DATA EVALUATION RECORD
ALGAL TOXICITY TEST
GUIDELINE OPPTS 850.5400 (TIERS I AND II)

1. **CHEMICAL:** Proxitane WW-12 (Peracetic acid – 12%, Hydrogen peroxide – 18.5%)
   PC Code No.: Peracetic acid-000595, Hydrogen peroxide- 063201

2. **TEST MATERIAL:** Proxitane WW-12 **Purity**: Peracetic acid 12.12%

3. **CITATION:**
   Author: Holberg, James R.
   Title: Proxitane WW-12-Acute Toxicity to the freshwater Diatom, *Navicula pelliculosa*
   Study Completion Date: October 2, 2006
   Laboratory: Springborn Smithers Laboratories
   Sponsor: Solvay Chemicals
   Laboratory Report ID: 13857.6104
   Sponsor Protocol: 0600804/OPPTS/SA-Navicula
   DP Barcode: D334873

4. **REVIEWED BY:**

   Signature: Richard C. Petrie, Agronomist/Team Leader Date: 7/03/07
   OPP/AD/RASSB

5. **APPROVED BY:**

   Signature: **Norm Cook, Chief** **Date:** 7/9/07
   OPP/AD/RASSB

6. **STUDY PARAMETERS:**

   Definitive Test Duration: August 30 to September 12 2006, 14 Days (including recovery period)

   Type of Concentrations:
   0.12, 0.32, 0.78, 1.9, 4.8 and 12 mg a.i./mL (Nominal Stock)
   0.12, 0.34, 0.79, 2.0, 5.0 and 15 mg a.i./mL (Measured Stock)
   0.10, 0.26, 0.64, 1.6, 4.0, and 10 mg/L (Nominal Test)

7. **CONCLUSIONS:**

Results Synopsis:

	EC_{50} (mg/L)	95% Confidence Limits (mg/L)	NOEC (mg/L)
96-Hour Cell Density:	0.56	0.44 - 0.74	0.10

8. ADEQUACY OF THE STUDY:

A. Classification: **Core**

B. Rationale:

C. Repairability:

9. GUIDELINE DEVIATIONS:

The following guideline deviations were based on EPA OPPTS Guideline 850.5400:

- The geometric series of test chemical concentrations had a ratio of 2.5X, whereas the guideline ratio is between 1.5 and 2X.
- Number of replicates for range-finding test was not reported.
- Continuous lighting may have facilitated loss of test chemical due to volatilization and photolysis (MSDS No. PAA1215-1103, revised 11/10/03 indicates potential for photolysis).
- Small deviations for test conditions, such as pH and photoperiod; these deviations are not considered large enough to significantly affect the results of the study.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ *Navicula pelliculosa*	▪ *Navicula pelliculosa,* strain 664
Initial Number of Cells ▪ 10,000 cells/mL minimum.	▪ 1.0×10^4 cells/mL
Stock Culture ▪ 3 to 7 days old	▪ 5 days old
Nutrients ▪ Standard formula (ASTM E1218-20) ▪ pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) ▪ Freshly prepared	▪ Freshly prepared ▪ Algal Assay Procedure medium prepared with sterile, deionized water (composition provided on page 23, table 1) ▪ pH 7.5 ± 0.1

B. Test System

Guideline Criteria	Reported Information
Solvent ▪ Upper limit - 0.5 mL/L	▪ Not Reported
Temperature ▪ 20° ± 2°C (*Skeletonema*) ▪ Recorded hourly	▪ 24° ± 2°C ▪ Measured Continuously
Light Intensity ▪ 4.3 K lx (± 10%) (*Navicula*) ▪ Photosynthetically active radiation approx. 66.5 ± 10% µEin/m^2/sec	▪ 3900 to 4700 lux ▪ 40-80 µE/m^2/sec
Photoperiod ▪ 14-hr light/10-hr dark for *Skeletonema* only. Continuous lighting for *Navicula pelliculosa.*	▪ Continuous illumination
pH ▪ 8.1 ± 0.1 (*Skeletonema*) ▪ Measured at beginning and end of test	▪ pH 6.8 ± 0.2 ▪ Measured at beginning and end of test
Oscillation Rates ▪ 60 cycles/min (*Skeletonema*)	▪ 100 ± 10 rpm
Test Containers ▪ 125-500 mL Erlenmeyer flasks ▪ Cleaned/sterilized (solvent and acid) and conditioned ▪ Test solution volume 50% of flask volume	▪ Sterile 250 mL Erlenmeyer flasks ▪ 100 mL liquid ▪ Flasks covered in stainless steel caps that permitted gas exchange
Dilution Water ▪ Sufficient quality (e.g., ASTM Type I) ▪ Saltwater - commercial or modified synthetic formulation added to distilled/deionized water (30 ppt or 24-35 g/kg)	▪ Purified (sterile and deionized) reagent water

C. Test Design

Guideline Criteria	Reported Information
Range-Finding Test ▪ Water solubility and physical-chemical properties of test chemical determined? ▪ Validated analytical method developed? ▪ Expose algae to widely spaced (e.g. log interval) chemical concentration series ▪ Lowest value should be at detection limit ▪ Upper value, for water soluble compounds, should be at saturation concentration ▪ Minimum of 3 replicates ▪ Algae should be exposed for 96 hours ▪ If highest concentration (saturation concentration or 100 mg/L) results in <50% reduction in growth, definitive test may not be necessary ▪ If lowest concentration (detection limit) results in >50% reduction, definitive test necessary	▪ Number of replicates not reported ▪ Nominal concentrations of 1.0, 2.0, 4.0, 8.0, 16, 32, and 64 mg/L tested ▪ Percent inhibition of cell density in the 1.0 mg/L solution relative to the control was 61%
Dose Range ▪ 1.5X -2X progression	▪ 2.5X progression
Doses ▪ 5 or more concentrations of test substance in a geometric series ▪ > 90% growth inhibited or stimulated at highest concentration or concentrations bracket expected EC_{50}	▪ 6 concentrations tested in a geometric series ▪ 98% of control growth inhibited at highest concentration
Controls ▪ Negative and/or solvent each test ▪ Positive - zinc chloride (periodically)	▪ Negative controls employed ▪ No positive control reported
Replicates Per Dose ▪ 3 or more (4 or more for *Navicula*)	▪ 4 replicates
Duration of Test ▪ 96-hr	▪ 96-hr
Growth ▪ Logarithmic growth (controls) by 96-hr or repeat test ▪ 1.5 x 10^6 cells/mL (*Skeletonema*)	▪ 96-hour cell density in control averaged 38.19 x 10^4 cells/mL
Daily Observations?	▪ Yes.

Method of Observations ▪ Direct - microscopic cell count of at least 400 cells/flask ▪ Indirect - spectrophotometry, electronic cell counter, dry weight, etc; calibrated by microscopic count ▪ Qualitative and descriptive	▪ Direct cell counts using a hemacytometer and compound microscope ▪ At least 400 algal cells/flask counted
Cell Separation ▪ Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula)*	▪ Orbital shaker table provided a shaking rate of 100 ± 10 rpm
Algistatic and algicidal effects differentiated?	▪ Yes, the chemical was determined to have algistatic effect

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	▪ Yes, pages 3 and 4.
Detailed information on test organisms included (scientific name, method of verification, strain, and source)?	▪ Yes, page 11.
Growth in controls reported?	▪ Yes, page 26 (table 4)
Description of test system and test design included?	▪ Yes.
Initial and final chemical concentrations and pH measured?	▪ Yes, page 24 (table 2)
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	▪ Yes, page 26 (table 4)
96-hr EC_{50} and when sufficient data generated 24-, 48-, and 72-hr EC_{50}, and 95% C.I. reported?	▪ Yes, page 27 (table 5)
Raw data included?	▪ Yes, page 26 (table 4)
Methods and data records reported?	▪ Yes.
Statistical Analysis ▪ Mean and standard deviation calculated and plotted? ▪ Goodness-of-fit determined?	▪ Yes/No. Mean and standard deviation plotted page 28 (figure 1) and calculated page 26 (table 4). Goodness-of-fit not determined

Dose Response

Table 1. Summary of Cell Density after 24, 48, 72, and 96 hours					
Nominal Concentration (mg/L)	Cell Density ($x10^4$ cells/mL)(SD)				Percent Inhibition
	24-hr	48-hr	72-hr	96-hr	
Control	2.50(1.50)	2.19(0.52)	11.13(3.63)	38.19(7.73)	NA
0.10	1.50(0.46)	3.50(1.37)	6.19(1.05)	32.94(2.57)	14
0.26	1.94(1.40)	2.56(0.55)	6.31(2.52)	29.69(7.32)	22
0.64	2.00(1.02)	2.19(1.66)	3.25(1.37)	16.06(3.27)	58
1.6	1.31(1.33)	1.00(0.68)	2.94(2.58)	6.44(1.91)	83
4.0	0.75(0.41)	1.00(0.41)	0.75(0.20)	0.31(0.24)	99
10	0.69(0.24)	0.75(0.35)	0.56(0.43)	0.63(0.32)	98

Statistical Results

Statistical Method:
Data were checked for normality using the Shapiro Wilks' Test and for homogeneity of variance using the Bartlett's Test. If the data sets passed the tests for homogeneity and normality, then Williams' Test was used to determine the NOEC. If the data sets did not pass the tests for homogeneity and normality, then Kruskal-Wallis' Test was used to determine the NOEC. All statistical determinations were at the 95% level of certainty, expect for Shapiro Wilks and Bartlett's Tests, which were at the 99% level of certainty. The computer program TOXSTAT Version 3.5 was used to calculate both the EC_{50} values and 95% confidence limits. If less than the required response was observed, the EC_{50} value was empirically estimated to be greater than the highest concentration.

Results Synopsis:

	EC_{50} (mg/L)	95% Confidence Limits (mg/L)	NOEC (mg/L)
24-Hour Cell Density:	2.2	0.45 - 5.6	Not Reported
48-Hour Cell Density:	1.3	0.52 - 2.9	Not Reported
72-Hour Cell Density:	0.37	0.12 - 0.60	Not Reported
96-Hour Cell Density:	0.56	0.44 - 0.74	0.10
72-Hour Biomass:	Not Reported	Not Reported	Not Reported
72-Hour Growth Rate:	Not Reported	Not Reported	Not Reported

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Results were verified using the TOXANAL screening program.

Results Verification Synopsis:

	EC_{50} (mg/L)	95% Confidence Limits (mg/L)	NOEC (mg/L)
96-Hour Cell Density:	.50	.33 and .72	Unable to be calculated.
72-Hour Biomass:	Unable to be calculated.	Unable to be calculated.	Unable to be calculated.
72-Hour Growth Rate:	Unable to be calculated.	Unable to be calculated.	Unable to be calculated.

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SPAN          G                LC50          95 PERCENT CONFIDENCE LIMITS
4              1.706694E-02                    .5042142       .4263768
.5913441


RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS    G                H              GOODNESS OF FIT PROBABILITY
3              9.593944E-02                    2.57148        3.582229E-02

SINCE THE PROBABILITY IS LESS THAN 0.05. RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE     =      1.874914
95 PERCENT CONFIDENCE LIMITS  = 1.294177     AND     2.455652

LC50 =           .4982037
95 PERCENT CONFIDENCE LIMITS = .3322929 AND  .7204733

LC10 =           .1047232
95 PERCENT CONFIDENCE LIMITS = 4.305126E-02 AND  .1764655
**********************************************************************
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
?
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14. REVIEWER'S COMMENTS:

The study provided adequate data and closely followed GUIDELINE OPPTS 850.5400 (TIERS I AND II). Photodegradation and volatilization may have occurred due to continuous lighting. Adjustments to lighting may be necessary in further tests of this chemical.