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 Freshwater Blue-Green Alga, *Anabaena flos-aquae*
   Study Completion Date: September 26 2006
   Laboratory: Springborn Smithers Laboratory
   Sponsor: Solvay Chemicals
   Laboratory Report ID: 13857.6105
   Sponsor Protocol: 110404/OPPTS/SA-Anabaena
   DP Barcode: D334873
   MRID No.: 46966606

4. **REVIEWED BY:**

   Signature: **Richard C. Petrie, Agronomist/Team Leader** **Date:** 7/05/07
   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 20 2006, 11 Days (including recovery period)
   Type of Concentrations:
   1.2, 2.4, 4.8, 9.7 and 19 mg a.i./mL (Nominal Stock)
   0.83, 2.4, 4.2, 7.7 and 17 mg a.i./mL (Measured Stock)
   1.0, 2.0, 4.0, 8.0 and 16 mg/L (Nominal Test)

7. **CONCLUSIONS:**

Results Synopsis:

	EC_{50} (mg/L)	95% Confidence Limits (mg/L)	NOEC (mg/L)
96-Hour Cell Density:	1.5	1.2 to 1.7	1.0

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 replicates in the range finding study was not reported.
- Zinc Chloride was not used as a positive control.
- Upper limit of the solvent was not reported.
- Following the 96-hour exposure period, the recovery phase was inadvertently initiated with a composite of the next lowest concentration to that which completely inhibited algal growth; this deviation had no effect on the results or interpretation of this test.
- Logarithmic growth was not reported at 96 hours.
- Final chemical concentration at 96 hours was not reported.
- Continuous illumination was used, however, this factor is not expected to have resulted in significant loss of test chemical to photodegradation and/or volatilization.

10. SUBMISSION PURPOSE: Registration

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species ▪ *Selenastrum capricornatum (Raphidocelis subcapitata)* ▪ *Skeletonema costatum* ▪ *Anabaena flos-aquae* ▪ *Navicula pelliculosa*	▪ *Anabaena flos-aquae*, strain 1444
Initial Number of Cells ▪ 10,000 cells/mL (*Skeletonema*)	▪ Inoculum of 8.3×10^4 cells/mL ▪ Initial number of cells (at 0 hour) were approximately 1.0×10^4 cells/mL
Stock Culture ▪ 3 to 7 days old	▪ 3 days old
Nutrients ▪ Standard formula (ASTM E1218-20) ▪ pH 7.5 ± 0.1 (*Selenastrum, Navicula, Anabaena*), 8.1 ± 0.1 (*Skeletonema*) ▪ Freshly prepared	▪ Algal Assay Procedure medium ▪ pH 7.5 ± 0.1 ▪ Freshly prepared

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 ▪ Continuously recorded
Light Intensity ▪ 4.3 K lx (± 10%) (*Selenastrum, Skeletonema, Navicula*) ▪ Photosynthetically active radiation approx. 66.5 ± 10% µEin/m^2/sec	▪ Methods indicate that the light intensity would range from 1600 to 2700 lux (150 to 250 footcandles) ▪ Reported light intensity during the experiment ranged from 1600 to 2300 lux (150 to 210 footcandles) (Table 2).
Photoperiod ▪ 14-hr light/10-hr dark (*Skeletonema*). Continuous soft white light is acceptable for other test species.	▪ Continuous illumination
pH ▪ 8.1 ± 0.1 (*Skeletonema*) ▪ Measured at beginning and end of test	▪ Measured at beginning and end of test ▪ pH 6.5 to 7.5
Oscillation Rates ▪ 60 cycles/min (*Skeletonema*)	▪ Shaking rate of 100 ± 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 sterile Erlenmeyer flasks containing 100 mL medium ▪ Covered with stainless steel caps which 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 deionizied) reagent water ▪ Similar to culture medium, tested for toxic compounds in agreement with ASTM guidelines (2002). ▪ Ppt was not reported.

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	▪ Nominal concentrations of 0.0010, 0.010, 0.10, 1.0, 10 and 100 mg/L ▪ Number of replicates not reported for preliminary test ▪ 96-hour exposure ▪ The highest concentration tested, 100 mg/L, had a 100% reduction of growth compared to control values. ▪ The lowest concentration tested, .0010 mg/L, had a 0% reduction of growth compared to control values
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}	▪ 5 concentrations in a geometric series ▪ 100% growth inhibited at highest concentrations
Controls ▪ Negative and/or solvent each test ▪ Positive - zinc chloride (periodically)	▪ Solvent control. ▪ Positive zinc chloride control not reported (note that 100% growth inhibited at highest concentration)
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*)	▪ Cell density of 5.825 x 10^5 cells/mL (control) were reached at 96-hr ▪ Cell growth is plotted in Figure 1 (page 29), and after a brief visual inspection, it does not seem like there was logarithmic growth.

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 Shaking rate of 100 ± 10 rpm
Algistatic and algicidal effects differentiated?	▪ Yes, the test substance was determined to have an algalstatic 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 organism's scientific name, strain, and source. ▪ Method of verification is not reported.
Growth in controls reported?	▪ Yes/No Growth in negative control reported on page 27 (Table 4); Positive control with zinc chloride was not reported. ▪ Note that highest concentration resulted in 100% growth inhibition (Table 4).
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 25 (Table 2). ▪ Initial chemical concentrations of stock solutions are reported on page 26 (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 27 (Table 4). Adverse effects, such as small and/or pale cells, is also reported in Table 4. ▪ Initial cell density for each replicate is not reported. It is estimated to 1.0×10^4 (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 28 (Table 5)

Raw data included?	▪ Yes, page 27 (Table 4). However, initial cell density for each replicate is not reported. It is estimated to 1.0 x 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 determined, page 27 (Table 4) ▪ Mean density is plotted in Figure 1 (page 29). ▪ 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 (x 10^4 cells/mL) (Standard Deviation)				Percent Inhibition
	24-hr	48-hr	72-hr	96-hr	
Control	2.29(1.13)	2.42(2.44)	14.71(11.14)	58.25(31.29)	NA
1.0	2.67(2.32)	2.83(1.48)	28.13(19.83)	59.08(22.34)	-1
2.0	2.88(2.55)	0.67(1.15)	3.08(1.61)	10.38(3.57)	82
4.0	0.00(0.00)	0.08(0.14)	0.00(0.00)	0.00(0.00)*	100
8.0	0.00(0.00)	0.33(0.58)	0.00(0.00)	0.00(0.00)*	100
16	0.00(0.00)	0.67(1.15)	0.00(0.00)	0.00(0.00)*	100

* Significantly reduced as compared to the control, based on Williams' test.

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 desired 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:	2.7	1.5-3.0	Not reported
48-Hour Cell Density:	1.8	1.2-3.1	Not reported
72-Hour Cell Density:	1.6	1.5-1.7	Not reported
96-Hour Cell Density:	1.5	1.2-1.7	1.0
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:	1.6	Unable to be Calculated	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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CONC.          NUMBER          NUMBER          PERCENT         BINOMIAL
               EXPOSED         DEAD            DEAD            PROB.(PERCENT)
 16             100             100             100             0
 8              100             100             100             0
 4              100             100             100             0
 2              100             82              82              0
 1              100             0               0               0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE
CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL TEST WOULD BE
UNRELIABLE.  USE THE CONFIDENCE INTERVALS FROM THE OTHER TESTS.


AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1.604223


WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE
PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

**********************************************************************
DO YOU WISH TO RUN ANOTHER DATA SET?
ENTER Y OR N.
? _
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14. REVIEWER'S COMMENTS:

The authors clearly presented their study and associated results.

Our verification statistics indicated that the EC50 for the 96-hour cell density was 1.6 mg/L, which is well 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).

Excessive photodegradation and volatilization may have occurred due to continuous illumination. A MSDS sheet for Peroxitane Peracetic Acid No. PPA1215-1103, revised 11/10/03 (on the internet) indicates that Peroxitane is not significantly volatile in air, and that it

has significant photolysis in air. Excessive photolysis may have occurred, however, it is not expected that a significant loss of test chemical occurred because the test vessels are covered during the test.