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Toxicology & Product Regulatory Compliance  
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Attn: TSCA Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics (OPPT)  
U.S. Environmental Protection Agency  
401 M Street, S.W.  
Washington, DC 20460

**CONTAINS NO CBI**

Attention: **TSCA SECTION 8(E) COORDINATOR**

REFERENCE: **8EHQ-99-14433**



**8EHQ-99-14433**

Dear Sir/Madam:

As a follow-up to our previous 8(e) submission dated April 20, 1999, for a commercial cationic terpolymer dispersion which contained ~20% terpolymer, ~24% ammonium sulfate, ~8% of a commercial polyamine and ~48% water, I am enclosing a copy of the following final reports entitled:

“Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions” – Received May 6, 1999

“Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions as Mitigated by Dissolved Organic Carbon” – Received July 1, 1999

“Toxicity To The Freshwater Green Alga, *Selenastrum capricornutum*, Under Static Test Conditions” – Received May 19, 1999

These reports **does not** contain confidential business information.

If you have any questions please contact me at (973) 357-3375.

Sincerely,

Patricia Ann Vernon  
Product Regulatory Compliance  
Manager, Asia-Pacific



**89990000262**

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**STUDY TITLE**

CT-648-98:  
Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*,  
Under Static Test Conditions

**DATA REQUIREMENT**

OECD Guideline 203

**AUTHOR**

Walter J. Ellenberg, Ph. D.  
Scott A. Glover

**STUDY INITIATION DATE**

February 19, 1999

**STUDY COMPLETION DATE**

May 4, 1999

**SPONSOR**

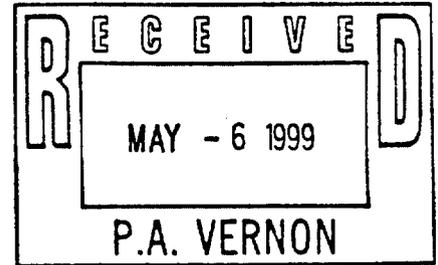
Cytec Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424

**PERFORMING LABORATORY**

Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477

**LABORATORY PROJECT ID**

99J0002c



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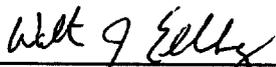
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**STATEMENT OF GOOD LABORATORY PRACTICES COMPLIANCE**

Test Substance: (CT-648-98)

Title: Acute Toxicity to Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions.

This study was conducted in accordance with and fully complies with published Good Laboratory Practices (GLP) regulations for tests of substances as promulgated by the OECD Guidelines for Testing of Chemicals (OECD, 1984) except the stability, purity and characterization of the test substance were not performed according to GLP Guidelines.

  
\_\_\_\_\_  
Walter J. Ellenberg, Ph.D.  
Study Director  
Toxikon Corporation

5-4-99  
\_\_\_\_\_  
Date

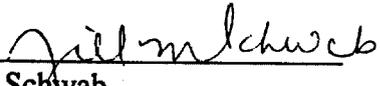
## STATEMENT OF QUALITY ASSURANCE

Test Substance: (CT-648-98)

Title: Acute Toxicity to Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions.

This study was examined for conformance with Good Laboratory Practices as published by the Organization For Economic Co-Operation And Development (OECD). The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed 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:	03-24-99	03-26-99
Study Data Review:	04-22-99	04-22-99
Draft Report Review:	04-22-99	04-22-99
Final Report Review:	05-04-99	05-04-99

  
\_\_\_\_\_  
Jill M. Schwab  
Quality Assurance Officer  
Toxikon Corporation

05-04-99  
\_\_\_\_\_  
Date

## LIST OF SCIENTIFIC PERSONNEL

Test Substance: (CT-648-98)

Title: Acute Toxicity to Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions.

Study Director: Walter J. Ellenberg, Ph. D.

Biologists: Patricia Rielly  
Scott Glover  
Amy S. Lang  
Lisa Parsons

Aquaculturist: Matthew W. Bolt

## SUMMARY

Sponsor: Cytec Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424

Study Director: Walter J. Ellenberg, Ph. D.

Location of Study: Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477

Location of Raw Data  
and Final Report: Cytec Industries  
West Paterson, New Jersey

Test Substance: (CT-648-98) Lot no. 99-2

Test Species: Juvenile rainbow trout, *Oncorhynchus mykiss*;  $29 \pm 1.81$ mm  
average standard length and  $0.305 \pm 0.058$  g average wet  
weight.

Source of Organisms: Thomas Fish Company,  
Anderson, CA

Condition at  
Study Initiation: Rainbow trout appeared to be in good physical condition  
at study initiation; mortality was 0% during the 7-day period  
prior to test initiation.

Dilution Water: Filtered freshwater with an initial hardness and alkalinity of 52  
and 6 mg/L as CaCO<sub>3</sub>, respectively; test temperature range of  
14.5 to 16.5°C.

Nominal  
Concentration: Control, 0.0625, 0.125, 0.25, 0.50 and 1.0 mg wm/L

Test Dates: March 22-26, 1999

Study Length: 96 hours

**Results:**

The 96-hour LC<sub>50</sub> was 0.66 mg wm/L (based on measured concentrations). The no-observable-effect concentration (NOEC) was 0.250 mg wm/L based on the lack of mortality and sublethal effects at this and lower test concentrations.

## 1.0 INTRODUCTION

A static freshwater toxicity test was conducted at Toxikon Corporation, Jupiter, Florida, to determine the acute toxicity of (CT-648-98) to rainbow trout, *Oncorhynchus mykiss*. The criterion for effect was death. Results of the test are expressed as a 96-hour median lethal concentration ( $LC_{50}$ ), the concentration of (CT-648-98) calculated to result in death to 50 percent of the test population at the specified time.

## **2.0 MATERIALS AND METHODS**

### **2.1 TEST SUBSTANCE**

The test substance, (CT-648-98) (Lot No. 99-2) was received at Toxikon Corporation on February 17, 1999 from Cytec Industries in a 1000-mL Nalgene bottle labeled "CT #648-98; 1200 grams; Lot # 99-2." The test substance was a milky-white viscous liquid and was stored in the dark at room temperature. The test substance was reported by Cytec Industries to be completely soluble in water.

Test concentrations are reported as milligrams (mg) of (CT-648-98) as whole material (wm) per liter (L) of freshwater.

### **2.2 TEST SPECIES**

Juvenile rainbow trout, *Oncorhynchus mykiss*, were received on March 9, 1999 at Toxikon Corporation from Thomas Fish Company Anderson, California. *O. mykiss* were maintained in filtered laboratory freshwater at a temperature of 12.6 to 13.0°C during the 13-day period prior to test initiation. *O. mykiss* were maintained on a diet of salmon starter (Ziegler Brothers, Inc., Gardners, PA) up until 24 hours before test initiation, but were not fed after that time or during the test. *O. mykiss* appeared to be in good physical condition at test initiation. Mortality during the 7-day period prior to test initiation was zero percent.

*O. mykiss* used for the test ranged from 26 to 33 millimeters (mm) standard length (mean and standard deviation =  $29 \pm 1.81$  mm) and from 0.183 to 0.413 grams (g) wet weight (mean and standard deviation =  $0.305 \pm 0.058$  g) as measured from the control fish at test termination. Loading was calculated to be 0.34 g of fish tissue per liter of test solution.

### **2.3 TEST WATER**

The dilution water was a moderately hard freshwater. The water originated from the Town of

Jupiter and was treated by vigorous aeration, filtered to 20 micrometers, passed through activated carbon, and re-aerated prior to use. The dilution water, at test initiation, possessed a hardness of 52 mg/L as calcium carbonate (CaCO<sub>3</sub>), alkalinity of 6 mg/L as CaCO<sub>3</sub>, and specific conductivity of (mean) 410 microSiemens (μS) (408 Rep A and 412 Rep B).

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

## 2.4 TEST METHODS

Methods used for the 96-hour static test were those described in Toxikon Corporation's test protocol entitled: "Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions."

A 96-hour static range-finding test was conducted prior to the definitive test. Five fish were exposed at nominal concentrations of 0.10, 1.00, 10.0, and 100 mg wm/L. After 96 hours of exposure, mortality in the control, and the 0.10 mg wm/L test concentration was zero percent. Mortality was 100 percent in the 1.0, 10.0, and 100.0 mg wm/L test concentrations. Fish utilized in the range-finding test averaged 32.2 mm in standard length and 0.300 g in wet weight as measured from the control fish at test termination. Based upon these preliminary results, nominal test concentrations of 0.0625, 0.125, 0.250, 0.50 and 1.0 mg wm/L were selected for the definitive test.

The test solutions were prepared by serial dilution of the CT-648-98 stock (100 mg wm/L) to 9-L volumes of dilution water and mixed thoroughly. A dilution water control was maintained concurrently with the test solutions.

The 96-hour definitive test was initiated on March 22, 1999 with the impartial addition of rainbow trout, by ones and twos, to all test chambers until 10 rainbow trout were distributed to each

chamber following the measurement of the initial water quality parameters. All treatments were duplicated resulting in a total of 20 fish per treatment. The test chambers were 10-L glass jars (22-cm diameter x 30-cm height) containing 9 L of dilution water and providing a final water depth of approximately 25 centimeters. All test chambers were covered throughout the exposure period to reduce evaporation. The test chambers were positioned in a water bath under fluorescent lighting regulated to a photo period of 16 hours light and 8 hours darkness. The light intensity ranged between 3.3 to 5.3 micromols per square meter per second as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a  $2\pi$  quantum sensor at the surface of the test solutions.

Survival of rainbow trout was monitored daily. The fish were also monitored for any abnormalities in their behavior or appearance. Test solutions were gently aerated from day two (after approximately 48 hours) until test termination in order to maintain dissolved oxygen concentrations above 60 percent for the remainder of the test.

Water quality (i.e., temperature, pH and dissolved oxygen concentration) was measured in each replicate daily. The diurnal range of the water bath temperature was continuously monitored using a minimum/maximum thermometer and recorded daily. Specific conductivity, total alkalinity, and total hardness of the dilution water were measured at test initiation and termination. 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). Dissolved oxygen concentrations and chamber temperatures were determined using a YSI Model 58 oxygen meter utilizing a membrane electrode. The pH was measured with a Corning Model pH 40 meter.

## 2.5 STATISTICAL ANALYSES

Based on results of the test, the 24- hr  $LC_{50}$  was equal to 0.76 mg wm/L (95% confidence limit of

0.62 and 0.92). The 48-, 72- and 96-hour LC<sub>50</sub> value was equal to 0.66 mg wm/L ( 95 percent confidence limits 0.52 to 0.84).

## **2.6 ARCHIVES**

The final report and all data related to this study will be archived at Cytex Industries, West Paterson, New Jersey.

### **3.0 RESULTS AND DISCUSSION**

The nominal concentrations of CT-648-98 at test initiation were 0.0625, 0.125, 0.25, 0.50, and 1.0 mg wm/L (Actual calculated concentrations were 0.064, 0.125, 0.25, 0.50, and 1.0 mg wm/L. No undissolved test substance was observed in the test chambers during the entire exposure.

Mortality of rainbow trout exposed for 96 hours to (CT-648-98) was zero percent at test concentrations of 0.0625, 0.125, and 0.25 mg wm/L, 25% at test concentrations 0.50, and 85% at 1.0 mg wm/L; control mortality was zero percent (Table 1). For sublethal effects, dark coloration was noted in 0.50 and 1.0 mg wm/L during the test. The 96-hour LC<sub>50</sub> was >1.0 mg wm/L. The no-observed-effect concentration (NOEC) was 0.250 mg wm/L based on a lack of mortality and sublethal effects at this and lower test concentrations.

The test temperature during the 96-hour exposure ranged from 14.5 to 16.5°C (Table 2). The dilution water possessed an initial hardness and alkalinity of 52 and 6 mg/L as calcium carbonate (CaCO<sub>3</sub>), respectively, and a conductivity of 410 µS (Table 3). The hardness, alkalinity and conductivity of dilution water at test termination were 44 mg/L as CaCO<sub>3</sub>, 12 mg/L as CaCO<sub>3</sub>, and 389 µS (mean), respectively (actual conductivity: 379 Rep A, and 399 Rep B) (Table 3). The dissolved oxygen concentrations in control and all test solutions at test initiation ranged from 8.6 to 10.3 mg/L (85 to 102 percent of saturation). The dissolved oxygen concentrations ranged from 6.0 to 9.6 mg/L (59 to 94.4 percent of saturation) for the remainder of the test in all test chambers (Table 4). After approximately 48 hours, the dissolved oxygen concentration had fallen to 60 percent in treatment 4 B and treatment 2 B. Therefore, gentle aeration was initiated in all test chambers from that point and continued for the duration of the test to maintain dissolved oxygen concentrations at ≥60 percent of saturation. Initial pH values of the control ranged from 6.5 to 7.0; the pH values for all test solutions ranged from 6.4 to 7.2.

#### **4.0 PROTOCOL DEVIATIONS**

One protocol deviation occurred during the conduct of the test. The temperature reached values greater than  $15 \pm 1$  °C in treatment 5 (0.50 mg wm/L) on the first and second day of the study. The highest recorded temperature was 16.5 °C, a deviation of 0.5 °C (Table 2). Based on the scientific opinion of the Study Director, the 0.5 degree deviation would have minimal effects on the results of the study.

## REFERENCES

American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environmental Federation(WEF). 1992. Standard Methods for the Examination of Water and Wastewater, 18th Edition.

Truesdale, G.A., A.L. Downing and G.F. Lowden. 1955. The Solubility of Oxygen in Pure Water and Seawater. Journal of Applied Chemistry, London, 5:53-63.

Table 1. Mortality of Rainbow Trout, *Oncorhynchus mykiss*, During a 96-Hour Exposure to CT-648-98 Under Static Test Conditions.

Nominal Concentrations (mg wm/L)	Cumulative Number Dead (Percent Mortality)							
	24 Hour		48 Hour		72 Hour		96 Hour	
Control	0	(0)	0	(0)	0	(0)	0	(0)
0.0625	0	(0)	0	(0)	0	(0)	0	(0)
0.125	0	(0)	0	(0)	0	(0)	0	(0)
0.250	0	(0)	0	(0)	0	(0)	0	(0)
0.50	4	(20) <sup>b</sup>	5	(25)	5	(25) <sup>b</sup>	5	(25) <sup>c</sup>
1.0	14	(70) <sup>a</sup>	17	(85) <sup>a</sup>	17	(85) <sup>a</sup>	17	(85)

<sup>a</sup> One fish showed dark coloration.

<sup>b</sup> Two fish showed dark coloration.

<sup>c</sup> Three fish showed dark coloration.

Table 2. Daily Temperature Values During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to CT-648-98.

Exposure Period (Days)	Temperature Range <sup>a</sup> (°C)
0	14.5 - 15.0
1	15.3 - 16.4
2	15.3 - 16.5
3	14.6 - 15.0
4	14.6 - 15.1

<sup>a</sup> Daily temperature values reported are the ranges of temperature taken in all test chambers each day.

Note: The diurnal temperature of the water bath ranged from 13.2 to 14.2°C as measured by a minimum/maximum thermometer during the test.

Table 3. Hardness, Alkalinity, and Conductivity Measurements During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to CT-648-98.

Day	Hardness* (mg/L)	Alkalinity* (mg/L)	Conductivity ( $\mu$ S)
0	52	6	410
4	44	12	389

\* Hardness and alkalinity measurements expressed as milligrams per liter as CaCO<sub>3</sub>.

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

Table 4. Dissolved Oxygen Concentrations During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to CT-648-98.

Nominal Concentrations (mg wm/L)	R E P	Dissolved Oxygen Concentration (mg/L)				
		0 HR	24 HR	48 HR <sup>b</sup>	72 HR	96 HR
Control	A	10.1	7.5	6.7	9.5	8.5
	B	9.8	7.3	6.6	9.5	8.5
0.0625	A	10.0	7.5	6.7	9.6	8.3
	B	9.6	7.2	6.5	9.5	8.5
0.125	A	10.2	7.8	7.5	9.4	8.4
	B	9.7	7.2	6.0	9.3	8.7
0.25	A	10.1	7.5	7.3	9.3	7.8
	B	10.0	7.2	6.6	9.5	8.4
0.5	A	10.3	7.2	6.2	9.5	8.5
	B	8.9	6.3	6.0	9.3	8.8
1.0	A	8.6	7.2	7.1	9.4	9.3
	B	10.3	7.7	7.7	---	---
O <sub>2</sub> Saturation <sup>a</sup>		10.1	10.0	10.0	10.3	10.1

<sup>a</sup> Dissolved oxygen saturation values are based on the average temperature in the control tanks at the specified times (Truesdale et al. 1955).

<sup>b</sup> After approximately 48 hours, gentle aeration was initiated in all test chambers to maintain the dissolved oxygen concentrations  $\geq 60$  percent of saturation.

Table 5. The pH Values During a 96-Hour Static Exposure of Rainbow Trout, *Oncorhynchus mykiss*, to CT-648-98.

Nominal Concentrations (mg wm/L)	R E P	pH				
		0 HR	24 HR	48 HR	72 HR	96 HR
Control	A	6.5	6.8	6.6	6.8	7.1
	B	6.8	6.4	6.5	7.0	7.1
0.0625	A	6.6	6.7	6.7	6.9	7.1
	B	6.8	6.5	6.5	7.0	7.1
0.125	A	6.6	6.7	6.7	7.0	7.1
	B	6.9	6.6	6.6	7.0	7.1
0.25	A	6.7	6.7	6.7	7.0	7.0
	B	6.9	6.6	6.6	7.0	7.0
0.5	A	6.8	6.7	6.6	7.0	7.0
	B	6.9	6.6	6.6	7.0	7.1
1.0	A	6.8	6.7	6.7	7.1	7.2
	B	7.0	6.6	6.7	---	---

**APPENDIX A**  
**DILUTION WATER CHARACTERIZATION**

FRESHWATER CHARACTERIZATION<sup>a</sup>

Parameter	Concentration <sup>b</sup>	Historical Range <sup>c</sup>
Aluminum	<0.079 mg/L	ND - 0.102 mg/L
Arsenic	<0.005 mg/L	ND
Boron	0.159 mg/L	ND - 0.159 mg/L
Beryllium	<0.004 mg/L	ND
Bromide	<0.10 mg/L	ND - 30 mg/L
Cadmium	<0.005 mg/L	ND
Calcium	22.5 mg/L	8.89 - 24.6 mg/L
Chloride	92 mg/L	60 - 108 mg/L
Chromium (hexavalent)	<0.010 mg/L	ND
Chromium (total)	<0.005 mg/L	ND
Cobalt	<0.005 mg/L	ND
Copper	<0.005 mg/L	ND - 0.009 mg/L
Fluoride	<0.10 mg/L	ND - 0.826 mg/L
Iodide	<0.050 mg/L	ND
Iron	0.047 mg/L	ND - 0.154 mg/L
Lead	<0.005 mg/L	ND
Manganese	<0.005 mg/L	ND
Magnesium	3.26 mg/L	0.789 - 9.91 mg/L
Mercury	<0.0005 mg/L	ND
Molybdenum	<0.020 mg/L	ND - 0.054 mg/L
Nickel	<0.005 mg/L	ND
Potassium	1.70 mg/L	1.14 - 5.50 mg/L
Selenium	<0.005 mg/L	ND
Silver	<0.005 mg/L	ND
Sodium	47.5 mg/L	27.4 - 74.0 mg/L
Tin	<0.05 mg/L	ND
Zinc	0.012 mg/L	ND - 0.043 mg/L
Ammonia (total)	<0.040 mg/L	ND - 0.676 mg/L
Cyanide (total)	<0.010 mg/L	ND
Nitrates (total as N)	1.20 mg/L	ND - 1.38 mg/L
Nitrites (total as N)	0.058 mg/L	ND - 0.058 mg/L
Phosphates (total)	<0.010 mg/L	ND - 0.12 mg/L
Sulfide (total)	<0.1 mg/L	ND
Sulfate (total)	33.2 mg/L	15 - 52 mg/L
TDS	296 mg/L	150 - 552 mg/L
TOC	4.36 mg/L	ND - 6.0 mg/L
TSS	<4.0 mg/L	ND - 16 mg/L
COD	32.6 mg/L	ND - 54.6 mg/L
Total organophosphorus pesticides	<1.0 µg/L	ND
Total phenoxy herbicides	<1.12 µg/L	ND
Total organochlorine pesticides	<0.01 µg/L	ND
PCBs	<0.100 µg/L	ND

<sup>a</sup> The characterized freshwater is carbon-treated Jupiter, Florida, town water which is aerated following carbon treatment.

<sup>b</sup> Sample of freshwater collected June 30, 1998.

<sup>c</sup> Historical range for laboratory freshwater.

**STUDY TITLE**

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CT-648-98:  
Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*,  
Under Static Test Conditions as Mitigated by Dissolved Organic Carbon

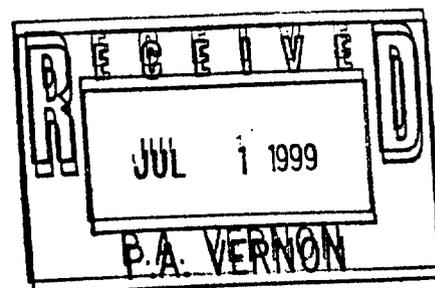
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**DATA REQUIREMENT**

TSCA Environmental Effects Testing Guideline 850.1085

**AUTHOR**

Walter J. Ellenberg, Ph. D.



**STUDY INITIATION DATE**

February 19, 1999

**STUDY COMPLETION DATE**

June 30, 1999

**SPONSOR**

Cytec Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424

**PERFORMING LABORATORY**

Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477

**LABORATORY PROJECT ID**

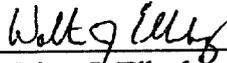
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## COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Test Substance: CT-648-98

Title: CT-648-98: Acute Toxicity to Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

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 except the stability, purity and characterization of the test substance were not performed according to GLP Guidelines.

  
\_\_\_\_\_  
Walter J. Ellenberg, Ph. D.  
Study Director  
Toxikon Corporation

6-30-99  
Date

## STATEMENT OF QUALITY ASSURANCE

Test Substance: CT-648-98

Title: CT-648-98: Acute Toxicity to Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

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 Quality Assurance activities on this study are listed 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:	4-20-99	4-23-99
	5-7-99	5-10-99
Study Data Review:	6/3-4/99	6-07-99
Draft Report Review:	6/3-4/99	6-07-99
Final Report Review:	6/30/99	6/30/99

  
\_\_\_\_\_  
Jill Schwab  
Quality Assurance Manager  
Toxikon Corporation

6-30-99  
\_\_\_\_\_  
Date

## LIST OF SCIENTIFIC PERSONNEL

Test Substance: CT-648-98

Title: CT-648-98: Acute Toxicity to Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

Study Director: Walter J. Ellenberg, Ph.D.

Biologists: Scott Glover  
Tricia Rielly  
Amy Lang  
Lisa Parsons

Aquaculturist: Matt Bolt

## SUMMARY

Sponsor: Cyttec Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424

Study Director: Walter J. Ellenberg, Ph. D.

Location of Study: Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477

Location of Raw Data  
and Final Report: Cyttec Industries  
West Paterson, New Jersey

Test Substance: CT-648-98 Lot No. 99-2

Test Species: Juvenile rainbow trout, *Oncorhynchus mykiss*

**Definitive Test 1:** Average length  $30.7 \pm 2.98$  mm  
Average wet weight  $0.359 \pm 0.104$  g

**Definitive Test 2:** Average length  $45.6 \pm 2.95$  mm  
Average wet weight  $0.924 \pm 0.23$  g

Source of Organisms: Thomas Fish Company,  
Anderson, CA

Condition at  
Study Initiation: Rainbow trout appeared to be in good physical condition  
at study initiation; mortality was 0% during the 7-day period  
prior to test initiation.

Dilution Water: **Definitive Test I:** Filtered freshwater with an initial hardness  
and alkalinity of 52 and 14 mg/L as CaCO<sub>3</sub>, respectively; the 96  
hour test temperature ranged from 13.9 to 15.4°C.

**Dilution Water:** **Definitive Test II:** Filtered freshwater with an initial hardness and alkalinity of 54 and 12 mg/L as CaCO<sub>3</sub>, respectively; the 96 hour test temperature ranged from 14.0 to 15.4°C.

**Nominal Concentrations:** **Definitive Test I:** Control, humic acid control (20 mg/L), 0.625, 1.25, 2.5, 5.0 and 10.0 mg wm/L CT-648-98 (20 mg/L Humic acid was added to each treatment except the control)

**Definitive Test II:** Control, humic acid control (20 mg/L), 12.9, 21.6, 36.0, 60.0, and 100 mg wm/L, CT-648-98 (20 mg/L Humic acid was added to each treatment except the control)

**Experimental Test Dates:** **Definitive Test I:** April 19 to 23, 1999  
**Definitive Test II:** May 4 to 8, 1999

**Study Length:** 96 hours

**Results:** **Definitive Test I:** The presence of humic acid (20 mg/L) greatly decreased the LC<sub>50</sub> of CT- 648-98 to Rainbow trout using test concentrations of 0.625, 1.25, 2.5, 5.0 and 10.0 mg wm/L. As a result, the 96-hour LC<sub>50</sub> and NOEC of CT-648-98 was greater than 10.0 mg wm/L.

**Definitive Test 2:** Based on these data and at the direction of the study sponsor, the protocol was amended to state that the concentration of humic acid to be used in the second test remain as 20 mg/L and the test concentrations of CT-648-98 would be increased. The nominal concentrations used in the second definitive test were 12.9, 21.6, 36.0, 60.0, and 100 mg wm/L, CT-648-98. The results of the second definitive test indicated that the 96-hour LC<sub>50</sub> of CT-648-98 to rainbow trout in the presence of 20 mg/L humic acid was 44.9 mg/L with 95% confidence limits of 36 and 60 mg wm/L. Based on the lack of

Results:

significant mortality and sublethal effects, the no-observed-effect-concentration (NOEC) was < 36 mg wm/L. These results indicate that the presence of dissolved organic carbon altered the LC<sub>50</sub> of the compound CT-648-98 by more than 20 times when compared to the LC<sub>50</sub> values of the same compound obtained in the acute rainbow trout toxicity test conducted in the absence of dissolved organic carbon (Toxikon Study Number 99J0002c).

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## 1.0 INTRODUCTION

Two static freshwater toxicity tests were conducted at the Toxikon Corporation, Jupiter, Florida, to determine the acute toxicity of CT-648-98 to the rainbow trout, *Oncorhynchus mykiss*, under static test conditions as mitigated by the presence of dissolved organic carbon (DOC). The criterion for effect was death.

Definitive Test 1 was conducted by exposing rainbow trout to nominal concentrations of CT-648-98. The concentrations used for the first definitive test were 0.625, 1.25, 2.50, 5.0, and 10.0 mg wm/L. Humic acid (20 mg/L) was added to each treatment as the source of dissolved organic carbon. Two controls were run in the first definitive test. A normal control (containing no additional dissolved organic carbon) and a humic acid control (containing 20 mg/L humic acid) were run concurrent with the chemical treatments for 96 hours.

Definitive Test 2 was conducted by exposing rainbow trout to nominal concentrations of CT-648-98. Per client request, the concentrations used for the second definitive test were 12.9, 21.6, 36.0, 60.0, and 100 mg wm/L. Humic acid (20 mg/L) was added to each treatment as the source of dissolved organic carbon. Two controls were run in the second definitive test. A normal control (containing no additional dissolved organic carbon) and a humic acid control (containing 20 mg/L humic acid) were run concurrent with the chemical treatments for 96 hours.

Results of each test are expressed as a 96-hour median lethal concentration ( $LC_{50}$ ) (the concentration of CT-648-98 in the presence of 20 mg/L humic acid calculated to result in mortality of 50 percent of the test population at the specified time).

## **2.0 MATERIALS AND METHODS**

### **2.1 TEST SUBSTANCE**

The test substance, CT-648-98 (Lot No. 99-2) was received at Toxikon Corporation on February 17, 1999 from Cytec Industries in a 1000-mL Nalgene bottle labeled "CT -648-98; 1200 grams; Lot # 99-2." The test substance was a milky-white viscous liquid and was stored in the dark at room temperature. The test substance was reported by Cytec Industries to be completely soluble in water.

Test concentrations are reported as milligrams (mg) of CT-648-98 as whole material (wm) per liter (L) of freshwater.

### **2.2 TEST SPECIES**

Rainbow trout, *Oncorhynchus mykiss*, used for testing were obtained from Thomas Fish Company, Anderson, CA and maintained in laboratory freshwater prior to test initiation. The fish used in Definitive Test 1 were received at Toxikon Corporation on 4-2-99. The holding water temperature ranged from 12.5 to 13.1 °C during the 10 days immediately prior to test initiation for Definitive Test 1. The temperature of the holding water was 12.5 °C when the fish were removed for Definitive Test 1.

During the 10 days immediately prior to test initiation for Definitive Test 2, the holding water temperature ranged from 12.8 to 13.5 °C. The temperature of the holding water was 13.0 °C when the fish were removed for Definitive Test 2.

Prior to each of the definitive tests, fish were fed once daily with salmon starter (Zeigler Brothers, Gardners, PA). No diseases were observed or treated during the 14-day period prior to

conducting each test, and mortality was zero percent during the 48-hour period prior to the initiation of each definitive test. Fish were not fed during the 48-hour period immediately prior to test initiation or during the test.

Rainbow trout used for the first definitive test ranged from 25 to 38 millimeters (mm) standard length (mean length and standard deviation =  $30.7 \pm 2.98$  mm). The mass of the fish used in Definitive Test 1 ranged from 0.16 to 0.63 grams (g) wet weight (mean and standard deviation =  $0.36 \pm 0.10$  g). The fish used for the second definitive test ranged from 40 to 54 millimeters (mm) standard length (mean length and standard deviation =  $45.6 \pm 2.95$  mm). The mass of those animals used in the second definitive test ranged from 0.53 to 1.5 grams (g) wet weight (mean and standard deviation =  $0.92 \pm 0.23$  g).

### 2.3 TEST WATER

The dilution water used for each definitive test was a moderately hard freshwater. The water originated from the Town of Jupiter and was treated by vigorous aeration, filtered to 20 micrometers, passed through activated carbon and re-aerated prior to use. At test initiation of Definitive Test 1, the dilution water possessed a hardness of 52 mg/L as CaCO<sub>3</sub>, alkalinity of 14 mg/L as CaCO<sub>3</sub>, and mean specific conductivity for the control replicates was 404 microsiemens (μS). At test termination, the dilution water possessed a hardness and alkalinity of 54 and 11 mg/L as CaCO<sub>3</sub>, respectively. The mean conductivity for the control replicates was 414 (μS) at test termination.

The dilution water for the second definitive test possessed a hardness of 54 mg/L as CaCO<sub>3</sub>, alkalinity of 12 mg/L as CaCO<sub>3</sub>. The mean specific conductivity for control replicates in the second definitive test at test initiation was 404 μS. At test termination, the dilution water for the controls of the second definitive test possessed a hardness and alkalinity of 60 and 14 mg/L as

CaCO<sub>3</sub>, respectively. The mean specific conductivity for the control replicates was 453 µS at test termination.

In each of the definitive tests, the mean total organic carbon (TOC) concentrations of dilution water used for Definitive Test 1 was 2.75 mg /L and 3.38 mg/L for Definitive Test 2. Chemical characterization of a representative batch of dilution water is presented in Appendix A.

## 2.4 TEST METHODS

Methods used for the 96-hour static tests were those described in Toxikon Corporation's test protocol entitled "CT-648-98: Acute Toxicity To Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions As Mitigated by Dissolved Organic Carbon." The methods are based on the U.S. EPA Toxic Substances Control Act (TSCA) Ecological Effects Test Guideline 850.1085.

In a previous study conducted at Toxikon Corporation, the toxicity of CT-648-98 to rainbow trout was examined in clean dilution water (humic acid-free) (Toxikon Study Number 99J0002c). In that study, a 96 hour exposure of rainbow trout to CT-648-98 at concentrations of 0.0625, 0.125, 0.250, 0.500 and 1.000 mg wm/L produced an LC<sub>50</sub> of 0.660 mg wm/L.

Based on these results, it was determined that the concentrations used in Definitive Test 1 ( in the presence of 20 mg/L humic acid) would be 0.625, 1.25, 2.50, 5.00, and 10.0 mg wm/L.

Test solutions were prepared by directly adding a calculated aliquot of the stock solution (1017 mg wm/L) to each test chamber and brought to a total volume of 9 liters with the addition of dilution water. The mixture was then vigorously stirred. A stock solution of humic acid (100,000 mg/L) was prepared by adding 10 g humic acid to 100 mL of dilution water. Prior to adding CT-648-98 to the dilution water, 1.80 mL of the humic acid stock solution were added to each test

chamber, except the dilution water control, to yield a humic acid concentration of 20 mg/L. Both a dilution water control and a humic acid control (20 mg humic acid/L) were maintained concurrently with the test solutions. All treatments were duplicated.

Definitive Test 1 was initiated on April 19, 1999 with the impartial addition of rainbow trout, by ones or twos, to all test chambers until 10 fish were distributed to each replicate, 20 per treatment. Test fish were added following the preparation of the targeted test concentrations and measurement of the initial water quality parameters. The test chambers were 10-L glass jars (22-cm diameter x 30-cm height) containing 9 L of dilution water or test solution. The depth of water in each test chamber was approximately 25 centimeters. The loading in all of the test chambers was 0.400 g of fish tissue per liter of test solution, as determined from control fish at test termination for Definitive Test 1. Test chambers were positioned in a temperature-controlled waterbath and illuminated with fluorescent lighting regulated to a photoperiod of 16 hour light and 8 hours dark.

Light intensity for Definitive Test 1 ranged from 3.1 to 8.4 micromols per second per square meter as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a  $2\pi$  quantum sensor.

The results of Definitive Test 1 indicated that the toxicity of CT-648-98 was significantly altered by the presence of dissolved organic carbon (DOC). Following Definitive Test 1, a second definitive test was conducted. Per client request, the protocol was amended so that the test would be conducted using elevated levels of the test compound in the presence of 20 mg/L humic acid. Nominal test concentrations selected for the second definitive test were 12.9, 21.6, 36.0, 60.0, and 100 mg wm/L. These concentrations were 60 percent of the next higher concentration. Test solutions were prepared by directly measuring and adding a calculated aliquot of CT-648-98

stock solution (10190 mg wm/L) to each test chamber. Dilution water was added to each test chamber to provide a 9 liter volume and then stirred vigorously. A stock solution of humic acid (100,000 mg/L) was prepared by weighing 10 g humic acid into a 100-mL volumetric flask and bringing it to volume with dilution water in Definitive Test 2. Prior to adding CT-648-98 to the dilution water, 1.80 mL of the humic acid stock solution were added to each test chamber, except the dilution water control, to yield a humic acid concentration of 20 mg/L. Both a dilution water control and a humic acid control (20 mg/L) were maintained concurrently with the test solutions. All treatments were duplicated.

Definitive Test 2 was initiated on May 4, 1999 with the impartial addition of rainbow trout, by ones or twos, to all test containers until 10 fish were distributed to each replicate, 20 per treatment. Test fish were added following the preparation of test concentrations and measurement of the initial water quality parameters. The test chambers were 10-L glass jars (22-cm diameter x 30-cm height) containing 9 L of dilution water. The water depth of each chamber was approximately 25 centimeters. The loading for the second definitive test was 2.05 g of rainbow trout tissue per liter of test solution, as determined from control fish at the termination of the test. Test chambers were positioned in a temperature-controlled waterbath under fluorescent illumination regulated to a photoperiod of 16 hours light and 8 hours dark. Light intensity ranged from 7.3 to 8.3 micromols per second per square meter as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a  $2\pi$  quantum sensor.

In each definitive test, the survival of rainbow trout was monitored daily and dead fish were removed (if present). Abnormalities in the behavior or physical appearance of the rainbow trout was also noted. Fish were not fed for 48 hours prior to test initiation, nor were they fed during the test. No aeration was required to maintain acceptable dissolved oxygen concentrations during Definitive Test 1. During Definitive Test 2, gentle aeration was initiated on day 2 (May 6, 1999)

to maintain dissolved oxygen concentrations above 4.5 mg/L in each test chamber.

The specific conductivity, total alkalinity, and total hardness were measured in the dilution water control at test initiation and termination in each of the definitive tests. Temperature was measured in all of the test chambers daily and the diurnal temperature range of the water bath was monitored daily by a minimum/maximum thermometer. In each of the definitive tests, the dissolved oxygen concentration, pH and temperature were measured every 24 hours in all replicates for all controls and test concentrations with surviving fish. Specific conductivity was measured using a Corning Checkmate 90 digital conductivity meter. Water hardness and alkalinity were determined by EDTA and potentiometric titrations, respectively (APHA et al., 1992). Dissolved oxygen concentrations were determined using a YSI Model 58 (YSI-2) oxygen meter utilizing a membrane electrode. The pH was measured with a Corning pH 40 meter.

## **2.5 CHEMICAL SAMPLING AND ANALYSIS**

No water samples were collected or analyzed for the test substance. Nominal concentrations were used during each of the definitive tests.

Three water samples (midway between the top, bottom and sides of the test chambers) of the dilution water controls and humic acid adjusted controls were collected and analyzed for total organic carbon (TOC) prior to test initiation using EPA Method 415.1.

## **2.6 STATISTICAL ANALYSIS**

Based on results of the test, the 24-, 48- 72- and 96-hour LC<sub>50</sub> values and their 95 percent confidence limits were calculated only for Definitive Test 2 since there were no observed mortalities in Definitive Test 1. The LC<sub>50</sub> values were estimated by a computer program (Wheat, 1989) using the following statistical methods: moving average angle, probit, logit and non-linear interpolation. Confidence limits for LC<sub>50</sub> values determined by non-linear interpolation were

calculated by binomial probability (Table 9). The method selected for reporting the test results was determined by the characteristics of the data, i.e., the presence or absence of 0-percent and 100-percent mortality and the number of concentrations in which mortalities between 0 and 100 percent occurred (Stephan, 1977).

## **2.7 ARCHIVES**

The final report and all raw data related to this study will be maintained in archive at Cytec Industries Inc., West Paterson, NJ.

### **3.0 RESULTS AND DISCUSSION**

#### **3.1 Definitive Test 1**

Mortality of rainbow trout exposed for 96 hours to CT-648-98 was zero percent in all test concentrations including the controls and the 96-hour LC<sub>50</sub> could not be calculated (Tables 1 & 2). The no-observed-effect-concentration (NOEC) was greater than 10.0 mg wm/L based on the lack of significant mortality and sublethal effects at this test concentration. The results indicated that the dissolved organic carbon significantly altered the toxicity of CT-648-98 to rainbow trout.

The mean TOC concentration was 2.75 mg/L in the dilution water control and 4.71 mg/L (corrected for the dissolved organic carbon in the dilution water) in the humic acid control (Table 3). In the presence of humic acid each of the test solutions possessed a transparent brown coloration; however over time several of the solutions produced a flocculent material which settled to the bottom of the affected chambers during the test.

The temperature ranged from 13.9 to 15.4°C (Table 4). The dilution water was a moderately hard freshwater. Basic characterization of the dilution water during the test is presented in Table 5. The dissolved oxygen concentrations for each of the control replicates was 10.2 mg/L (106 percent of saturation) at test initiation. The overall dissolved oxygen concentrations ranged from 6.3 to 8.0 mg/L (66 to 83 percent of saturation) in the controls and treatments for the remainder of the test. No aeration was required in the first definitive test. The pH values ranged from 7.1 to 7.3 in all test solutions at test initiation and from 6.6 to 7.0 thereafter (Table 7). The pH of the controls ranged from 7.1 to 7.2 at test initiation and ranged from 6.5 to 7.1 for the remainder of the test.

### 3.2 Definitive Test 2

The results of the second definitive test indicated that the mortality of rainbow trout exposed for 96 hours to CT-648-98 ranged from 0 percent at 12.9 mg/L to 100 percent at test concentrations  $\geq 60.0$  mg/L. The mortality in both the dilution water and humic acid controls was zero percent (Table 8). The 96-hour LC<sub>50</sub> was calculated to be 44.9 mg/L (based on nominal concentrations) with 95% confidence limits of 36.0 and 60.0 mg wm/L (Table 9). The NOEC was  $< 36$  mg wm/L based on the lack of significant mortality and sublethal effects at this test and lower concentrations.

The mean TOC concentration was 3.38 mg/L in the dilution water control and 5.34 mg/L (corrected for the dissolved organic carbon in the dilution water) in the humic acid control (Table 10) in the second definitive test. As in the first definitive test, the presence of humic acid in each of the test solutions produced a transparent brown coloration; however, over time several of the solutions produced a flocculent material and settled to the bottom of each of the affected chambers during the course of the test.

The test temperature for the second definitive 96-hour test ranged from 14.0 to 15.4°C (Table 11). The dilution water was a moderately hard freshwater. Basic characterization of the dilution water during the test is presented in Table 12. At test initiation, the dissolved oxygen concentrations in the test solutions ranged from 7.3 to 10.2 mg/L (76 to 106 percent of saturation) and ranged from 4.1 to 9.7 mg/L (43 to 101 percent of saturation) for the remainder of the test (Table 13). It should be noted that gentle aeration was initiated to each of the test chambers on day 2. The dissolved oxygen concentrations of the controls (including the humic acid control) ranged from 9.5 – 10.0 mg/L (99 and 100 percent of saturation) at test initiation and from 4.4 to 8.7 mg/L (46 to 91 percent of saturation) in the control treatments

throughout the remainder of the test. The dissolved oxygen concentrations ranged from to 4.1 to 10.2 mg /L through the entire experiment. The pH of the controls ranged from 6.9 to 7.4 at test initiation and ranged from 6.4 to 6.9 during the test. The pH values ranged from 7.0 to 7.4 in all test solutions at test initiation and from 6.4 to 7.2 during the remainder of the test (Table 14).

#### 4.0 CONCLUSIONS

The 96-hour  $LC_{50}$  for CT-648-98 in the presence of 20 mg/L humic acid could not be calculated in the first definitive test due to the absence of mortality. The second definitive test measured the toxicity of higher concentrations of CT-648-98 in the presence of 20 mg/L humic acid. The 96 hour  $LC_{50}$  for the second definitive test was calculated to be 44.6 mg wm/L. These results indicate that the presence of dissolved organic carbon greatly decreased the toxicity of the CT-648-98 to the rainbow trout under static conditions.

## 5.0 PROTOCOL DEVIATIONS

There were three deviations from the test protocol during the conduct of this study.

- 1) Per study protocol, TSS measurements should have been acquired at test initiation and termination. Samples were not analyzed for TSS.
- 2) The test temperature ranged from 13.9 to 14.1°C on the final day of the first test, a deviation from  $15.0 \pm 1.0$  °C as stated in the protocol.
- 3) The concentrations used for the first definitive test were prepared as 50 % of the next higher concentration other rather than 60% of the next higher concentration as stated in the protocol. The concentrations used in Definitive test 2 were 60 % of the next higher concentration.

In the scientific judgement of the Study Director, these deviations were minor and did not affect the outcome or validity of the test results.

## REFERENCES

- American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF). 1989. Standard Methods for the Examination of Water and Wastewater, 18th Edition.
- Stephan, C.E. 1977. Methods for Calculating an LC50. IN: American Society for Testing and Materials (ASTM). Aquatic Toxicology and Hazard Evaluation, pp. 65-84, F.L. Mayer and J.L. Hamelink, Editors. ASTM STP 534, Philadelphia, Pennsylvania.
- Truesdale, G.A., A.L. Downing and G.F. Lowden. 1955. The Solubility of Oxygen in Pure Water and Saltwater. *Journal of Applied Chemistry*, London, 5:53-63.
- 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. Definitive Test I: Mortality of Rainbow trout, *Oncorhynchus mykiss*, Exposed to CT-648-98 for 96 Hours Under Static Conditions As Mitigated by Dissolved Organic Carbon

Nominal Concentration (mg wm/L)	Cumulative Number Dead (Percent Mortality)			
	24 Hours	48 Hours	72 Hours	96 Hours
Control	0 (0)	0 (0)	0 (0)	0 (0)
Humic Acid Control	0 (0)	0 (0)	0 (0)	0 (0)
0.625	0 (0)	0 (0)	0 (0)	0 (0)
1.25	0 (0)	0 (0)	0 (0)	0 (0)
2.5	0 (0)	0 (0)	0 (0)	0 (0)
5.0	0 (0)	0 (0)	0 (0)	0 (0)
10.0	0 (0)	0 (0)	0 (0)	0 (0)

Table 2. Definitive Test I: Results of LC<sub>50</sub> Calculations Based on Nominal Concentrations for Rainbow trout, *Oncorhynchus mykiss*, Exposed to CT-648-98 for 96 Hours Under Static Conditions As Mitigated by Dissolved Organic Carbon

Exposure Period (Hour)	LC <sub>50</sub> (mg wm/L)	Confidence Limits (mg wm/L)	95-Percent Statistical Method
24	> 10.0	--	Binomial
48	> 10.0	--	Binomial
72	> 10.0	--	Binomial
96	> 10.0	--	Binomial

Note: The LC<sub>50</sub> Values could not be calculated due to the absence of mortality.

Table 3. Definitive Test I: Total Organic Carbon (TOC) Concentrations During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

Treatment	Replicate	TOC Concentration (mg/L)		
		0 HR	Rep. Mean	Mean
Control A	1	2.78		
	2	2.75		
	3	2.78	2.77	
Control B	1	2.78		
	2	2.69		
	3	2.69	2.72	2.75
HA Control A	1	7.17		
	2	7.68		
	3	7.51	7.45	
HA Control B	1	7.52		
	2	7.50		
	3	7.38	7.47	7.46
<b>Total Dissolved Organic Carbon</b>				<b>4.71</b>

Table 4. Definitive Test I: Daily Temperature Ranges During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Conditions As Mitigated by Dissolved Organic Carbon

Exposure Period (Hour)	Temperature Range <sup>a</sup> (°C)
0	14.4 – 14.8
24	14.5 – 14.9
48	14.4 – 15.3
72	14.4 – 15.4
96	13.9 – 14.1

<sup>a</sup> Daily temperature ranges reported are the minimum and maximum of the temperatures recorded from all the controls and treatments.

NOTE: The diurnal temperature of the water bath ranged from 13.1 to 14.6°C as measured by a minimum/maximum thermometer during the test.

Table 5. Definitive Test I: Hardness, Alkalinity, and Conductivity Measurements During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Conditions As Mitigated by Dissolved Organic Carbon

Day	Hardness* (mg/L)	Alkalinity* (mg/L)	Mean Conductivity ( $\mu$ mhos/cm)
0	52	14	404
4	54	11	414

\* Hardness and alkalinity measurements expressed as milligrams per liter as CaCO<sub>3</sub>.

\*\* Mean conductivity was obtained from the average of the control replicates.

Table 6. Definitive Test I: Dissolved Oxygen Concentrations During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, Exposed to CT-648-98 Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

Nominal Concentration (mg wm/L)	R E P	0 HR	Dissolved Oxygen Concentration (mg/L)			
			24 HR	48 HR	72 HR	96 HR
Control	A	10.2	7.5	6.9	6.8	7.2
	B	10.2	7.8	7.2	6.7	6.8
Humic Acid Control	A	8.6	7.4	6.9	6.8	7.6
	B	8.6	7.5	7.1	6.6	6.7
0.625	A	10.1	7.9	7.3	7.4	7.5
	B	9.8	7.8	7.3	6.8	7.4
1.25	A	10.2	7.4	7.4	7.4	7.3
	B	9.7	7.6	7.0	6.3	7.1
2.5	A	9.7	7.7	7.0	7.0	7.2
	B	9.8	8.0	7.4	6.5	6.7
5.0	A	9.8	7.8	7.0	7.0	7.2
	B	9.2	7.2	7.3	6.7	6.8
10.0	A	8.7	7.4	6.9	7.0	7.3
	B	9.4	6.9	6.7	6.3	6.9
Saturation <sup>a</sup>		9.6	9.6	9.6	9.6	9.7

<sup>a</sup> Dissolved oxygen saturation values based on the temperature in a control replicate at the specified times (Truesdale et al. 1955).

Table 7. Definitive Test I: pH Values During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

Nominal Concentration (mg wm/L)	R E P	pH				
		0 HR	24 HR	48 HR	72 HR	96 HR
Control	A	7.2	6.7	6.5	6.8	6.7
	B	7.1	6.8	6.6	7.1	6.7
Humic Acid Control	A	7.2	6.8	6.6	6.9	6.8
	B	7.1	6.8	6.7	7.1	6.8
0.625	A	7.3	6.8	6.6	6.9	6.8
	B	7.2	6.8	6.7	7.0	6.8
1.25	A	7.3	6.8	6.6	6.9	6.8
	B	7.2	6.8	6.7	7.0	6.8
2.5	A	7.3	6.8	6.6	6.9	6.8
	B	7.2	6.9	6.7	7.0	6.8
5.0	A	7.3	6.8	6.6	6.9	6.8
	B	7.2	6.9	6.7	7.0	6.8
10.0	A	7.2	6.8	6.6	6.9	6.8
	B	7.2	6.9	6.7	6.9	6.8

Table 8. Definitive Test II: Mortality of Rainbow trout, *Oncorhynchus mykiss*, Exposed to CT-648-98 for 96 Hours Under Static Conditions As Mitigated by Dissolved Organic Carbon

Nominal Concentration (mg wm/L)	Cumulative Number Dead (Percent Mortality)			
	24 Hours	48 Hours	72 Hours	96 Hours
Control	0 (0)	0 (0)	0 (0)	0 (0)
Humic Acid Control	0 (0) <sup>b</sup>	0 (0) <sup>b</sup>	0 (0) <sup>b</sup>	0 (0)
12.9	0 (0) <sup>b</sup>	0 (0) <sup>b</sup>	0 (0) <sup>b</sup>	0 (0)
21.6	0 (0) <sup>c</sup>	0 (0) <sup>c</sup>	0 (0)	0 (0)
36.0	1 (5)	1 (5)	1 (5)	1 (5)
60.0	20 (100)	20 (100)	20 (100)	20 (100)
100	20 (100)	20 (100)	20 (100)	20 (100)

<sup>a</sup> Two fish exhibited a partial loss of equilibrium.

<sup>b</sup> Fish were brown in color.

<sup>c</sup> Fish were light brown in color.

Table 9. Definitive Test II: Results of LC<sub>50</sub> Calculations Based on Nominal Concentrations Rainbow trout, *Oncorhynchus mykiss*, Exposed to CT-648-98 for 96 Hours Under Static Conditions As Mitigated by Dissolved Organic Carbon

Exposure Period (Hour)	LC <sub>50</sub> (mg wm/L)	95-Percent Confidence Limits (mg wm/L)	Statistical Method
24	44.9	36 - 60	Binomial
48	44.9	36 - 60	Binomial
72	44.9	36 - 60	Binomial
96	44.9	36 - 60	Binomial

Table 10. Definitive Test II: Total Organic Carbon (TOC) Concentrations During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

Treatment	Replicate	Concentration (mg/L)		
		0 HR	Rep. Mean	Mean
Control A	1	3.55		
	2	3.47		
	3	3.33	3.45	
Control B	1	3.41		
	2	3.29		
	3	3.20	3.30	3.38
HA Control A	1	8.62		
	2	8.85		
	3	8.98	8.82	
HA Control B	1	8.62		
	2	8.68		
	3	8.59	8.63	8.72
<b>Total Organic Carbon</b>				5.34

Table 11. Definitive Test II: Daily Temperature Ranges During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Conditions As Mitigated by Dissolved Organic Carbon

Exposure Period (Hour)	Temperature Range <sup>a</sup> (°C)
0	14.3 – 14.7
24	14.0 – 15.4
48	14.0 – 14.7
72	14.2 – 14.3
96	14.2 – 14.3

<sup>a</sup> Daily temperature ranges reported are the minimum and maximum of the temperatures recorded from all the controls and treatments.

NOTE: The diurnal temperature of the water bath ranged from 13.0 to 14.5 °C as measured by a minimum/maximum thermometer during the test.

Table 12. Definitive Test II: Hardness, Alkalinity, and Conductivity Measurements During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Conditions As Mitigated by Dissolved Organic Carbon

Day	Hardness* (mg/L)	Alkalinity* (mg/L)	Mean Conductivity ( $\mu$ mhos/cm)**
0	54	12	404
4	60	14	453

\* Hardness and alkalinity measurements expressed as milligrams per liter as CaCO<sub>3</sub>.

\*\* Mean conductivity was obtained from the average of the control replicates.

Table 13. Definitive Test II: Dissolved Oxygen Concentrations During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

Nominal Concentration (mg wm/L)	R E P	Dissolved Oxygen Concentration (mg/L)				
		0 HR	24 HR	48 HR <sup>c</sup>	72 HR	96 HR
Control	A	9.5	6.2	5.5	8.1	8.1
	B	9.6	5.9	5.3	8.7	8.6
Humic Acid Control	A	9.8	6.2	5.8	7.7	7.5
	B	10.0	5.1	4.4	7.4	7.4
12.9	A	10.2	6.3	5.8	7.7	7.9
	B	9.4	5.3	4.3	7.6	7.5
21.6	A	10.0	5.8	5.1	7.7	7.1
	B	9.7	5.7	4.7	6.4	6.6
36	A	9.4	5.8	4.5	6.2	6.8
	B	8.2	5.0	4.1	5.8	6.7
60	A	9.7	9.6	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>
	B	7.3	7.8	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>
100	A	8.0	8.2	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>
	B	9.6	9.7	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>
Saturation <sup>a</sup>		9.6	9.6	9.6	9.6	9.6

<sup>a</sup> Dissolved oxygen saturation values based on the temperature in a control replicate at the specified times (Truesdale et al. 1955).

<sup>b</sup> No measurements taken due to 100% mortality in the test chambers.

<sup>c</sup> Aeration was initiated.

Table 14. Definitive Test II: pH Values During a 96-Hour Exposure of Rainbow trout, *Oncorhynchus mykiss*, to CT-648-98 Under Static Test Conditions As Mitigated by Dissolved Organic Carbon

Nominal Concentration (mg wm/L)	R E P	0 HR	24 HR	pH		
				48 HR	72 HR	96 HR
Control	A	7.1	6.4	6.5	6.7	6.7
	B	6.9	6.6	6.5	6.9	6.7
Humic Acid Control	A	7.4	6.6	6.5	6.7	6.7
	B	7.1	6.7	6.5	6.8	6.7
12.9	A	7.4	6.6	6.5	6.7	6.7
	B	7.2	6.7	6.5	6.8	6.7
21.6	A	7.3	6.6	6.5	6.7	6.7
	B	7.2	6.7	6.5	6.8	6.7
36.0	A	7.3	6.6	6.5	6.7	6.7
	B	7.2	6.7	6.5	6.7	6.7
60.0	A	7.3	7.2	-- <sup>a</sup>	-- <sup>a</sup>	-- <sup>a</sup>
	B	7.0	7.0	-- <sup>a</sup>	-- <sup>a</sup>	-- <sup>a</sup>
100	A	7.1	7.2	-- <sup>a</sup>	-- <sup>a</sup>	-- <sup>a</sup>
	B	7.1	7.1	-- <sup>a</sup>	-- <sup>a</sup>	-- <sup>a</sup>

<sup>a</sup> No measurements taken due to 100% mortality in the test chambers.

**APPENDIX A**  
**DILUTION WATER CHARACTERIZATION**

LABORATORY FRESHWATER CHARACTERIZATION<sup>a</sup>

Parameter	Concentration <sup>b</sup>	Historical Range <sup>c</sup>
Aluminum	<0.100 mg/L	ND - 0.102 mg/L
Arsenic	<0.010 mg/L	ND
Boron	0.110 mg/L	ND - 0.159 mg/L
Beryllium	<0.004 mg/L	ND
Bromide	<0.10 mg/L	ND - 30 mg/L
Cadmium	<0.005 mg/L	ND
Calcium	19.0 mg/L	8.89 - 24.6 mg/L
Chloride	77.5 mg/L	60 - 108 mg/L
Chromium (hexavalent)	<0.010 mg/L	ND
Chromium (total)	<0.01 mg/L	ND
Cobalt	<0.010 mg/L	ND
Copper	<0.010 mg/L	ND - 0.009 mg/L
Fluoride	<0.10 mg/L	ND - 0.826 mg/L
Iodide	<0.050 mg/L	ND
Iron	< 0.100 mg/L	ND - 0.154 mg/L
Lead	<0.005 mg/L	ND
Manganese	<0.010 mg/L	ND
Magnesium	3.30 mg/L	0.789 - 9.91 mg/L
Mercury	<0.0002 mg/L	ND
Molybdenum	<0.020 mg/L	ND - 0.054 mg/L
Nickel	<0.020 mg/L	ND
Potassium	1.60 mg/L	1.14 - 5.50 mg/L
Selenium	<0.005 mg/L	ND
Silver	<0.005 mg/L	ND
Sodium	36.0 mg/L	27.4 - 74.0 mg/L
Tin	<0.30 mg/L	ND
Zinc	0.059 mg/L	ND - 0.059 mg/L
Ammonia (total)	0.033 mg/L	ND - 0.676 mg/L
Cyanide (total)	<0.010 mg/L	ND
Nitrates (total as N)	0.956 mg/L	ND - 1.38 mg/L
Nitrites (total as N)	<0.050 mg/L	ND - 0.058 mg/L
Phosphates (total)	0.010 mg/L	ND - 0.12 mg/L
Sulfide (total)	<0.04 mg/L	ND
Sulfate (total)	28.4 mg/L	15 - 52 mg/L
TDS	260 mg/L	150 - 552 mg/L
TOC	2.32 mg/L	ND - 6.0 mg/L
TSS	4.00 mg/L	ND - 16 mg/L
COD	9.62 mg/L	ND - 54.6 mg/L
Total organophosphorus pesticides	<1.0 µg/L	ND
Total phenoxy herbicides	<1.12 µg/L	ND
Total organochlorine pesticides	<0.04 µg/L	ND
PCBs	<0.100 µg/L	ND

<sup>a</sup> The characterized freshwater is carbon-treated Jupiter, Florida, town water which is aerated following carbon treatment.

<sup>b</sup> Sample of freshwater collected June 8, 1999.

<sup>c</sup> Historical range for laboratory freshwater.

**STUDY TITLE**

CT-648-98:  
Toxicity To The Freshwater Green Alga,  
*Selenastrum capricornutum*, Under Static Test Conditions

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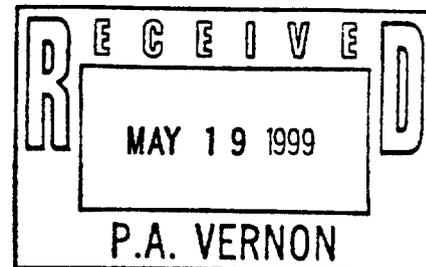
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**DATA REQUIREMENT**

OECD Guideline 201

**AUTHORS**

Walt Ellenberg, Ph. D.  
Scott Glover, BS



**STUDY INITIATION DATE**

February 19, 1999

**STUDY COMPLETION DATE**

May 13, 1999

**SPONSOR**

Cytec Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424

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**PERFORMING LABORATORY**

Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477

**LABORATORY PROJECT ID**

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## COMPLIANCE WITH GOOD LABORATORY PRACTICE STANDARDS

Test Substance: CT-648-98

Title: CT-648-98: Toxicity to The Freshwater Green Alga, *Selenastrum capricornutum*, Under Static Test Conditions

This study was conducted in accordance with and fully complies with published Good Laboratory Practices (GLP) regulations for tests of substances as promulgated by the OECD Guidelines for Testing of Chemicals (OECD, 1984) except the stability, purity and characterization of the test substance were not performed according to GLP Guidelines.

  
\_\_\_\_\_  
Walt Ellenberg, Ph. D.  
Study Director  
Toxikon Corporation

5-13-99  
\_\_\_\_\_  
Date

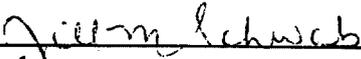
## STATEMENT OF QUALITY ASSURANCE

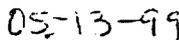
Test Substance: CT-648-98

Title: CT-648-98: Toxicity to The Freshwater Green Alga, *Selenastrum capricornutum*, Under Static Test Conditions

This study was examined for conformance with Good Laboratory Practices as published by the Organization For Economic Co-Operation And Development (OECD). The final report was determined to be an accurate reflection of the data obtained. The dates of Quality Assurance activities on this study are listed 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	3-25-99	3-26-99
Study Data Review:	5-10-99	5-10-99
Draft Report Review:	5-10-99	5-10-99
Final Report Review:	5-13-99	5-13-99

  
\_\_\_\_\_  
Jill M. Schwab  
Quality Assurance Officer  
Toxikon Corporation

  
\_\_\_\_\_  
Date

## LIST OF SCIENTIFIC PERSONNEL

Test Substance: CT-648-98

Title: CT-648-98: Toxicity to The Freshwater Green Alga, *Selenastrum capricornutum*,  
Under Static Test Conditions

Study Director: Walt Ellenberg

Biologists: Patricia Rielly  
Scott Glover  
Amy Lang  
Lisa Parsons

Aquaculturist: Patricia Rielly

## SUMMARY

Sponsor: Cytex Industries  
Five Garret Mountain Plaza  
West Paterson, NJ 07424

Study Director: Walt Ellenberg, Ph. D.

Location of Study: Toxikon Corporation  
106 Coastal Way  
Jupiter, Florida 33477

Location of Raw Data and Final Report: Cytex Industries  
West Paterson, New Jersey

Test Substance: CT-648-98; Lot No. 99-2

Test Species: Green Alga (*Selenastrum capricornutum*);  
5-day old culture at test initiation

Source of Organisms: Toxikon Corporation,  
Jupiter, FL

Dilution Medium: Freshwater algal growth medium with an initial pH of 7.0; test  
temperature ranged from 24.0 to 26.0°C.

Nominal Concentrations: Control, 0.0625, 0.125, 0.25, 0.5, and 1.0 mg wm/L

Test Dates: March 22-25, 1999

Study Length: 72 hours

Results: The  $E_bC_{50}$  (0-72 hours), based on nominal test concentrations, was 0.16 mg wm/L. The  $E_rC_{50}$  (24-72 hours) could not be calculated. The no-observable-effect concentration (NOEC) was 0.0625mg wm/L based on a lack of significant reduction in growth (cell density) at this test concentration.

## 1.0 INTRODUCTION

A phytotoxicity test was conducted at Toxikon Corporation, Jupiter, Florida, to determine the effect of CT-648-98 on the freshwater green alga, *Selenastrum capricornutum*. The criterion for effect was reduction in cell growth. Test results are expressed as  $E_bC_{50}$  and  $E_rC_{50}$  values, the concentrations of CT-648-98 estimated to be effective in reducing algal growth (based on biomass and growth rates) by 50 percent at the specified times. A no-observed-effect concentration (NOEC) was also calculated as the highest concentration tested at which growth was not statistically reduced from that of the control following 72 hours of exposure.

## **2.0 MATERIALS AND METHODS**

### **2.1 TEST SUBSTANCE**

The test substance, CT-648-98 (Lot No. 99-2) was received at Toxikon Corporation on February 17, 1999 from Cytec Industries in a 1000-mL Nalgene bottle labeled CT-648-98; Quantity Shipped: 1200 g; SPS Lot # 99-2. The test substance was a white/milky viscous liquid and was stored in the dark at room temperature. The test substance was reported by Cytec Industries to be soluble in water.

Test concentrations are reported as milligrams (mg) CT-648-98 as whole material (wm) per liter (L) of freshwater.

### **2.2 TEST SPECIES**

The freshwater alga tested was the green alga, *Selenastrum capricornutum* (CBS #SEC 23B). The culture originated from an inoculum received from the Carolina Biological Supply Co. and maintained since October 29, 1998. The algae were cultured in freshwater algal medium (ASTM, 1990) under continuous illumination yielding approximately 60 micromols per square meter per second ( $\mu\text{mol}/\text{m}^2/\text{s}$ ). Two weeks before the test, the algae culture was transferred to a higher light intensity at approximately 120 micromols per square meter per second ( $\mu\text{mol}/\text{m}^2/\text{s}$ ). Cultures were maintained at approximately  $24 \pm 2^\circ\text{C}$  prior to test initiation. The inoculum culture was 5 days old at test initiation.

### **2.3 TEST MEDIUM**

The base water for the test medium was deionized water. The base water was enhanced with reagent-grade nutrients as described in ASTM (1990). The pH of the test medium at test initiation was 7.0. The test medium was filtered through a sterilized 0.45  $\mu\text{m}$  membrane filter prior to use.

Chemical characterization of a representative batch of the deionized water is presented in Appendix A.

## 2.4 TEST METHODS

Methods for the test with *Selenastrum capricornutum* were those described in Toxikon Corporation's test protocol entitled: CT-648-98: Toxicity To The Freshwater Green Alga, *Selenastrum capricornutum*, Under Static Test Conditions."

A static range-finding test was conducted at nominal test concentrations of 0.1, 1.0, 10, 100 and 1000 mg wm/L prior to performing the definitive test. Since results indicated that the algal growth in the experimental control was inconsistent when compared to the alga test treatments, algal growth in 0.1 mg wm/L was used for the inhibition calculation. Inhibition of algal growth in the range-finding test was 7% at 0.10 mg wm/L, 78% at 1.0 mg wm/L, and 100% at 10.0, 100.0, and 1000.0 mg wm/L, respectively. Based upon the results of these tests, nominal test concentrations of 0.0625, 0.125, 0.25, 0.5, and 1.0 mg wm/L were selected for the definitive test. The definitive exposure was conducted under static conditions in a temperature controlled chamber set to provide a test temperature of  $24 \pm 2^{\circ}\text{C}$ .

All test solutions were prepared by mixing the appropriate amount of CT-648-98 stock with algal medium. Approximately 0.0464 g of the chemical was mixed with 0.500 liters of algae medium to prepare the stock and highest test solution (92.8 mg wm/L). The following amounts of stock (5.0, 2.5, 1.25, 0.625, and 0.3125 mL) were used to make the test concentrations by mixing with 495, 497.5, 498.8, 500, and 500 mL of freshwater algal media individually. A dilution water control was maintained concurrently with the five test solutions. Following mixing and initial pH measurements, four (seven for the control) 100-mL aliquots of each test solution were transferred

to sterile 250-mL glass Erlenmeyer flasks. This produced three replicates (six for controls) for each test concentration plus a chemical "blank" flask which would not be inoculated with algae. All flasks were covered with gas exchange caps.

The definitive test was initiated on March 22, 1999, with the inoculation of approximately 10,000 cells/mL to each test chamber (0.158 mL of an inoculum culture with a cell density of  $630 \times 10^4$  cells/mL was added to each 100-mL volume of freshwater algal medium). The test chambers were randomly positioned in a temperature-controlled chamber under continuous fluorescent lighting and continuously agitated on an orbital shaker table at approximately 100 rpm. Test chambers were randomly re-positioned daily to minimize any position effects. Light intensity was measured daily during the 72-hour exposure period at the level of the test solutions. Light intensity ranged between 57 and 106  $\mu\text{mol}/\text{m}^2/\text{s}$  as measured by a LI-COR, Inc. Model LI-189 light meter equipped with a  $2\pi$  quantum sensor at the surface of the test solutions.

Algal growth was measured for each testing flask including the chemical control "blank" replicates by direct cell counts using a 0.1 mm deep hemocytometer (Reichert, Buffalo, NY) coupled with a compound microscope. Algal counts were conducted on day one and every 24 hours thereafter. Morphological observations were also conducted on each test treatment using a compound microscope to detect abnormal cell morphology and coloration as compared to the control.

Temperature was measured in one uninoculated flask of test medium daily during the test. The temperature range of the environmentally-controlled chamber was monitored using a minimum/maximum thermometer and the diurnal temperature ranges recorded daily. The pH was measured at test initiation and termination in all control and test solutions with an Corning CH3 pH meter.

## 2.5 STATISTICAL ANALYSES

EC<sub>50</sub> values were calculated based on both biomass growth (comparison of areas under the growth curves) and on growth rates. EC values and their 95 percent confidence limits were estimated by an EPA computer program (U.S. EPA, 1994) for calculating EC values by probit analysis.

In addition to the EC values, a no-observed-effect concentration (NOEC) was calculated by analysis of variance (ANOVA) with statistical differences between cell density means determined by Dunnett's procedure (U.S.EPA, 1988). Statistical differences were determined at a probability level of 0.05.

### 2.5.1 Comparison of Areas Under the Growth Curves

Algal growth (as area under the growth curve) was calculated at the end of the test as follows:

$$A = \left( \frac{N_1 - N_0}{2} \times t_1 \right) + \left( \frac{N_1 + N_2 - 2N_0}{2} \right) \times (t_2 - t_1) \\ + \left( \frac{N_{n-1} + N_n - 2N_0}{2} \right) \times (t_n - t_{n-1})$$

where

- A = area
- N<sub>0</sub> = nominal number of cells/mL at time t<sub>0</sub>
- N<sub>1</sub> = measured number of cells/mL at time t<sub>1</sub>
- N<sub>n</sub> = measured number of cells/mL at time t<sub>n</sub>
- t<sub>1</sub> = time of first measurement after beginning of test
- t<sub>n</sub> = time of n<sup>th</sup> measurement after beginning of test

The percent inhibition of the cell growth at each test substance concentration (I<sub>A</sub>) was calculated as the difference between the area under the control growth curve (A<sub>c</sub>) and the area under the

growth curve at each test substance concentration ( $A_t$ ) as:

$$I_A = \frac{A_c - A_t}{A_c} \times 100$$

Based on results of the test (and above calculations), the  $E_bC_{50}$  (0-72 hour) value and its 95 percent confidence limits were calculated.

### 2.5.2 Comparison of Growth Rates

The average specific growth rate ( $\mu$ ) for each treatment was calculated as:

$$\mu = \frac{\ln N_n - \ln N_1}{t_n - t_1}$$

The percent reduction in average growth rate at each test substance concentration compared to the control was calculated and  $E_rC_{50}$  (24-72 hour) and its 95 percent confidence limits were calculated.

## 2.6 Archive

The final report and all raw data related to this study will be archived at Cytec Industries, West Paterson, New Jersey.

### **3.0 RESULTS AND DISCUSSION**

After 72 hours of exposure to CT-648-98, the percentage inhibition of cell growth (based upon area under growth curve) compared to the control ranged from 3.24 percent at nominal concentration of 0.065 mg wm/L to 99.9 percent at 1.0 mg wm/L (Table 1). Daily cell counts for all control and test solutions are presented in Table 1 while replicate counts are presented in Appendix B. Growth curves for the control and all CT-648-98 treatments are presented in Figure 1. The growth curves of the controls exhibited a pattern of exponential growth during the 72-hour growth period. Observations of cell morphology detected no changes in CT-648-98 exposed cells as compared to cells in control media.

The  $E_bC_{50}$  (0-72 h) was 0.16 mg wm/L with 95 percent confidence limits of 0.14 and 0.19 mg wm/L. The  $E_rC_{50}$  (24-72 h) could not be calculated. The no-observed-effect concentration (NOEC) was determined to be 0.0625 mg wm/L, the highest concentration at which cell growth (based upon cell numbers) was not statistically reduced from that of the control.

The measured test temperature during the 72-hour exposure ranged from 24 to 26°C (Table 3). The initial pH of the control and all test solutions ranged from 7.0 to 7.2 (Table 4). After 72 hours, the pH ranged from 7.0 to 7.8 in the control and all test solutions (Table 4).

#### **4.0 PROTOCOL DEVIATIONS**

There was no deviation from the test protocol during the conduct of this study.

## REFERENCES

American Society for Testing and Materials (ASTM). 1990. Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae. ASTM Designation E1218-90.

OECD (Organization for Economic Co-Operation and Development). 1984. Alga, Growth Inhibition Test, Method 201, Adopted June 7, 1984. 14 pp.

U.S. Environmental Protection Agency (USEPA). 1988. EPA Dunnett's Program, Version 1.1.

U.S. Environmental Protection Agency (USEPA). 1994. EPA Probit Analysis Program Used For Calculating LC/EC Values, Version 1.5.

Table 1. Cell Numbers During a 72-hour Static Exposure of *Selenastrum capricornutum* to CT 648-98

Nominal Concentration (mg wm/L)	Mean Cell Numbers ( $\times 10^4$ )/mL <sup>a</sup>			Percent Change <sup>b</sup> (IA)
	24 hr	48 hr	72 hr	
Control	1.00 (0.16)	10.5 (3.85)	92.5 (22.9)	---
0.063	0.11 (0.06)	6.1 (0.66)	89.5 (20.3)	-3.24
0.125	0.13 (0.133)	2.4 (0.35)	60 (4.64)	-35.1 <sup>c</sup>
0.250	0.22 (0.20)	0.9 (0.4)	15.6 (13.9)	-83.1 <sup>c</sup>
0.500	0.02 (0.03)	0.2 (0)	5.33(0.2)	-94.2 <sup>c</sup>
1.00	0 (0)	0 (0)	0.11 (0.11)	-99.9 <sup>c</sup>

<sup>a</sup> Values are means of triplicate test chambers, (six replicates for controls) the standard deviations are presented in parentheses. Measurements for individual replicates are presented in Appendix B.

<sup>b</sup> Percent inhibition (-) or stimulation (+) as determined against the control at 72 hours based upon area under algal growth curve.

<sup>c</sup> Growth was statistically less than the control ( $\alpha = 0.05$ ).

Figure 1. Growth Curves for the Freshwater Green Alga, *Selenastrum capricornutum*, During An 72-hour Exposure to CT 648-98 Under Static Test Conditions

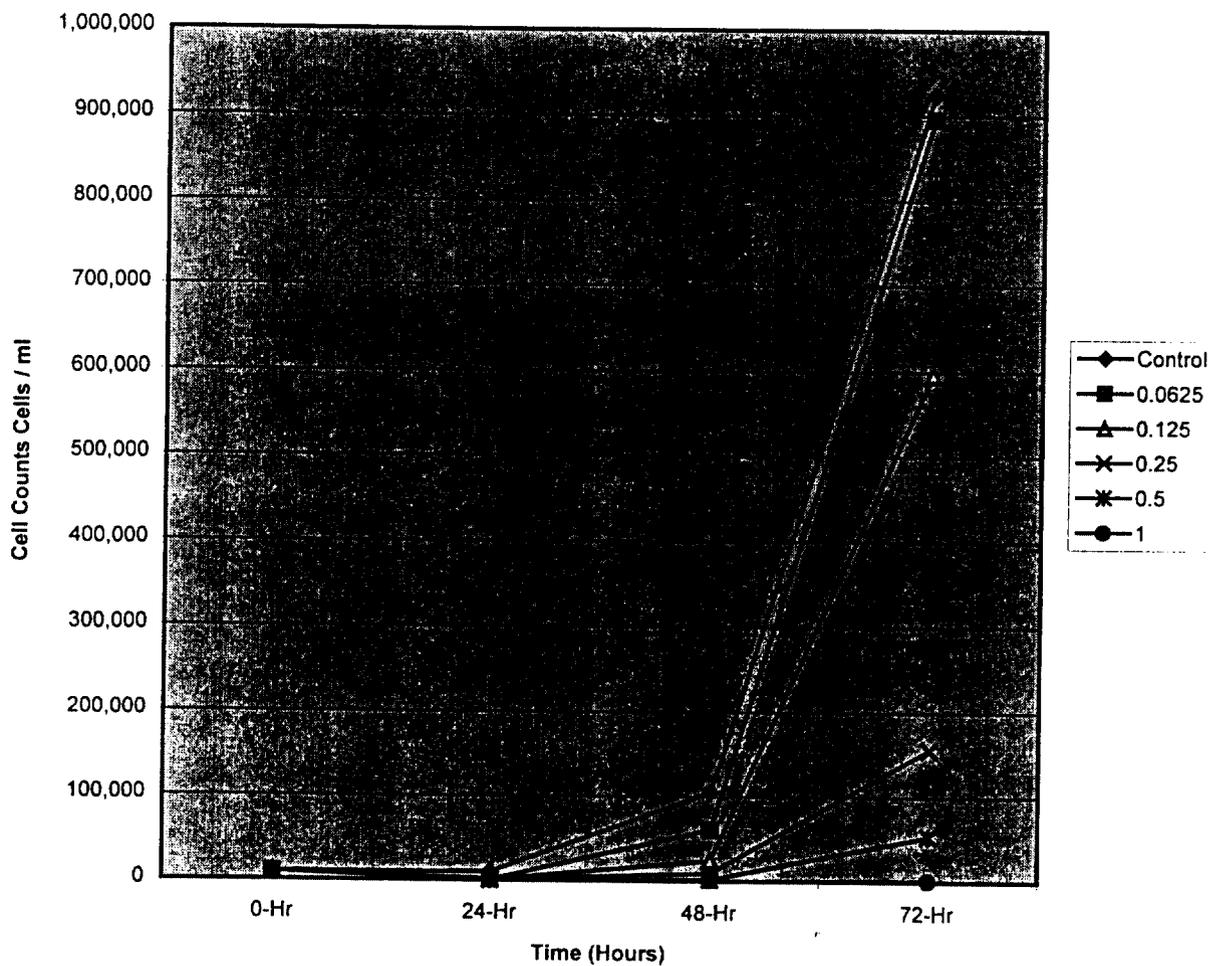


Table 2. Daily Temperature During a 72-hour Static Exposure of the Green Alga, *Selenastrum capricornutum*, to CT 648-98

Exposure Period (Hour)	Temperature (°C)
0	26.0
24	24.0
48	25.9
72	25.0

Note: Temperature measured in uninoculated test container. The diurnal temperature range of the temperature controlled chamber was 24.3 to 26.9°C during the conduct of the test as measured continuously by a minimum/maximum thermometer.

Table 3. The pH Values During a 72-Hour Static Exposure of the Green Alga, *Selenastrum capricornutum*, to CT 648-98

Nominal Concentration (mg wm/L)	Replicate	pH	
		0 Hours <sup>a</sup>	72 Hours
Control	A	7.0	7.5
	B		7.6
	C		7.6
	D		7.5
	E		7.6
	F		7.6
0.0625	A	7.2	7.7
	B		7.8
	C		7.5
0.125	A	7.1	7.7
	B		7.7
	C		7.6
0.25	A	7.1	7.7
	B		7.5
	C		7.1
0.5	A	7.0	7.0
	B		7.0
	C		7.0
1.0	A	7.0	7.0
	B		7.1
	C		7.1

<sup>a</sup> Measurements taken from solutions prior to distribution to test vessels.

**APPENDIX A**  
**DEIONIZED WATER CHARACTERIZATION**

DEIONIZED WATER CHARACTERIZATION<sup>a</sup>

Parameter	Concentration <sup>b</sup>
Aluminum	0.060 mg/L
Arsenic	<0.005 mg/L
Boron	0.209 mg/L
Beryllium	<0.004 mg/L
Bromide	<0.10 mg/L
Cadmium	<0.005 mg/L
Calcium	0.133 mg/L
Chloride	<1.00 mg/L
Chromium (hexavalent)	<0.01 mg/L
Chromium (total)	<0.005 mg/L
Cobalt	<0.005 mg/L
Copper	<0.005 mg/L
Fluoride	<0.10 mg/L
Iodide	<0.050 mg/L
Iron	0.020 mg/L
Lead	<0.005 mg/L
Manganese	<0.005 mg/L
Magnesium	<0.050 mg/L
Mercury	<0.0002 mg/L
Molybdenum	<0.020 mg/L
Nickel	<0.008 mg/L
Potassium	<0.500 mg/L
Selenium	<0.005 mg/L
Silver	<0.005 mg/L
Sodium	0.537 mg/L
Tin	<0.050 mg/L
Zinc	0.074 mg/L
Ammonia (total)	<0.03 mg/L
Cyanide (total)	<0.010 mg/L
Nitrates (total as N)	<0.0047 mg/L
Nitrites (total as N)	<0.050 mg/L
Phosphates (total)	<0.01 mg/L
Sulfide (total)	<0.004 mg/L
Sulfate (total)	<2.0 mg/L
TDS	<12 mg/L
TOC	<1.0 mg/L
TSS	<4 mg/L
COD	<5.0 mg/L
Total organophosphorus pesticides	<1.0 µg/L
Total phenoxy herbicides	<1.2 µg/L
Total organochlorine pesticides	<0.01 µg/L
PCBs	<0.10 µg/L

<sup>a</sup> The characterized deionized water is Jupiter, Florida, town water which has been deionized by a U.S. Filter ion exchange system and passed through activated carbon.

<sup>b</sup> Sample of deionized water collected June 30, 1998.

**APPENDIX B**  
**CELL COUNT RAW DATA**

Table B-1. Daily Cell Counts of the Green Alga, *Selenastrum capricornutum*, During a 72-Hour Exposure to CT 648-98 Under Static Test Conditions

Nominal Concentration (mg/L)	Cell Numbers ( $\times 10^4$ )/mL			
	Replicate	24 Hours	48 Hours	72 Hours
Control	A	0.9	6.1	88.9
	B	1.0	11.6	71.1
	C	1.2	13.4	70.0
	D	0.8	8.3	129.0
	E	1.1	7.5	86.7
	F	1.2	16.1	109.0
0.063	A	0.1	6.2	77.8
	B	0.05	6.7	113.0
	C	0.17	5.4	77.8
0.125	A	0.05	2.4	63.3
	B	0.05	2.8	61.1
	C	0.28	2.1	54.4
0.250	A	0	0.9	26.7
	B	0.28	0.5	20.0
	C	0.39	1.3	0
0.500	A	0	0.2	5.22
	B	0.05	0.2	5.56
	C	0	0.2	5.22
1.00	A	0	0	0.11
	B	0	0	0.22
	C	0	0	0.0