MRID # 402369-03

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 - Skeletonema costatum.

4. **CITATION:** Hughes, J.S. 1987. The Toxicity of Glyphosate Technical to Skeletonema costatum. Project No. 1092-02-1100-3. Prepared by Malcolm Pirnie, Inc. White Plains, NY. Submitted by Monsanto Agricultural Company, Chesterfield, MO. MRID No. 402369-03.

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: 1/9/90

7. **CONCLUSIONS:** This study appears scientifically sound. However, the pH of the algal test medium was not reported in the medium preparation procedure nor at test initiation. This is required in the guidelines for aquatic plant nontarget phytotoxicity testing. As a result, this study has been rated as supplemental. The 7-day EC50 for Glyphosate Technical was 0.77 mg/L.

Reclassified core CRL 9/19/91

Day 4 EC50 0.85 mg/L (0.46 to 1.81 mg/L) CRL 9/19/91

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

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

2009176

11. **MATERIALS AND METHODS:** An algal assay bottle test on Skeletonema costatum, 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 Psycrotherm Controlled Environment Incubator Shaker, Model G-27. The test flasks were continuously shaken at 100 oscillations per minute with illumination of 4306 ± 650 lumens/m^2 provided by cool-white fluorescent lights with a photoperiod of 14 hours light: 10 hours dark. Temperature was maintained at 20 ± 2 °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 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/L were prepared by diluting appropriate volumes of a 0.05 mg a.i./mL, 0.5 mg a.i./ml, or 5.0 mg a,i,/ml stock solution to 50 mL volumes with sterile-filtered synthetic marine algal assay nutrient medium. Test and control solutions were inoculated with algae from a 7-day old stock culture to give an initial cell count of 10,000 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 to remove algae cells 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 values for the test concentrations that were stimulatory were omitted from the regression analysis.

12. **REPORTED RESULTS**: Mean standing crop (cells/mL) and Percent Inhibition, Relative to Control, for Skeletonema costatum Exposure to Glyphosate Technical

Mean Measured Percent Concentration[a] mg/L	Day 2	Day 3	Day 4	Day 7	Inhib.[b]	
<0.05 (0)	16000	36333	76667	360667	--	Day 4
0.24 (0.1)	14000	30000	64333	327333	9.2	16 %
0.28 (0.2)	16333	36667	81333	410667	-13.6	-6 %
0.48 (0.4)	14333	24667	46000	250667	30.5	40 %
0.94 (0.8)	13333	20333	27000	76333	78.8	65 %
1.79 (1.6)	12000	15333	17333	24000	93.3	77 %
3.42 (3.2)	11333	12667	14000	15667	95.7	82 %

[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 0.37 mg/L (95% confidence limits 0.10 - 0.95 mg/L) and the 7-day EC50 was 0.64 mg/L (95% confidence limits 0.21 - 1.70 mg/L). The measured concentrations on day 7 yielded an average of 171.6% of the nominal concentrations. The day 7 measured concentrations were substantially greater than the day 0 measured concentrations in the two lowest test treatments and a significant amount of glyphosate was detected in the control sample on day 7 (i.e., control, 0.1, and 0.2 mg/L concentrations were 0.61, 0.39, and 0.34 mg/L). These high recovery values for day 7 are thought to be artifacts resulting from cross-contamination during the day 7 sampling (filtration) process, as no glyphosate was introduced at any point into the test system after day 0.

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. The following items were not included in the report and are required in the guidelines:

o The pH of the initial test medium was not stated. CRL

The following items deviated from the Guidelines: normally 8.1 -

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

o Photoperiod used during the test was 14 hours light: 10 hours dark. SEP states a photoperiod of 16 hours light: 8 hours dark will be used. TOSCA Jerry Smith CRL

B. Statistical Analysis: The reviewer used linear regression analysis to calculate the EC25 and EC50 values of 0.45 mg/L and 0.77 mg/L, respectively. These calculations are attached. The calculated values are slightly higher then the reported values (i.e., EC25 0.37 mg/L and EC50 0.64 mg/L). The reported values were calculated with the omission of the stimulatory concentration. This omission results in artificially low EC values. The review 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. Due to "cross-contamination", the day 7 measured concentrations were substantially greater than the day 0 measured concentrations in the two lowest test treatments, however, by using day 7 reported results a more conservative estimate of the EC values would be obtained. The reviewer does not feel the conservative EC values invalidate the study. But, the missing data (i.e., pH values) due critically affect the validation of this study.

D. Adequacy of the Study:

CRL 9/19/91

(1) **Classification:** ~~Supplemental~~ CORE

(2) **Rationale:** This study is classified as supplemental because of the departure from the recommended protocol as described in 14.A.

(3) **Repairability:** Yes, If the information missing under 14.A. is provided, and considered scientifically acceptable, this study can be upgraded to core.

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} 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:	____	____
8-Day Dietary LC_{50} Species Lab Acc	____	LC50 = pp__ (95% C.L.) Contr. Mort.(%)= Sol. Contr. Mort.(%)= Slope= # Animals/Level= 96-Hour Dose Level ppm /(%Mortality) Temperature = ( ), ( ), ( ), ( ), ( ) Comments:	____	____
7-day EC50 Species Skeletonema costatum 96.6 Lab Malcolm Pirnie Acc MRID 40236903		EC50 = 0.77 ppm* (95% C.L. NA) Con. Mor(%)=NA Sol. Con. Mor.(%)=NA Slope= NA # Animals/Level= N A Temp.= 20±2°C BAR 11/30/88 7-Day Dose Level ppm*/% Inhibition 0.24(9.2) 0.28(13.6) 0.48(30.5) 0.94(78.8) 1.79(93.3) 3.42(95.7) Comments: Underlined Inhibition values are % stimulation * = mean measured concentration.		____
96-Hour LC_{50} Species Lab Acc.	____	LC50 = pp__ (95% C.I.) Con. Mort.(%)= Sol. Con. Mort.(%)= Slope= # Animals/Level= 96-Hour Dose Level ppm /(%Mortality) Temp.= ( ), ( ), ( ), ( ), ( ) Comments:	____	____

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JOB ____
SHEET NO. ____ OF ____
CALCULATED BY ____ DATE ____
CHECKED BY ____ DATE ____
SCALE ____

Acc. No. 402369-03

Linear Regression

Glyphosate Technical — Skeletonema costatum

Measured conc.	log	% inhibition
<0.05		—
0.24	−0.61978	9.2
0.28	−0.5528420	−13.6
0.48	−0.318758	30.5
0.94	−0.026872	78.8
1.79	0.25285	93.3
3.42	0.534026	95.7

omitted stimulatory conc.:
EC 25 = 0.36 mg/L
EC 50 = 0.69 mg/L

Reported: EC25 = 0.37 mg/L
EC50 = 0.64 mg/L

all conc.:
EC 25 = 0.45
EC 50 = 0.77

BAR 11/29/88

HES-30778

lewis glyphosate skeletonema 4-day

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3.42	100	82	82	0
1.79	100	77	77	0
.94	100	65	65	0
.48	100	40	40	0
.28	100	0	0	0
.24	100	16	16	0

THE BINOMIAL TEST SHOWS THAT .48 AND .94 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 .6271849

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.605283E-02	.8729462	.783385	.9704216

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.3676475	8.883656	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 = 2.067895
95 PERCENT CONFIDENCE LIMITS = .8140489 AND 3.321742

LC50 = .8482726
95 PERCENT CONFIDENCE LIMITS = .4585991 AND 1.805181

LC10 = .2062455
95 PERCENT CONFIDENCE LIMITS = .023682 AND .3991199

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