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: Hoberg, James R.
   Title: Proxitane WW-12 – Acute Toxicity to the Marine Diatom, *Skeletonema costatum,* Under Static Conditions
   Study Completion Date: 25 September 2006
   Laboratory: Springborn Smithers Laboratories
   Sponsor: Solvay Chemicals
   Laboratory Report ID:13857.6103
   Sponsor Protocol: 091704/OPPTA/SA-Skeletonema
   DP Barcode: D334873
   MRID No.: 46966607

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 10 to 18 2006, 9 days (including the recovery period)

   Type of Concentrations:
   1.2, 2.4, 4.8, 9.7, 19, 39, and 78 mg a.i./mL (Nominal Stock)
   0.83, 2.4, 4.2, 7.7, 17, 31, and 74 mg a.i./mL (Measured Stock)
   1.0, 2.0, 4.0, 8.0, 16, 32, and 64 mg/L (Nominal Test)

7. **CONCLUSIONS:**

Results Synopsis:

	EC_{50} (mg/L)	95% Confidence Limits (mg/L)	NOEC (mg/L)
96-Hour Cell Density:	27	22 - 32	16

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:

- Number of initial cells should be a minimum of 10,000. Logarithmic growth (1.5×10^6 cells/mL) should be reached in 96 hours. After 96 hours in this study, the mean cell density was reported as 1.0×10^6 cells/mL. While this deviation is not considered significant enough to invalidate this test, future studies should start with a higher initial cell count or be extended to 120 hours to compensate for slow growth rate.
- Temperature range was 1 degree Celsius lower than the range defined in the guidelines; this deviation is not considered large enough to significantly affect the results of the study.
- Upper limit of the solvent was not reported
- Final chemical concentrations and method of strain verification were not reported.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ *Skeletonema costatum*	▪ *Skeletonema costatum*, strain CCMP 1332
Initial Number of Cells ▪ 10,000 cells/mL (*Skeletonema*), minimum	▪ Initial number of cells were approximately 7.7×10^4 cell/mL
Stock Culture ▪ 3 to 7 days old	▪ 6 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 ▪ Artificially Enriched Seawater (AES) ▪ pH 8.1 ± 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	▪ 19° ± 2°C ▪ Monitored continuously
Light Intensity ▪ 4.3 K lx (± 10%) (*Selenastrum, Skeletonema, Navicula*) ▪ Photosynthetically active radiation approx. 66.5 ± 10% µEin/m^2/sec	▪ 360-440 footcandles (3.9 to 4.7 K lx) ▪ Photosynthetically active radiation ranged from 62 to 77 µEin/m^2/sec
Photoperiod ▪ 14-hr light/10-hr dark (*Skeletonema*)	▪ 14-hr light/10-hr dark
pH ▪ 8.1 ± 0.1 (*Skeletonema*) ▪ Measured at beginning and end of test	▪ 7.2 to 8.9 ▪ Measured at beginning and end of test
Oscillation Rates ▪ 60 cycles/min (*Skeletonema*)	▪ 60 ± 10 rpm
Test Containers ▪ 125-500 mL Erlenmeyer flasks ▪ Cleaned/sterilized (solvent and acid) and conditioned ▪ Test solution volume 50% of flask volume	▪ 250 mL sterilized Erlenmeyer flasks ▪ Flasks were fitted with stainless steel caps which permitted gas exchange ▪ Test solution volume did not exceed 50% of flask volume
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)	▪ AES ▪ Purified (sterile and deionizied) 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	▪ Concentrations of 0.0083, 0.083, 0.83, 8.3 and 83 mg/L ▪ Two exposures were established for each concentration and the control ▪ 96-hour exposure ▪ The highest concentration tested, 83 mg/L, had a 100% reduction of growth compared to control values. ▪ The lowest concentration tested, 0.00083 mg/L, had a 17% reduction of growth compared to the control.
Dose Range ▪ 1.5X -2X progression	▪ 2X 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}	▪ 7 concentrations of test substance in a geometric series ▪ 100% growth was inhibited at the highest concentration tested
Controls ▪ Negative and/or solvent each test ▪ Positive - zinc chloride (periodically)	▪ Untreated AES medium used for negative control ▪ Positive control not reported
Replicates Per Dose ▪ 3 or more (4 or more for *Navicula*)	▪ 3 replicates per dose
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*)	▪ 1.0 x 10^6 cells/mL at 96 hr
At 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 cells counts of at least 400 cells, or until four fields were counted, using a hemacytometer and compound microscope
Cell Separation ▪ Manual or rotary shaking only (*Selenastrum, Skeletonema, Navicula)*	▪ Orbital table shaker at 60 ± 10 rpm.
Algistatic and algicidal effects differentiated?	▪ Yes, the substance was determined to have an 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/No, page 11 contains the organisms' scientific name, strain, and source. ▪ Method of verification is not reported.
Growth in controls reported?	▪ Yes/No, Growth in negative control reported on page 26 (table 4); Positive control with zinc chloride was not reported.
Description of test system and test design included?	▪ Yes.
Initial and final chemical concentrations and pH measured?	▪ Yes/No; Initial and final pH measurements reported on page 24 (table 2). ▪ Initial chemical concentrations of stock solutions are reported on page 25 (table 3). ▪ Final chemical concentrations not reported.
Initial, 24-, 48-, 72- and 96-hr cell densities measured? % of inhibition or growth and other adverse effects reported?	▪ Yes/No, percent inhibition and cell densities at 24-, 48-, 72-, and 96-hr are reported on page 26 (table 4). Initial cell density for each replicate is not reported. It is estimated to 7.7×10^4 (page 14).
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). However, initial cell density for each replicate is not reported. It is estimated to 7.7×10^4 (page 14).
Methods and data records reported?	▪ Yes.

Statistical Analysis	
▪ Mean and standard deviation calculated and plotted? ▪ Goodness-of-fit determined?	▪ Mean and standard deviation reported on page 26 (table 4) and the means are plotted on page 28 (Figure 1); Standard deviation is not plotted ▪ Goodness-of-fit not determined.

Dose Response

Table 1. Summary of Cell Density after 24, 48, 72, and 96 hours

Nominal Concentration (mg/L)	Mean Cell Density ($x10^4$ cells/mL)(SD)				Percent Inhibition
	24-hr	48-hr	72-hr	96-hr	
Control	17.75(2.61)	24.08(4.40)	65.67(13.50)	99.67(23.03)	NA
1.0	17.58(5.27)	32.67(4.65)	57.08(7.29)	93.58(18.96)	6
2.0	17.17(5.49)	24.58(4.86)	55.33(3.69)	84.92(17.67)	15
4.0	20.92(3.76)	30.67(4.65)	69.42(20.25)	86.92(18.15)	13
8.0	14.67(3.39)	22.58(2.47)	36.00(11.79)	89.75(30.62)	10
16	10.67(2.79)	13.50(1.52)	32.92(7.50)	98.00(3.03)	2
32	1.08(1.01)	3.50(2.84)	7.42(6.69)	29.83(22.62)	70
64	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	100

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 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:	18	14-21	Not reported
48-Hour Cell Density:	15	14-18	Not reported
72-Hour Cell Density:	13	7.0-20	Not reported
96-Hour Cell Density:	27	22-32	16
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:	22	4.2 and 1.7 $x10^{38}$	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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RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS     G                H                GOODNESS OF FIT PROBABILITY
 5              .9898366        28.74733          0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

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

SLOPE      =       1.61263
95 PERCENT CONFIDENCE LIMITS  = 8.215785E-03      AND     3.217045

LC50 =             21.50518
Overflow
95 PERCENT CONFIDENCE LIMITS = 4.2364 AND  1.701412E+38

LC10 =             3.507599
Overflow
95 PERCENT CONFIDENCE LIMITS = 5.877472E-39 AND  11.12947
**************************************************************************
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
? _
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14. REVIEWER'S COMMENTS:

As previously noted, there were small deviations from the protocol, which we do not believe would impact the conclusions of the study.

The authors clearly presented their study and associated results.

Our verification statistics indicated that the EC50 for the 96-hour cell density was 22 mg/L, which is just within the 95% confidence interval that was calculated by the study's author.

With the exception of the instances noted above, the author's study followed guideline OPPTS 850.5400 (TIERS I AND II).

The MSDS sheet Peroxitane Peracetic Acid, No. PAA1215, revised 11/10/03 indicates that Peroxitane may be sensitive to photolysis. However, test vessels were covered during this test.

Cell density should be increased to 10,000 cells or greater or extend the test to 120 hours in order to achieve proper growth rate.