MRID # 402369-04

DATA EVALUATION RECORD

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 - Anabaena flos-aquae.

4. **CITATION:** Hughes, J.S. 1987. The Toxicity of Glyphosate Technical to Anabaena flos-aquae. Project No. 1092-02-1100-4. Prepared by Malcolm Pirnie, Inc. White Plains, NY. Submitted by Monsanto Agricultural Company, Chesterfield, MO. MRID No. 402369-04.

5. **REVIEWED BY:**

Bruce A. Rabe
Aquatic Toxicologist
Hunter/ESE

Signature: Bruce A. Rabe
Date: 12/6/88

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: Dec. 6, 1988

Henry Craven
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 1/9/89

7. **CONCLUSIONS:** This study appears scientifically sound. However, a significant difference was found between the reported 7-day EC50 value and the reviewer's 7-day EC50 calculation (i.e., reported EC50 4.4 mg/L, calculated EC50 16.5 mg/L). In addition, the reported EC25 of 2.1 mg/L was outside the reported 95 percent confidence interval of 52.0 - 161.6 mg/L.
Day 4 EC50 11.72 mg/L (10.91 to 12.48 mg/L)

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

2009177

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

11. **MATERIALS AND METHODS**: An algal assay bottle test on Anabaena flos-aquae, 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 manually shaken each working day with continuous illumination of 2153 ± 323 lumens/m^2 provided by cool-white fluorescent lights. Temperature was maintained at 24 ± 2 ^{o}C.

Test bottles utilized were sterile 500-mL Erlenmeyer flasks fitted with foam stoppers. Three replicates were used for each concentration.

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 100 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. Prior to counting, each sample was sonicated in a water bath ultrasonic cleaning machine for 5 minutes to reduce the length of the algal filaments. 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.

The value for the lowest test concentration, which was stimulatory, was omitted from the regression analysis.

12. REPORTED RESULTS: Mean standing crop (cells/mL) and Percent Inhibition, Relative to Control, for Anabaena flos-aquae Exposure to Glyphosate Technical

Mean Measured Percent Concentration[a] mg/L	Day 2	Day 3	Day 4	Day 7	Inhib.[b]	
<0.05 (0)	21333	80000	383333	1486667	--	Day 4
9.7 (10)	16333	82000	271333	1566667	-5.4	2%
18.1 (18)	10667	32000	32667	300000	79.8	92%
32.6 (32)	8333	10333	8667	10000	99.3	98%
55.1 (56)	7667	7667	6667	8333	99.4	98%
102.2 (100)	6333	8667	7333	7667	99.5	98%

[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 2.1 mg/L (95% confidence limits 52.0 - 161.6 mg/L) and the 7-day EC50 was 14.4 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 104.5% of the nominal concentrations.

typo 14.4 mg/L

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. Test Procedure: 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 linear regression analysis to calculate the EC25 and EC50 values of 12.7 mg/L and 16.5 mg/L, respectively. These calculations are attached. The calculated values are higher then the reported values (i.e., EC25 2.1 mg/L and EC50 4.4 mg/L). In addition, the reported EC25 of 2.1 mg/L was outside the reported 95 percent confidence limits (52.0 - 161.6 mg/L). The reported values were calculated with the omission of the stimulatory concentration. This omission results in artificially low EC values. The reviewer believes that the EC25 and EC50 values calculated by linear regression are a more representative set of values because stimulatory as well as inhibitory values are included in the EC determinations.

C. Discussion/Results: The study results appear to be scientifically valid. However, based on percent inhibition, the reported 7-day EC25 and EC50 values and/or the 95 percent confidence limits for the EC25 value appear to be incorrect.

D. Adequacy of the Study:

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

(2) **Rationale:** N/A

(3) **Repairability:** N/A

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

No. _____ Chemical Name Glyphosate Technical Chemical Class ____ Page 1 of ____

Study/Species/Lab/ Succession	Chemical % a.i	Results	Reviewer/ Date	Validation Status
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.(%)=		
Species		Sol. Contr. Mort.(%)= Slope= # Animals/Level=		
Lab	____	96-Hour Dose Level pp__ /(%Mortality) Temperature = ( ), ( ), ( ), ( ), ( )	____	____
Acc		Comments:		
7-day EC50		EC50 = 16.5 ppm* (95% C.L. NA) Con. Mor(%)=NA		
Species Anabaena flos-aquae		Sol. Con. Mor.(%)=NA Slope= NA # Animals/Level= N A	BAR	____
Lab Malcolm Pirnie 96.6		Temp.= 24±2°C	11/30/88	
Acc. MRID 40236904		7-Day Dose Level ppm*/%inhibition 9.7(5.4) 18.1(79.8) 32.6(99.3) 55.1(99.4) 102(99.5) * = mean measured concentration		
		Comments: Underlined Inhibition values are % stimulation		
96-Hour LC_{50}		LC50 = pp__ (95% C.I.) Contr. Mort.(%)=		
Species		Sol. Contr. Mort.(%)= Slope= # Animals/Level=		
Lab	____	96-Hour Dose Level pp__ /(%Mortality) Temp.= ( ), ( ), ( ), ( ), ( )	____	____
Acc.		Comments:		

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CHECKED BY DATE
SCALE

Acc. No. 402369-04

Linear Regression

Glyphosate Technical — Anabaena flos-aquae

Measured concentrations (mg/L)	Log	% inhibition
< 0.05		—
9.7	0.9867717	-5.4
* 18.1	1.2576766	79.8
* 32.6	1.5132176	99.3
55.1	1.7411516	99.4
102.2	2.0094509	99.5

omitted stimulatory conc.: EC25 = 0.72 mg/L; EC50 = 3.12 mg/L

Regraded: EC25 2.1 mg/L; EC50 4.4 mg/L

* 2 conc.: EC25 3.46 mg/L; EC50 7.36 mg/L

all conc.: EC25 = 14.1 mg/L; EC50 = 21.2 mg/L

first 3 conc.: EC25 = 12.7 mg/L; EC50 = 16.5 mg/L

Bruce A. Kahn
11/29/86

Lewis glyphosate anabaena 4-day

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
102.2	100	98	98	0
55.1	100	98	98	0
32.6	100	98	98	0
18.1	100	92	92	0
9.7	100	29	29	0

THE BINOMIAL TEST SHOWS THAT 9.7 AND 18.1 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 11.72472

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	3.838476E-02	11.72472	10.91027	12.47753

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	5.426955	47.23138	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 = 3.534478
95 PERCENT CONFIDENCE LIMITS =-4.69938 AND 11.76834

LC50 = 11.0957
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 4.851064
95 PERCENT CONFIDENCE LIMITS = 0 AND 16.03778

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