, Jupiter, Florida, to determine the chronic effects of C-1771 (3-Benzotriazoloyl-THPE) to the water flea, *Daphnia magna*. The criteria for effect were first generation mortality, growth, and reproduction (i.e., number of offspring produced). Results of the test are expressed as the maximum acceptable toxicant concentration (MATC), that is, the C-1771 (3-Benzotriazoloyl-THPE) concentration which is bound at the lower end by the highest concentration in the test that produced no effect (NOEC, no-observable-effect concentration) on the test organisms and at the higher end by the lowest concentration tested that produced a statistically significant effect (LOEC, lowest-observable-effect concentration) on test organisms as compared to the controls.

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 bottle labeled: "C-1771 (3-Benzotriazoloyl-THPE)" from Hoechst Celanese Corporation. The test substance was a white powder which was stored at ambient room temperature in the dark. The purity of the test substance was reported to be 99.2 percent and C-1771 was reported to be insoluble in water and highly soluble in acetone by Hoechst Celanese Corporation (Appendix A).

Measured test concentrations are reported as micrograms (μg) of C-1771 (3-Benzotriazoloyl-THPE), as active ingredient (ai), per liter (L) of freshwater or parts per billion (ppb).

2.2 Test Species

Daphnia magna used for testing were obtained from Toxikon Environmental Sciences' cultures established from animals originally received from the U.S. Environmental Protection Agency, Duluth, Minnesota in October 1989. A subculture of adults was isolated from these cultures and maintained prior to testing. The subculture was fed the green alga, *Selenastrum capricornutum*, and a mixture of cereal leaves (YCT) daily. Less than 24 hours prior to test initiation, the adults were re-isolated in dilution water. Neonates (<24 hours old) collected from subcultures on July 6, 1995 were used for test initiation. All subcultures were maintained in moderately hard freshwater (74 mg/L as CaCO_3) at $23 \pm 3^\circ\text{C}$. No ephippia were produced and no disease treatments were administered during culture. Test organisms appeared to be in good physical condition at test initiation.

2.3 Test Water

The dilution water was carbon-treated Town of Jupiter, Florida, freshwater of moderate hardness. The town water was treated by aeration, filtered to 5 micrometers, passed through activated carbon and a biofilter and finally vigorously re-aerated by an air stone prior to delivery to the exposure system.

Chemical characterization of a recent representative batch of freshwater is presented in Appendix B.

2.4 Test Methods

Methods for the 21-day flow-through chronic test with the water flea were those described in Toxikon Environmental Sciences' test protocol and amendments entitled: "C-1771 (3-Benzotriazoloyl-THPE): Chronic Life-Cycle Toxicity To The Water Flea, *Daphnia magna*, Under Flow-Through Test Conditions." These methods were based on those described in U.S. EPA Office of Pollution Prevention and Toxics (OPPT) Daphnid Chronic Toxicity Test Guideline, 40 CFR 797.1330.

A flow-through preliminary chronic test (10 days) was conducted from May 30 to June 9, 1995 at nominal concentrations of 12.5, 50.0 and 200 $\mu\text{g ai/L}$ using 20 daphnids per concentration. After a 10-day exposure, mortality of water fleas was 0% at 12.5 and 50.0 $\mu\text{g ai/L}$, and 5% at 200 $\mu\text{g ai/L}$. Numbers of neonates produced among different concentrations were not significantly different among treatments, however, there was a higher neonate mortality (neonates produced dead especially on days 9 & 10 of the test) in the two higher test concentrations (i.e., 50 and 200 $\mu\text{g ai/L}$). A summary of the data was submitted to the client and EPA for review containing all preliminary test results. Based upon the results of the preliminary flow-through test, the

limited water solubility of the test substance and solvent limitations, the 48-hour static EC_{50} value of 400 $\mu\text{g ai/L}$ (nominal concentration), and the recommendations of the EPA, nominal test concentrations of C-1771 (3-Benzotriazoloyl-THPE) selected for the chronic test were 3.75, 7.50, 15.0, 30.0, 60.0, and 120 $\mu\text{g ai/L}$. The definitive exposure was first initiated on June 30, 1995 and was prematurely terminated (Day 3) due to unacceptable solvent control survival. The data collected during this aborted test are included in the raw data for this study.

The definitive exposure was conducted under flow-through conditions in a modified proportional vacuum-siphon diluter system based on the original design of Mount and Brungs (1967). The diluter system was constructed of glass, silicone adhesive, and silicone tubing. The test system was volumetrically calibrated to provide a test concentration series with a 50 percent dilution and equal solvent concentrations in all test solutions.

Two C-1771 (3-Benzotriazoloyl-THPE) stock solutions were prepared during the course of the test. Each diluter stock solution (approximately 1,200 mg ai/L) was prepared by weighing approximately 0.121 g of test material into a 100-mL volumetric flask and then bringing to volume with solvent dimethylformamide (DMF). A total volume of 49.3 or 49.4 microliters (μL) of stock solution was injected into the chemical mixing chamber (total volume of 495 mL) at each diluter cycle providing a high nominal test concentration of approximately 120 $\mu\text{g ai/L}$. This test solution was proportionally diluted in the diluter system to provide the five lower test concentrations (i.e., 60.0, 30.0, 15.0, 7.50, and 3.75 $\mu\text{g ai/L}$). Both a solvent (DMF) control and a dilution water control were maintained concurrently with the

six test concentrations. The solvent (DMF) control was prepared by injecting 123.7 or 123.8 μL of DMF into the solvent mixing chamber (total volume of 1240 mL) during each diluter cycle providing a solvent concentration of 100 μL of DMF/L in the solvent (DMF) control and all C-1771 treatment solutions. The dilution water control did not contain solvent or test substance.

A test solution volume of approximately 250 mL was delivered to each test tank during every cycle; the total volume was split into four replicates (approximately 62.5 mL) via a splitter box prior to entry into the test chambers. Splitter flows were calibrated and maintained within 10 percent of the desired volumes. Test tanks were 11.3-L glass tanks equipped with glass overflow tubes positioned to provide a maximum depth of 6.0 centimeters and volume of approximately 5.4 L. Within each test tank, four replicate test chambers were positioned to receive incoming flow of test solution from the splitter box. Two types of retention chambers were used during this study. The first type (used from day 0 through day 6) were designed to minimize floating of neonates. These retention chambers consisted of a 51- by 70-millimeter (mm) glass tube with a 355-micrometer mesh size screen floor located 10 mm from the bottom of the tube. This chamber rested on two pieces of 6-mm glass inside a 80- by 100-mm crystallizing dish. On day 6, surviving animals were transferred to the second type of retention chamber. These retention chambers consisted of 100- X 50-millimeter (mm) glass crystallizing dishes with a 30-mm high collar of 355-micrometer mesh Nitex screening attached with silicone sealant to the rim of the dish. Each test chamber maintained a 300-mL volume of test solution. The diluter cycled at an average rate of 3.5 cycles per hour providing approximately 18 volume additions every 24 hours.

The 21-day test was initiated on July 6, 1995 with the impartial addition of daphnids, by twos, to the retention chambers until 10 daphnids were distributed to each of four replicate retention chambers for a total of forty daphnids per treatment. The retention chambers located within glass crystallizing dishes were then randomly transferred into the test tanks and positioned under the splitter box to receive incoming test solution. During the test, the replicate retention chambers were systematically re-positioned on a daily basis to eliminate any effects due to replicate positions within the test chamber. The test tanks were randomly positioned (by lottery) in a water bath under fluorescent lighting regulated to an overall photoperiod of 16 hours light and 8 hours dark. A 15-minute transition period of lower intensity incandescent lighting was provided at the initiation and termination of each light period to simulate dawn and dusk. The light intensity ranged between 3.4 and 4.8 microEinsteins per square meter per second as measured by a LICOR, Inc. Model LI-189 light meter equipped with a 2π quantum sensor.

Survival and reproduction of the water fleas was monitored daily and any dead removed. All young produced were counted and discarded. Any abnormalities in the behavior or physical appearance of the daphnids were also noted. During the test, *Daphnia magna* were fed the green alga, *Selenastrum capricornutum*, and the prepared food YCT. The algae was grown in a mass laboratory culture (established from a culture originally obtained from the University of Texas at Austin). The YCT was prepared at Toxikon Environmental Sciences according to procedures outlined in U.S. EPA (1985). The YCT [yeast, cereal leaves (Cerophyll®), and trout chow], preparation, which was fed throughout the test, possessed a concentration of 5 g of dried

constituents/L. During the first six days of the test, approximately 4 mL of concentrated algae (approximately 4×10^5 cells/mL) were added to each test chamber three times each day. This provided a daily algal concentration of approximately 900 cells/mL. Beginning on day 6, the non-concentrated mass algal culture was metered into the dilution water headbox (volume = 2350 mL) during each diluter cycle. The volume of algae metered into the headbox was based upon the cell density of the mass culture, but was set to provide 1.0 to 2.0×10^5 cells/mL of dilution water. A sample of test solution was taken daily from a control chamber and cell density was determined giving an average feeding concentration of 2.1×10^5 cells/mL throughout the test. In addition to the algae, 90 μ L of the YCT solution was added three times a day yielding a YCT concentration of approximately 0.3 mg/L per replicate test chamber. In order to maintain water quality and minimize the amounts of debris, the retention chambers were cleaned three times per week. Cleaning of the system was also conducted periodically to reduce the amount of C-1771 (3-Benzotriazoloyl-THPE) flocculent (precipitate) in the diluter system in response to the insolubility of the test substance in laboratory freshwater. No solvents were used in the cleaning process in order to minimize the desorption of any test material from the glass.

Test water quality was monitored periodically during the test. Water temperature in the dilution water control was monitored and recorded hourly with a Data-Mentor data logger and spot checked with a thermocouple thermometer daily. The temperature of the water bath was continuously monitored using a minimum/maximum thermometer and the diurnal temperature range recorded daily. Dissolved oxygen concentrations and pH were measured in all test replicates at test initiation and on days 7, 14, and 21 (test

termination); while specific conductivity, hardness, and alkalinity were measured in the control and the low, middle, and high concentrations at test initiation and on days 7, 14 and 21 (test termination). Water hardness and alkalinity were measured by EDTA and potentiometric titrations respectively (Standard Method procedures 2340 C and 2320 B, respectively; APHA et al., 1992). Dissolved oxygen concentrations 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. Conductivity measurements were taken with a Corning Checkmate 90 digital conductivity meter equipped with a conductivity probe.

At the termination of the 21-day exposure, all surviving *Daphnia* were removed from their retention chambers and preserved in a 5% buffered formalin solution until length determination could be performed. Individual lengths (apex of helmet to base of spine) were measured to the nearest 0.02 mm. The measurements were performed with an Olympus binocular dissecting scope equipped with an ocular by placing the *Daphnia* directly on a stage micrometer.

2.5 Chemical Sampling

Prior to test initiation, water samples (approximately 1 to 5 milliliters in volume) were collected from all C-1771 (3-Benzotriazoloyl-THPE)-containing test solutions to verify proper diluter function. At test initiation and generally once every week thereafter throughout the test, water samples (approximately 1 to 5 milliliters in volume) were collected from each replicate control and C-1771 (3-Benzotriazoloyl-THPE) test solution and composited to monitor actual exposure concentrations. Samples were analyzed for confirmation analysis of the parent compound by high performance liquid chromatography (HPLC). All water samples

were collected from mid-depth in the water column, using a volumetric glass pipet. Concentrations of C-1771 were measured by HPLC analysis following the methods presented in Toxikon Environmental Sciences' (J9409010a) report entitled: "C-1771: Analytical Method Validation In Freshwater" (Appendix C).

2.6 Statistical Analyses

Based on results of the test, the 21-day EC_{50} value and its 95 percent confidence limits was calculated. The EC_{50} value was estimated by a computer program (Wheat, 1989) using the following statistical methods: moving average angle, probit, logit and non-linear interpolation. Confidence limits for EC_{50} 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).

After termination of the chronic test, mortality of control and solvent control *Daphnia* was statistically compared using Fisher's Exact Test. Student's t-test was used for evaluating control reproduction and growth. If no significant difference was detected between the two controls, the control data were pooled prior to statistical evaluation with the C-1771 treatments. If a significant difference was detected, only the solvent control data were used for statistical evaluation with the treatments. Reproduction was evaluated following normalization of the number of offspring produced per replicate by dividing by the total number of young produced by the number of reproductive days. Reproductive days were determined by summing the products of the

numbers of adult *Daphnia* alive from the observation of the first young in a test chamber (which occurred on day 7) through the end of the test and the number of days during that period that each adult was alive. Statistical differences in survival between the control and treatment groups were calculated using Fisher's Exact Test. Statistical differences in growth and reproduction between the control and treatment groups were calculated by analysis of variance (ANOVA) followed with Dunnett's multiple comparison test. All statistical calculations were performed with PC-SAS (1989) at the 0.05 probability level.

The MATC was calculated as the geometric mean between the lowest measured test substance concentration that had a statistically significant effect and the highest measured test substance concentration that had no significant effect on daphnid survival, growth, or reproduction. The most sensitive test criterion was used to derive the maximum acceptable toxicant concentration (MATC). A point estimate of the MATC was calculated as the geometric mean of the NOEC and LOEC values (logarithm of NOEC plus logarithm of LOEC divided by two and then converted back to a concentration by the antilogarithm).

2.7 Archives

The final report and all raw data related to this study will be maintained in archive by Hoechst Celanese Corporation at Pathology Associates Incorporated, Frederick, Maryland.

3.0 RESULTS AND CONCLUSION

3.1 Analytical Chemistry Results

Prior to initiating the definitive test, the diluter test system was operated for twenty-four days with C-1771 (3-Benzotriazoloyl-THPE) delivered at the desired nominal concentrations of 3.75, 7.50, 15.0, 30.0, 60.0 and 120 $\mu\text{g ai/L}$. The diluter functioned properly throughout the entire 21-day test based upon daily calculations of test substance delivery and observations of diluter solution delivery. The test was initiated after day 0 measured concentrations of C-1771 (3-Benzotriazoloyl-THPE) were determined to be 47 to 107 percent of nominal concentrations. Measured concentrations of C-1771 (3-Benzotriazoloyl-THPE) and corresponding quality control samples are presented in Table 1. Mean measured concentrations of C-1771 (3-Benzotriazoloyl-THPE) during the 21-day test ranged from 2.74 to 78.1 $\mu\text{g ai/L}$ and from 60 to 78 percent of nominal concentrations (Table 1). The stock solutions utilized during the test were determined to average 100% of the nominal concentration throughout the test (Table 1). Undissolved test substance was observed in chemical mixing box but not in the test chambers during the entire exposure. Because the diluter prepares all test solutions by mixing up the high test concentration and diluting this solution, the precipitate observed was due to exceeding solubility of the test material in dilution water at the high concentration. This solubility problem also explains the low measured values obtained for the chemical results which was due to the fact that initially only approximately 60 to 70% of the chemical was able to go into solution. The higher percent of nominal values obtained at the lower test concentrations was likely due to some of the chemical (precipitate) going into solution at the lower test concentrations.

3.2 Biological Results

After 21 days of exposure, mortality of water fleas exposed to

C-1771 (3-Benzotriazoloyl-THPE) ranged from 5 percent at 21.2 $\mu\text{g/L}$ to 65 percent at 78.1 $\mu\text{g/L}$; mortality in the control and solvent control was 15 and 18 percent, respectively (Table 2). A statistically significant increase in mortality as compared to the pooled controls was detected at 78.1 $\mu\text{g/L}$ C-1771 (3-Benzotriazoloyl-THPE) concentration. The 21-day EC_{50} value was 62.8 $\mu\text{g/L}$ (Table 3).

Neonates were first observed on day 7 in all concentrations, except 78.1 $\mu\text{g/L}$ at which neonates were first observed on day 8. Therefore, day 7 was used to calculate the number of adult reproductive days (Appendix D). The total numbers of young produced by first generation daphnids exposed to the test concentrations ranged from a high of 4851 at 2.74 $\mu\text{g/L}$ to a low of 2070 at 78.1 $\mu\text{g/L}$. The control and solvent control produced 4576 and 4248 young, respectively (Table 4). The average numbers of young per adult reproductive day (YPARD) ranged from 4.4 at 78.1 $\mu\text{g/L}$ to 9.0 at 2.74 $\mu\text{g/L}$. The control and solvent control produced 8.2 and 8.3 young per adult reproductive day, respectively (Table 5). The numbers of young per female reproductive day was determined for all chambers based upon release of the first young on day 7 (a total of 15 reproductive days - see Appendix D). Comparison of the pooled control to the test substance treatments at test termination yielded a statistically significant reduction of YPARD at test concentration 78.1 $\mu\text{g/L}$ C-1771 (3-Benzotriazoloyl-THPE) (Table 5).

Individual lengths (helmet-to-spine) of surviving water fleas are presented in Table 6. Mean lengths of surviving water fleas ranged from 3.42 at 78.1 $\mu\text{g/L}$ to 4.15 mm at 2.74 $\mu\text{g/L}$; mean length of control and solvent control water fleas was 4.19 and 4.14 mm, respectively (Table 7). Mean length was statistically reduced from that of the pooled controls at test concentrations $\geq 9.85 \mu\text{g/L}$.

The no-observed-effect concentration (NOEC) was 5.83 $\mu\text{g/L}$ based upon the lack of significant mortality, reproductive or growth effects at this and lower test concentrations as compared to the pooled controls. The lowest-observed-effect concentration (LOEC) was 9.85 $\mu\text{g/L}$ based upon a significant reduction in growth (length). Therefore, the MATC for C-1771 (3-Benzotriazoloyl-THPE) was $>5.83 < 9.85 \mu\text{g/L}$ with a point estimate (geometric mean) of 7.58 $\mu\text{g/L}$.

3.3 Water Quality Parameters

The test temperature during the 21-day exposure ranged from 19.2 to 20.8°C (Table 8). The mean temperature was 19.6°C with a standard deviation of 0.3°C. The specific conductivity of the dilution water and test solutions ranged from 371 to 471 $\mu\text{mhos/cm}$ (Table 9). The hardness and alkalinity ranges of the dilution water and test solutions were 64 to 96 mg/L and 12 to 69 mg/L as CaCO_3 , respectively (Tables 10 and 11, respectively). The dissolved oxygen saturation values at the temperatures recorded when dissolved oxygen measurements were taken are presented in Table 12. Dissolved oxygen concentrations remained ≥ 6.5 and ≥ 4.0 mg/L (≥ 72 and ≥ 44 percent of saturation) in the dilution water control and solvent control chambers, respectively, and ≥ 2.5 (≥ 27 percent of saturation) in C-1771 (3-Benzotriazoloyl-THPE) treatment chambers throughout the test (Table 13). The pH values ranged from 6.8 to 7.7 in all test chambers during the test (Table 14).

4.0 SUMMARY OF RESULTS

The MATC for *Daphnia magna* exposed to C-1771 (3-Benzotriazoloyl-THPE) during a 21-day chronic test was $>5.83 <9.85 \mu\text{g/L}$ with a geometric mean of $7.58 \mu\text{g/L}$. The MATC was based on a reduction in growth (length) of first generation *Daphnia* at mean measured C-1771 (3-Benzotriazoloyl-THPE) concentrations $\geq 9.85 \mu\text{g/L}$. Growth was the most sensitive biological indicator of toxicity during the chronic test. The no-observed-effect concentration (NOEC) was $5.83 \mu\text{g/L}$ based upon the lack of significant growth effect at this and lower test concentrations.

5.0 PROTOCOL DEVIATIONS

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

- 1) Although several methods (i.e., increasing the cycle rate, cleaning the test chambers, and aeration) were used in order to increase the dissolved oxygen concentrations, the dissolved oxygen concentrations still fell below the 60% saturation level as stated in the protocol.
- 2) On average, 2.54 neonates were produced per adult per day during the 7 days prior to test initiation. Although this number is below the 3 minimum stated in the test protocol, the neonates were health as evidenced by the lack of significant mortality in both sets of controls and in the five lowest test concentrations. The reproductive value was low at least in part due to the use of animals collected from subcultures in which the animals were ≤ 14 days old and not yet producing their largest broods.
- 3) The average temperature of the cultures during the 7-day period prior to test initiation was $24.5 \pm 1.3^{\circ}\text{C}$. This average exceeded the protocol stipulation of $20 \pm 2^{\circ}\text{C}$.

These deviations from the test protocol were minor and, in the scientific opinion of the Study Director, did not affect the outcome or validity of the test results.

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- 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. Measured Concentrations of C-1771 (3-Benzotriazoloyl-THPE) During a 21-Day Chronic Exposure of Water Flea, *Daphnia magna*, Under Flow-Through Conditions

Nominal Concentration (µg/L; ppb)	Measured Concentration (µg/L; ppb)					Percent of Nominal
	Day 0	Day 7	Day 15	Day 21	Mean (±SD)	
Control ^a	<1.52	<1.20	<1.01	<1.40	<1.52 (---)	---
Solvent ^a Control	<1.52	<1.20	<1.01	<1.40	<1.52 (---)	---
3.75	4.01	2.85	3.05	1.06 ^b	2.74 (1.10)	73
7.50	5.30	5.35	6.04	6.62	5.83 (0.64)	78
15.0	8.93	9.24	11.6	9.68	9.85 (1.23)	66
30.0	17.9	22.9	23.7	20.3	21.2 (1.76)	71
60.0	28.2	40.3	39.9	36.2	36.2 (2.26)	60
120	75.4	66.1	93.8	77.0	78.1 (11.5)	65

1200 (ppm) (Stock)	1120	1160	1280	1220	1195 (70.0)	100

MATRIX SPIKE RECOVERY DATA						
MS 5.00 (Low)	5.15	4.39	4.43	4.60	4.64 (0.11)	93
MS 50.0 (mid)	42.6	46.5	50.7	43.8	45.9 (3.49)	92
MS 150 (High)	139	141	149	138	142 (5.78)	94

The level of detection (LOD) as determined in the freshwater validation of C-1771 (3-Benzotriazoloyl-THPE) is 1.01 ug/L.

MS = Matrix Spike
 SD = Standard Deviation

NOTE: Matrix Spikes consisted of test substance in dilution water.

^a Values indicated are the minimum quantified level, which are equal to one-half the lowest standard response time the dilution factor of the lowest test concentration

^b Value below detectable level so it is only an estimated value.

Table 2. Mortality of Water Flea, *Daphnia magna*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Test Conditions

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Rep	Cumulative Number Dead (% Mortality)				Treatment ^a		
		Day 7	Day 14	Day 21	Treatment ^a			
Control	A	0	(0)	1	(10)	2	(20)	
	B	0	(0)	1	(10)	2	(20)	
	C	0	(0)	1	(10)	2	(20)	
	D	0	(0)	0	(0)	0	(0)	6 (15)
Solvent Control	A	6	(60)	6	(60)	6	(60)	
	B	0	(0)	0	(0)	0	(0)	
	C	0	(0)	0	(0)	0	(0)	
	D	0	(0)	0	(0)	1	(10)	7 (18)
2.74	A	0	(0)	0	(0)	1	(10)	
	B	0	(0)	0	(0)	0	(0)	
	C	0	(0)	0	(0)	0	(0)	
	D	4	(40)	4	(40)	4	(40)	5 (13)
5.83	A	5	(50)	5	(50)	5	(50)	
	B	0	(0)	0	(0)	0	(0)	
	C	0	(0)	0	(0)	0	(0)	
	D	0	(0)	0	(0)	1	(10)	6 (15)
9.85	A	0	(0)	0	(0)	1	(10)	
	B	0	(0)	1	(10)	1	(10)	
	C	0	(0)	0	(0)	0	(0)	
	D	0	(0)	1	(10)	1	(10)	3 (8)
21.2	A	0	(0)	0	(0)	0	(0)	
	B	0	(0)	0	(0)	0	(0)	
	C	0	(0)	1	(10)	1	(10)	
	D	0	(0)	1	(10)	1	(10)	2 (5)
36.2	A	0	(0)	0	(0)	0	(0)	
	B	0	(0)	0	(0)	4	(40)	
	C	0	(0)	0	(0)	1	(10)	
	D	0	(0)	1	(10)	1	(10)	6 (15)
78.1	A	1	(10)	1	(10)	5	(50)	
	B	0	(0)	0	(0)	4	(40)	
	C	0	(0)	2	(20)	8	(80)	
	D	0	(0)	0	(0)	9	(90)	26 (65) ^b

^a The total number of animals exposed in each treatment was 40.

^b Mean mortality is statistically greater than the pooled control ($\alpha = 0.05$).

Table 3. Results of EC₅₀ Calculations for *Daphnia magna* During a 21-Day Chronic Exposure to C-1771 (3-Benzotriazoloyl-THPE)

Test Day	LC ₅₀ Value ^a	LCL ^b	UCL ^c	Method Used
7	>78.1	---	---	---
14	>78.1	---	---	---
21	62.8	36.2	78.1	Binomial

- ^a LC₅₀ values and confidence limits expressed in μg C-1771/L.
^b Lower 95 percent confidence limit.
^c Upper 95 percent confidence limit.

Table 4. Number of Young Produced by Water Flea, *Daphnia magna*, During a 21-Day Chronic Flow-Through Exposure to C-1771 (3-Benzotriazoloyl-THPE)

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Number of Young Produced				Treatment
	Rep A	Rep B	Rep C	Rep D	
Control	1102	1038	1246	1190	4576
Solvent Control	701	1311	1131	1105	4248
2.74	1302	1253	1484	812	4851
5.83	621	1270	1375	1206	4472
9.85	840	995	1117	1138	4090
21.2	994	1112	1061	955	4122
36.2	1343	843	1007	991	4184
78.1	508	624	433	505	2070

Table 5. Number of Young Produced by Water Flea, *Daphnia magna*, Per Reproductive Day During a 21-Day Chronic Flow-Through Exposure to C-1771 (3-Benzotriazoloyl-THPE)

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Number of Young Per Reproductive Day				
	Rep A	Rep B	Rep C	Rep D	Treatment
Control	8.3	7.6	8.8	7.9	8.2
Solvent Control	11.7	8.7	7.5	7.4	8.3
2.74	8.8	8.4	9.9	9.0	9.0
5.83	8.3	8.5	9.2	8.3	8.6
9.85	5.9	7.3	7.4	8.1	7.2
21.2	6.6	7.4	7.6	6.9	7.1
36.2	9.0	6.2	6.9	7.2	7.3
78.1	4.3	4.7	4.1	4.5	4.4 ^a

^a Statistically significant ($\alpha = 0.05$) reduction in the number of young produced per adult reproductive day from the pooled control.

Table 6. Lengths of Water Flea, *Daphnia magna*, after 21 Days of Flow-Through Exposure to C-1771

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Rep	Helmet-Spine Length (mm)											
		A	B	C	D	A	B	C	D	A	B	C	D
Control	A	4.48	4.16	4.32	4.38	3.96	4.08	4.26	4.18				
	B	4.18	4.28	4.06	4.04	4.10	4.20	4.16	4.24				
	C	4.20	3.82	4.16	4.20	4.36	4.34	4.16	4.08				
	D	4.32	4.24	4.10	4.38	4.26	4.14	4.28	4.16	4.22	4.08		
Solvent Control	A	4.46	4.48	4.10	4.34								
	B	4.26	4.12	4.10	4.18	3.82	4.10	4.02	4.28	4.26	4.12		
	C	4.20	4.22	4.24	4.16	4.32	4.16	4.08	4.16	3.84	4.14		
	D	3.94	4.10	4.04	4.08	4.12	4.02	4.06	3.98	4.00			
2.74	A	4.14	4.16	4.08	4.02	3.90	4.18	4.12	4.10	3.98			
	B	4.16	4.22	4.08	4.22	4.28	4.16	4.26	4.18	4.26	4.14		
	C	4.18	4.36	4.02	4.54	4.08	4.02	4.32	4.28	4.20	4.22		
	D	4.42	4.08	4.12	3.78	4.10	3.98						
5.83	A	4.10	4.08	4.14	4.10	3.26							
	B	4.08	4.02	4.24	4.14	4.06	3.94	4.20	4.22	4.28	3.94		
	C	3.74	4.32	4.04	4.46	4.06	4.12	4.10	4.24	4.08	4.34		
	D	4.12	4.32	4.28	4.02	4.04	4.06	4.32	4.08	3.98			
9.85	A	3.12	3.98	3.88	3.84	4.06	4.10	4.12	3.92	4.10			
	B	3.60	3.88	4.22	4.28	3.96	4.08	4.14	4.06	3.98			
	C	3.90	4.06	3.64	4.00	4.16	4.08	4.02	4.06	3.96	4.14		
	D	4.16	4.44	4.04	4.28	4.22	4.18	4.18	4.16	4.20			

Table 6 (Cont.). Lengths of Water Flea, *Daphnia magna*, after 21 Days of Flow-Through Exposure to C-1771 (3-Benzotriazoloyl-THPE)

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Rep	Helmet-Spine Length (mm)											
		A	B	C	D	A	B	C	D	A	B	C	D
21.2	A	3.54	4.26	3.84	3.88	3.68	4.08	3.60	3.88	4.22	4.14		
	B	4.08	3.98	3.94	3.96	3.92	4.10	3.86	3.66	4.12	4.26		
	C	4.00	3.90	3.88	4.04	4.14	4.18	3.86	4.08	4.06			
	D	3.72	3.86	3.88	3.86	3.58	4.14	3.72	3.84	4.28			
36.2	A	4.04	4.02	3.88	4.00	4.12	4.06	4.16	4.02	4.18	3.78		
	B	3.78	4.06	3.94	3.86	3.96	3.98						
	C	4.18	3.56	3.92	3.84	4.02	3.84	3.92	3.78	3.70			
	D	3.88	3.44	4.04	3.96	4.00	3.90	3.66	4.04	4.08			
78.1	A	3.86	3.50	3.46	3.28	3.08							
	B	3.32	3.54	3.40	3.40	3.56	3.72						
	C	3.18	3.20										
	D	3.34											

Table 7. Mean Lengths of *Daphnia magna*, after 21 Days of Exposure to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Meas. Conc. ($\mu\text{g/L}$)	Mean Length (mm)				Mean ^b	(\pm SD)
	Rep A	Rep B	Rep C	Rep D		
Control	4.23	4.16	4.17	4.22	4.19	0.13
Solvent Control	4.35	4.13	4.15	4.04	4.14	0.15
2.74	4.08	4.20	4.22	4.08	4.15	0.14
5.83	3.94	4.11	4.15	4.14	4.10	0.21
9.85	3.90	4.02	4.00	4.21	4.03 ^a	0.23
21.2	3.91	3.99	4.02	3.88	3.95 ^a	0.20
36.2	4.03	3.93	3.86	3.89	3.93 ^a	0.17
78.1	3.44	3.49	3.19	3.34	3.42 ^a	0.21

^a Significantly ($\alpha = 0.05$) reduced from the pooled control.

^b Mean lengths based on individual lengths of *Daphnia*.

SD = Standard Deviation

Table 8. Daily Temperature Range During a 21-Day Chronic Flow-Through Exposure of Water Flea, *Daphnia magna*, to C-1771 (3-Benzotriazoloyl-THPE)

Exposure Period (Day)	Temperature (°C)	
	Range ^a	Mean
1	20.0 - 20.8	20.3
2	19.7 - 20.1	19.9
3	19.3 - 20.0	19.5
4	19.2 - 19.5	19.3
5	19.3 - 19.7	19.4
6	19.3 - 19.6	19.4
7	19.2 - 19.5	19.3
8	19.2 - 19.5	19.3
9	19.3 - 19.5	19.4
10	19.3 - 19.5	19.4
11	19.3 - 19.6	19.4
12	19.2 - 19.7	19.4
13	19.3 - 19.7	19.4
14	19.2 - 19.6	19.4
15	19.5 - 19.7	19.6
16	19.4 - 20.1	19.5
17	19.5 - 19.7	19.6
18	19.5 - 20.0	19.6
19	19.6 - 19.9	19.8
20	19.6 - 19.8	19.7
21	19.6 - 19.7	19.6

^a Daily temperature ranges reported represent the minimum and maximum hourly temperatures recorded by the data logger in the control tank during each test day. The average temperature and standard deviation for the 21-day exposure was $19.6 \pm 0.3^{\circ}\text{C}$.

NOTE: The diurnal temperature of the waterbath ranged from 18.8°C to 20.4°C as measured by a minimum/maximum thermometer during the test.

Table 9. Specific Conductivity During a 21-Day Chronic Flow-Through Exposure of Water Flea, *Daphnia magna*, to C-1771 (3-Benzotriazoloyl-THPE)

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Specific Conductivity ($\mu\text{mhos/cm}$)			
	Day 0	Day 7	Day 14	Day 21
Control	371	378	453	376
2.74	398	388	471	447
9.85	385	396	451	462
78.1	392	409	453	445

Table 10. Water Hardness During a 21-Day Flow-Through Chronic Exposure of Water Flea, *Daphnia magna*, to C-1771 (3-Benzotriazoloyl-THPE)

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Water Hardness (mg/L as CaCO_3)			
	Day 0	Day 7	Day 14	Day 21
Control	80	78	92	66
2.74	84	82	90	64
9.85	82	80	90	64
78.1	80	82	96	64

Table 11. Water Alkalinity During a 21-Day Flow-Through Chronic Exposure of Water Flea, *Daphnia magna*, to C-1771 (3-Benzotriazoloyl-THPE)

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Water Alkalinity (mg/L as CaCO_3)			
	Day 0	Day 7	Day 14	Day 21
Control	12	14	46	13
2.74	17	17	66	59
9.85	12	21	69	62
78.1	14	27	64	55

Table 12. Temperature Measurements in the Control Treatment with Corresponding 100 Percent Dissolved Oxygen Saturation Concentrations During a 21-Day Chronic Flow-Through Exposure of *Daphnia magna* to C-1771 (3-Benzotriazoloyl-THPE)

Day	Temperature (°C) ^a	100% DO Saturation (mg/L)
0	20.5	8.9
7	19.2	9.3
14	19.4	9.3
21	19.6	9.1

^a Temperature measured by the data logger.

Table 13. Dissolved Oxygen Concentrations During a 21-Day Chronic Flow-Through Exposure of *Daphnia magna* to C-1771 (3-Benzotriazoloyl-THPE)

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)	Replicate	Dissolved Oxygen Concentration (mg/L)			
		Day 0	Day 7	Day 14 ^a	Day 21
Control	A	6.8	7.3	6.8	7.0
	B	6.7	7.0	7.1	6.5
	C	6.6	6.9	7.1	7.2
	D	6.7	7.2	7.0	6.9
Solvent Control	A	7.3	7.6	5.4	5.1
	B	6.9	7.6	4.1	4.0
	C	6.9	6.9	4.8	4.1
	D	6.6	7.2	5.2	4.5
2.74	A	7.3	7.5	3.9	2.8
	B	7.4	7.5	3.8	3.1
	C	7.1	7.5	4.5	3.2
	D	7.2	7.5	4.4	3.2
5.83	A	7.3	7.5	5.3	3.2
	B	7.2	7.6	4.5	3.1
	C	7.1	7.4	4.7	3.1
	D	7.2	7.5	5.0	2.5
9.85	A	7.2	7.6	5.7	2.7
	B	7.1	7.4	4.9	3.4
	C	7.2	7.2	5.6	2.9
	D	7.1	7.3	4.9	3.1
21.2	A	7.0	7.2	4.5	3.3
	B	6.9	7.4	4.6	3.8
	C	7.0	7.3	4.9	2.3
	D	7.0	7.3	4.1	2.8
36.2	A	6.9	6.9	5.4	2.8
	B	6.8	7.1	5.3	3.3
	C	6.6	7.1	4.4	2.8
	D	6.6	7.0	5.0	3.2
78.1	A	6.7	7.2	5.7	3.4
	B	6.3	7.1	4.9	3.7
	C	5.9	7.0	5.6	3.6
	D	5.8	7.1	5.8	3.4

^a Diluter rate increased as well as aeration added to all splitter trays in order to increase dissolved oxygen concentrations.

Table 14. The pH Values During a 21-Day Chronic Flow-Through Exposure of *Daphnia magna* to C-1771 (3-Benzotriazoloyl-THPE)

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)	R e p	pH			
		Day 0	Day 7	Day 14	Day 21
Control	A	6.8	7.0	7.3	7.0
	B	6.8	7.0	7.3	7.0
	C	6.8	7.0	7.4	7.0
	D	6.8	7.0	7.3	7.0
Solvent Control	A	7.0	7.2	7.4	7.4
	B	7.2	7.2	7.5	7.5
	C	7.1	7.0	7.5	7.4
	D	7.4	7.1	7.5	7.4
2.74	A	7.1	7.1	7.3	7.5
	B	7.1	7.1	7.3	7.4
	C	7.0	7.1	7.3	7.4
	D	7.0	7.1	7.3	7.5
5.83	A	7.0	7.2	7.3	7.5
	B	7.0	7.2	7.3	7.5
	C	7.0	7.2	7.3	7.5
	D	7.0	7.1	7.3	7.6
9.85	A	7.0	7.2	7.3	7.6
	B	7.0	7.2	7.3	7.6
	C	7.0	7.2	7.3	7.7
	D	7.0	7.1	7.3	7.6
21.2	A	7.0	7.1	7.2	7.6
	B	6.9	7.1	7.2	7.7
	C	6.9	7.1	7.2	7.7
	D	6.9	7.1	7.3	7.5
36.2	A	7.0	7.1	7.2	7.5
	B	6.9	7.1	7.2	7.4
	C	6.9	7.1	7.2	7.5
	D	6.9	7.1	7.2	7.5
78.1	A	7.0	7.1	7.2	7.5
	B	6.9	7.1	7.2	7.6
	C	6.9	7.1	7.2	7.3
	D	6.9	7.1	7.2	7.5

APPENDIX A
CERTIFICATE OF ANALYSIS

HOECHST CELANESE CORPORATION

Certificate of Analysis
3-Benzotriazolyl-1',1',1'-tris(4-hydroxyphenyl)ethane
(THPE BZT)

HCC Sample <u>Number</u>	Date <u>Shipped</u>	Quantity <u>Shipped</u>
SN 11311	09/14/94	200 g

Destination: Toxikon Environmental Sciences

Assay

Analytical Results^a

THPE BZT, wt%

99.2

^aHPLC - Lab Prepared External Standard

Linda L. Jones
Analyst

APPENDIX B
DILUTION WATER CHARACTERIZATION

FRESHWATER CHARACTERIZATION*

Parameter	Concentration ^b	Historical Range ^c
Aluminum	0.101 mg/L	ND - 0.102 mg/L
Arsenic	<0.005 mg/L	ND
Boron	0.132 mg/L	ND - 0.158 mg/L
Beryllium	<0.005 mg/L	ND
Bromide	<0.50 mg/L	ND - 30 mg/L
Cadmium	<0.001 mg/L	ND
Calcium	21.8 mg/L	8.89 - 24.4 mg/L
Chloride	73.5 mg/L	60 - 108 mg/L
Chromium (hexavalent)	<0.05 mg/L	ND
Chromium (total)	<0.01 mg/L	ND
Cobalt	<0.01 mg/L	ND
Copper	<0.001 mg/L	ND - 0.009 mg/L
Fluoride	<0.10 mg/L	ND - 0.826 mg/L
Iodide	<0.050 mg/L	ND
Iron	0.022 mg/L	ND - 0.154 mg/L
Lead	<0.001 mg/L	ND
Manganese	<0.010 mg/L	ND
Magnesium	3.43 mg/L	0.789 - 9.91 mg/L
Mercury	<0.0002 mg/L	ND
Molybdenum	<0.010 mg/L	ND - 0.054 mg/L
Nickel	<0.003 mg/L	ND
Potassium	1.14 mg/L	1.14 - 5.50 mg/L
Selenium	<0.003 mg/L	ND
Silver	<0.0001 mg/L	ND
Sodium	36.4 mg/L	27.4 - 74.0 mg/L
Tin	<0.01 mg/L	ND
Zinc	<0.01 mg/L	ND - 0.043 mg/L
Ammonia (total)	0.164 mg/L	ND - 0.549 mg/L
Cyanide (total)	<0.020 mg/L	ND
Nitrates (total as N)	1.23 mg/L	ND - 1.38 mg/L
Nitrites (total as N)	<0.05 mg/L	Not Established
Phosphates (total)	0.016 mg/L	ND - 0.12 mg/L
Sulfide (total)	<0.1 mg/L	ND
Sulfate (total)	33.7 mg/L	15 - 52 mg/L
TDS	260 mg/L	150 - 552 mg/L
TOC	3.98 mg/L	ND - 6.0 mg/L
TSS	4.0 mg/L	ND - 16 mg/L
COD	14.4 mg/L	ND - 54.6 mg/L
Total organophosphorus pesticides	<1.0 µg/L	ND
Total phenoxy herbicides	<1.2 µg/L	ND
Total organochlorine pesticides	<0.50 µg/L	ND
PCBs	<0.25 µg/L	ND

* The characterized freshwater is carbon-treated Jupiter, Florida, town water which is aerated following carbon treatment.

^b Sample of freshwater collected July 24, 1995.

^c Historical range for laboratory freshwater.

APPENDIX C
ANALYTICAL METHOD VALIDATION

STUDY TITLE

C-1771:
Analytical Method Validation
in Freshwater

AUTHOR

Kelly L. Eyler

STUDY COMPLETION DATE

April 27, 1995

SPONSOR

Hoechst Celanese Corporation
Route 202-206, P.O. Box 2500
Somerville, NJ 00876-1258

PERFORMING LABORATORY

Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

LABORATORY PROJECT ID

J9409010a

STATEMENT OF GLP COMPLIANCE

Test Substance: C-1771

Title: C-1771: Analytical Method Validation in Freshwater

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) by the U.S. Environmental Protection Agency (40 CFR Part 792).

Kelly L. Eyer
Kelly L. Eyer
Study Director

4-27-95
Date

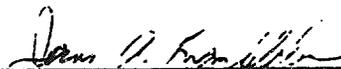
STATEMENT OF QUALITY ASSURANCE

Test Substance: C-1771

Title: C-1771: Analytical Method Validation in Freshwater

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

<u>Type of Audit</u>	<u>Date of Audit</u>	<u>Date Reported to the Study Director and to Management</u>
In-Study Audit:	02-21-95	02-21-95
Study Data Review:	04-24-95	04-24-95
Draft Report Review:	04-24-95	04-24-95
Final Report Review:	04-27-95	04-27-95



James A. Kranzfelder, Ph.D.
Quality Assurance Auditor
Toxikon Environmental Sciences

4-27-95
Date

LIST OF SCIENTIFIC PERSONNEL

Test Substance: C-1771

Title: C-1771: Analytical Method Validation in Freshwater

Chemist: Kelly L. Eyler

Study Director: Kelly L. Eyler

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1.0 INTRODUCTION

An analytical method validation study was conducted at Toxikon Environmental Sciences (TES), Jupiter, Florida, to determine the precision and accuracy of a procedure to analyze C-1771 in freshwater. An HPLC method, received from Hoechst-Celanese Corporation, was modified at TES with the objective of minimizing sample analysis time and using standard laboratory materials.

Quantitation of C-1771 was performed by liquid chromatography (LC) using a UV/VIS detector and the external standard technique. The method was validated by fortifying laboratory freshwater with C-1771 at two concentrations which encompass the range of test concentrations expected to be utilized in toxicity tests of freshwater organisms. This study was conducted February 21, 1995.

2.0 MATERIALS AND METHODS

2.1 Test Methods

The methods for the analytical validation of C-1771 in freshwater were those described in Toxikon Environmental Sciences' test protocol entitled: "C-1771 (3-Benzotriazoloyl-THFE): Analytical Method Validation In Freshwater."

2.2 Apparatus And Materials

High Pressure Liquid Chromatograph: Shimadzu LC600

HPLC Detector: Shimadzu SPD10AV (340 nm)

Autosampler: Perkin Elmer ISS 200 (100- μ L injection volume)

HPLC Column: Phenomenex IB Sil 5 Phenyl, 4.6 mm x 25 cm

Volumetric Flasks: 10-, 50-, and 100-milliliter (mL), class A with ground glass stoppers

Volumetric Pipettes: 1-, 2-, and 3-mL capacity, calibrated to deliver, and 500- μ L glass syringes

Glassware: General assortment of laboratory glassware

Solvents and Reagents:

- a. Water: Modulab PureOne (TES)
- b. Acetonitrile: HPLC grade (B&J)
- c. Glacial Acetic Acid: Baker Analyzed (Baker)

Liquid Chromatographic Mobile Phase (HPLC): Added 370 mL acetonitrile, 200 mL water, and 1 mL acetic acid to a 1-L flask. Degassed by magnetic stirring under a vacuum.

Test Substance: C-1771, Lot # SN-11311, 99.2%, received from Hoechst Celanese (Appendix A)

Matrix: Laboratory freshwater with the following characteristics: pH 7.02; temperature 21.8°C; hardness of approximately 60 to 80 mg/L as CaCO₃

2.3 Preparation Of Stock and Standard Solutions

A primary test substance stock solution was prepared by weighing 0.0104 gram (g) of C-1771 (99.2% purity) into a 100-mL volumetric flask and bringing to volume with acetonitrile. The solution was

thoroughly mixed. The resulting concentration of this primary stock solution was 103.2 mg/L C-1771. A secondary stock solution was prepared by pipetting 1.0 mL of the primary analytical stock into a 10-mL volumetric flask and bringing to volume with acetonitrile. The solution was thoroughly mixed. The resulting concentration of this secondary stock was 10.32 mg/L. A tertiary stock solution was prepared by pipetting 1.0 mL of the secondary stock solution into a 100-mL volumetric flask, bringing to volume with 50:50 acetonitrile:water and mixing thoroughly. The concentration of this tertiary stock solution was 0.1032 mg/L C-1771.

A series of five working calibration standards were prepared by adding the appropriate volumes of the tertiary stock solution to 10-mL volumetric flasks and diluting to volume with 50:50 acetonitrile:water, as shown in Table 1.

2.4 Preparation Of Spike Samples

Low concentration level spike samples were prepared by adding 0.45 mL of the 0.1032 mg/L tertiary stock solution to a 20-mL scintillation vial and diluting to 10 mL with freshwater. The resulting concentration of the low level spike samples was 4.64 $\mu\text{g/L}$. Ten milliliters of acetonitrile was added to each spike sample in preparation for HPLC analysis. High concentration level spike samples were prepared by adding 0.1 mL of the 10.32 mg/L secondary stock solution to a 50-mL volumetric flask and diluting to 5 mL with freshwater. The resulting concentration of the high level spike samples was 206 $\mu\text{g/L}$. The high level spike samples were brought to a total volume of 50 mL with 50:50 acetonitrile:water in preparation for HPLC analysis.

Each spike level was prepared in triplicate. A matrix blank was prepared from an unfortified 10-mL aliquot of freshwater, diluted

with 10 mL of acetonitrile.

2.5 Liquid Chromatographic Analysis

The LC600 pump and SPD10 A UV/VIS detector were set with the following conditions:

Column:	Phenomenex IB Sil 5 Phenyl 4.6-mm x 25-cm column (room temperature)
Detector Wavelength:	340 nm
Mobile Phase:	65:35 ACN:0.5% HOAc in H ₂ O
Flow Rate:	1.0 mL/min, isocratic
Chart Speed:	0.5 cm/min

After equilibration of the system and attainment of a stable baseline on the integrator, quality control samples (method blank and calibration standards) were analyzed along with the validation spike samples to assess the accuracy and precision of the method. A matrix blank of unspiked freshwater was analyzed to determine the limit of detection.

2.6 Quantitation

The standard response curve (linear regression curve) of C-1771 concentration versus peak area (integrator response) was generated from the data obtained during the validation (Figure 1). The equation of the curve is:

$$\text{C-1771 mg/L} = (\text{Peak Area} + 17.04) / 195.12,$$

with a correlation coefficient of 0.998. The C-1771 concentration found in the samples was calculated using the following equation:

$$\text{mg/L C-1771 from std curve} * \text{dil factor} = \text{mg/L C-1771}$$

2.7 Example Calculation

Run Date: February 21, 1995

Run Report#: 14 (MV7; 206 µg/L)

Response = 4114

Dilution Factor = 10X

$$\begin{aligned} \text{C-1771 } \mu\text{g/L} &= (4114 + 17.04) / 195.12 \\ &= 21.17 \mu\text{g/L} * 10 = 212 \mu\text{g/L} \end{aligned}$$

2.8 Limit of Detection

The limit of detection for C-1771 was calculated from a matrix blank and the lowest concentration standard (2.06 $\mu\text{g/L}$ C-1771). The signal(S)-to-noise(N) ratio for the 2.06 $\mu\text{g/L}$ standard and matrix blank was 7. Extrapolation to a S/N ratio of 3 and multiplication by the dilution factor (DF) of 2 is 1.77 $\mu\text{g/L}$ which is the limit of detection (LOD).

Matrix blank = 1.0 mm
2.06 $\mu\text{g/L}$ = 7.0 mm

S/N = 7
DF = 20/10 = 2

$$(2)(3/7) = \text{LOD } \mu\text{g/L} / 2.06 \mu\text{g/L} \quad \text{LOD} = 1.77 \mu\text{g/L}$$

2.9 Archives

The final report and all raw data related to this study will be maintained in archive by Hoechst Celanese Corporation at Pathology Associates Incorporated, Frederick, Maryland.

3.0 RESULTS AND DISCUSSION

Recovery data from the fortified freshwater samples analyzed during this method validation study are presented in Table 2. Samples with a spike concentration of 4.64 $\mu\text{g/L}$ had an average recovery of 99% with a standard deviation of 6%. Samples with a spike concentration of 206 $\mu\text{g/L}$ had an average recovery of 102% with a standard deviation of 2%. The overall average recovery was 101% with a standard deviation of 4%. The limit of detection was 1.77 $\mu\text{g/L}$.

4.0 CONCLUSION

The method described is suitable for the analysis of C-1771 in freshwater over a concentration range of 4.64 to 206 $\mu\text{g/L}$.

Table 1. Preparation of Working Calibration Standards for C-1771

Standard Designation	Volume of Standard (mL)	Stock Concentration (mg/L)	Final Volume (mL)	Standard Concentration ($\mu\text{g/L}$)
Std 1	0.20	0.1032	10	2.06
Std 2	0.50	0.1032	10	5.16
Std 3	1.00	0.1032	10	10.32
Std 4	2.00	0.1032	10	20.64
Std 5	3.00	0.1032	10	30.96

Table 2. Recovery Data for C-1771 From Freshwater During Method Validation

Spike Level	Dilution Factor	$\mu\text{g/L}$ Added	$\mu\text{g/L}$ Found	Percent Recovery*
Method Blank	2X	0.0	<1.77	N/A
Low Rep 1	2X	4.64	4.32	93
Low Rep 2	2X	4.64	4.60	99
Low Rep 3	2X	4.64	4.84	104
Mean \pm Standard Deviation = 99 \pm 6%				
High Rep 1	10X	206	214	104
High Rep 2	10X	206	206	100
High Rep 3	10X	206	212	103
Mean \pm Standard Deviation = 102 \pm 2%				
Grand Mean \pm Standard Deviation = 101 \pm 4%				

*Percent Recovery = $(\mu\text{g/L found} \div \mu\text{g/L added}) \times 100\%$

Figure 1. Calibration Curve for Validation of C-1771 in
Freshwater

C-1771 Method Validation J9409010a
KE February 21, 1995

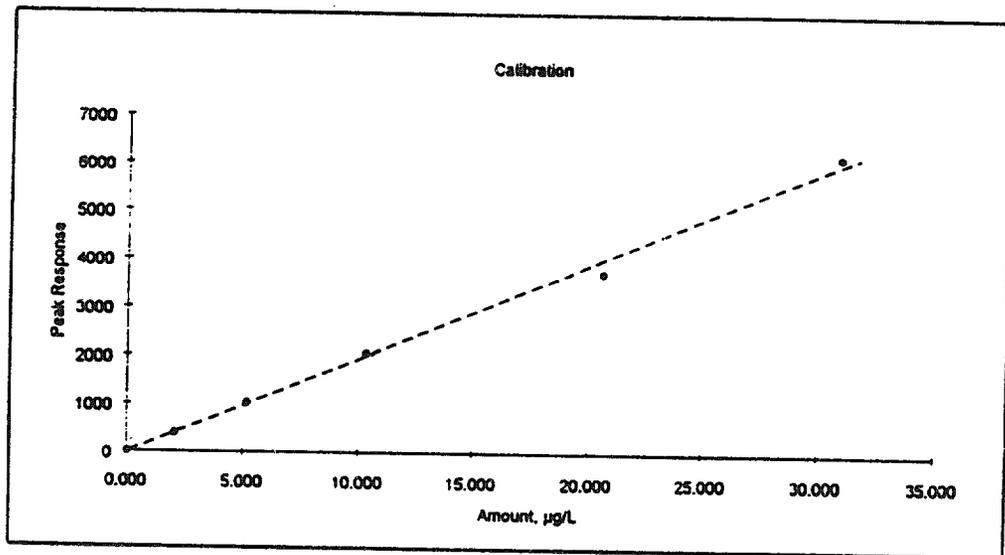
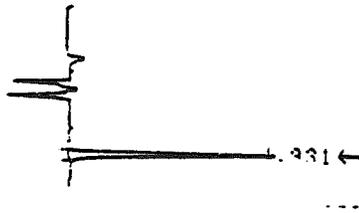


Figure 2. Typical Chromatogram of C-1771 (arrow denotes the retention time of C-1771)

a. Standard-4: 20.63 $\mu\text{g/L}$ C-1771

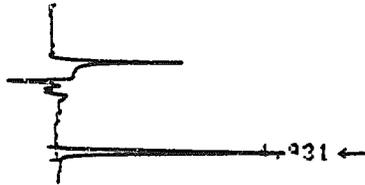


b. Reagent Blank



Figure 2 (Cont.) Typical Chromatogram of C-1771

c. MV7: high spike replicate 3 C-1771; 206 $\mu\text{g/L}$ in
freshwater



d. MV1: Freshwater method blank



APPENDIX A
CERTIFICATE OF ANALYSIS

HOECHST CELANESE CORPORATION

Certificate of Analysis
3-Benzotriazolyl-1',1',1'-tris(4-hydroxyphenyl)ethane
(THPE BZT)

<u>HCC Sample</u>	<u>Date</u>	<u>Quantity</u>
<u>Number</u>	<u>Shipped</u>	<u>Shipped</u>
SN 11311	09/14/94	200 g

Destination: Toxikon Environmental Sciences

Assay

Analytical Results^a

THPE BZT, wt%

99.2

^aHPLC - Lab Prepared External Standard

Linda Allen Farn
Analyst

APPENDIX D
RAW DATA TABLES

Table D-1. Number of Surviving Daphnids During the Reproductive Period

Day	# Daphnids Alive							
	Control				0.314 mg/L			
	A	B	C	D	A	B	C	D
7	10	10*	10	10*	4	10*	10	10*
8	10*	10	10*	10	4*	10	10*	10
9	10	10	10	10	4	10	10	10
10	9	10	10	10	4	10	10	10
11	9	10	10	10	4	10	10	10
12	9	10	10	10	4	10	10	10
13	9	10	10	10	4	10	10	10
14	9	9	9	10	4	10	10	10
15	9	9	9	10	4	10	10	10
16	8	9	9	10	4	10	10	10
17	8	8	9	10	4	10	10	10
18	8	8	9	10	4	10	10	10
19	8	8	9	10	4	10	10	10
20	8	8	9	10	4	10	10	10
21	8	8	8	10	4	10	10	9
Total^a	133	137	141	150	60	150	150	149

* Day young first observed in retention chamber.

^a Number of Total Reproductive Days.

Table D-1. cont. Number of Surviving Daphnids During the Reproductive Period

Day	# Daphnids Alive							
	2.74 $\mu\text{g/L}$				5.83 $\mu\text{g/L}$			
	A	B	C	D	A	B	C	D
7	10*	10*	10*	6	5	10*	10*	10
8	10	10	10	6	5*	10	10	10*
9	10	10	10	6*	5	10	10	10
10	10	10	10	6	5	10	10	10
11	10	10	10	6	5	10	10	10
12	10	10	10	6	5	10	10	10
13	10	10	10	6	5	10	10	10
14	10	10	10	6	5	10	10	10
15	10	10	10	6	5	10	10	10
16	10	10	10	6	5	10	10	10
17	10	10	10	6	5	10	10	9
18	10	10	10	6	5	10	10	9
19	10	10	10	6	5	10	10	9
20	9	10	10	6	5	10	10	9
21	9	10	10	6	5	10	10	9
Total ^a	148	150	150	90	75	150	150	145

* Day young first observed in retention chamber.

^a Number of Total Reproductive Days.

Table D-1. cont. Number of Surviving Daphnids During the Reproductive Period

Day	# Daphnids Alive							
	9.85 µg/L				21.2 µg/L			
	A	B	C	D	A	B	C	D
7	10	10	10	10*	10	10*	10	10
8	10*	10*	10	10	10*	10	10*	10*
9	10	9	10*	10	10	10	10	10
10	10	9	10	10	10	10	10	9
11	10	9	10	10	10	10	10	9
12	10	9	10	10	10	10	9	9
13	10	9	10	9	10	10	9	9
14	10	9	10	9	10	10	9	9
15	9	9	10	9	10	10	9	9
16	9	9	10	9	10	10	9	9
17	9	9	10	9	10	10	9	9
18	9	9	10	9	10	10	9	9
19	9	9	10	9	10	10	9	9
20	9	9	10	9	10	10	9	9
21	9	9	10	9	10	10	9	9
Total ^a	143	137	150	141	150	150	140	138

* Day young first observed in retention chamber.

^a Number of Total Reproductive Days.

Table D-1. cont. Number of Surviving Daphnids During the Reproductive Period

Day	# Daphnids Alive							
	36.2 $\mu\text{g/L}$				78.1 $\mu\text{g/L}$			
	A	B	C	D	A	B	C	D
7	10	10	10*	10	9	10	10	10
8	10*	10*	10	10*	9	10*	10*	10
9	10	10	10	10	9	10	10	10
10	10	10	10	9	9*	10	10	10*
11	10	10	10	9	9	10	9	10
12	10	10	10	9	9	10	8	10
13	10	10	10	9	9	10	8	10
14	10	10	10	9	9	10	8	10
15	10	10	10	9	9	10	8	10
16	10	10	10	9	9	10	8	10
17	10	10	10	9	8	10	8	5
18	10	7	9	9	6	10	2	4
19	10	7	9	9	5	10	2	2
20	10	6	9	9	5	10	2	1
21	10	6	9	9	5	10	2	1
Total ^a	150	136	146	138	119	132	105	113

* Day young first observed in retention chamber.

^a Number of Total Reproductive Days.

DRAFT

STUDY TITLE

C-1771 (3-Benzotriazoloyl-THPE):
Toxicity to Embryos and Larvae of the
Rainbow Trout, *Oncorhynchus mykiss*,
Under Flow-Through Test Conditions

DATA REQUIREMENT

TSCA Environmental Effects Testing Guideline 797.1600

AUTHOR

Jay W. Davis

STUDY INITIATION DATE

December 29, 1994

STUDY COMPLETION DATE

1995

Contains No CBI

SPONSOR

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

PERFORMING LABORATORY

Toxikon Environmental Sciences
106 Coastal Way
Jupiter, Florida 33477

LABORATORY PROJECT ID

J9409010f

STATEMENT OF GLP COMPLIANCE

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

Title: C-1771 (3-Benzotriazoloyl-THPE): Toxicity to Embryos
and Larvae of the Rainbow Trout, *Oncorhynchus mykiss*,
Under Flow-Through 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

Date

STATEMENT OF QUALITY ASSURANCE

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

Title: C-1771 (3-Benzotriazoloyl-THPE): Toxicity to Embryos and Larvae of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through 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:		
In-Life Audit		
Study Data Review:		
Draft Report Review:		
Final Report Review:		

James A. Kranzfelder, Ph.D.
Quality Assurance Auditor
Toxikon Environmental Sciences

Date

LIST OF SCIENTIFIC PERSONNEL

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

Title: C-1771 (3-Benzotriazoloyl-THPE): Toxicity to Embryos and Larvae of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Test Conditions

Study Director: Jay W. Davis, M.S.

Biologists: Hui (Jeff) Liu
Flynn J. Cunningham
Leslie D. Hartman
Michael R. Dunham
Michael B. Malone

Aquaculturists: Keith Ferris
Jonathan Spalding

Chemists: Kelly Eyler
Lynn Masson
Matthew W. Pike
Timothy Z. Kendall

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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: Rainbow Trout; *Oncorhynchus mykiss*

Source of Organisms: Mount Lassen Trout Farm
Red Bluff, California

**Condition at
Study Initiation:** The fertilized eggs appeared healthy and
undamaged at test initiation.

Dilution Water: Filtered freshwater with a hardness range
of 60 to 78 mg/L as CaCO₃; test temperature
range of 8.9 to 14.8°C (mean and standard
deviation of 10.9 ± 0.9°C)

**Nominal (Measured)
Concentrations:** ^{why this value?} Control (<5.21), Solvent (DMF) Control
(<5.21), 9.38 (8.57), 18.8 (15.2), 37.5
(28.2), 75.0 (56.6), 150 (123) µg a.i./L

**Experimental
Test Dates:** March 31 to July 5, 1995

Study Length: 96 days total; 61 days post-hatch

Results:

The maximum acceptable toxicant concentration (MATC) was $>15.2 < 28.2 \mu\text{g/L}$ C-1771 (3-Benzotriazoloyl-THPE) (measured concentration) based upon survival. The MATC calculated as the geometric mean of the lower and upper limit was $20.7 \mu\text{g/L}$ C-1771 (3-Benzotriazoloyl-THPE). The no-observed-effect concentration was $15.2 \mu\text{g/L}$ C-1771 (3-Benzotriazoloyl-THPE).

1.0 INTRODUCTION

A 96-day flow-through toxicity test was conducted at Toxikon Environmental Sciences, Jupiter, Florida, to estimate the chronic effects of C-1771 (3-Benzotriazoloyl-THPE) to the rainbow trout, *Oncorhynchus mykiss*. The criteria for effects in the early life stage test were hatching success of embryos, and growth and survival of juveniles. Results of the test are reported as a maximum acceptable toxicant concentration (MATC), that is, the C-1771 (3-Benzotriazoloyl-THPE) concentration which is bounded at the lower end by the highest concentration in the test that produced no effect (NOEC, no observed effect concentration) and at the higher end by the lowest concentration tested that produced a statistically significant effect (LOEC, lowest observed effect concentration). A point estimate of the MATC was calculated as the geometric mean of the NOEC and LOEC values.

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.

Measured test concentrations are reported as micrograms (μg) C-1771, as active ingredient (ai), per liter (L) or parts per billion (ppb).

2.2 Test Species

Rainbow trout (*Oncorhynchus mykiss*) eggs and milt were obtained from Mt. Lassen Trout Farm, Red Bluff, CA. The eggs and milt for the definitive test were received at Toxikon Environmental Sciences on March 30, 1995. Upon receipt at the laboratory, the gametes were allowed to acclimated to the test system temperature. The eggs from three females were then placed in a plastic pan and the milt was added (the milt was a collection from three males, mixed together at Mt. Lassen Trout Farm). Following gentle mixing for about thirty seconds, "egg wash" solution was added to the pan to activate the sperm and the eggs and gently mixed for approximately ten seconds. The "egg wash" consisting of tris buffer, glycerin and salt, then remained undisturbed for 20 to 30 seconds. Prior to initiating the test, the eggs were cleaned with three cold dilution water rinses (approximately 2 L each) and allowed to harden in dilution water (1 L) for approximately two hours. The eggs were then split into two batches and held in dilution water at test temperature with

slight aeration overnight in preparation for test initiation the following day. The eggs were distributed to test chambers within the test system approximately 19.5 hours after fertilization.

2.3 Test Water

The dilution freshwater was Town of Jupiter water which was vigorously aerated to remove chlorine, filtered through a five-micrometer filter, and then passed through activated carbon beds before being pumped into the laboratory. The water was re-aerated prior to use. During the test, the dilution water (a moderately hard freshwater) possessed a hardness of 60 to 78 mg/L as calcium carbonate (CaCO₃), an alkalinity of 9 to 20 mg/L as CaCO₃, and a specific conductivity of 351 to 447 micromhos per centimeter (μ mhos/cm). The dilution water had a total suspended solid content of ≤ 6.0 mg/L and a mean total organic carbon content of 2.57 mg/L.

Chemical characterization of a recent representative batch of dilution water is presented in Appendix A.

2.4 Test Methods

Methods for the 96-day early life stage test with rainbow trout were those described in Toxikon Environmental Science's test protocol entitled: "C-1771 (3-Benzotriazoloyl-THPE): Toxicity to Embryos and Larvae of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Test Conditions". These methods were based on and were consistent with those described in U.S. EPA Office of Toxic Substances (OTS) Fish Early Life Stage Toxicity Test Guideline, 40 CFR 797.1600 (1988).

The nominal concentrations of C-1771 (3-Benzotriazoloyl-THPE) selected for the early life stage test were 9.38, 18.8, 37.5,

75.0, and 150 $\mu\text{g ai/L}$. These concentrations were selected based on a static acute test with rainbow trout using the test substance (TES Project Number J9409010b) and a range-finding test initiated with embryos and terminated after a total exposure of 15 days. The 96-hour LC_{50} value for rainbow trout, based upon nominal concentrations of C-1771, was 350 $\mu\text{g ai/L}$ with a NOEC of 250 $\mu\text{g ai/L}$. The range-finding embryo-sac fry exposure was conducted under static conditions at nominal concentrations of 10.0, 25.0, 100, 250, and 500 $\mu\text{g ai/L}$ between February 1 and 16, 1995. The exposure was initiated with 20 embryo per test concentration and controls. Hatch rate was unaffected by the presence of the test substance with the percent hatch ranging from 80 to 100% in the test treatments and controls. However, after 15 days of exposure, survival was affected by the presence of the test substance. Mortality of hatched fry was 0% in the controls and test treatments ≤ 100 $\mu\text{g ai/L}$, 61% at 250 $\mu\text{g ai/L}$ and 100% at 500 $\mu\text{g ai/L}$. Therefore, based upon the results of the acute and preliminary studies, the test concentrations selected for the early life stage test ranged from 9.38 to 150 $\mu\text{g ai/L}$.

The definitive exposure was conducted under flow-through conditions in a modified proportional vacuum-siphon diluter system based on the original design of Mount and Brungs (1967). The diluter system was constructed of glass, silicone adhesive, and silicone tubing. A special chemical mixing box equipped with a blender was utilized to enhance solubility of the test substance. The test system was volumetrically calibrated to provide a test concentration series with a 50 percent dilution.

Five C-1771 (3-Benzotriazoloyl-THPE) stock solutions were used during the course of the definitive test. Stock solutions were prepared as needed and the new stock applied to the diluter. The stock solutions (approximately 15 g ai/L) were prepared by

transferring approximately 1.5 g C-1771 (3-Benzotriazoloyl-THPE) to a 100-mL volumetric flask and bringing to volume with dimethylformamide (DMF). The DMF, lot number 4929 KMDA, was Mallinckrodt brand and analytical reagent grade. A total volume of approximately 39 microliters (μL) of stock solution was pumped into the chemical mixing chamber (total volume of 3,863 mL) at each diluter cycle providing a high nominal test concentration of 150 $\mu\text{g ai/L}$. This test solution was proportionally diluted in the diluter system to provide the four lower test concentrations (i.e., 9.38, 18.8, 37.5, and 75.0 $\mu\text{g ai/L}$). A dilution water control and a solvent (DMF) control were maintained concurrently with the five test solutions. The dilution water control did not contain any test substance or solvent. A solvent concentration of approximately 10 $\mu\text{L/L}$ was maintained in the solvent control and all test substance concentrations by injecting approximately 81 μL of DMF into the solvent mixing box (total volume of 8,127 mL) with each diluter cycle. Each treatment solution was split by a splitter box into two portions for delivery into the duplicate test chambers.

A test solution volume of approximately 1000 mL was delivered to each test chamber during every cycle. Test chambers were 24-L glass tanks (40-cm x 29.5-cm x 20-cm) equipped with an overflow standpipe providing a maximum depth of 13 centimeters and a volume of approximately 15.3 L. The diluter cycled at an average rate of 4.3 cycles per hour during the test providing approximately 6.7 volume additions every 24 hours.

The test was initiated with the impartial addition of embryos, one to two at a time, to embryo incubation chambers (13-X 150-mm diameter glass petri dishes encircled by 355- μm Nitex mesh with a height of 17 cm and attached with silicone sealant) until 20 embryos were distributed to each chamber. Two embryo incubation

chambers were positioned within each treatment replicate tank and were maintained stationary. One additional incubation chamber containing 20 embryos was added to each replicate control tank for determination of fertilization success. Test solutions were split by Y-tubes as they entered each replicate test chamber to direct water flow into the embryo incubation chambers. Incubation chambers were rotated under different flows within each replicate twice weekly to eliminate any position effect. A total of 40 embryos were added per replicate (20 per embryo chamber) and 80 embryos per treatment.

The test chambers were randomly positioned in a single water bath in order to maintain a target temperature of $10 \pm 2^{\circ}\text{C}$ under banks of fluorescent and incandescent lights and surrounded by opaque black curtains. The embryos were maintained in total darkness until the eggs hatched and the fry rainbow trout reached the "swim-up" stage. Temperature of the test system was slowly increased to a target of $12 \pm 2^{\circ}\text{C}$ after embryos hatched into alevins. At swim-up, a light regime was initiated with a 4-day transition period. At first, only the incandescent lights were lit for 10 to 12 hours a day with increasing intensity from 50 to 90 percent intensity during the first two days of the transition period, respectively. On days three and four of the transition period, fluorescent lighting on a 12-hour and 14-hour light regime including a 15-minute transition period of incandescent lighting at the initiation and termination of each light period was set up to complete the transition, respectively. Following the transition period, a full light cycle was initiated with fluorescent lighting on a 14-hour light and 10-hour dark regime including a 15-minute transition period of incandescent lighting at the initiation and termination of each light period to simulate dawn and dusk. At full lighting, the light intensity ranged between 3.5 and 4.8 microEinsteins per second per square

meter as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a 2π quantum sensor.

Survival of embryos was recorded daily until hatching was complete. Complete hatch was considered to be attained when 95 percent or more of all live embryos in the controls had hatched. After hatching, fish were not released from the incubation chambers into the test tanks until closer to the "swim up" date. Survival was monitored daily until test termination at 61 days post-hatch. Any abnormalities in the behavior or physical appearance of the fish during the exposure period were also noted.

Fish were fed live brine shrimp (Aquarium Products, Glen Burnie, MD) nauplii and salmon starter of various sizes (Zeigler Bros. Inc., Gardners, PA) four times a day (two times on weekends and holidays) following swim-up until test termination. Analyses of feeds are presented in Appendix B. Feeding was terminated the day prior to test termination. Test aquaria were generally siphoned three times per week (at least twice per week) to remove excess quantities of uneaten food and feces. At test termination, all fish were measured individually to determine standard length and wet weight.

Basic water quality was monitored during the test. Water temperature was measured in a dilution water control chamber hourly during the test via thermocouple and recorded by a data logger. The diurnal range of the water bath temperature was continuously monitored using a minimum/maximum thermometer and recorded daily. Temperature was also monitored in the dilution water control daily. Dissolved oxygen concentrations, test aquaria temperature, and pH were measured at test initiation and once a week thereafter in all controls and test solutions.

Dissolved oxygen concentrations and chamber temperatures were determined using a YSI Model 58 oxygen meter utilizing a membrane electrode. The pH was measured with Fisher Scientific Accumet® 1001 and 1002 pH meters. Water hardness, alkalinity, specific conductivity, total organic carbon (TOC), and total suspended solids (TSS) of the dilution water and high test concentration were measured initially and once a week thereafter. 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) and the total suspended solids using EPA Method 160.2 (U.S. EPA, 1983).

2.5 Chemical Sampling

Prior to test initiation, water samples (approximately 1 to 5 milliliters in volume) were collected from all C-1771 (3-Benzotriazoloyl-THPE)-containing test solutions to verify proper diluter function. At test initiation and generally once every week thereafter throughout the test, water samples (approximately 1 to 5 milliliters in volume) were collected from each replicate control and C-1771 (3-Benzotriazoloyl-THPE) test solution to monitor actual exposure concentrations. Each new stock solution was also analyzed during the test. Samples were analyzed for confirmation analysis of the parent compound by high performance liquid chromatography (HPLC). All water samples were collected from mid-depth in the water column, using a volumetric glass pipet. Concentrations of C-1771 were measured by HPLC analysis following the methods presented in Toxikon Environmental Sciences' (J9409010a) report entitled: "C-1771: Analytical Method Validation In Freshwater" (Appendix C).

2.6 Statistical Analyses

At the termination of the test, hatching success and survival of the control and solvent control embryos were statistically compared using Fisher's Exact Test. A Student's t-Test was used for comparing control and solvent control growth (standard lengths and wet weights) of the fry. If no significant difference was detected between the replicate means of the two controls, the control data were pooled prior to statistical evaluation with the C-1771 (3-Benzotriazoloyl-THPE) treatments. If a significant difference was detected, only the solvent control data were used for statistical evaluation with the treatments.

Statistical differences in hatching and survival between the replicate means of the control and treatment groups were calculated using Fisher's Exact Test. Statistical differences in growth (standard lengths and wet weights) between the replicate means of the control and treatment groups were calculated by analysis of variance (ANOVA) followed with Dunnett's multiple comparison test (one-tailed). All statistical calculations were performed at the replicate level with PC-SAS at the 0.05 probability level. The most sensitive test criterion was used to derive the maximum acceptable toxicant concentration (MATC). A point estimate of the MATC was calculated as the geometric mean of the NOEC and LOEC values (logarithm of NOEC plus logarithm of LOEC divided by two and then converted back to a concentration by the antilogarithm).

2.7 ARCHIVES

The final report and all raw data related to this study will be maintained in archive by Hoechst Celanese Corporation at Pathology Associates, Incorporated, Frederick, Maryland.

3.0 RESULTS AND DISCUSSION

3.1 Analytical Chemistry Results

Prior to initiating the definitive test, the diluter test system was operated for ten days with C-1771 (3-Benzotriazoloyl-THPE) delivered at nominal concentrations of 9.38, 18.8, 37.5, 75.0, and 150 $\mu\text{g ai/L}$ as well as the desired solvent concentration. Due to the limited solubility of the test compound in dilution water, several adjustments were performed during the study in order to optimize the diluter delivery. Analytical data associated with the adjustments can be found in Appendix E. The test was initiated after day 0 sampling, and C-1771 (3-Benzotriazoloyl-THPE) were measured to be 63 to 84 percent of the nominal concentrations (Table 1). Minor adjustments were made to the diluter system to correct any problems associated with the limited solubility of the test compound in an attempt to obtain more consistent analytical results. After approximately a five-hour period the treatments were reanalyzed and found to be 71 to 109% of nominal concentrations indicating the solubility problem had been improved. Since the post-hatch period was the time where developing rainbow trout were most sensitive to the toxic effects of C-1771 (3-Benzotriazoloyl-THPE), and since both a clear effect and no-effect concentration were observed in the study (see Biological Results Section), the fluctuation in test concentrations observed during the test did not compromise the study or the results.

The diluter functioned properly during the entire 96-day definitive test based upon daily calculations of test substance delivery and observations of diluter solution delivery with minor exceptions. On several occasions calculations revealed lower than expected chemical delivery and parts to the injector pump were replaced as well as test chamber concentrations analyzed when appropriate. Finally, when observations indicated the

blender component of the chemical mixing box was not functioning properly, either the relative parts or the enter motorized base was replaced.

The measured values of C-1771 concentrations were slightly lower than the nominal concentrations. Undissolved test substance was observed in chemical mixing box but not in the test chambers during the entire exposure. It was also observed that the test substance was precipitating out of solution not only in the chemical mixing chamber, but was forming on the tip of the injection line as well. Because the diluter prepares all test solutions by mixing up the high test concentration and diluting this solution, the precipitate observed was due to exceeding solubility of the test material in dilution water at the high concentration. This solubility problem also explains the lower measured values obtained for the chemical results which was due to the fact that initially only approximately 70 to 90% of the chemical was able to go into solution. The higher percent of nominal values obtained at the lower test concentrations was likely due to some of the chemical (precipitate) going into solution at the lower test concentrations. Since the high test concentration was close to maximum solubility and the observed precipitation and seeding could be expected. These observations would also account for the slightly lower chemical concentrations measured throughout the test. In order to minimize the "seeding" effect of the precipitate, the injection line and chemical mixing box were cleaned several times throughout the test. A blender was also placed on the diluter system in which the test compound (C-1771) was mixed thoroughly before entering the chemical cells.

Mean measured concentrations of C-1771 (3-Benzotriazoloyl-THPE) during the 96 day test ranged from 8.57 to 123 $\mu\text{g ai/L}$ and from 75 to 91 percent of the nominal concentrations (Table 1). All

new stock solutions were sampled, analyzed, and were determined by HPLC to be approximately (on average) 102% of the nominal concentration.

3.2 Biological Results

Fertilization success was determined from the extra eggs maintained in the control test chambers. Fertilization of these extra eggs was determined to be approximately 97 percent. Rainbow trout embryos began hatching on day 29 of the test and all hatching was completed by day 41 (a total of 12 days). The day selected for hatch completion was day 35, the day when ≥ 95 percent of all viable control embryos had hatched. Hatching success throughout the test ranged from 39 percent at 123 $\mu\text{g/L}$ to 96 percent at 28.2 $\mu\text{g/L}$; hatching success was 94 percent in both the dilution water and solvent controls (Table 2). There was no statistical difference in hatching success between the control and solvent control. A statistical difference in hatching success between test treatment 123 $\mu\text{g/L}$ only and the pooled control was detected (Table 2).

Survival of juvenile fish during the 61-day post-hatch period ranged from 0 percent at 123 $\mu\text{g/L}$ to 97 percent at 8.57 $\mu\text{g/L}$; survival of fish in the dilution water and solvent controls was 100 and 97 percent, respectively (Table 3 and Appendix D). Survival for each treatment replicate was calculated as the number of juvenile fish alive at test termination divided by the number of fry which successfully hatched. There was no statistical difference in survival between the control and solvent control. Survival in the C-1771 (3-Benzotriazoloyl-THPE) test solutions was significantly different from that of the pooled controls at concentrations ≥ 28.2 $\mu\text{g/L}$.

Mean fish standard length at test termination ranged from 43.6 mm at 8.57 $\mu\text{g/L}$ to 45.5 mm at 56.6 $\mu\text{g/L}$ (Table 4 and Appendix D). Mean length of the control and solvent control fish were 43.3 and 43.7 mm, respectively. There was no statistical difference in standard length between the control and solvent control fish. Standard length in the C-1771 (3-Benzotriazoloyl-THPE) test solutions was not significantly different (statistically) from that of any of the test treatments.

Mean wet weight of fish at test termination ranged from 1.10 g at 8.57 $\mu\text{g/L}$ to 1.28 g at 56.6 $\mu\text{g/L}$ (Table 4 and Appendix D). Mean wet weight of the control and solvent control fish was 1.01 and 1.11 g, respectively. The relative standard deviation of fish weight for all surviving fish at test termination in the control and solvent control replicates was $\leq 23.1\%$ (Table 5). There was a statistical difference in wet weight between the control and solvent control fish; thus, weight comparisons were made to the solvent control. No significant reductions in fish weight were detected at any C-1771 (3-Benzotriazoloyl-THPE) concentration tested.

The MATC for C-1771 (3-Benzotriazoloyl-THPE) was $>15.2 <28.2 \mu\text{g/L}$ based upon a reduction in survival. Based upon the geometric mean of the MATC limits, a point estimate of the MATC was calculated to be 20.7 $\mu\text{g/L}$. The NOEC was 15.2 $\mu\text{g/L}$.

3.3 Water Quality Parameters

The water temperature during the 96-day exposure ranged from 8.9 to 14.8°C (Table 6). Overall mean test temperature was 10.9°C with a standard deviation of 0.9°C. Dissolved oxygen concentrations remained $\geq 7.0 \text{ mg/L}$ (≥ 65 percent of saturation) in the control chambers throughout the test (Table 8). The dissolved oxygen concentrations ranged from 7.4 to 11.7 (70 to

>100 percent of saturation) in the C-1771 (3-Benzotriazoloyl-THPE) test solutions during the test (Table 8). The pH values ranged from 6.7 to 7.5 in all test chambers during the test (Table 9). The dilution water hardness ranged from 60 to 78 mg/L as CaCO₃, the alkalinity ranged from 9 to 20 mg/L as CaCO₃, and the specific conductivity ranged from 351 to 447 μmhos/cm (Table 10). The high test treatment solution (123 μg/L) hardness ranged from 62 to 78 mg/L as CaCO₃, the alkalinity ranged from 9 to 20 mg/L as CaCO₃, and the specific conductivity ranged from 349 to 460 μmhos/cm (Table 10). The dilution water control had a total suspended solid (TSS) concentration of ≤6.0 mg/L while the high test treatment (123 μg/L) had a TSS concentration of ≤7.0 mg/L (Table 11). The dilution water control and high test treatment (123 μg/L) had a mean (± standard deviation) total organic carbon (TOC) content of 2.57 ± 1.16 mg/L and 5.91 ± 1.26 mg/L, respectively (Table 11).

4.0 SUMMARY

The MATC for rainbow trout embryos and juveniles exposed to C-1771 (3-Benzotriazoloyl-THPE) during a 96-day early life stage test was >15.2 <28.2 $\mu\text{g/L}$ with a geometric mean of 20.7 $\mu\text{g/L}$. The MATC was based on a reduction in survival of juvenile fish following a 61-day post-hatch exposure. C-1771 (3-Benzotriazoloyl-THPE) exposure resulted in no reduction of fish growth as standard length and wet weight at test concentrations ≤ 56.6 $\mu\text{g/L}$. Hatching success was reduced only at a C-1771 (3-Benzotriazoloyl-THPE) concentration of 123 $\mu\text{g/L}$.

APPENDIX E
RAW CHEMISTRY DATA TABLES

5.0 PROTOCOL DEVIATIONS

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

The test temperature in the system ranged from 8.9 to 14.8°C according to the data logger. These values exceeded the protocol test temperature range of $10 \pm 2^\circ\text{C}$ from day 0 to 35 on one occasion and exceeded $12 \pm 2^\circ\text{C}$ from day 36 to 96 on four occasions. The deviations were generally associated with a mechanical waterbath pump and/or a chiller unit malfunction and were corrected upon identifying the problem. All temperature changes were gradual in nature. The mean temperature and standard deviation was $10.9 \pm 0.9^\circ\text{C}$.

This deviation from the protocol was minor, and in the scientific judgement of the Study Director, it did not affect the outcome or validity of the test results.

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Table 1. Measured Concentrations of C-1771 (3-Benzotriazoloyl-THPE) During a 96-Day Early Life Stage Exposure of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Conditions

Nominal Concentration (µg/L; ppb)	R E P	Measured Concentration (µg/L)				
		Day 0 ^a	Day 0 ^b	Day 7	Day 12	Day 13
Control	A	<1.77 ^c	---	<1.77 ^c	<1.77 ^c	<1.77 ^c
	B	<1.77 ^c	---	<1.77 ^c	<1.77 ^c	<1.77 ^c
Solvent Control	A	<1.77 ^c	---	<1.77 ^c	<1.77 ^c	<1.77 ^c
	B	<1.77 ^c	---	<1.77 ^c	<1.77 ^c	<1.77 ^c
9.38	A	7.07	9.06	8.80	12.4	10.1
	B	7.86	10.2	8.78	8.46	8.00
18.8	A	14.4	15.6	17.4	15.7	16.3
	B	15.2	16.4	17.2	13.2	17.1
37.5	A	26.9	33.2	33.3	35.6	54.6
	B	27.3	33.8	34.6	28.1	33.1
75.0	A	49.6	53.4	63.2	55.5	68.7
	B	52.9	60.8	70.5	47.7	57.3
150	A	97.0	127	159	193	123
	B	95.0	118	137	95.0	151
-----		-----				
Stock (~15 g/L)		13.1	---	---	16.4	15.1
-----		-----				

MATRIX SPIKE RECOVERY DATA

10.0 (low)	8.54	8.62	10.1	9.85	9.77
75.0 (mid)	---	---	---	---	---
150 (high)	122	118	128	128	103

^a Samples taken and analyzed prior to adding embryos to the test system.

^b Samples taken and analyzed after embryos were added to the test system.

^c Value determined from method validation in freshwater.

Table 1 (cont.). Measured Concentrations of C-1771 (3-Benzotriazoloyl-THPE) During a 96-Day Early Life Stage Exposure of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Conditions

Nominal Concentration ($\mu\text{g/L}$; ppb)	R E P	Measured Concentration ($\mu\text{g/L}$)			
		Day 13 ^a	Day 14	Day 20	Day 27
Control	A	---	<1.77 ^b	<1.77 ^b	<4.40 ^c
	B	---	<1.77 ^b	<1.77 ^b	<4.40 ^c
Solvent Control	A	---	<1.77 ^b	<1.77 ^b	<4.40 ^c
	B	---	<1.77 ^b	<1.77 ^b	<4.40 ^c
9.38	A	6.63	10.6	10.9	17.3
	B	5.49	7.75	9.53	10.7
18.8	A	---	18.9	19.3	21.8
	B	---	18.3	19.7	21.3
37.5	A	21.5	35.7	37.0	34.1
	B	23.1	33.7	35.0	28.5
75.0	A	36.6	73.0	73.1	59.1
	B	62.9	68.2	67.6	61.6
150	A	---	188	173	119
	B	---	177	158	119
-----		---	---	---	---
Stock (~15 g/L)		---	---	---	---
-----		---	---	---	---

MATRIX SPIKE RECOVERY DATA

10.0 (low)	---	8.75	10.1	7.47
75.0 (mid)	---	---	---	---
150 (high)	132	150	162	147

^a Samples taken and analyzed for several treatment replicates on Day 13.

^b Value determined from method validation in freshwater.

^c Value indicated is the minimum quantified level, which is equal to one-half the lowest standard response times the dilution factor of the lowest test concentration.

Table 1 (cont.). Measured Concentrations of C-1771 (3-Benzotriazoloyl-THPE) During a 96-Day Early Life Stage Exposure of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Conditions

Nominal Concentration ($\mu\text{g/L}$; ppb)	R E P	Measured Concentration ($\mu\text{g/L}$)			
		Day 28 ^a	Day 34	Day 41	Day 48
Control ^b	A	---	<5.05	<4.52	<4.28
	B	---	<5.05	<4.52	<4.28
Solvent Control ^b	A	---	<5.05	<4.52	<4.28
	B	---	<5.05	<4.52	<4.28
9.38	A	9.14	13.0	8.21	10.3
	B	4.95	8.73	6.80	10.7
18.8	A	---	16.9	11.2	17.0
	B	---	16.4	10.8	16.6
37.5	A	---	27.8	20.2	28.2
	B	---	27.3	19.3	42.7
75.0	A	---	64.4	54.3	81.5
	B	---	58.3	79.7	67.2
150	A	---	116	176	121
	B	---	115	175	122

Stock (~15 g/L)		--	--	14.2	16.4

MATRIX SPIKE RECOVERY DATA					
10.0 (low)		8.67 & 8.85	7.52	8.10	11.9
75.0 (mid)		---	56.4	62.8	72.1
150 (high)		---	143	130	168

^a Samples for treatment one sampled and analyzed.
^b Value indicated is the minimum quantified level, which is equal to one-half the lowest standard response times the dilution factor of the lowest test concentration.

Table 1 (cont.). Measured Concentrations of C-1771 (3-Benzotriazoloyl-THPE) During a 96-Day Early Life Stage Exposure of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Conditions

Nominal Concentration ($\mu\text{g/L}$; ppb)	R E P	Measured Concentration ($\mu\text{g/L}$)			
		Day 55	Day 60	Day 66	Day 74
Control ^a	A	<4.76	<4.65	<5.21	<3.81
	B	<4.76	<4.65	<5.21	<3.81
Solvent Control ^a	A	<4.76	<4.65	<5.21	<3.81
	B	<4.76	<4.65	<5.21	<3.81
9.38	A	9.28	6.30	2.61	4.57
	B	6.90	7.40	8.19	7.41
18.8	A	13.1	12.3	10.8	11.7
	B	12.9	13.5	10.5	10.7
37.5	A	15.6	22.4	18.1	19.7
	B	18.5	16.6	20.6	21.4
75.0	A	20.1	25.4	45.6	48.4
	B	17.5	31.4	40.9	39.5
150	A	54.7	35.3	91.2	88.6
	B	56.5	42.2	98.1	111

Stock (~15 g/L)		24.2 ^b	17.7	13.3	17.3

MATRIX SPIKE RECOVERY DATA

10.0 (low)	11.9	9.70	10.5	9.42
75.0 (mid)	62.1	69.4	59.0	63.7
150 (high)	173	121	125	138

^a Value indicated is the minimum quantified level, which is equal to one-half the lowest standard response times the dilution factor of the lowest test concentration.

^b Same stock as analyzed on days 48, 60 and 66; thus, value suspect and attributed to a dilution error. Value not used in calculating mean stock concentration.

Table 1 (cont.). Measured Concentrations of C-1771 (3-Benzotriazoloyl-THPE) During a 96-Day Early Life Stage Exposure of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Conditions

Nominal Concentration ($\mu\text{g/L}$; ppb)	R E P	Measured Concentration ($\mu\text{g/L}$)		
		Day 82	Day 90	Day 96
Control ^a	A	<4.04	<3.90	<3.66
	B	<4.04	<3.90	<3.66
Solvent Control ^a	A	<4.04	<3.90	<3.66
	B	<4.04	<3.90	<3.66
9.38	A	6.38	7.02	8.95
	B	5.76	7.64	11.0
18.8	A	10.9	12.0	16.9
	B	10.6	12.2	23.9
37.5	A	18.8	23.0	38.9
	B	20.9	23.1	40.8
75.0	A	45.4	63.9	67.2
	B	79.2	49.8	89.7
150	A	100	112	207
	B	118	114	160

Stock (~15 g/L)		15.6	14.8 ^b	14.1

MATRIX SPIKE RECOVERY DATA				
10.0 (low)		10.0	9.29	8.62
75.0 (mid)		54.2	71.7	61.0
150 (high)		134	140	130

^a Value indicated is the minimum quantified level, which is equal to one-half the lowest standard response times the dilution factor of the lowest test concentration.
^b Stock analysis on day 89 of the study.

Table 1 (cont.). Measured Concentrations of C-1771 (3-Benzotriazoloyl-THPE) During a 96-Day Early Life Stage Exposure of the Rainbow Trout, *Oncorhynchus mykiss*, Under Flow-Through Conditions

Nominal Concentration ($\mu\text{g/L}$; ppb)	R E P	Replicate			Treatment		
		Mean	Std	%Nom.	Mean	Std	%Nom.
Control	A	<5.21	-.--	--	<5.21	-.--	--
	B	<5.21	-.--	--			
Solvent Control	A	<5.21	-.--	--	<5.21	-.--	--
	B	<5.21	-.--	--			
9.38	A	9.05	3.24	97	8.57	2.58	91
	B	8.11	1.72	87			
18.8	A	15.1	3.25	80	15.2	3.54	81
	B	15.4	3.90	82			
37.5	A	28.7	9.64	76	28.2	8.60	75
	B	27.8	7.67	74			
75.0	A	55.2	16.0	74	56.6	16.7	76
	B	58.0	17.6	77			
150	A	127	47.6	85	123	41.9	82
	B	120	36.4	80			

Stock (~15 g/L)		--	--	--	15.3	1.6	102

MATRIX SPIKE RECOVERY DATA							
10.0 (low)		---	---	---	9.38	1.21	94
75.0 (mid)		---	---	---	63.2	6.16	84
150 (high)		---	---	---	136	17.7	91

Table 2. Hatching Success of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	R E P	Number of Fish Hatched (% Hatch)							
		Subreplicate				Rep		Treatment	
		1	2	Total	Total	Total	Total		
Control	A	19	(95)	18	(90)	37	(93)	75	(94)
	B	19	(95)	19	(95)	38	(95)		
Sol. Control	A	17	(85)	20	(100)	37	(93)	75	(94)
	B	19	(95)	19	(95)	38	(95)		
Pooled Cont.	A	36	(90)	38	(95)	74	(93)	150	(94)
	B	38	(95)	38	(95)	76	(95)		
8.57	A	16	(80)	20	(100)	36	(90)	74	(93)
	B	19	(95)	19	(95)	38	(95)		
15.2	A	17	(85)	18	(90)	35	(88)	71	(89)
	B	17	(85)	19	(95)	36	(90)		
28.2	A	20	(100)	20	(100)	40	(100)	77	(96)
	B	17	(85)	20	(100)	37	(93)		
56.6	A	18	(90)	17	(85)	35	(88)	70	(88)
	B	17	(85)	18	(90)	35	(88)		
123	A	7	(35)	5	(25)	12	(30)	31*	(39)
	B	8	(40)	11	(55)	19	(48)		

Note: Each replicate initially had 40 embryos (20 in each of two chambers) for a total of 80 embryos per treatment.

* Denotes that the mean number of embryos which hatched is significantly reduced as compared to the pooled control ($\alpha = 0.05$).

Table 3. Mortality of Rainbow Trout, *Oncorhynchus mykiss*, Exposed To C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Number of Fish Alive/Total Exposed ^a (% Survival)					
	Replicate A		Replicate B		Treatment	
Control	37/37	(100)	38/38	(100)	75/75	(100)
Solvent Control	35/37	(95)	38/38	(100)	73/75	(97)
Pooled Control	72/74	(97)	76/76	(100)	148/150	(99)
8.57	36/36	(100)	36/38	(95)	72/74	(97)
15.2	33/35	(94)	35/36	(97)	68/71	(96)
28.2	32/40	(80)	25/37	(68)	57/77	(74)*
56.6	0/35	(0)	6/35	(17)	6/70	(9)*
123	0/12	(0)	0/19	(0)	0/31	(0)*

^a The values presented represent the number of surviving fish at test termination (61 days after hatching of the embryos) divided by the total number exposed for the post-hatch period.

* Denotes that the mean survival is significantly reduced as compared to the pooled control ($\alpha = 0.05$).

Table 4. Growth of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) for 96 Days During the Early Life Stages of Development Under Flow-Through Conditions

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Growth		
	Mean Length ^a (mm)		Mean Wet Weight ^b (g)
Control	43.3	(2.7)	1.01 (0.22)
Solvent Control	43.7	(2.2)	1.11 (0.22)
Pooled Control	43.5	(2.4)	1.06 (0.22)
8.57	43.6	(2.2)	1.10 (0.20)
15.2	43.8	(2.0)	1.15 (0.22)
28.2	43.7	(2.5)	1.17 (0.26)
56.6	45.5	(3.2)	1.28 (0.27)
123	---	(--)	--- (--)

^a Standard lengths for individual fish are presented in Appendix D.

^b Wet weights for individual fish are presented in Appendix D.

NOTE: Numbers in parenthesis are the standard deviations of the means.

There was no significant difference detected in the mean lengths and mean weights of the fish of any C-1771 (3-Benzotriazoloyl-THPE) treatment when compared to the pooled control and solvent control, respectively ($\alpha = 0.05$).

Table 5. Relative Standard Deviations for Control and Solvent Control Rainbow Trout, *Oncorhynchus mykiss*, Weights at Termination of 96-Day Early Life-Stage Toxicity Test to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Treatment	Replicate	RSD (%)
Control	A	19.7
	B	23.1
Solvent Control	A	18.1
	B	20.1

RSD = relative standard deviation = $\frac{\text{standard deviation}}{\text{mean}} \times 100$

Table 6. Daily Temperature Ranges (in °C) During a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Test Day	Temperature ^a		Test Day	Temperature ^a		Test Day	Temperature ^a	
	Min	Max		Min	Max		Min	Max
0	8.9	9.5	33	9.8	10.1	66	10.8	11.7
1	9.2	9.5	34	9.8	10.3	67	11.4	11.8
2	9.2	9.3	35	9.7	11.6	68	11.1	11.8
3	9.1	9.4	36	11.2	11.5	69	11.4	11.9
4	9.2	9.5	37	11.2	11.5	70	11.5	11.9
5	9.2	9.8	38	11.1	11.4	71	11.5	11.8
6	9.2	10.2	39	11.1	11.4	72	11.5	11.8
7	9.5	10.2	40	11.2	11.5	73	11.4	11.8
8	9.9	10.1	41	11.3	11.7	74	11.4	11.7
9	9.8	10.1	42	11.3	12.0	75	11.3	11.7
10	9.8	10.3	43	11.5	11.9	76	11.5	12.0
11	10.0	10.5	44	11.4	11.9	77	11.3	11.9
12	9.9	10.5	45	11.0	11.9	78	10.6	11.7
13	9.9	10.3	46	11.4	13.3	79	9.9*	11.7
14	9.9	10.9	47	11.4	12.8	80	11.4	11.8
15	9.8	10.3	48	11.3	13.5	81	11.5	11.8
16	10.0	10.3	49	11.1	14.8*	82	10.9	11.8
17	9.8	10.3	50	10.9	11.1	83	11.3	11.7
18	9.9	10.5	51	10.8	11.2	84	11.4	11.8
19	9.9	10.3	52	10.9	11.6	85	11.4	11.8
20	10.1	11.0	53	11.0	11.5	86	11.4	11.7
21	10.1	14.1*	54	11.1	11.6	87	11.4	11.8
22	9.5	11.5	55	11.2	11.6	88	11.4	11.7
23	9.4	10.0	56	11.1	11.9	89	9.8*	11.7
24	9.7	10.1	57	11.0	11.3	90	9.7*	12.9
25	9.7	9.9	58	11.0	11.4	91	12.5	12.8
26	8.9	9.9	59	11.0	11.3	92	12.4	12.6
27	9.6	10.2	60	11.0	11.3	93	12.4	12.7
28	9.7	10.1	61	10.9	11.3	94	12.4	12.7
29	9.7	9.9	62	11.0	11.3	95	12.5	12.8
30	9.7	10.0	63	10.6	11.3	96	12.5	12.7
31	9.7	10.4	64	11.0	11.4			
32	9.8	10.0	65	10.9	11.2	Overall	8.9	14.8

^a Daily temperature ranges reported represent the minimum and maximum hourly temperature recorded by the data logger during each test day.

* Denotes periods in which temperature deviated outside the specified range of 10 ± 2°C (from day 0 to 35) and 12 ± 2°C (from day 36 to 96).

NOTE: Overall test temperature mean was 10.9°C with a standard deviation of 0.9°C.

Table 7. Temperature Measurements in the Control Treatment with Corresponding 100 Percent Dissolved Oxygen Saturation Concentrations During a 96-Day Early Life Stage Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Test Day	Temperature (°C)	100% DO Saturation (mg/L) ^a
0	9.4	11.6
7	9.5	11.3
14	9.9	11.3
21	12.8	10.5
28	9.8	11.3
35	9.8	11.3
42	11.3	11.0
49	11.9	10.8
56	11.1	11.0
63	11.1	11.0
70	11.7	10.8
77	11.6	10.8
84	11.5	10.8
91	12.6	10.5
96	12.5	10.5

From: Truesdale, et al., 1955.

^a Saturation values are based upon temperature as recorded by the data-mentor values as measured in the control.

Table 8. Dissolved Oxygen Concentrations During a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)	R e p	Dissolved Oxygen Concentration (mg/L)				
		Day 0	Day 7	Day 14	Day 21	Day 28
Control	A	9.6	11.1	10.8	10.1	10.8
	B	9.8	11.0	11.0	10.2	10.8
Solvent Control	A	9.7	11.3	10.9	10.2	10.8
	B	9.7	11.3	10.8	10.2	10.8
8.57	A	9.4	11.4	11.1	10.3	10.9
	B	9.6	11.3	11.0	10.3	10.8
15.2	A	9.4	11.2	11.0	10.2	10.7
	B	9.5	11.4	11.1	10.4	10.8
28.2	A	9.6	11.4	11.1	10.4	11.0
	B	9.4	11.2	11.0	10.4	10.9
56.6	A	9.8	11.3	11.0	10.4	11.0
	B	9.9	11.4	11.0	10.3	10.8
123	A	10.4	11.5	11.3	10.5	11.1
	B	10.6	11.5	11.0	10.4	11.0

Table 8 (cont.). Dissolved Oxygen Concentrations During a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)	R e p	Dissolved Oxygen Concentration (mg/L)				
		Day 35	Day 42	Day 49	Day 56	Day 63
Control	A	11.2	10.2	10.4	9.2	9.8
	B	11.4	10.2	10.4	9.4	9.9
Solvent Control	A	11.6	10.3	10.5	9.1	9.6
	B	11.8	10.3	10.6	8.8	9.4
8.57	A	11.6	10.5	10.6	9.4	9.7
	B	11.7	10.5	10.7	8.8	9.3
15.2	A	11.3	10.3	10.5	9.2	9.4
	B	11.3	10.4	10.8	9.3	9.5
28.2	A	11.6	10.6	10.9	9.4	9.2
	B	11.6	10.5	10.6	9.0	9.1
56.6	A	11.5	10.5	10.7	9.9	10.3
	B	11.6	10.6	10.9	9.6	10.0
123	A	11.6	10.8	11.0	10.9	10.4
	B	11.5	10.6	11.0	10.7	10.2

Table 8 (cont.). Dissolved Oxygen Concentrations During a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)	R e p	Dissolved Oxygen Concentration (mg/L)				
		Day 70	Day 77	Day 84	Day 91	Day 96
Control	A	9.3	9.4	8.1	8.1	9.0
	B	9.3	9.6	8.4	8.1	9.4
Solvent Control	A	8.8	8.6	7.0	8.2	9.3
	B	8.5	8.8	7.2	8.1	9.3
8.57	A	8.8	8.7	7.6	7.4	8.7
	B	8.8	8.8	7.7	7.7	9.0
15.2	A	8.3	9.2	8.2	8.2	9.2
	B	8.4	8.2	7.7	7.8	8.9
28.2	A	8.4	8.4	7.6	7.5	9.3
	B	8.6	8.8	7.8	8.0	9.2
56.6	A	10.2	10.9	10.4	10.5	10.5
	B	9.2	9.5	8.8	9.2	10.0
123	A	10.6	11.1	10.7	10.8	10.8
	B	10.4	10.8	10.4	10.7	10.6

Table 9. The pH Values During a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)	R e p	pH				
		Day 0	Day 7	Day 14	Day 21	Day 28
Control	A	7.3	7.2	7.2	7.3	7.2
	B	7.5	7.2	7.3	7.4	7.2
Solvent Control	A	7.3	7.2	7.2	7.4	7.2
	B	7.5	7.2	7.3	7.4	7.2
8.57	A	7.3	7.2	7.3	7.4	7.3
	B	7.5	7.3	7.3	7.4	7.2
15.2	A	7.3	7.2	7.3	7.4	7.2
	B	7.5	7.3	7.3	7.4	7.2
28.2	A	7.3	7.2	7.3	7.4	7.3
	B	7.4	7.3	7.3	7.5	7.2
56.6	A	7.3	7.2	7.3	7.5	7.3
	B	7.4	7.3	7.3	7.5	7.2
123	A	7.3	7.3	7.3	7.5	7.3
	B	7.4	7.3	7.3	7.5	7.3

Table 9 (cont.). The pH Values During a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)	R e p	pH				
		Day 35	Day 42	Day 49	Day 56	Day 63
Control	A	7.2	7.2	7.2	7.2	7.1
	B	7.2	7.1	7.2	7.3	7.2
Solvent Control	A	7.2	7.2	7.2	7.2	7.1
	B	7.3	7.1	7.3	7.3	7.2
8.57	A	7.3	7.2	7.3	7.2	7.1
	B	7.4	7.1	7.4	7.3	7.3
15.2	A	7.3	7.2	7.3	7.3	7.2
	B	7.3	7.1	7.4	7.3	7.3
28.2	A	7.3	7.2	7.4	7.3	7.2
	B	7.4	7.0	7.4	7.3	7.3
56.6	A	7.3	7.2	7.4	7.3	7.3
	B	7.4	7.2	7.4	7.3	7.3
123	A	7.3	7.3	7.5	7.4	7.3
	B	7.4	7.2	7.5	7.3	7.3

Table 9 (cont.). The pH Values During a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)	R e p	pH				
		Day 70	Day 77	Day 84	Day 91	Day 96
Control	A	6.8	6.9	6.8	6.6	6.7
	B	6.9	6.9	6.8	6.7	6.9
Solvent Control	A	6.8	6.9	6.7	6.7	6.8
	B	6.8	6.8	6.8	6.7	6.9
8.57	A	6.8	6.9	6.7	6.7	6.8
	B	6.9	6.9	6.8	6.7	6.9
15.2	A	6.8	6.9	6.8	6.7	6.8
	B	6.8	6.8	6.8	6.7	6.8
28.2	A	6.8	6.8	6.7	6.7	6.8
	B	6.9	6.9	6.8	6.7	6.9
56.6	A	7.0	7.2	7.1	7.0	7.1
	B	6.9	6.9	6.9	6.8	6.9
123	A	7.2	7.3	7.2	7.2	7.1
	B	7.1	7.2	7.2	7.0	7.1

Table 10. Hardness, Alkalinity, and Conductivity Measurements From a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Day	Replicate	Hardness* (mg/L)	Alkalinity* (mg/L)	Conductivity ($\mu\text{mhos/cm}$)
Control	0	B	64	15	447
123			68	14	453
Control	7	A	68	15	384
123			68	15	386
Control	14	B	78	15	398
123			64	14	413
Control	21	A	64	15	419
123			64	15	428
Control	28	B	70	11	397
123			62	12	405
Control	35	A	60	11	417
123			66	13	460
Control	42	B	68	10	413
123			64	10	416
Control	49	A	68	20	389
123			72	20	392
Control	56	B	64	16	391
123			64	14	413
Control	63	A	66	12	411
123			68	13	425
Control	70	B	68	9	397
123			66	9	397
Control	77	A	68	9	351
123			74	12	349
Control	84	B	78	14	374
123			76	12	364
Control	91	A	74	12	373
123			76	13	369
Control	96	B	76	12	370
123			78	12	375

* Hardness and alkalinity measurements expressed as milligrams per liter as CaCO_3 .

Table 11. Total Organic Carbon (TOC) and Total Suspended Solid (TSS) Measurements From a 96-Day Early Life Stage Test of Rainbow Trout, *Oncorhynchus mykiss*, Exposed to C-1771 (3-Benzotriazoloyl-THPE) Under Flow-Through Conditions

Mean Measured Concentration ($\mu\text{g/L}$; ppb)	Day	Replicate	TSS (mg/L)	TOC (mg/L)
Control	0	B	ND	2.50
123			ND	2.82
Control	7	A	ND	1.60
123			ND	5.59
Control	14	B	ND	1.69
123			ND	5.95
Control	21	A	ND	1.71
123			ND	5.96
Control	28	B	ND	2.21
123			ND	6.28
Control	35	A	ND	3.42
123			ND	4.78
Control	42	B	ND	2.56
123			ND	6.49
Control	49	A	ND	3.58
123			ND	7.77
Control	56	B	ND	2.80
123			ND	6.70
Control	63	A	ND	2.19
123			ND	5.29
Control	70	B	ND	1.61
123			ND	5.30
Control	77	A	6.0	2.70
123			ND	5.44
Control	84	B	4.0	4.72
123			7.0	6.86
Control	91	A	ND	4.65
123			ND	5.31
Control	96	B	ND	--- ^a
123			ND	8.04

^a Sample broken at the analytical laboratory; no value was obtained.

ND = Not detected.

NOTE: The detection limit for TSS was 4.0 mg/L and for TOC was 1.0 mg/L. The Mean TOC in the control was 2.57 ± 1.16 mg/L while in test concentration 123 $\mu\text{g/L}$ it was 5.91 ± 1.26 mg/L.

APPENDIX A
FRESHWATER CHARACTERIZATION

FRESHWATER CHARACTERIZATION*

Parameter	Concentration ^b	Historical Range ^a
Aluminum	0.094 mg/L	ND - 0.102 mg/L
Arsenic	<0.005 mg/L	ND
Boron	0.106 mg/L	ND - 0.158 mg/L
Beryllium	<0.005 mg/L	ND
Bromide	8.99 mg/L	ND - 30 mg/L
Cadmium	<0.001 mg/L	ND
Calcium	24.3 mg/L	8.89 - 24.4 mg/L
Chloride	85.0 mg/L	mg/L 60 - 108
Chromium (hexavalent)	N/A mg/L	ND
Chromium (total)	<0.01 mg/L	ND
Cobalt	<0.01 mg/L	ND
Copper	<0.001 mg/L	ND - 0.009 mg/L
Fluoride	0.106 mg/L	ND - 0.826 mg/L
Iodide	<0.050 mg/L	ND
Iron	<0.030 mg/L	ND - 0.154 mg/L
Lead	<0.003 mg/L	ND
Manganese	<0.010 mg/L	ND
Magnesium	4.09 mg/L	0.789 - 9.91 mg/L
Mercury	<0.0002 mg/L	ND
Molybdenum	<0.010 mg/L	ND - 0.054 mg/L
Nickel	<0.008 mg/L	ND
Potassium	1.43 mg/L	1.32 - 5.50 mg/L
Selenium	<0.003 mg/L	ND
Silver	<0.0001 mg/L	ND
Sodium	29.7 mg/L	27.4 - 74.0 mg/L
Tin	N/A mg/L	ND
Zinc	N/A mg/L	ND - 0.043 mg/L
Ammonia (total)	0.549 mg/L	ND - 0.549 mg/L
Cyanide (total)	<0.020 mg/L	ND
Nitrates (total as N)	N/A mg/L	ND - 1.38 mg/L
Nitrites (total as N)	N/A mg/L	Not Established
Phosphates (total)	0.014 mg/L	ND - 0.12 mg/L
Sulfide (total)	<0.1 mg/L	ND
Sulfate (total)	31.4 mg/L	15 - 52 mg/L
TDS	250 mg/L	mg/L 150 - 552
TOC	2.76 mg/L	ND - 6.0 mg/L
TSS	<4 mg/L	mg/L ND - 16 mg/L
COD	<5.0 mg/L	ND - 54.6 mg/L
Total organophosphorus pesticides	<1.0 µg/L	ND
Total phenoxy herbicides	<1.2 µg/L	ND
Total organochlorine pesticides	<0.01 µg/L	ND
PCBs	<0.10 µg/L	ND

* The characterized freshwater is carbon-treated Jupiter, Florida, town water which is aerated following carbon treatment.

^b Sample of freshwater collected January 24, 1995.

^a Historical range for laboratory freshwater.

APPENDIX B
CHEMICAL CHARACTERIZATION
OF ANIMAL FEEDS

CHEMICAL ANALYSIS OF ANIMAL FEEDS

<u>Feed</u>	<u>Parameter</u>	<u>Concentration</u>
Aquarium Products	TPH	6330 mg/kg
Brine shrimp nauplii (Lot #BS-180)	TCP	<0.10 mg/kg
	PCB	<0.10 mg/kg
Zeigler Brothers, Inc.	TPH	138,000 mg/kg
Salmon Starter - Biodiet I (Lot #BD95-2-7-1)	TCP	<0.10 mg/kg
	PCB	<0.10 mg/kg
Zeigler Brothers, Inc.	TPH	48,200 mg/kg
Salmon Starter - Biodiet 2 (Lot #BD95-2-7-2)	TCP	<0.10 mg/kg
	PCB	<0.10 mg/kg
Zeigler Brothers, Inc.	TPH	152,000 mg/kg
Salmon Starter (Lot #94-11-28)	TCP	<0.10 mg/kg
	PCB	<0.10 mg/kg

TPH = Total Petroleum Hydrocarbons
TCP = Total Chlorinated Pesticides
PCB = Total PCBs

Total petroleum hydrocarbons were analyzed by IR following EPA Methods 418.1 (Water) and 9071/9073 (Solids); Methods for Chemical Analysis of Water and Wastes. EPA 600/4-79-020 (Revised, March 1983). EPA/EMSL, Cincinnati, OH and Test Methods for Evaluating Solid Waste: Physical/Chemical Methods. EPA SW-846 (Third Edition) 1986. Office of Solid Waste, USEPA.

Total chlorinated pesticides and total PCBs were determined by GC following Guidance for Performing Tests on Dredged Material to be Disposed of in Ocean Waters. U.S. Army Corps of Engineers.
21 December 1984.

APPENDIX C
ANALYTICAL METHOD VALIDATION

APPENDIX D
RAW BIOLOGY DATA TABLES

Table D-1. Daily Count of Rainbow Trout, *Oncorhynchus mykiss*, Fry During the 61-Day Post-Hatch Exposure

Number of Fish Alive									
Test Day (Post Hatch)		Control		Sol. Control		8.57 µg/L		15.2 µg/L	
		Rep A	Rep B	Rep A	Rep B	Rep A	Rep B	Rep A	Rep B
35	(0)	37	37	37	38	36	38	34	36
36	(1)	37	37	37	38	36	38	35	36
37	(2)	37	38	37	38	36	38	35	36
38	(3)	37	38	37	38	36	38	35	36
39	(4)	37	38	37	38	36	38	35	36
40	(5)	37	38	37	38	36	38	35	36
41	(6)	37	38	37	38	36	38	35	36
42	(7)	37	38	37	38	36	38	35	36
43	(8)	37	38	37	38	36	38	35	36
44	(9)	37	38	37	38	36	38	35	36
45	(10)	37	38	37	38	36	38	35	36
46	(11)	37	38	37	38	36	38	35	36
47	(12)	37	38	37	38	36	38	34	36
48	(13)	37	38	37	38	36	38	34	36
49	(14)	37	38	37	38	36	38	34	35
50	(15)	37	38	37	38	36	38	34	35
51	(16)	37	38	37	38	36	38	34	35
52	(17)	37	38	37	38	36	38	34	35
53	(18)	37	38	37	38	36	38	34	35
54	(19)	37	38	37	38	36	38	34	35
55	(20)	37	38	37	38	36	38	34	35
56	(21)	37	38	36	38	36	38	34	35
57	(22)	37	38	35	38	36	38	34	35
58	(23)	37	38	35	38	36	37	34	35
59	(24)	37	38	35	38	36	37	34	35
60	(25)	37	38	35	38	36	37	34	35
61	(26)	37	38	35	38	36	36	34	35
62	(27)	37	38	35	38	36	36	34	35
63	(28)	37	38	35	38	36	36	34	35
64	(29)	37	38	35	38	36	36	34	35
65	(30)	37	38	35	38	36	36	34	35
66	(31)	37	38	35	38	36	36	34	35
67	(32)	37	38	35	38	36	36	34	35
68	(33)	37	38	35	38	36	36	34	35

Table D-1. (cont.) Daily Count of Rainbow Trout, *Oncorhynchus mykiss*, Fry During the 61-Day Post-Hatch Exposure

Number of Fish Alive									
Test Day (Post Hatch)	Control		Sol. Control		8.57 μ g/L		15.2 μ g/L		
	Rep A	Rep B	Rep A	Rep B	Rep A	Rep B	Rep A	Rep B	
69	(34)	37	38	35	38	36	36	34	35
70	(35)	37	38	35	38	36	36	34	35
71	(36)	37	38	35	38	36	36	34	35
72	(37)	37	38	35	38	36	36	34	35
73	(38)	37	38	35	38	36	36	33	35
74	(39)	37	38	35	38	36	36	33	35
75	(40)	37	38	35	38	36	36	33	35
76	(41)	37	38	35	38	36	36	33	35
77	(42)	37	38	35	38	36	36	33	35
78	(43)	37	38	35	38	36	36	33	35
79	(44)	37	38	35	38	36	36	33	35
80	(45)	37	38	35	38	36	36	33	35
81	(46)	37	38	35	38	36	36	33	35
82	(47)	37	38	35	38	36	36	33	35
83	(48)	37	38	35	38	36	36	33	35
84	(49)	37	38	35	38	36	36	33	35
85	(50)	37	38	35	38	36	36	33	35
86	(51)	37	38	35	38	36	36	33	35
87	(52)	37	38	35	38	36	36	33	35
88	(53)	37	38	35	38	36	36	33	35
89	(54)	37	38	35	38	36	36	33	35
90	(55)	37	38	35	38	36	36	33	35
91	(56)	37	38	35	38	36	36	33	35
92	(57)	37	38	35	38	36	36	33	35
93	(58)	37	38	35	38	36	36	33	35
94	(59)	37	38	35	38	36	36	33	35
95	(60)	37	38	35	38	36	36	33	35
96	(61)	37	38	35	38	36	36	33	35

Table D-1. (cont.) Daily Count of Rainbow Trout, *Oncorhynchus mykiss*, Fry Following Culling During the 61-Day Post-Hatch Exposure

Test Day (Post Hatch)		Number of Fish Alive					
		28.2 $\mu\text{g/L}$		56.6 $\mu\text{g/L}$		123 $\mu\text{g/L}$	
		Rep A	Rep B	Rep A	Rep B	Rep A	Rep B
35	(0)	39	37	33	33	7	15
36	(1)	40	37	31	34	9	15
37	(2)	40	37	31	33	8	14
38	(3)	40	37	31	33	8	12
39	(4)	40	37	29	33	8	10
40	(5)	40	37	28	33	7	8
41	(6)	40	37	25	31	7	6
42	(7)	40	36	15	29	2	1
43	(8)	40	36	12	21	0	0
44	(9)	39	35	8	16	0	0
45	(10)	39	35	7	15	0	0
46	(11)	39	35	4	11	0	0
47	(12)	38	35	1	6	0	0
48	(13)	37	35	0	6	0	0
49	(14)	36	34	0	6	0	0
50	(15)	36	34	0	6	0	0
51	(16)	36	33	0	6	0	0
52	(17)	36	31	0	6	0	0
53	(18)	35	31	0	6	0	0
54	(19)	35	30	0	6	0	0
55	(20)	35	30	0	6	0	0
56	(21)	35	30	0	6	0	0
57	(22)	35	29	0	6	0	0
58	(23)	35	29	0	6	0	0
59	(24)	34	27	0	6	0	0
60	(25)	34	27	0	6	0	0
61	(26)	33	27	0	6	0	0
62	(27)	33	27	0	6	0	0
63	(28)	33	26	0	6	0	0
64	(29)	32	26	0	6	0	0
65	(30)	32	26	0	6	0	0
66	(31)	32	26	0	6	0	0
67	(32)	32	26	0	6	0	0

Table D-1. (cont.) Daily Count of Rainbow Trout, *Oncorhynchus mykiss*, Fry During the 61-Day Post-Hatch Exposure

		Number of Fish Alive					
Test Day (Post Hatch)		28.2 $\mu\text{g/L}$		56.6 $\mu\text{g/L}$		123 $\mu\text{g/L}$	
		Rep A	Rep B	Rep A	Rep B	Rep A	Rep B
68	(33)	32	26	0	6	0	0
69	(34)	32	26	0	6	0	0
70	(35)	32	26	0	6	0	0
71	(36)	32	25	0	6	0	0
72	(37)	32	25	0	6	0	0
73	(38)	32	25	0	6	0	0
74	(39)	32	25	0	6	0	0
75	(40)	32	25	0	6	0	0
76	(41)	32	25	0	6	0	0
77	(42)	32	25	0	6	0	0
78	(43)	32	25	0	6	0	0
79	(44)	32	25	0	6	0	0
80	(45)	32	25	0	6	0	0
81	(46)	32	25	0	6	0	0
82	(47)	32	25	0	6	0	0
83	(48)	32	25	0	6	0	0
84	(49)	32	25	0	6	0	0
85	(50)	32	25	0	6	0	0
86	(51)	32	25	0	6	0	0
87	(52)	32	25	0	6	0	0
88	(53)	32	25	0	6	0	0
89	(54)	32	25	0	6	0	0
90	(55)	32	25	0	6	0	0
91	(56)	32	25	0	6	0	0
92	(57)	32	25	0	6	0	0
93	(58)	32	25	0	6	0	0
94	(59)	32	25	0	6	0	0
95	(60)	32	25	0	6	0	0
96	(61)	32	25	0	6	0	0

Table D-2. Individual Fish Standard Lengths (in millimeters) at Test Termination

Mean Measured Concentrations ($\mu\text{g/L}$; ppb)														
Fish No.	Control		Solvent Control		8.57		15.2		28.2		56.6		123	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	46.5	42.8	44.0	44.0	49.0	45.4	45.0	46.0	48.0	48.2	*	51.0	*	*
2	46.8	43.0	42.8	43.3	46.5	45.3	46.4	45.0	43.0	45.0		47.1		
3	42.2	45.0	44.1	41.2	45.8	47.8	44.8	43.8	46.8	48.0		44.6		
4	43.0	38.9	43.2	46.9	46.2	43.1	43.4	46.2	43.2	47.8		46.0		
5	43.9	45.1	45.2	47.7	44.5	44.9	46.4	45.0	43.6	48.1		41.0		
6	45.0	45.2	42.1	46.5	45.2	42.8	44.1	43.8	38.0	49.2		34.0		
7	45.0	41.8	41.4	43.2	41.0	42.5	42.5	41.0	49.0	46.3				
8	48.1	46.3	45.1	42.0	46.7	48.0	43.0	42.1	46.2	46.5				
9	42.0	48.2	42.8	45.8	47.0	43.2	46.3	42.3	45.2	49.0				
10	41.9	43.2	47.4	44.0	42.2	45.2	48.0	43.0	44.9	45.0				
11	42.3	43.4	42.0	41.0	48.0	41.8	40.2	42.0	46.8	47.5				
12	44.8	41.9	45.0	42.0	44.8	39.5	42.8	39.0	44.2	45.2				
13	45.0	43.4	45.2	43.4	41.5	43.0	42.3	42.5	42.0	45.8				
14	42.5	47.2	45.4	42.0	44.0	43.1	42.2	45.0	45.0	47.0				
15	43.6	44.9	46.2	43.8	44.8	44.1	46.9	42.4	41.8	43.8				
16	41.1	49.0	42.8	45.8	44.5	45.0	44.5	43.0	40.2	45.0				
17	46.0	39.5	42.9	42.0	45.1	39.0	43.2	44.2	43.0	43.2				
18	42.2	43.8	47.0	45.0	45.2	46.2	43.5	42.6	43.2	49.0				
19	42.1	45.2	43.1	48.2	45.3	46.8	44.0	39.8	45.0	47.0				
20	46.0	43.8	42.9	43.1	41.8	42.0	41.0	48.9	39.6	46.8				
21	43.1	42.9	44.8	42.9	43.0	43.8	44.8	41.8	44.8	43.0				
22	43.0	42.9	43.1	47.0	43.1	41.0	47.0	43.8	46.0	45.5				
23	45.2	45.2	43.0	42.6	44.0	41.5	45.0	41.0	40.2	44.6				
24	46.0	42.0	48.8	41.0	41.8	44.0	47.0	46.0	44.5	43.0				
25	44.8	43.0	45.1	44.5	40.0	42.0	46.4	42.8	39.8	41.8				
26	43.0	43.0	48.2	43.0	44.0	41.8	41.8	42.3	43.2					
27	45.3	42.2	44.0	42.0	44.1	43.2	44.5	41.8	43.0					
28	39.8	42.1	43.2	44.8	42.7	42.5	46.0	43.3	46.3					
29	46.5	43.3	42.0	42.5	43.5	42.3	43.4	46.8	43.0					
30	43.1	40.8	43.0	42.8	43.9	39.5	44.2	41.5	43.0					
31	42.4	40.0	44.2	44.0	43.2	44.5	43.2	43.2	43.2	45.1				
32	44.0	40.1	47.0	39.0	44.1	42.3	45.1	44.0	41.8					
33	47.0	41.8	41.5	40.5	41.0	42.0	40.1	44.8						
34	41.8	46.0	46.2	44.8	41.2	42.8		41.8						
35	38.7	33.0	37.5	43.8	42.0	42.1		43.8						
36	37.9	46.2		41.6	39.0	43.0								
37	37.8	41.8		41.2										
38		42.2		40.9										

* No surviving fish at test termination.

Table D-3. Individual Fish Weights (in grams) at Test Termination

Fish No.	Mean Measured Concentrations ($\mu\text{g/L}$; ppb)													
	Control		Solvent Control		8.57		15.2		28.2		56.6		123	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	1.34	1.03	1.29	1.14	1.65	1.18	1.27	1.47	1.72	1.43		1.89		
2	1.12	1.03	0.96	1.12	1.38	1.33	1.34	1.13	0.98	1.27		1.27		
3	0.84	1.19	1.23	0.80	1.12	1.39	1.32	1.07	1.53	1.48		1.02		
4	0.99	0.76	1.09	1.37	1.20	1.10	1.29	1.32	1.04	1.41		1.21		
5	0.91	1.31	1.24	1.59	1.03	1.09	1.45	1.13	1.09	1.56		0.82		
6	0.93	1.19	1.01	1.45	1.20	1.04	1.19	1.06	0.72	1.59		0.51		
7	1.07	0.90	0.92	1.13	0.93	0.85	1.04	0.90	1.77	1.39				
8	1.40	1.33	1.23	0.85	1.38	1.52	1.21	0.98	1.49	1.50				
9	0.87	1.50	1.01	1.28	1.39	0.99	1.28	0.84	1.26	1.54				
10	0.87	0.96	1.46	1.25	1.12	1.22	1.76	1.05	1.21	1.15				
11	1.06	1.00	1.00	0.77	1.62	0.87	0.82	1.07	1.45	1.46				
12	1.13	0.84	1.15	0.99	1.27	0.74	1.08	0.82	0.91	1.12				
13	1.21	0.99	1.24	1.10	0.95	0.96	1.01	0.90	0.97	1.34				
14	0.86	1.36	1.26	0.86	1.18	1.02	0.99	1.14	1.10	1.29				
15	1.06	1.09	1.43	1.14	1.30	1.04	1.42	1.07	0.94	1.16				
16	0.78	1.65	0.95	1.16	1.22	1.11	1.17	0.91	0.80	1.11				
17	1.22	0.76	1.07	0.95	1.40	0.73	1.15	1.13	1.08	1.05				
18	0.90	0.89	1.45	1.12	1.22	1.29	1.33	0.94	1.15	1.78				
19	1.04	1.21	1.22	1.50	1.21	1.29	1.27	0.71	1.22	1.47				
20	1.14	1.14	1.09	1.06	0.97	0.82	0.91	1.71	0.98	1.32				
21	1.03	0.92	1.22	0.91	0.98	1.04	1.27	0.83	1.40	1.04				
22	0.97	0.98	1.26	1.28	1.16	0.88	1.65	1.21	1.35	1.20				
23	1.02	1.07	0.99	0.86	1.15	0.90	1.33	1.20	0.78	1.19				
24	1.31	0.85	1.65	0.86	1.10	1.07	1.40	1.27	1.21	1.13				
25	0.93	0.90	1.18	1.21	0.92	1.03	1.50	0.98	0.88	1.00				
26	0.93	1.04	1.62	0.98	1.17	0.96	0.99	1.06	1.17					
27	1.17	0.91	1.37	0.96	1.16	1.19	1.26	0.87	1.00					
28	0.70	0.93	1.06	1.04	1.05	1.05	1.54	1.16	1.54					
29	1.24	0.97	0.91	1.07	1.39	0.99	1.06	1.28	1.17					
30	0.87	0.82	1.01	0.95	1.18	0.80	1.16	0.85	1.03					
31	1.03	0.71	1.14	1.04	1.06	1.15	1.03	1.05	1.32					
32	1.02	0.78	1.48	0.75	1.32	0.96	1.16	1.10	1.02					
33	1.24	0.98	1.04	0.83	0.93	0.94	0.81	1.26						
34	0.89	1.27	1.25	1.16	0.99	0.92		0.89						
35	0.66	0.41	0.65	0.96	0.97	1.01		1.16						
36	0.58	1.30		0.81	0.74	0.95								
37	0.62	0.87		0.85										
38		0.94		0.77										

^a No surviving fish at test termination.

APPENDIX E
RAW CHEMISTRY DATA TABLES

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