

**DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
§123-2 (TIER II)**

1. **CHEMICAL:** Fentin hydroxide PC Code No.: 083601

2. **TEST MATERIAL:** TPTH Purity: 97.5%

3. **CITATION:**

Author: Hoberg, J. R.

Title: TPTH Technical - Acute Toxicity to the Freshwater Green Alga, *Pseudokirchneriella subcapitata*

Study Completion Date: November 9, 2000

Laboratory: Springborn Laboratories, Inc.
790 Main Street
Wareham, MA 02571-1075

Sponsor: Landis International, Inc.
3185 Madison Highway
Valdosta, GA 31603-5126

Laboratory Report ID: Springborn Study No. 13733.6103

MRID No.: 45276003

DP Barcode: D271849

4. **REVIEWED BY:** Dana Worcester, Senior Staff Scientist, Dynamac Corporation

Signature:

Date: 4/17/01

APPROVED BY: Kathleen Ferguson, Ph.D., Senior Staff Scientist, Dynamac Corporation

Signature:

Date: 4/17/01

5. **APPROVED BY:** *Contractor Draft copy*

Signature:

Date:



6. STUDY PARAMETERS

Scientific Name of Test Organism: *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*)

Definitive Test Duration: 96 hours

Study Method: Static

Type of Concentrations: Nominal

7. CONCLUSIONS:

In this algae EC₅₀ test, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) were exposed to TPTH at nominal concentrations of 1.0, 2.6, 6.4, 16, 40, and 100 µg/L; mean measured concentrations were 0.82, 2.5, 5.5, 20, 38, and 92 µg/L, respectively. By 96 hours, cell density was increased in the mean measured 0.82 µg/L treatment group by 2.3%, compared to the pooled control; cell density was reduced in the mean measured 2.5, 5.5, 20, 38, and 92 µg/L treatment groups at a rate of 4.1%, 13%, 18%, 96%, and 99%, respectively, compared to the pooled control group. **The 96-hour NOEC value for this study was 0.82 µg/L. The study author determined the 96-hour EC₅₀ value for cell density to be 14 µg/L.** This value is lower than that estimated by the reviewer (24 µg/L) and, so, the reviewer recommends using the lower estimate.

The study author failed to assess and report conclusions on both biomass and growth rate endpoints therefore, the reviewer was unable to statistically verify the study author's NOEC, LOEC, and EC₅₀ estimates pertaining to biomass (area under the curve) and growth rate. The study author only assessed data for one endpoint, cell density, which was statistically verified by the reviewer.

This study is classified as CORE. This study supports the requirements for an algae EC₅₀ test (Subdivision J, §123-2 (TIER II)).

Results Synopsis

Cell density:

EC₅₀: 14 µg/L

95% C.I.: 3.5-55 µg/L

NOEC: 0.82 µg/L

Probit Slope: 2.7

Biomass (Area Under the Curve): Not analyzed; data were not provided.

EC₅₀:

95% C.I.:

NOEC:

Probit Slope:

Growth rate: Not analyzed; data were not provided

EC₅₀:

95% C.I.:

NOEC:

Probit Slope:

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The maximum labeled rate was not reported.

10. SUBMISSION PURPOSE: R (NC)

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Pseudokirchneriella subcapitata</i> (formerly <i>Selenastrum capricornutum</i>)
Initial number of cells: 3,000 - 10,000 cells/mL	10,000 cells/mL
Nutrients: Standard formula	Algal Assay Procedure medium

B. Test System

Guideline Criteria	Reported Information
Solvent:	0.10 mL/L dimethylformamide
Temperature: <i>Skeletonema</i> : 20°C Others: 24-25°C	24°C

Guideline Criteria	Reported Information
Light Intensity: <i>Anabaena</i> : 2.0 Klux ($\pm 15\%$) Others: 4.0-5.0 Klux ($\pm 15\%$)	4.3-5.4 Klux
Photoperiod: <i>Skeletonema</i> : 14 h light, 10 h dark, or 16 h light, 8 h dark Others: Continuous	Continuous
pH <i>Skeletonema</i> : approx. 8.0 Others: approx. 7.5	Initial 7.1-7.4; Final 7.9-9.9

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	2.5x
Doses: at least 5	Nominal: 1.0, 2.6, 6.4, 16, 40, and 100 $\mu\text{g/L}$
Controls: Negative and/or solvent	Negative and solvent control
Replicates per dose: 3 or more	3 replicates
Duration of test: 120 hours	96 hours
Daily observations were made?	Yes
Method of observations:	The number of cells was determined using a hemacytometer and microscope.
Maximum labeled rate:	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Initial and 96 h cell densities were measured.
Control cell count at 120 hr \geq2x initial count?	Control cell count at 96 hr \geq 2x initial count.
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response:**Cell Density**

Initial Measured Concentration (µg/L)	Avg. Cell Density (x 10 ⁴ cells/mL)	% Reduction ¹	96-hour pH
Control	240	---	9.8
Solvent control	236	---	9.8
Pooled control	238	---	---
0.82	243	<2.3>	9.9
2.5	228	4.1	9.8
5.5	206	13	9.6
20	196	18	9.4
38	9.1	96	8.3
92	2.2	99	7.9

<> Represents an increase in cell density relative to the pooled control.

¹ % reduction values are based on raw data provided by the study author.

Other Significant Results: After 96 hours, cells exposed to 38 µg/L were reported to be bloated and cell fragments were present.

Statistical Results

Statistical Method: The negative and solvent controls were compared using the Student's T-test. EC₅₀ values were determined by a linear regression estimation procedure. Cell density data were evaluated for normality using Shapiro-Wilks' test and for homogeneity of variances using Bartlett's test. The NOEC for algal growth was determined using Williams' test. Calculations were performed with a computer program developed by the performing laboratory (Springborn).

Cell density:

EC₅₀: 14 µg/L

NOEC: 0.82 µg/L

95% C.I.: 3.5-55 µg/L

Probit Slope: 2.7

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Only algal cell density data were analyzed statistically because replicate data were not provided for area under the growth curve or growth rate. After confirming normality and homogeneity of variance, negative and solvent control data were pooled because a two-tailed *t*-test revealed no significant difference. Treatment effects were assessed via Bonferroni-adjusted *t*-tests. An EC₅₀ estimate was performed using the method of Bruce and Versteeg via Nuthatch software.

Cell density:

EC ₅₀ : 24 µg/L	95% C.I.: 14 and 42 µg/L
NOEC: 0.82 µg/L	Probit Slope: 6.85

Biomass (Area Under the Curve): Replicate data not provided; values could not be determined.

EC ₅₀ :	95% C.I.:
NOEC:	Probit Slope:

Growth rate: Replicate data not provided; values could not be determined.

EC ₅₀ :	95% C.I.:
NOEC:	Probit Slope:

14. REVIEWERS' COMMENTS:

The reviewer's conclusions could only be compared to those of the study author for one of three endpoints, cell density. The author's EC₅₀ estimate of 14 µg a.i./L for cell density is the lower than that calculated by the reviewer, 24 µg a.i./L and, so, the reviewer recommends using the study author's estimate. The NOEC and LOEC reported by the study author were equivalent to those reported by the reviewer. The two endpoints, biomass and growth rate could not be statistically verified due the fact that the study author did not assess these endpoints during the study period or these data were not included within the study report.

According to the October 21, 1994 USEPA, OPPTS memorandum on "Closure on Nontarget Plant Phytotoxicity Policy Issues", Aquatic Plant Growth Studies (122-2, 123-2), four or five day algal studies will be accepted for review by USEPA.

According to the October 21, 1994 USEPA, OPPTS memorandum on "Closure on Nontarget Plant Phytotoxicity Policy Issues", Aquatic Plant Growth Studies (122-2, 123-2), an initial cell density of 10,000 cells/mL is acceptable.

The maximum labeled rate was not reported.

This study was conducted in accordance with USEPA Good Laboratory Practice regulations and included a Quality Assurance Statement.

15. REVIEWER'S STATISTICAL RESULTS:

File: 6003d Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	222151.837	37025.306	1080.148
Within (Error)	17	582.728	34.278	
Total	23	222734.565		

Critical F value = 2.70 (0.05,6,17)
 Since F > Critical F REJECT Ho:All groups equal

File: 6003d Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL		TRANSFORMED	ISOTONIZED
		N	MEAN	MEAN	MEAN
1	GRPS 1&2 POOLED	6	238.000	238.000	239.778
2	0.82 3	3	243.333	243.333	239.778
3	2.5 3	3	228.333	228.333	228.333
4	5.5 3	3	206.667	206.667	206.667
5	20 3	3	195.667	195.667	195.667
6	38 3	3	9.200	9.200	9.200
7	92 3	3	2.183	2.183	2.183

6003d

File: 6003d Transform: NO TRANSFORMATION

WILLIAMS TEST (isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. MEAN	SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
GRPS 1&2 POOLED	239.778					
0.82	239.778	0.429	*	1.74	k= 1, v=17	
2.5	228.333	2.335	*	1.82	k= 2, v=17	
5.5	206.667	7.569	*	1.85	k= 3, v=17	

DP Barcode: D271849

MRID No: 45276003

20	195.667	10.226	*	1.87	k= 4, v=17
38	9.200	55.267	*	1.87	k= 5, v=17
92	2.183	56.961	*	1.88	k= 6, v=17

s = 5.855

Note: df used for table values are approximate when $v > 20$.