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Document Title	INITIAL SUBMISSION: ACUTE TOXICITY OF MIXED METHYLTIN CHLORIDES TO THE FRESHWATER ALGA, SELENASTRUM CAPRICORNUTUM (RANGE FINDING TEST), WITH COVER LETTER DATED 12/22/2000		
Chemical Category	MIXED METHYLTIN CHLORIDES		

A 03

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42491

BEHQ-1200-14841

December 22, 2000



Document Processing Center (TS-790)  
Attention: Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

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Dear Coordinator:

Rohm and Haas Company submits this notice in accordance with Section 8(e) of the Toxic Substances Control Act.

This letter transmits results of a acute toxicity test indicating EC50 < 1 mg/L to freshwater alga effects for trichloromethylstannane (CAS No. = 993-16-8) and dichlorodimethylstannane (CAS No. 753-73-1).

Rohm and Haas Company does not consider the exact identity of this chemical to be Confidential Business Information (CBI)

If you have any questions concerning this submittal, my telephone number is (215) 592-2986.

Sincerely,

  
George J. Powell  
TSCA Manager  
EHS Corporate Center



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Attachment 1

Acute Toxicity of Mixed Methyltin Chlorides to the  
Freshwater Alga, *Selenastrum capricornutum*

- Range Finding Test -

Sponsor

Morton International, Inc.  
100 North Riverside Plaza  
Chicago, Illinois 60606-1596

CAS# 993-16-8  
753-73-1

Attachment 1

Acute Toxicity of Mixed Methylin Chlorides to the  
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I. TITLE PAGE

Title: Acute Toxicity of Mixed Methyltin Chlorides  
to the Freshwater Alga, *Selenastrum  
capricornutum*

Sponsor: Morton International, Inc.  
100 North Riverside Plaza  
Chicago, Illinois 60606-1596

Testing Facility T.R. Wilbury Laboratories, Inc.  
40 Doaks Lane  
Marblehead, Massachusetts 01945

Reported Purity: 45%

Study Initiation Date: July 14, 1995

Study Completion Date: January 4, 1996

Authors: Timothy J. Ward  
Jeanne P. Magazu  
Robert L. Boeri

Data Requirement: TSCA 797.1050 (US.EPA, 1993)  
OECD 201 (OECD, 1984)

## II. SUMMARY

The toxicity of Mixed Methyltin chlorides to the freshwater alga, *Selenastrum capricornutum*, was investigated during a range-finding study conducted at T.R. Wilbury Laboratories, Inc., from August 11 to 15, 1995. The test was performed at  $24 \pm 1^\circ\text{C}$  under static conditions with a control and 0.10, 1.0, 10, 100, and 1,000 mg/L mixtures of test substance and water. The dilution water was sterile synthetic media adjusted to a pH of 7.5. Nominal concentrations of test substance were used for all calculations. There was no insoluble material observed during the test. The test was performed using nominal concentrations of whole test substance and results are reported both as active ingredient (reported purity = 45% a.i.) and whole test substance.

Algae used for the test (*Selenastrum capricornutum*, UTEX 1648) was from a culture originally procured from the Culture Collection of Algae at the University of Texas at Austin on April 11, 1995. The culture was transferred to sterile enriched media identical to media used for this test and maintained at test conditions for at least 14 days before the definitive test. Algae was incubated under continuous light on a rotary shaker adjusted to 100 rpm. Cell counts were made at 0, 24, 72 and 96 hours with a haemocytometer.

Results of the test with Mixed Methyltin Chlorides are presented below:

Test Results	Whole Test Substance		Active Ingredient	
	72-hour (mg/L)	96-hour (mg/L)	72-hour (mg/L)	96-hour (mg/L)
Based on Cell Density:				
EC50	0.56	0.19	0.25	0.086
NOEC	0.10	<0.10	0.045	<0.045
Based on Growth Rate:				
EC50	3.8	1.0	1.7	0.45
NOEC	0.10	0.10	0.045	0.045

Regrowth of inhibited cultures from the 10 and 100 mg/L (whole test substance) test levels revealed that the effect at 10 mg/L was algistatic and at 100 mg/L was algicidal.

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**V. INTRODUCTION**

This study was sponsored by Morton International, Inc. It was conducted by Jeanne Magazu, Peter Kowalski, Jacqueline Nevius, Peter Nevius, Keven Stevens, Robert Boeri, and Timothy Ward.

The objective of the study was to determine the acute toxicity (72 and 96 hour EC50 and NOEC values) of Mixed Methyltin Chlorides to *Selenastrum capricornutum*, a freshwater alga. The report contains sections that describe the methods and materials employed in the study, and the results of the investigation.

**VI. METHODS AND MATERIALS****TEST SUBSTANCE:**

The sample of Mixed Methyltin Chlorides (T.R. Wilbury Laboratories sample number 507) used for the toxicity test was delivered on June 23, 1995. It was contained in a 100 ml glass bottle that was shipped in a cardboard box at ambient temperature. The label attached to the container included the following information: "Reference #: 1681-101, Date: June 16, 1995, Mixed Methyltin Chlorides, Mono/Di Methyltin Chlorides".

Mixed Methyltin Chlorides (a clear liquid) was supplied by Morton International, Inc., Cincinnati, Ohio. Prior to use the test substance was stored at room temperature in the dark and unused test substance is returned to the sponsor. The test substance was assumed to be 100% active ingredient and to be stable under test conditions. Subsequent correspondence from the sponsor indicated that the concentration of Mixed Methyltin Chlorides in the sample tested was 45%.

**DILUTION WATER:**

Water used for acclimation of test organisms and for all toxicity testing was sterile enriched media (U.S. EPA, 1978) with a pH of 7.5. A representative sample of water used to prepare media was analyzed for pesticides, metals, and total organic carbon and results of these analyses are presented in Table 1. Test media from a control vessel contained 34 mg/L particulate matter at the start of the test and 27 mg/L at the end of the test.

**TEST ORGANISM:**

Algae used for the test (*Selenastrum capricornutum*, UTEX 1648) was from a culture originally procured from the Culture Collection of Algae at the University of Texas at Austin and delivered to T.R. Wilbury on April 11, 1995. The identity of the algae was verified using an appropriate taxonomic key. The culture was transferred to sterile enriched media identical to media used for this test and maintained at test conditions for at least 14 days before the definitive test. The inoculum used for the test was from a single source that had been growing for 7 days prior to testing.

**TOXICITY TESTING:**

The range finding test was conducted for 96 hours from August 11 to 15, 1995. The toxicity test was performed according to T.R. Wilbury Laboratories protocol number 859-MO (Acute Toxicity of Mixed Methyltin Chlorides To The Freshwater Alga, *Selenastrum capricornutum* - Range Finding Test -), which was signed by the study director on July 14, 1995. The protocol was based on procedures of the U.S. EPA (1993) and the OECD (1984).

A 1,000 mg/L stock solution was formulated by the addition of 0.250 g test substance to sterile dilution water in a class A volumetric flask and adjusting the final water volume to 250 ml. Test media was prepared at 0.10, 1.0, 10, 100, and 1,000 mg/L (whole test substance) by combining the appropriate volume of the 1,000 mg/L stock solution and dilution water.

The test was conducted at a target temperature of  $24 \pm 1^\circ\text{C}$  with five concentrations of test substance and a dilution water control. Algae was distributed among 2 replicates of each treatment at the rate of approximately 10,000 cells/ml. The test was performed in 250 ml glass erlenmeyer flasks that contained 100 ml of test solution. Test vessels were randomly arranged on a rotary shaker adjusted to 100 rpm in an incubator during the test (a random numbers table was used to select the location of each vessel). A 24 hour light and 0 hour dark photoperiod was automatically maintained with cool-white fluorescent lights that provided a light intensity of approximately 400 footcandles.

The number of algal cells/ml in each test vessel and the occurrence of relative size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells was determined visually by means of direct microscopic examination with a haemocytometer. Cell counts were made and recorded at the beginning of the test and after 72, and 96 hours.

Temperature of the incubator was measured and recorded daily and temperature in a beaker of water incubated among the test vessels was recorded continuously from approximately 1 hour to the end of the test, and pH (Beckman Model PHI 12 meter; instrument number 124) was determined in each test vessel at the beginning and end of the test. Particulate material was measured in media from a control vessel at the beginning and end of the test.

At the conclusion of the test a 0.5 ml subsample of test media from each flask containing the 10 and 100 mg/L solutions of Mixed Methyltin Chlorides were combined with 100 ml of fresh media to determine whether algicidal or algistatic effects had occurred. These flasks were incubated under test conditions for 216 hours and examined for the presence of algal cells.

#### **CALCULATIONS AND STATISTICAL METHODS:**

The average specific growth rate was calculated as the natural log of the number of cells/ml at 72 and 96 hours minus the natural log of the number of cells/ml at 0 hours divided by the exposure period. The percent change from the control was calculated by subtracting the treatment average specific growth rate from the control average specific growth rate, dividing the difference by the average specific growth rate in the control, and multiplying that value by 100. The EC50 and NOEC values were calculated using nominal concentrations of test substance, when warranted. The values were computed twice, once using the average number of cells/ml at each concentration expressed as a percent of the control and a second time using the average specific growth rate expressed as the percent change from the control.

The binomial/nonlinear interpolation method (Stephan, 1983) was used to calculate EC50 values. The slope of the 96-hour dose response curve (cell density) was computed using the probit method. The no observed effect concentration (NOEC) is the highest concentration of test substance that allowed cell growth equal to at least 90% of the control growth.

## VII. RESULTS

No insoluble material was noted in test vessels containing Mixed Methyltin Chlorides. Water quality data generated by the acute toxicity test are presented in Table 2. The pH of test media was decreased for the test substance and the pH at 100 and 1,000 mg/L test substance may have been low enough to cause toxicity. The algal population grew well during the test, resulting in an average of 1,800,000 cells per ml in the control.

Results of the exposure of freshwater algae to the 5 concentrations of Mixed Methyltin Chlorides are presented in Tables 3 and 4. The slope of the 96 hour dose response curve is 1.5, based on cell density. Results of the test with Mixed Methyltin Chlorides are presented below:

Test Results	Whole Test Substance		Active Ingredient	
	72-hour (mg/L)	96-hour (mg/L)	72-hour (mg/L)	96-hour (mg/L)
Based on Cell Density:				
EC50	0.56	0.19	0.25	0.086
NOEC	0.10	<0.10	0.045	<0.045
Based on Growth Rate:				
EC50	3.8	1.0	1.7	0.45
NOEC	0.10	0.10	0.045	0.045

No effects (size differences, unusual cell shapes, colors, flocculations, adherence of cells to test containers, or aggregation of cells) were noted. After 216 hours, examination of media from the flask containing a 0.50 ml subsample of test media from the flask containing the 10 mg/L Mixed Methyltin Chlorides solution (whole test substance) and 100 ml of fresh media contained 2,124,000 algal cells/ml, indicating that the effect of the test substance at this concentration was algistatic and the flask containing a 0.50 ml subsample of test media from the flask containing the 100 mg/L Mixed Methyltin Chlorides solution (whole test substance) and 100 ml of fresh media contained <10,000 cells/ml, indicating that the effect of the test substance at this concentration was algicidal.

VIII. REFERENCES

- APHA. 1985. Standard Methods for the Examination of Water and Wastewater. Sixteenth Edition. Washington, DC.
- OECD. 1981. OECD Guidelines for Testing of Chemicals. Annex 2. OECD Principals of Good Laboratory Practice. Adopted 1 June 1981.
- OECD. 1984. OECD Guidelines for Testing of Chemicals. Section 2: Effects on Biotic Systems. Method 201. Algal Growth Inhibition Test. Adopted 4 April 1984.
- Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
- U.S. EPA. 1993. 40 CFR Part 797. Toxic Substances Control Act Test Guidelines; Final Rules. Section 797.1050.
- U.S. EPA. 1993. 40 CFR Part 792. Toxic Substances Control Act (TSCA); Good Laboratory Practice Standards; Final Rule.

Table 1. Chemical characterization of a representative sample of water used to formulate media for the toxicity test with freshwater alga, *Selenastrum capricornutum*, and Mixed Methyltin Chlorides.

Parameter <sup>1</sup>	Unit of Measurement	Detection Limit	Measured Value
<b>Metals</b>			
Aluminum	mg/L	0.1	ND <sup>2</sup>
Arsenic	mg/L	0.01	ND
Boron	mg/L	0.5	ND
Cadmium	mg/L	0.0002	ND
Chromium	mg/L	0.01	ND
Cobalt	mg/L	0.03	ND
Copper	mg/L	0.005	ND
Iron	mg/L	0.03	0.03
Lead	mg/L	0.005	ND
Mercury	mg/L	0.0003	ND
Nickel	mg/L	0.03	ND
Silver	mg/L	0.02	ND
Zinc	mg/L	0.02	ND
Nitrate	mg/L as N	0.05	ND
Chloride	mg/L	1	
Fluoride	mg/L	0.1	ND
Total Organic Carbon	mg/L	1	ND
Total Phosphorus	mg/L	0.03	ND
<b>Organochlorine</b>			
Pesticides	µg/L	0.5	ND
Toxaphene	µg/L	2	ND
<b>Organophosphorus</b>			
Pesticides	µg/L	0.5	ND
Dimethoate	µg/L	2.0	ND
TEPP	µg/L	2.0	ND
Monocrotophos	µg/L	2.0	ND
PCBs	µg/L	0.5	ND

Notes: 1. Parameters were measured in deionized water (used to formulate dilution water) that was collected during August, 1995 and analyzed by Pace, Inc. as part of routine water quality testing.

2. ND = not detected at or above the detection limit.

Table 2. Water quality data from the toxicity test with Mixed Methyltin Chlorides and the freshwater alga, *Selenastrum capricornutum*.

Hour of Exposure	Temperature of Incubator (°C)
0	23.8
24	23.6
48	23.6
72	23.6
96	23.4

Table 2. Water quality data from the toxicity test with Mixed Methyltin Chlorides and the freshwater alga, *Selenastrum capricornutum*. (cont.)

Nominal Concentration of Whole Test Substance (mg/L)	replicate	pH	
		Initial	Final
0 (control)	1	7.5	8.6
	2	7.5	10.2
0.10	1	7.6	9.7
	2	7.6	9.4
1.0	1	7.6	8.7
	2	7.6	8.6
10	1	7.6	8.5
	2	7.6	8.4
100	1	3.9	3.7
	2	3.9	3.7
1,000	1	2.5	2.3
	2	2.5	2.3

Table 3. Cell growth data from the acute toxicity test with Mixed Methyltin Chlorides and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of Whole Test Substance (mg/L)	Replicate	Number of Cells/ml x 10 <sup>3</sup>		
		0 hr	72 hr	96 hr
0 (control)	1	10	260	1,722
	2	10	532	1,878
	Mean	10	396	1,800
	% Control	--	--	--
0.10	1	10	422	1,144
	2	10	432	1,354
	Mean	10	427	1,249
	% Control	100	108	69
1.0	1	10	118	146
	2	10	90	128
	Mean	10	104	137
	% Control	100	26	<10
10	1	10	50	20
	2	10	36	34
	Mean	10	43	27
	% Control	100	11	<10
100	1	10	20	<10
	2	10	12	<10
	Mean	10	16	<10
	% Control	100	4	<1
1,000	1	10	<10	<10
	2	10	<10	<10
	Mean	10	<10	<10
	% Control	100	<1	<1

Table 4. Average specific growth rate and percent change from the control from the acute toxicity test with Mixed Methyltin chlorides and the freshwater alga, *Selenastrum capricornutum*.

Nominal Concentration of Whole Test Substance (mg/L)	Average Specific Growth Rate		Percent Change From Control	
	72hr	96hr	72hr	96hr
0 (control)	0.051	0.054	--	--
0.10	0.052	0.050	0	7
1.0	0.033	0.027	36	50
10	0.020	0.010	60	81
100	0.007	0.000	87	100
1,000	0.000	0.000	100	100