MRID # 402369-01

DATA EVALUATION RECORD

uses propylamine salt

1. **CHEMICAL:** N-(phosphonomethyl) glycine
   Shaughnessey No. 103601.

2. **TEST MATERIAL:** Technical glyphosate, 96.6% a.i., Lot No.
   NBP-3594465, CAS No. 1071-83-6, a white solid

3. **STUDY TYPE:** Nontarget area phytotoxicity, aquatic plant
   growth - <u>Selenastrum</u> <u>capricornutum</u>.
   or Kirchneria subcapitata

4. **CITATION:** Hughes, J.S. 1987. The Toxicity of Glyphosate
   Technical to <u>Selenastrum</u> <u>capricornutum</u>. Project No. 1092-
   02-1100-1. Prepared by Malcolm Pirnie, Inc. White Plains,
   NY. Submitted by Monsanto Agricultural Company,
   Chesterfield, MO. Mrid No. 402369-01.

5. **REVIEWED BY:**

   Bruce A. Rabe   Signature: *[signature]*
   Aquatic Toxicologist
   Hunter/ESE   Date: 12/6/88

                                    [signature] /sw/

6. **APPROVED BY:**

   Prapimpan Kosalwat, Ph.D.   Signature: P. Kosalwat
   Staff Toxicologist
   KBN Engineering and   Date: Dec. 6, 1988
   Applied Sciences, Inc.

   Henry Craven   Signature: *[signature]* Henry T Craven
   Supervisor, EEB/HED
   USEPA   Date: 1/9/90

7. **CONCLUSIONS:** This study appears scientifically sound. The
   7-day EC50 for Glyphosate Technical was 13.8 mg/L.
   4-day EC50   12.54 mg/L   CRL   9/19/91

8. **RECOMMENDATIONS:** N/A   *(11.91 to 13.56 m,/L)*

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS**: An algal assay bottle test on _Selenastrum_ _capricornutum_, obtained from stock cultures, was conducted by the laboratory of Malcolm Pirnie, White Plains, New York. The test was conducted for 7 days in a Sherer Model RI-32-LLTP Incubator. The test flasks were continuously shaken at 100 oscillations per minute with continuous illumination of 4306 $\pm$ 650 lumens/m^2 provided by cool-white fluorescent lights. Temperature was maintained at 24 $\pm$ 2 $^{\circ}$C.

Test bottles utilized were sterile 250-mL Erlenmeyer flasks fitted with foam stoppers. Three replicates were used for each treatment.

Nominal tests concentrations of 10, 18, 32, 56, and 100 mg/L were prepared by diluting appropriate volumes of a 5.0 mg a.i./mL stock solution to 50 mL volumes with sterile-filtered AAP medium. Test and control solutions were inoculated with algae from a 7-day old stock culture to give an initial cell count of 3000 cells/mL.
Growth, as measured by cell counts, was determined on test days 2, 3, 4, and 7 using a Coulter Counter Model ZBI equipped with a C-1000 Channelyzer and MHR Computer. Three counts per replicate were made. All counts were multiplied by the appropriate conversion factors (for sample dilution and volume counted) to yield cells/mL.

Samples were analyzed by Monsanto Company, Chesterfield, MO for actual concentrations of glyphosate on test days 0 and 7. Samples on day 0 before inoculation and samples passed through a 0.8-micron membrane filter on day 7 were placed in polyethylene bottles and frozen prior to shipment to Monsanto Company. Samples were analyzed by a high pressure liquid chromatograph (HPLC) equipped with an o-phthalaldehyde (OPA) post-column reactor (PCR) and fluorescence detector.

The EC25 and EC50 values for glyphosate were calculated by plotting the log of average measured concentration (x-axis) against the percent inhibition expressed as probit (y-axis). Inverse estimation least squares linear regression was used to determine the line of best fit, the concentrations corresponding to 25 and 50 percent inhibition and associated 95% confidence limits. Parameters of the regression line were determined using the SAS statistical package. Only the values of the three lowest test concentrations were used in the regression analysis.

12. **REPORTED RESULTS:** Mean standing crop (cells/mL) and Percent Inhibition, Relative to Control, for <u>Selenastrum capricornutum</u> Exposure to Glyphosate Technical

Mean Measured Concentration[a] mg/L	Day 2	Day 3	Day 4	Day 7	Percent Inhib.[b]	
<0.05 (0)	119333	741333	3333333	7000000	--	*Day 4*
10.6 (10)	113000	616667	2696667	6346667	9.3	*19*
19.6 (18)	20000	29000	37000	168333	97.6	*98.8*
35.2 (32)	1000	13667	11667	11000	99.8	*99.6*
58.8 (56)	9667	120000	10000	9333	99.9	*99.7*
104 (100)	7667	10000	8667	8333	99.9	*99.7*

a   The nominal concentrations are given in parentheses
b   The percent inhibition is based on day 7 values

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
Based on mean standing crop, the 7-day EC25 was 11.4 mg/L and the 7-day EC50 was 13.8 mg/L. The 95% confidence limits for these EC values could not be determined from the data since an error condition arises in the calculations as a result of an attempt to take the square root of a negative number. The measured concentrations on day 7 yielded an average of 109.9% of the nominal concentrations.

The study was conducted following the intent of the Good Laboratory Practice Regulations and the final report was reviewed by Malcolm Pirnies' Quality Assurance Unit. A Quality Assurance Statement was included and signed by the Quality Assurance Officer.

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

A. <u>Test Procedure</u>: The procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

o Growth observations were only taken on days 2, 3, 4, and 7 instead of daily as recommended.

B. Statistical Analysis: The reviewer used the Toxanal computer program to calculate the EC50 value. These calculations are attached. The moving average method provides a 7-day EC50 value of 13.8 mg/L with a 95 percent confidence interval of 13.3 to 14.3 mg/L which is similar to that reported by the author.

C. Discussion/Results: The study results appear to be scientifically valid. The 7-day EC50 values based upon measured concentrations was estimated to be 13.8 mg/L.

D. Adequacy of the Study:

    (1) Classification: ~~(to be added)~~ Core 9/19/91 CRC

    (2) Rationale: N/A

    (3) Repairability: N/A

15. Completion OF ONE-LINER FOR STUDY: Yes, 11-30-88

No. ____						Reviewer/ Date	Validation Status
Study/Species/Lab/ Succession	Chemical % a.i			Results			

14-Day Single Dose Oral LD$_{50}$

LD50 = __ mg/kg (‾‾‾‾ 95% C.L ‾‾‾‾) Contr. Mort.(%)=

Species ____   Slope= __ # Animals/Level= __ Age(Days)= __ Sex =

Lab ____   14-Day Dose Level mg/kg/(% Mortality)
( ), ( ), ( ), ( ), ( )

Acc. ____   Comments:

14-Day Single Dose Oral LD$_{50}$

LD50 = __ mg/kg (‾‾‾‾ 95% C.L ‾‾‾‾) Contr. Mort.(%)=

Species ____   Slope= __ # Animals/Level= __ Age(Days)= __ Sex =

Lab ____   14-Day Dose Level mg/kg/(% Mortality)
( ), ( ), ( ), ( ), ( )

Acc. ____   Comments:

8-Day Dietary LC$_{50}$

LC50 = __ ppm (‾‾‾‾ 95% C.L. ‾‾‾‾) Contr. Mort.(%)=

Species ____   Slope= __ # Animals/Level= __ Age(Days)= __ Sex =

Lab ____   8-Day Dose Level ppm/(% Mortality)
( ), ( ), ( ), ( ), ( )

Acc. ____   Comments:

8-Day Dietary LC$_{50}$

LC50 = __ ppm (‾‾‾‾ 95% C.L ‾‾‾‾) Contr. Mort.(%)=

Species ____   Slope= __ # Animals/Level= __ Age(Days)= __ Sex =

Lab ____   8-Day Dose Level ppm/(% Mortality)
( ), ( ), ( ), ( ), ( )

Acc. ____   Comments:

8-Day Dietary LC$_{50}$

LC50 = __ pp__ (‾‾‾‾ 95% C.L. ‾‾‾‾) Contr. Mort.(%)= Sol. Contr. Mort.(%)=

Species ____   Slope= __ # Animals/Level= __ Temperature =

Lab ____   96-Hour Dose Level pp /(% Mortality)
( ), ( ), ( ), ( )

Acc ____   Comments:

7-day EC$_{50}$
Species *Selenastrum*
capricornutum 96.6

Lab Malcolm Pirnie

Acc. MRID 40236901

EC50 = 13.8 ppm (* ‾‾‾ 95% C.L. ‾‾‾ 13.3-14.3) Con. Mor(%)=NA Sol. Con. Mor.(%)=NA

Slope= NA # Animals/Level= N A   Temp.=24±2°C   BAR 11/30/88

7-Day Dose Level ppm/% Inhibition *
10.6(9.3)19.6(97.6)35.2(99.8)58.8(99.9)104(99.9)

Comments: * Mean measured concentration.

96-Hour LC$_{50}$

LC50 = __ pp__ (‾‾‾‾ 95% C.I. ‾‾‾‾) Contr. Mort.(%)= Sol. Contr. Mort.(%)=

Species ____   Slope= __ # Animals/Level= __ Temp.=

Lab ____   96-Hour Dose Level pp /(% Mortality)
( ), ( ), ( ), ( ), ( )

Acc. ____   Comments:

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
104	100	100	100	0
56	100	100	100	0
32	100	100	100	0
18	100	99	99	0
10	100	19	19	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 12.15491

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	1.928673E-02		12.15491	11.61468
12.67096				

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
104	100	100	100	0
58.8	100	100	100	0
35.2	100	100	100	0
19.6	100	99	99	0
10.6	100	19	19	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 12.99974

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	1.928673E-02		12.99974	12.39616
13.57747				

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
GOODNESS OF FIT PROBABILITY		
12	5.995906E-02	1
.9999924		

SLOPE   =   12.00296
95 PERCENT CONFIDENCE LIMITS = 9.063854   AND   14.94207

LC50 =   12.54426
95 PERCENT CONFIDENCE LIMITS = 11.91481 AND 13.35648

LC10 =   9.831909
95 PERCENT CONFIDENCE LIMITS = 9.112126 AND 10.40034
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