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TDMS

DATA EVALUATION RECORD

PAGE 1 OF

CASE GS

Glyphosate

PM 25

CHEM 103601

Isopropylamine glyphosate (N-phosphon

BRANCH EEB

DISC

FORMULATION Technical 99.7%

FICHE/MASTER ID MCOGLY02 00124763

CITATION: Analytical Biochemistry Laboratories (198²?) Chronic Study of Glyphosate to Daphnia magna under Flow-Through Test Conditions, Project No. 28742, Study No. AB-82-036.

SUBST. CLASS=

OTHER SUBJECT DESCRIPTORS
PRIM:

| DIRECT RW TIME = | (MH) | START-DATE | END DATE |
|--------------------------------------|------|------------|--------------|
| REVIEWED BY: Dennis J. McLane | | | |
| TITLE: Wildlife Biologist | | | |
| ORG: EEB/HED | | | |
| LOC/TEL: | | | |
| SIGNATURE: <i>Dennis J. McLane</i> | | | DATE: 9-8-85 |
| APPROVED BY: Raymond W. Matheny | | | |
| TITLE: Head, Section 1 | | | |
| ORG: EEB/HED | | | |
| LOC/TEL: | | | |
| SIGNATURE: <i>Raymond W. Matheny</i> | | | DATE: 9-9-85 |

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2009160

DATA EVALUATION RECORD

1. Chemical: Glyphosate
2. Test Material: 99.7%
3. Study Type: Daphnia magna Flow-Through 21-Day Life Cycle Study
4. Study ID: McKee, M.M.; McAllister, W.A.; and Schofield, M. (1982) Chronic Toxicity of Glyphosate (AB-82-036) to Daphnia magna Under Flow-Through Test Conditions, Project No. 28742, Analytical Biochemistry Laboratories, Submitted by Monsanto Agricultural Project No. on December 27, 1982, for Registration No. 524-308, Accession No. 249160,
5. Reviewed by: Dennis J. McLane
Wildlife Biologist
EEB/HED
Signature: *Dennis J. McLane*
Date: *9-9-85*
6. Approved by: Raymond W. Matheny
Supervisory Biologist
EEB/HED
Signature: *Raymond W. Matheny*
Date: *9-9-85*
7. Conclusion:

This study is scientifically sound and meets guideline requirements. The MATC for Glyphosate is considered to be between 50 and 96 mg/L and the no-effect level is 50 mg/L.
8. Recommendation:
N/A
9. Background:

This review was made in connection with the Registration Standard for Glyphosate. The first DER was by W. Faatz in May 1983.
10. Discussion of Individual Tests:
N/A

11. Materials and Methods:

- a. Species- Daphnia magna
Source- ABC's own culture
Age- < 24 hours old (1st - instar)
Test system- (excerpted from study)

The system contained six sets of quadruplicate one liter test chambers, designated as control and level Nos. 1 through level No. 5.

- b. Design- Forty animals per level (4 replicates); 5 dose level plus control (0, 25, 50, 99, 199, and 397 mg/L).

Also, see W. Faatz DER which excerpted the entire study description.

- d. Statistics: (See attached DER by W. Faatz of excerpt from the study).

12. Reported Results: (see excerpt from W. Faatz review).

13. Study Author's Conclusion/QA Measures:

The study was conducted following the intent of the Good Laboratory Practice Regulations (12) and the final report was reviewed by Analytical Bio-Chemistry Laboratories.

14. Reviewer's Discussion and Interpretation of the Study:

- a. Test Procedures: The study followed the protocol suggested by the guidelines.
- b. Statistical Analysis: Faatz's review verified the results of the ANOVA for the young/adult/day data. It appears both the reported results and Faatz's indicated a decrease at the 99 and 199 mg/L levels.
- c. Discussion/Result: The study followed acceptable protocol following statistical and test procedures suggested by the guidelines. The reported MATC of 50 and 96 mg/L for young/adult/day, as reported by this study, the most sensitive parameter.

d. Adequacy of Study:

1. Category: Core

2. Rationale: The study followed guideline reference protocol.

3. Repairability: N/A

15. Completion of One-Liner for Study:

Completed August 23, 1985

16. CBI Appendix:

N/A

103601

MCOGLY02

DATA EVALUATION RECORD

- 1. Chemical: Roundup: Glyphosate
- 2. Formulation: Technical grade 99.7%
- 3. Citation:

Chronic Study of Glyphosate to Daphnia magna Under Flow-Through Test Conditions.

Analytical Biochemistry Laboratories
Columbia, Missouri 65205

Project No. 28742

Study No. AB-82-036

- 4. Reviewed by: Wayne C. Faatz, Ph.D.
- 5. Date Reviewed: 5/83
- 6. Test Type: Chronic Life Cycle
Test species: Daphnia magna
- 7. Reported Results:

"Chronic exposure of Daphnia magna to analytical glyphosate concentrations of 26, 50, 96, 186 and 378 mg/L resulted in reduced reproduction when compared with controls at the upper three concentration levels. No other adverse compound-related effects were observed. The maximum acceptable glyphosate concentration (MATC) in this study was considered to be between 50 and 96 mg glyphosate per liter of water and the no-effect level (NOEL) was considered to be 50 mg/l. It should be mentioned, however, that according to the proposed ASTM guidelines for chronic Daphnia sp. testing, acceptable control values for the number of young per adult per reproductive day are as low as 2.0. Thus, in the present study, only the reproduction values for the highest glyphosate - concentration group were below this standard. The potential environment impact of the intermediate glyphosate concentrations, then, may even be less than that indicated by the current study."

- 8. Reviewers Conclusion

The study is acceptable for a chronic life cycle for Daphnia magna. The inference that the potential for environment impact of glyphosate could be less than that indicated in the study should be discounted. A more comprehensive discussion is in the Reviewers Discussion/Results Section.

Material and Methods

The material/Methods is excerpted from the submitted report.

I. Test Organisms

Test specimens of Daphnia magna were obtained from an in-house daphnid culture which has been maintained by ABC for four years. The primary culture was obtained from the Columbia National Fisheries Research Laboratory (CNFRL), Columbia, Missouri, in 1977. A trace of the daphnid strain indicated that CNFRL acquired their culture from the U.S.F.W.S. Fish Control Laboratory, LaCrosse, Wisconsin, in 1960 and they obtained their culture from Pennsylvania State University in 1954. *from*

All daphnids were held in a temperature controlled area at $20 \pm 2^\circ\text{C}$. The lighting was 50-70 footcandles on a 16-hour daylight photoperiod. During the holding and test periods, the daphnids received a food preparation containing algae (Selenastrum capricornutum). Only first-instar daphnids (<24 hours old) were selected for testing.

II. Test System

A one-liter proportional diluter system described by Mount and Brungs, with modifications to allow intermittent delivery of large stock volumes of Glyphosate and diluent water into the test chambers was used. The system contained six sets of quadruplicate one liter test chambers, designated as control and level #1 through level #5. Flow-splitting chambers were utilized to thoroughly mix and divide each Glyphosate concentration for delivery to the test chambers. To minimize turbulence the influent water was introduced into the test chambers via duplicate 18 gauge hypodermic needles. One liter beakers with a notched drain covered with 50-mesh stainless steel screen to prevent escape of the daphnids were used as the test chambers. Daphnids were placed in each of the quadruplicate chambers by random assignment. A diagram of the diluter system is presented in Figure 4. Aerated ABC well water was delivered to each test chamber at a rate of 200 ml/aquarium every 120 minutes, an amount which was sufficient to replace the 1 liter rest volume 3 times in a 24 hour period. The test aquaria were immersed in a circulating water bath held at 20°C ($\pm 2^\circ\text{C}$) by thermostatically controlled heating elements.

III. Test Compound

The Glyphosate standard was received from Monsanto Chemical Company on February 26, 1982. The compound was observed to be a white powder. Compound purity was listed as 99.7%. The diluter stock solutions were prepared as needed on a weight: volume basis by dissolving in deionized water and were delivered to the diluter from a Mariotte bottle via a Teflon® delivery line.

IV. Test Procedure - Biological

The test concentrations were confirmed analytically after the test solution had been flowing through the aquaria for 24 hours. The biological phase of the study was initiated on April 5, 1982 by distributing 10 randomly selected Daphnia magna (first-instar less than 24 hours old) into each of the four exposure chambers, i.e. 40 daphnids per concentration. Reproductive success was measured by recording and discarding the offspring produced in each concentration every Monday, Wednesday and Friday for the duration of the study. Survival was also determined on these days.

In addition to the survival and reproduction data, growth of adult daphnids was determined at the termination of the test. The daphnia were placed on an absorbent background (filter paper) and measured from the apex of the helmet to the base of the spine using an ocular micrometer on a stereomicroscope. The measurement technique was accurate to the nearest 0.07 mm.

Throughout the study the test chambers were inoculated daily with algae (Selenastrum capricornutum). All chambers were cleaned every Monday, Wednesday and Friday. This was accomplished by gently removing adult daphnids from each chamber and pouring remaining water and young daphnids through a 50 mesh stainless steel screen into a clean one liter beaker. The young collected on the 50 mesh screen were placed in a petri dish to be counted before being discarded. Each chamber was cleaned with a nylon bristle brush and rinsed with ABC well water. The strained water and adult daphnids were returned to their respective chamber and returned to the test system.

V. Test Procedure - Chemical and Physical

Water quality parameters of temperature, dissolved oxygen and pH were measured on days 0, 4, 7, 14 and 21 of the study. Measurements of these selected parameters were made in the control, level #1, level #3 and level #5. In addition temperature was measured daily.

Prior to initiation of the test, the toxicant delivery system was calibrated through the use of volumetric measurement of the toxicant dispenser, thereby allowing the nominal concentrations of the test material to be calculated. The actual concentrations of the test material were determined on days 0, 4, 7, 14 and 21 during the study.

The actual concentrations were verified by the analysis of Glyphosate. The Glyphosate standard employed for spiking quality control samples was received from Monsanto Agricultural Products Company in January of 1982 as a white crystalline solid. This material was stored at -10°C prior to use. A primary stock solution of this material at 5.0 mg/ml in deionized water was prepared with correction for purity. Secondary solutions were prepared by dilution of the primary stock solution with deionized water. All solutions were stored at 4°C prior to use.

VI. Statistical Analyses

Measured parameters in the quadruplicate test chambers were analyzed using one-way analyses of variance (ANOVA). Interaction and block effects were tested by using two-way analysis of variance (ANOVA) prior to pooling data for one-way ANOVA. When treatment effects were indicated following a significant F-test of the means, a multiple means comparison test, Fisher's Protected Least Significant Difference (LSD), was used. All treatment means were compared to the control group. All differences were considered significant at $\alpha = 0.05$ (95% confidence level).

Analysis of reproduction data was accomplished by comparing the young/adult/reproductive day. This number was calculated by first determining the total number of young produced from day 7 (first day of reproduction) to day 21. The total number of adult reproduction days was determined by summing the number of surviving adults for each of the days during the reproduction period. Dividing the total number of young by the total number of adult reproduction days yields young/adult/reproductive day.

Example control chamber A:

Total number young = 744

Total adult reproductive days = (14 day X 10 adults) = 140.

Young/Adult/Reproductive day = $744/140 = 5.3$

Analysis of survival data was performed by transforming the percent survival using the arc sine transformation and then subjecting the data to a one-way ANOVA.

RESULTS

✓ A flow-through 21 day life cycle toxicity bioassay was conducted to determine the toxicity of Glyphosate to Daphnia magna. The test concentrations of Glyphosate were measured on days 0, 4, 7, 14 and 21 through the use of analytical methods outlined in the text of this report. The results are summarized in Table 2. The nominal concentrations of Glyphosate were 397 mg/l, 199 mg/l, 99 mg/l, 50 mg/l and 25 mg/l. The mean measured concentrations were 365 mg/l, 186 mg/l, 96 mg/l, 50 mg/l and 27 mg/l, respectively.

Water quality parameters of temperature, dissolved oxygen and pH were measured in the control, level #1, level #3 and level #5 and are presented in Table 3. Temperature was maintained between 18 and 20°C and dissolved oxygen ranged from 7.0 to 9.0 mg/l. The pH of the test concentrations decreased with increasing Glyphosate concentrations as indicated in Table 3.

No significant decreases in survival or length of adult daphnia were observed in organisms exposed to Glyphosate for 21 days (Table 4 and 5; Figures 2 and 3). Length of daphnids in the lowest (27 mg/l) and highest (365 mg/l) Glyphosate concentrations were significantly greater than controls.

Reproduction was significantly decreased at the three highest Glyphosate concentrations (96, 186 and 365 mg/l) (Tables 4 and 5;). The lowest level of Glyphosate (27 mg/l) effected increased reproduction compared to controls. The highest test concentration that did not result in decreased reproduction was 50 mg/l. In this study, the increased length and reproduction of daphnia at the lowest concentration was not considered to be deleterious and thus not used to estimate the MATC. Also, this increase in reproduction in the low test concentration, as well as the reproduction in the control, was within the range of production obtained in the controls of nine previous Daphnia magna chronic studies conducted at ABC Laboratories. This historical range of number of young/adult/reproductive day in the controls was 3.3-12.5.

Based on the statistical analysis of survival, length and young/adult/reproductive day from this 21 day Daphnia magna flow-through life cycle study, the MATC limits are estimated to be the Glyphosate mean measured concentrations of 50 and 96 mg/l.

The study was conducted following the intent of the Good Laboratory Practice Regulations (12) and the final report was reviewed by Analytical Bio-Chemistry Laboratories' Quality Assurance Unit. All original raw data was provided to Monsanto Chemical Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

Reviewers Evaluation

A. Test Procedure

The test procedure is sound and the results are adequate to characterize the toxicity of Roundup on an aquatic invertebrate.

B. Statistical Analysis

The ANOVA^{is} appropriate for this type of data.

EEB rechecked the data category young/adult/day with a one way ANOVA and generated the same variances (see attached sheet).

C. Discussion

The data supports the conclusion that the no effect level of glyphosate is 50 mg/l. Above 50 mg/l^e reproductive capacity can be expected to drop. The contractor proposed that the no effect level is much higher than the test indicate. This is based on an acceptance standard by ASTM of 2.0 young/adult/ reproductive day as being sufficient for an environmental test. Only in the 378 mg/l test level did the parameter drop below the 2.0 cutoff. Appealing as it sounds, this supposition is unacceptable. The control serves as a comparison standard within a given system. Controls can be expected to vary on a day by day, system to system basis. The 2.0 is the bottom line that a control can have where the system is still considered adequate for testing.

The submitted study indicates a good test system based on the reproductive capacities of the control animals. To use the 2.0 unacceptable limit as a comparative basis is inserting a control parameter taken completely out of context. Concurrent controls are requested to avoid such faulty comparisons out of context relative to the test system being used.

Conclusions

1. Category: Core
2. Rationale: N/A
3. Repairability: N/A

110062402

GENERAL LINEAR MODELS PROCEDURE

CLASS LEVEL INFORMATION

| CLASS | LEVELS | VALUES |
|-------|--------|-------------|
| TRT | 6 | A B C D E F |

NUMBER OF OBSERVATIONS IN DATA SET = 24

1 SAS 11:23 WEDNESDAY, JULY 6, 1983 2

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RESPONSE

| SOURCE | DF | SUM OF SQUARES | MEAN SQUARE | F VALUE |
|-----------------|----|----------------|-------------|---------|
| MODEL | 5 | 51.48708333 | 10.29741667 | 53.07 |
| ERROR | 18 | 3.49250000 | 0.19402778 | PR > F |
| CORRECTED TOTAL | 23 | 54.97958333 | | 0.0001 |

| R-SQUARE | C.V. | ROOT MSE | RESPONSE MEAN |
|----------|---------|------------|---------------|
| 0.936476 | 10.1164 | 0.44048584 | 4.35416667 |

| SOURCE | DF | TYPE I SS | F VALUE | PR > F |
|--------|----|-------------|---------|--------|
| TRT | 5 | 51.48708333 | 53.07 | 0.0001 |

| SOURCE | DF | TYPE III SS | F VALUE | PR > F |
|--------|----|-------------|---------|--------|
| TRT | 5 | 51.48708333 | 53.07 | 0.0001 |

1 SAS 11:23 WEDNESDAY, JULY 6, 1983 3

MC06402

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE: RESPONSE
 NOTE: THIS TEST CONTROLS THE TYPE I COMPARISONWISE ERROR RATE,
 NOT THE EXPERIMENTWISE ERROR RATE.
 ALPHA=0.05 DF=18 MSE=0.194028
 MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

| DUNCAN | GROUPING | MEAN | N | TRT |
|--------|----------|--------|---|-------|
| | A | 6.5250 | 4 | B 25 |
| | B | 5.0500 | 4 | C 30 |
| | B | 4.9000 | 4 | A 0 |
| | C | 4.1250 | 4 | D 99 |
| | C | 3.8250 | 4 | E 799 |
| 1 | D | 1.7000 | 4 | F 301 |

SAS 11:23 WEDNESDAY, JULY 6, 1983 4

| VARIABLE | N | MEAN | STANDARD DEVIATION | MINIMUM VALUE | MAXIMUM VALUE | STD. ERROR OF MEAN |
|-------------------|---|------------|--------------------|---------------|---------------|--------------------|
| ----- TRT=A ----- | | | | | | |
| RESPONSE | 4 | 4.90000000 | 0.42426407 | 4.30000000 | 5.30000000 | 0.21213203 |
| ----- TRT=B ----- | | | | | | |
| RESPONSE | 4 | 6.52500000 | 0.15000000 | 6.40000000 | 6.70000000 | 0.07500000 |
| ----- TRT=C ----- | | | | | | |
| RESPONSE | 4 | 5.05000000 | 0.49328829 | 4.50000000 | 5.60000000 | 0.24664414 |
| ----- TRT=D ----- | | | | | | |
| RESPONSE | 4 | 4.12500000 | 0.78049130 | 3.00000000 | 4.70000000 | 0.39024565 |
| ----- TRT=E ----- | | | | | | |
| RESPONSE | 4 | 3.82500000 | 0.09574271 | 3.70000000 | 3.90000000 | 0.04787136 |
| ----- TRT=F ----- | | | | | | |
| RESPONSE | 4 | 1.70000000 | 0.31622777 | 1.40000000 | 2.10000000 | 0.15811388 |

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