Accession No. 411114-04

DATA EVALUATION RECORD

1. **CHEMICAL:** Sulfosate
Shaughnessey No: 128501.

2. **TEST MATERIAL:** ICIA0224 (technical material), N-phosphonomethylglycine trimethylsulphonium salt, 57.5% active ingredient (w/w), batch reference WF1002/Lot 2, a yellow liquid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants.
Species Tested: Selenastrum capricornutum.

4. **CITATION:** Smyth, D.V. and J.F. Tapp. 1988. ICIA0224: Determination of Toxicity to the green alga Selenastrum capricornutum. Laboratory Project No. FT54/88. Conducted by ICI PLC, Brixham Laboratory, Brixham Devon TQ5 8BA, England. Submitted by ICI Americas Inc. EPA Accession No. 411114-04.

5. **REVIEWED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: July 27, 1989
9/7/89

6. **APPROVED BY:**

Isabel C. Johnson, M.S.
Principal Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Isabel C. Johnson
Date: August 4, 1989

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 9/8/89

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a Tier-2 growth and reproduction of a non-target green alga test. The 4-day EC50 value of ICIA0224 for Selenastrum capricornutum was calculated to be 21.6 mg/L nominal concentration. Based on the reduction of cell counts, the NOEC was estimated to be 8 mg/L nominal concentration. Study is core (4 day test is acceptable) C. Lewis 9/6/89

8. **RECOMMENDATIONS:** N/A.

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9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** Selenastrum capricornutum (Strain ATCC 22662) used in this test came from laboratory cultures at the testing facility, maintained under axenic conditions. A culture of the alga was grown in the test medium under the test conditions and it was used as inoculum for the test upon reaching the exponential growth phase.

B. **Test System:** Test vessels were 250-mL borosilicate glass conical flasks closed with polyurethane foam bungs. Each flask contained 100 ml of test solution. The cultures were incubated at 24 $\pm$ 1°C, under continuous illumination, with orbital shaking at 100 rpm. The culture medium used for the test was described in the report.

C. **Dosage:** Four-day growth and reproduction test.

D. **Design:** The nominal concentrations selected for the test were 1, 2, 4, 8, 16, 32, and 64 mg/L of the test substance as received. Each test solution was prepared by adding an aliquot of the appropriate stock solution to sterile culture medium. The control solution consisted of culture medium only.

Six replicates of the control and three replicates of each test concentration were employed in the test. One blank vessel (without algal inoculum) for each control and treatment was incubated concurrently. The positions of the vessels in the incubator were randomized by rows. Each replicate test vessel was inoculated with 0.255 ml of the inoculum culture to give a nominal density of 1.0 x 10^4 cells/ml. Three 100-ml volumes of Coulter electrolyte, inoculated in the same manner, had a mean measured cell density of 1.05 x 10^4 cells/ml, and this value was used for the growth calculations.

The algal cell densities of the inoculum and test cultures were determined by electronic particle counting. After 24, 48, 72, and 96 hours, samples were removed from each test and blank vessel. The appropriate blank particle count was subtracted from that of the test culture to obtain the cell density.

The pH of test solutions was measured at the start of the test in the excess remaining after filling the test vessels, and at the end of the test in two of the replicate test solutions and control. The temperature of the incubator was measured daily. The light intensity was measured once during the study. At the start of the test, samples were taken from each test solution using the excess remaining after filling the test vessels, and were analyzed for concentration of the test substance. At the end of the test, each blank solution was sampled and analyzed in the same manner. Analysis for the test substance was not carried out in the solutions containing algal cells.

E. **Statistics:** The area under the growth curve (Days 0 to 4) was calculated for each replicate vessel as follows:

$$\text{Area} = \frac{(N_0+N_1)-2N_0}{2} \times t_1 + \frac{(N_1+N_2)-2N_0}{2} \times (t_2-t_1) + \frac{(N_{n-1}+N_n)-2N_0}{2} \times (t_n-t_{n-1})$$

Where: N_0 = Cell density at start of test (x 10^4 cells/ml),
N_1 = Cell density at t_1,
N_n = Cell density at t_n,
t_1 = Time (days) of first measurement after start of test,
t_n = Time (days) of n^{th} measurement after start of test.

These areas were examined using one-way analysis of variance. Dunnett's test was used to identify significant differences ($p = 0.05$) from the control.

The mean areas under the growth curves were expressed as percentages of that of the control and were transformed to probability scale and analyzed by linear regression against log concentration, to estimate the median effective concentration (based on biomass curve area, E_bC50) and its 95% confidence limits.

The average (Days 0 to 4) growth rate of each replicate vessel was calculated using the following formula:

$$\text{Growth rate} = \frac{\ln (N_2/N_1)}{t}$$

Where: N_1 = Cell density at start (x 10^4 cells/ml),
N_2 = Cell density at end (x 10^4 cells/ml),
t = Time interval = 4 days.

These data were analyzed as described for the area method.

12. **REPORTED RESULTS:** The pH at the start and end of the test ranged from 6.6 to 7.1 and from 7.8 to 10.2, respectively. The daily temperature ranged from 23.9 to 24.2°C. The light intensity, measured once during the study, was 6,430 Lux. Mean measured concentrations of the test solutions during the study ranged from 94 to 106% of the nominal concentrations.

The algal cell densities measured at each time period, the mean areas under the growth curves, and the mean growth rates are shown in Tables 1, 2, and 3 (attached), respectively. Figure 1 (attached) presents the four-day growth curves of S. capricornutum at different test concentrations. The results are summarized as follows:

	Based on	
	Areas under the growth curves	Growth rates
NOEC (mg/L)	8	8
LOEC (mg/L)	16	16
EC50 (mg/L) (95% C.L.)	19 (11 - 33)	>64 (36 - >64)

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the authors. No quality assurance measures were reported. A statement was included, indicating that "this report is not subject to the requirements of 40 CFR Part 160."

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

o The maximum label rate was not provided in the report.

o The micronutrient stock solution used to prepare the culture and test medium contained 150 mg/L of $Na_2EDTA.2H_2O$. According to Subdivision J guidelines, EDTA should not be used in the experimental medium.

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o The pH of the nutrient medium (measured in the control solution at test initiation) was 7.1. The SEP recommends the pH of 7.5.

o The light intensity (measured once during the test) was 6.43 Klux, instead of the recommended 4.0 Klux.

o Each test solution was inoculated with an algal density of 10,500 cells/ml. The SEP recommends the initial cell concentration of 3,000 cells/ml.

o The SEP states that the duration of the test for algae must be a minimum of 5 days. This test was conducted for only 4 days.

B. **Statistical Analysis:** Analysis of variance and Dunnett's test were performed to compare cell counts at each treatment level to those of the control (attached). The results showed that nominal concentrations $\geq$ 16 mg/L reduced the cell counts of S. capricornutum at test termination (day 4). The NOEC was determined to be 8 mg/L. The results confirmed the analyses performed by the authors even though different parameters were used (Note: The authors analyzed the mean area under growth curves and mean growth rates, instead of the number of cell counts).

The reviewer calculated the percentage inhibition by comparing the mean number of cell counts per milliliter after 4 days of exposure to the mean control value and used EPA's Toxanal computer program to calculate the EC50 value (attached). The EC50 value calculated by the moving average method was 21.6 mg/L (95% C.L. = 19.2-24.6 mg/L) which is similar to the authors' calculation using the percentage inhibition based on the area under growth curves.

C. **Discussion/Results:** This study is scientifically sound. However, it does not fulfill the guideline requirements for a growth and reproduction test using non-target plants since the test was conducted for only 4 days. A minimum of 5 days is required for algae. The 4-day EC50 value of ICIA0224 for S. capricornutum was 21.6 mg/L nominal concentration. Based on the reduction of cell counts, the NOEC was determined to be 8 mg/L nominal concentration.

D. **Adequacy of the Study:**

(1) **Classification:** ~~Supplemental.~~ CORE CR Lewis 9/6/89

(2) **Rationale:** The duration of the test was only 4 days. A minimum of 5 days is required.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, July 27, 1989.

Analysis of Variance File: icia1 Date: 07-27-1989

FILTER: None

N's, means and standard deviations based on dependent variable: Cell DENSITY (cells/ml)

* Indicates statistics are collapsed over this factor

Factors: C	Nominal Conc (mg/L)	N	Mean	S.D.
*		27	2133407.5000	972424.1900
1	Control	6	2816666.8000	244185.7340
2	1	3	2863333.2000	240277.6090
3	2	3	2500000.0000	185202.5940
4	4	3	2673333.2000	344286.6900
5	8	3	2630000.0000	338673.8800
* 6	16	3	1756666.6200	650410.1200
* 7	32	3	989666.6900	368171.6200
* 8	64	3	154333.3280	35949.0390

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Fmax for testing homogeneity of between subjects variances: 327.34

Number of variances= 8 df per variance= 2.

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Analysis of Variance Dependent variable: DENSITY

Source	df	SS (H)	MSS	F	P
Between Subjects	26%	24585829.0000E+06			
C (CONC)	7%	22517410.0000E+06%	3216773.0000E+06	29.549	0.0000
Subj w Groups	19%	2068418920000.0000%	108864152000.0000		

Analysis of Variance File: icia1 Date: 07-27-1989

FILTER: None

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	2816666.800	6	1756666.620
2	2863333.200	7	989666.690
3	2500000.000	8	154333.328
4	2673333.200		
5	2630000.000		

Comparison	Dunnett	
1 < 2		
1 > 3		
1 > 4		
1 > 5		
1 > 6	0.0100	*
1 > 7	0.0100	*
1 > 8	0.0100	*
2 > 3	N.A.	
2 > 4	N.A.	
2 > 5	N.A.	
2 > 6	N.A.	
2 > 7	N.A.	
2 > 8	N.A.	
3 < 4	N.A.	
3 < 5	N.A.	
3 > 6	N.A.	
3 > 7	N.A.	
3 > 8	N.A.	
4 > 5	N.A.	
4 > 6	N.A.	
4 > 7	N.A.	
4 > 8	N.A.	
5 > 6	N.A.	
5 > 7	N.A.	
5 > 8	N.A.	
6 > 7	N.A.	
6 > 8	N.A.	
7 > 8	N.A.	

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

KOSALWAT ICIA0224 SELENASTRUM CAPRICORNUTUM 07-27-89

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
64	100	95	95	0
32	100	65	65	0
16	100	38	38	0
8	100	7	7	0
4	100	5	5	0
2	100	11	11	0
1	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 21.75181

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.578944E-02	21.6323	19.23378	24.56649

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.4046931	13.1494	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 = 2.092585

95 PERCENT CONFIDENCE LIMITS = .7613769 AND 3.423794

LC50 = 19.741

95 PERCENT CONFIDENCE LIMITS = 9.746961 AND 55.34234

LC10 = 4.880501

95 PERCENT CONFIDENCE LIMITS = .5003509 AND 9.855707

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Sulfosate ecological effects review

Page _____ is not included in this copy.

Pages 10 through 13 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

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Shaughnessy No. 128501 Chemical Name Sulfosate (ICIA0224) Chemical Class ____ Page 1 of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validati Status
14-Day Single Dose Oral LD_{50} Species ____ Lab Acc.		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)= Slope= # Animals/Level= Age(Days)= Sex = 14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( ) Comments:	____	____
14-Day Single Dose Oral LD_{50} Species ____ Lab Acc.		LD50 = mg/kg. (95% C.L) Contr. Mort.(%)= Slope= # Animals/Level= Age(Days)= Sex = 14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( ) Comments:	____	____
8-Day Dietary LC_{50} Species Lab ____ Acc.		LC50 = ppm (95% C.L.) Contr. Mort.(%)= Slope= # Animals/Level= Age(Days)= Sex = 8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( ) Comments:	____	____
8-Day Dietary LC_{50} Species Lab ____ Acc.		LC50 = ppm (95% C.L) Contr. Mort.(%)= Slope= # Animals/Level= Age(Days)= Sex = 8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( ) Comments:	____	____
48-Hour LC_{50} Species Lab ____ Acc.		LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)= Slope= # Animals/Level= Temperature = 48-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( ) Comments:	____	____
96-Hour EC_{50} Species Selenastrum Capricornutum 57.5 Lab ICI PLC, Brixham Lab. Acc. 411114-04		EC50 = 21.6 ppm * (95% C.L. 19.2 - 24.6) Con. Mor(%)= N/A Sol. Con. Mor.(%)= N/A Slope= N/A Cell density # Animals/Level= 10,500 cells/ml Temp.= 24C Inhibition 96-Hour Dose Level ppm/(%Mortality) 1 (0), 2 (11), 4 (5), 8 (7), 16 (38), 32 (65), 64 (95) Comments: Based on nominal concentrations	PK 7-27-89	Suppl
96-Hour LC_{50} Species Lab ____ Acc.		LC50 = pp__ (95% C.L) Con. Mort.(%)= Sol. Con. Mort.(%)= Slope= # Animals/Level= Temp.= 96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( ) Comments:	____	____