DATA EVALUATION RECORD
AQUATIC PLANT TOXICITY USING *LEMNA* SPP.
GUIDELINE OPPTS 850.4400

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 (Lot No. BT14974; CAS No. 79-21-0 peroxyacetic acid)
Purity: 12.08% peracetic acid

3. **CITATION:**

Author: James R. Hoberg
Title: Proxitane WW-12 – Toxicity to Duckweed, *Lemna gibba*
Study Completion Date: 20 September 2006
Laboratory: Springborn Smithers Laboratories, 790 Main Street, Wareham, Massachusetts 02571-1037
Sponsor: Solvay Chemicals. 1130 Battleground Road, LaPorte, Texas 77571
Study Report ID: Springborn Smithers Study No. 13857.6101
Laboratory Report ID: Springborn Smithers Protocol No.: 032803/OECD/OPPTS/SA-Lemna/Solvay
DP Barcode: D334873
MRID No.: 46966604

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**

Study Type: Aquatic Plant Toxicity Test
Definitive Study Duration: 7-day (21 April to 3 May which includes dry weight determination)

7. **CONCLUSIONS**

Results Synopsis:

Biomass: (mg/L)

EC50 = 230 (220-240), 7-day value.
EC05 = 33 (24-38)

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 850.4400:

- Positive controls were not examined
- Concentration response curves with 95% confidence limits and goodness-of-fit delineations were not provided.
- Volatilization and evaporation of the test solution were not reported and therefore we cannot determine if less than 20% of the test solution was lost. Photolysis is suspected due to continuous lighting.
- Stock culture information such as the age of the cultures was not reported.
- Small deviations for test conditions, such as light, photoperiod, pH, and ratio of the geometrically increasing concentrations for the dose range; 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: ▪ *L. gibba* G3 and *L. minor* ▪ Cultures obtained from laboratory or commercial sources. ▪ Stock culture grown from a single isolated plant should be used to inoculate all the flasks in a given test. ▪ Axenic stock cultures should be grown in an aquarium for 2 weeks prior to use.	▪ *Lemna gibba,* Strain 310 ▪ Springborn Smithers (Wareham) culture originally obtained on 7 October 2005 from the University of Toronto, Canada ▪ Inoculum two days since previous transfer in fresh media
Plants: ▪ Three to five plants consisting of three to four fronds each per replicate.	▪ 5 plants each with two to four fronds per replicate

B. Test System

Guideline Criteria	Reported Information

Nutrient Media: ▪ M-Hoagland's or 20X-AAP nutrient media ▪ Medium should be prepared prior to each transfer of *Lemna* cultures and for preparation of new test solutions during the course of the test. ▪ If M-Hoagland's medium is used, pH is adjusted to between 4.8 and 5.2 by addition of 0.1N or 1 N NaOH. ▪ If 20X-AAP medium is used pH is adjusted to 7.5 ± 0.1 with 0.1 N NaOH or HCL.	▪ 20X-APP nutrient media with pH adjusted to 7.5 ± 0.1 with dilute HCl or NaOH ▪ Prepared fresh on day of test initiation (day 0)
Test Container: ▪ At least three replicate containers should be used for each concentration, each containing 150 mL of test solution, or enough test solution to result in a volume-to-vessel size ratio of 2:5. ▪ Test containers may be 250-mL glass beakers or Erlenmeyer flasks, large enough to hold 150 mL of test solution and *Lemna* colonies without crowding for the duration of the test. ▪ The same number of replicates should be used for each test concentration and control. ▪ Test containers should be randomly placed in the environmental chamber.	▪ Three replicate 270-mL crystallizing dishes per concentration and control with each containing 100 mL dilution water and 0.10 mL test solution ▪ Test containers were randomly placed, based on a computer-generated random numbers calculation, in the environmental chamber and re-assigned positions on day 3 and 5
Test Apparatus: ▪ Controlled environment growth chamber or enclosed area capable of maintaining the specified number of growth chambers and test parameters required ▪ All glassware and equipment should be cleaned following good laboratory practice. Nytex screen or inoculating loops used for transferring the *Lemna* should be disposed of after use or thoroughly cleaned and sterilized before reuse.	▪ The test was conducted in a controlled environmental growth chamber ▪ A GLP document was provided and signed
Temperature: ▪ Environmental chamber maintained at 25 ± 2EC	▪ Temperature ranged from 23-24°C ▪ Continuously measured throughout experiment

pH: ▪ If M-Hoagland's medium is used pH is adjusted to between 4.8 and 5.2 ▪ If 20X-AAP medium is used pH is adjusted to 7.5 ± 0.1 ▪ Test solution pH may vary from the nutrient medium after addition of the test chemical and/or carrier. Changes should be recorded but not adjusted. ▪ Report pH of test chemical in test solutions prior to use and discarding on days 3, 5 and 7.	▪ pH adjust to 7.5 ± 0.1 using HCl or NaOH for the 20X-AAP medium ▪ During the 7-day study, the pH ranged from 5.7 to 7.8 (day 0) and 5.7 to 9.1 (day 7) for the test solution ▪ pH reported at the beginning and end of study
Photoperiod and Light Intensity: ▪ Continuous warm-white fluorescent lighting should be used to provide a light intensity in the range of 4,200 and 6,700 lux. ▪ Light intensity at each position in the incubation area should be measured and should not differ by more than 15 percent of selected light intensity.	▪ Lighting ranged from 625 to 800 footcandles (6700 to 8600 lux) ▪ Photosynthetically active radiation = 100 to 123 $\mu E/m^2/s$ ▪ 24-hr light cycle
Transfer of Colonies: ▪ The colonies should be transferred to test solution on day 0, and to replacement solutions on days 3 and 5 (to prevent nutrient limitation or depletion). ▪ No more than 20 percent of the test substance should be lost by volatilization (or other processes) between replacements. ▪ Transfer should be done in a clean, draft-free area as quickly as possible to minimize contamination of the colonies.	▪ Colonies were exposed to the test solution on day 0 and replacement solutions were not renewed during the course of the study as per the Sponsor's request ▪ Test solution was added to the test vessels by being spiked directly into the vessel and stirred gently ▪ Volatilization and evaporation data were not reported
Observation of Colonies: ▪ Observation of frond numbers and appearance should be made of the colonies on day 0, 3, 5 and 7.	▪ Observations were made on day 0, 3, and 7
Preparation of Stock Solutions or Growth Media ▪ Stock solutions or growth media should be prepared just prior to use and diluted with water of high quality such as glass-distilled, deionized water, or ASTM Type I to obtain the test solutions ▪ pH of test solutions should be measured prior to and after use. ▪ Stock solutions of substances with low aqueous solubility may be prepared by use of organic solvents	▪ The 20X-AAP media was prepared with sterile, deionized water which was periodically analyzed for pesticides, PCBs, toxic metals, and TOC concentration ▪ pH was tested on day 0 with a range of 5.7 to 7.8 and on day 7 with a range of 5.7 to 9.1 ▪ Test solution (830 mg/mL) was prepared on day 0 by placing 20.6977 g of the test material in a 25-mL flask and bringing it to volume with deionized water. It was then diluted accordingly to obtain the test concentrations to be used in the study

Solvents ▪ When solvent or carrier used, second set of controls should be prepared with highest concentration of substance ▪ Concentration should not exceed 0.5 mL/L	▪ The solvent used was sterile, deionized water

C. Test Design

Guideline Criteria	Reported Information
Replacement of Nutrient Media: ▪ Replace nutrient media on day 3 or 5, or as needed to prevent nutrient limitation or depletion of test chemical but not required. ▪ In 14 day test renewal may be necessary every 3 to 5 days.	▪ Media was not replaced during the course of the study as per the Sponsor's request
Doses/Dose Range: ▪ At least five concentrations of chemical, exclusive of controls, in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, 64 mg/L). ▪ The concentration range should be selected to define the concentration response curve between EC5 and EC90. ▪ The range of chemical concentrations should result in the highest concentration affecting at least 90 percent of the fronds and lowest concentration affecting no more than 5 percent of fronds compared with controls. Or, test concentrations should bracket the expected EC50 value.	▪ The nominal test concentrations used were 6.6, 13, 26, 52, 110, 210, 420, and 830 mg/L, which brackets the EC50 values. ▪ The nominal stock concentrations used were 0.80, 1.6, 3.1, 6.3, 13, 25, 50, and 100 mg/a.i./mL ▪ The measured stock concentrations were 0.80, 1.3, 26, 52, 110, 210, 420, and 830 mg/L ▪ Ratio is between 1.94 to 2.12

Preliminary (Range-Finding) Test: ▪ Perform range-finding test to establish whether a definitive test is necessary and to determine the concentrations for the definitive test. ▪ Expose *Lemna* to chemical concentration series (e.g., 0.1, 1.0, 10, 100, 1,000 mg/L) plus controls. ▪ Minimum of three replicates of 3 to 5 plants consisting of three to four fronds each should be added to each test chamber. ▪ Select plants of similar size and the number of plants and number of fronds should be identical or near identical as possible in each test chamber. ▪ At least 12, but no more than 16 fronds, per test chamber recommended. ▪ Plants exposed to equal volumes of each chemical concentration for 7 days. ▪ The highest test concentration should be at least 1,000 mg/L (except for pesticide testing under FIFRA). ▪ If range-finding test showed that the highest concentration of chemical tested (not less than 1,000 mg/L or the maximum pesticide label application rate) had no effect on *Lemna*, report the results and measured concentrations and a statement that the chemical is not phytotoxic. ▪ If range-finding test showed greater than 50 percent effect with a test concentration below the analytical detection limit, report the results and a statement that the chemical is phytotoxic below the analytical detection limit.	▪ *Lemna* exposed to 0.010, 0.1, 1.0, 10, and 100 mg/L concentrations of the test substance for a 7-day range-finding test ▪ There were 2 replicates per exposure including the control ▪ Frond density for each exposure was 271, 233, 279, 338, and 192 frond/replicate, respectively, and the control averaged 263 fronds/replicate ▪ All fronds were considered normal except those exposed to the highest concentration which were curled and smaller than the control ▪ The number of initial fronds per replicate were not reported

Controls: ▪ Controls consist of same nutrient medium, number of fronds, environmental conditions, and procedures as the test containers except that none of the chemical is added. ▪ If a solvent or carrier is used to dissolve or suspend the test chemical, additional controls containing the solvent or carrier should be included. ▪ The upper limit of the carrier volume is 0.5 mL/L and same amount of carrier should be added to each test concentration. ▪ Positive controls using zinc chloride should be run periodically.	▪ 3 controls with 20X-AAP media and 5 plants with two to four fronds were tested similarly to that of the test containers ▪ No positive control was tested
Replicates Per Dose: ▪ For each concentration and control at least three replicate containers should be used. ▪ Three to five plants consisting of three to four fronds each should be used. ▪ Fewer replicates, each containing a greater number of colonies, may be used. But the test containers and solution volumes will have to be adjusted accordingly.	▪ 3 replicates/dose were tested ▪ 5 plants with two to four fronds were used
Duration of Test: ▪ 7-days	▪ 7-days
Observations: ▪ Colonies should be inspected for changes in frond number and appearance at the beginning of day 0, days 3 and 5, and at the end of the exposure (day 7). ▪ On day 7 count the number of living and/or dead fronds.	▪ Examinations occurred on day 0, 3, 5 and 7 of the study

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements included in report?	Yes, pages 3 and 4
Concentration response curves should be plotted for total frond number, growth rate (as number of fronds per day) and mortality (percentage of dead fronds to total number of fronds).	Yes, pages 32 to 34 with pages 33 and 34 containing bar graphs rather than curves
Means and standard deviations for frond number, growth rate, and percent frond mortality calculated and plotted for each treatment and control.	Yes/No. Means and SDs were calculated for frond density/number, growth rate, and dry weight and only the means were plotted for each treatment and control, pages 26 to 34

Concentration response curves with 95 percent confidence limits delineated, goodness-of-fit determination, and EC5s, EC50s, and EC90s, LOECs, and NOECs identified.	Yes/No. EC5s, EC50s, EC90s, LOECs, NOECs, and 95 percent confidence limits were identified. Concentration response curves with 95 percent confidence limits and goodness-of-fit determinations were not provided, pages 27, 29, and 31
Report any change in frond development of appearance such as increase in number (a frond is counted regardless of size as long as it is visible adjacent to the parent frond), decrease in size, necrosis, chlorosis, etc. Also report any additional observations such as sedimentation of test solution, sinking of fronds, or other abnormalities.	Yes, pages 18-20, 26, 28, and 30

Dose Response The following tables provide a summary of the results.

Table 1. Summary of Frond Production and Density Results and Observations Reported

Mean measured concentration mg/L (mg a.i./mL)	Nominal concentration mg/L (mg a.i./mL)	Fronds/replicate				
		Replicate	Day 3	Day 5	Day 7	7-day Inhibition[a]
Control	Control	A	75	179	379	NA[c]
		B	57	140	332	
		C	55	130	287	
		Mean	**62**	**150**	**333**	
		SD[b]	**11**	**26**	**46**	
6.6 (0.80)	6.6 (0.80)	A	73	149	331	-1
		B	66	146	375	
		C	57	130	306	
		Mean	**65**	**142**	**337**	
		SD	**8**	**10**	**35**	
13 (1.3)	13 (1.6)	A	59	160	411	-14
		B	68	163	368	
		C	76	174	354	
		Mean	**68**	**166**	**378**	
		SD	**9**	**7**	**30**	
26 (2.9)	26 (3.1)	A	69	162	360	-15
		B	70	173	402	
		C	64	154	382	
		Mean	**68**	**163**	**381**	
		SD	**3**	**10**	**21**	
52 (6.3)	52 (6.3)	A	49	126	348	3
		B	46	112	321	
		C	49	126	297	
		Mean	**48[de]**	**121[de]**	**322**	
		SD	**2**	**8**	**26**	
110 (9.1)	110 (13)	A	42	122	291	12
		B	50	120	317	
		C	45	110	272	

Table 1. Summary of Frond Production and Density Results and Observations Reported

Mean measured concentration mg/L (mg a.i./mL)	Nominal concentration mg/L (mg a.i./mL)	Fronds/replicate				
		Replicate	Day 3	Day 5	Day 7	7-day Inhibition[a]
		Mean	**46^{def}**	**117^{def}**	**293**	
		SD	**4**	**6**	**23**	
210 (23)	210 (25)	A	51	105	207	42
		B	48	90	184	
		C	49	115	187	
		Mean	**49^{df}**	**103^{defg}**	**193^{gj}**	
		SD	**2**	**13**	**13**	
420 (37)	420 (50)	A	29	36	58	81
		B	38	45	71	
		C	31	44	65	
		Mean	**33^{defg}**	**42^{defh}**	**65^{defgj}**	
		SD	**5**	**5**	**7**	
830 (120)	830 (100)	A	15	15	9	97
		B	18	17	11	
		C	17	16	10	
		Mean	**17^{defhi}**	**16^{defhi}**	**10^{dfhij}**	
		SD	**2**	**1**	**1**	

Table found on page 26 of study report.

a Percent inhibition relative to control
b SD = standard deviation
c NA = not applicable
d Fronds were observed to be smaller compared to the control.
e Fronds were observed to be curled.
f Fronds were observed to have less root formation compared to the control.
g Fronds were observed to be slightly chlorotic.
h Fronds were observed to be chlorotic.
i Fronds were observed to be bleached.
j Significantly reduced compared to the control, based on Williams' Test.

Table 2. Summary of Growth Rates Results

Mean measured concentration mg/L (mg a.i./mL)	Nominal concentration mg/L (mg a.i./mL)	Average Growth Rate (days^{-1})				
		Replicate	Day 0-3	Day 0-5	Day 0-7	7-day Inhibition[a]
Control	Control	A	0.55	0.52	0.48	NA[c]
		B	0.46	0.47	0.46	
		C	0.45	0.46	0.44	
		Mean	**0.49**	**0.48**	**0.46**	
		SD[b]	**0.06**	**0.04**	**0.02**	
6.6 (0.80)	6.6 (0.80)	A	0.54	0.49	0.46	0
		B	0.51	0.48	0.48	
		C	0.46	0.46	0.45	
		Mean	**0.50**	**0.47**	**0.46**	
		SD	**0.04**	**0.02**	**0.02**	
13 (1.3)	13 (1.6)	A	0.47	0.50	0.49	-4
		B	0.52	0.50	0.48	

Table 2. Summary of Growth Rates Results

Mean measured concentration mg/L (mg a.i./mL)	Nominal concentration mg/L (mg a.i./mL)	Average Growth Rate ($days^{-1}$)				
		Replicate	Day 0-3	Day 0-5	Day 0-7	7-day Inhibition[a]
		C	0.56	0.52	0.47	
		Mean	**0.52**	**0.51**	**0.48**	
		SD	**0.04**	**0.01**	**0.01**	
26 (2.9)	26 (3.1)	A	0.53	0.50	0.47	-4
		B	0.53	0.52	0.49	
		C	0.50	0.49	0.48	
		Mean	**0.52**	**0.50**	**0.48**	
		SD	**0.02**	**0.01**	**0.01**	
52 (6.3)	52 (6.3)	A	0.41	0.45	0.47	0
		B	0.39	0.43	0.46	
		C	0.41	0.45	0.44	
		Mean	**0.40**	**0.44**	**0.46**	
		SD	**0.01**	**0.01**	**0.01**	
110 (9.1)	110 (13)	A	0.35	0.44	0.44	4
		B	0.41	0.44	0.45	
		C	0.38	0.42	0.43	
		Mean	**0.38**	**0.44**	**0.44**	
		SD	**0.03**	**0.01**	**0.01**	
210 (23)	210 (25)	A	0.42	0.41	0.39	17
		B	0.40	0.38	0.37	
		C	0.41	0.43	0.38	
		Mean	**0.41**	**0.41**	**0.38[d]**	
		SD	**0.01**	**0.03**	**0.01**	
420 (37)	420 (50)	A	0.23	0.19	0.20	52
		B	0.32	0.23	0.23	
		C	0.25	0.23	0.22	
		Mean	**0.27**	**0.22**	**0.22[d]**	
		SD	**0.05**	**0.03**	**0.02**	
830 (120)	830 (100)	A	0.00	0.00	-0.08	113
		B	0.06	0.03	-0.05	
		C	0.04	0.01	-0.06	
		Mean	**0.04**	**0.01**	**-0.06[d]**	
		SD	**0.03**	**0.01**	**0.01**	

Table found on page 28 of study report.
a Percent inhibition relative to the control.
b SD = standard deviation
c NA = not applicable
d Significantly reduced compared to the control, based on Williams' Test.

Table 3. Summary of Dry Weight (Biomass) Results

Mean measured concentration mg/L (mg a.i./mL)	Nominal concentration mg/L (mg a.i./mL)	Frond Dry Weight (mg)		
		Replicate	Day 7	7-day Inhibition[a]
Control	Control	A	36.6	NA[c]

Statistical Results

Statistical Method: The Shapiro-Wilks' and Bartlett's Tests were used to determine normality and homogeneity of variance, respectively. If the data sets passed for homogeneity and normality, the Williams' Test was used to determine the NOEC and LOEC. If the data sets did not pass for homogeneity and normality, the Kruskal-Wallis' Test was used to determine NOEC and LOEC. EC5s, EC50s, and EC90s were calculated using TOXSTAT version 3.5 (Gulley et al., 1996) as were LOEC and NOEC statistical determinations.

Results Synopsis:

Frond Density:

LOEC	= 210
NOEC	= 110
7-day EC05	= 39 (31-65)
7-day EC50	= 230 (210-250)
7-day EC90	= 640 (610-660)

Growth rate:

LOEC	= 210
NOEC	= 110
7-day EC05	= 85 (45-120)
7-day EC50	= 400 (380-410)
7-day EC90	= 670 (650-680)

Biomass:

LOEC	= 110
NOEC	= 52
7-day EC05	= 33 (24-38)
7-day EC50	= 230 (220-240)
7-day EC90	= 580 (520-630)

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: A statistical program called Toxanal was used to verify results. The screen caps from the results of the program appear below.

Results Verification Synopsis:

Frond production/density:

LOEC	= Not calculated in Toxanal
NOEC	= Not calculated in Toxanal
7-day EC10	= 84 (37-126)
7-day EC50	= 222 (154-324)
7-day EC90	= Not calculated in Toxanal

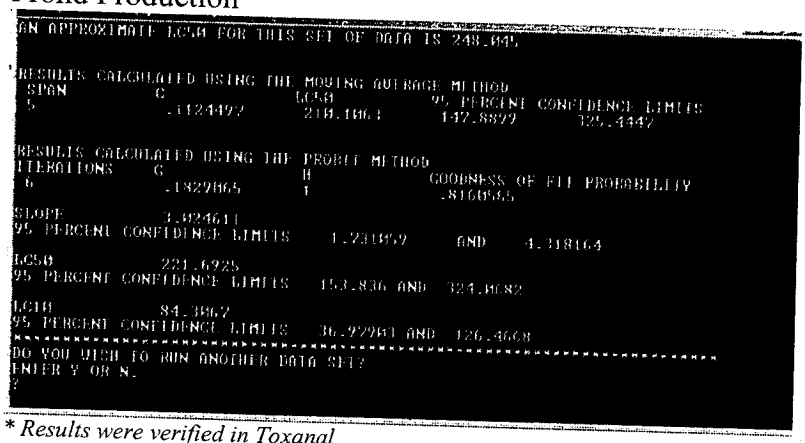
Growth rate:

LOEC	= Not calculated in Toxanal
NOEC	= Not calculated in Toxanal
7-day EC10	= 177 (124-221)
7-day EC50	= 351 (293-423)
7-day EC90	= Not calculated in Toxanal

Biomass:

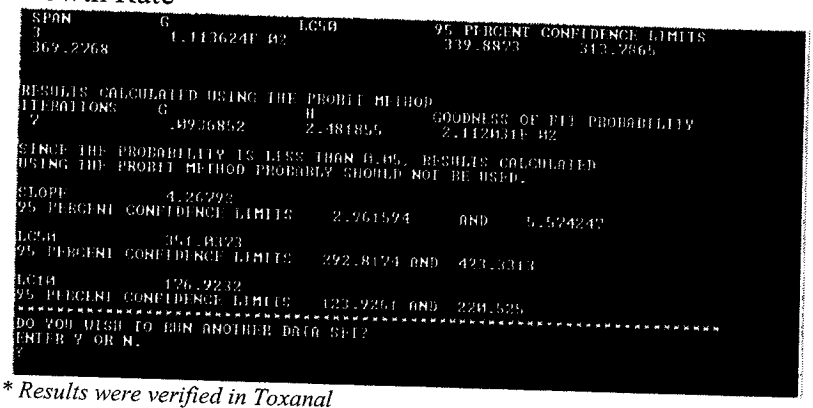
LOEC	= Not calculated in Toxanal
NOEC	= Not calculated in Toxanal
7-day EC10	= 72 (46-97)
7-day EC50	= 206 (164-261)
7-day EC90	= Not calculated in Toxanal

Frond Production



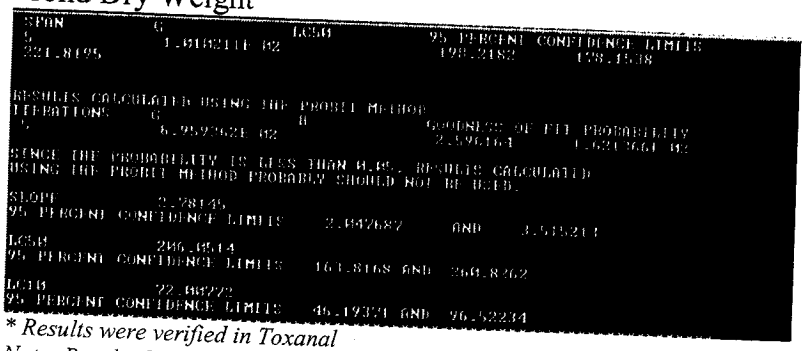
** Results were verified in Toxanal*
Note: Results for nominal concentrations

Growth Rate



** Results were verified in Toxanal*
Note: Results for nominal concentrations

Frond Dry Weight



** Results were verified in Toxanal*
Note: Results for nominal concentrations

14. REVIEWER'S COMMENTS:

The study deviates slightly from the guidelines as is mentioned above in section 9; however, the deviations probably did not interfere with the conclusions of the study. The quality assurance and GLP papers were given and the statistical results were similar to those calculated by the *Toxanal* program. Therefore, the conclusions were acceptable in estimating aquatic plant toxicity. Photodegradation of test chemical during the study is suspected due to continuous lighting (continuous lighting is not a deviation form guidelines, however, may have been a factor for this chemical (based on a review of MSDS No. PAA1215-1103, revised 11/10/03 – "Abiotic degradation – air – significant photolysis").

15. REFERENCES:

Gulley, D.D., Boetler, A.M. and Bergman, H.L. 1996 TOXSTAT Release 3.5. University of Wyoming, Laramie, Wyoming.