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00124760

DATA EVALUATION RECORD

1. Chemical: Glyphosate

2. Test Material: Roundup formulation 31.01% glyphosate (41.83% IPA salt of glyphosate)

3. Study Type: Ninety-six hour Lepomis macrochirus LC_{50}

4. Study ID: Forbis, A.D. (1982) Dynamic 96-Hour Acute Toxicity of Roundup to Bluegill Sunfish, Study No. AB-82-33, Project No. 28746, Analytical Biochemistry Laboratories, Inc., Columbia, MO 65205, Submitted on December 27, 1982, for EPA Registration No. 524-308 by Monsanto Agricultural Products Co., St. Louis, MO 63166. Accession No. 249159

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 can be used for hazard assessment purposes. Also, it meets the guideline requirements. Using the toxicity categories of Brooks et al. (1973) the acute LC_{50} of 5.8 (4.4 to 8.3) mg/L would place Roundup into the category of moderately toxic.

8. Recommendation:

N/A

9. Background:

This study was first received by RD on December 27, 1982, and reviewed by W. Faatz on March 4, 1983. The present review was initiated by the Registration Standard for Glyphosate.

10. Discussion of Individual Tests:

N/A

11. Material and Methods:

Test Procedure

Test Fish: The bluegill sunfish used in the test were obtained from Osage catfisheries, in Osage Beach, Missouri. They were identified to species using taxonomic keys. All fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. During the holding period the fish received a standard commercial fish food (Rangen's®) daily in an amount equivalent to 3 percent of their body weight. The test fish were not fed during the acclimation and test periods. The bluegill sunfish used for this experiment had a mean weight of 0.25 g and a mean standard length of 21 mm. Weight and length measurements were made on the control group of fish at termination of the test. As a quality check the bluegill sunfish were challanged with a reference compound, Antimycin A. The observed 96-hour LC_{50} and 95 percent confidence limits were within the 95 percent confidence limits reported in the literature, indicating the fish were in good condition.

Test System: A proportional diluter system was used for intermittent introduction of Roundup® and diluent water into the test aquaria. Aerated well water was delivered to glass aquaria at a rate of 150 ml/min/aquarium, an amount sufficient to replace the 30 liter test volume at least 7 times in a 24-hour period. The test aquaria were immersed in a circulating water bath held at 22 °C ($\pm$ 1°) b submerged heating elements.

Test Compound: The Roundup® standard was received on February 24, 1982, in good condition. The sample upon receipt was observed to be a yellow liquid and was refrigerated at 4 °C. The stock solution was prepared in deionized water on a weight/volume basis (mg/L).

Test Procedure: The test was initiated on March 22, 1982, by random assignment of 20 bluegill sunfish to each of the 30 liter test aquaria after test solutions had been flowing through the aquaria for 24 hours. The fish were exposed to the following nominal concentrations of Roundup®: 20 mg/L, 10 mg/L, 5.8 mg/L, 3.4 mg/L and 1.6 mg/L. The fish were observed once every 24 hours for the 96-hour test period. Dead individuals were removed at each observation. A computerized LC_{50} program by Stephan was used to calculate the LC_{50} values and their 95 percent confidence limits.

Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.

The actual Roundup® concentrations were verified by analysis of its active ingredient Glyphosate.

12. Reported Results: (excerpted from citation)

Table 1: The Estimated LC_{50} Values With Their 95% Confidence Limits for the 96-Hour Bluegill Sunfish Toxicity Study of Roundup®.

Hours of Exposure	Roundup® (mg/l) LC_{50}	Roundup® (mg/l) 95% Confidence Limits
24	10	8.3 to 18
48	7.0	4.4 to 8.3
72	6.3	4.4 to 8.3
96	5.8	4.4 to 8.3

13. Study Author's Conclusion/QA Measures: (excerpted from citation)

Table 1 presents the predicted LC_{50} values and 95% confidence intervals for Roundup®. These values were obtained by employing a computerized LC_{50} program developed by Stephan et al. (8) and are presented in Table 1. The mean measured concentration of Roundup® were used in the above calculations and were 18, 8.3, 4.4, 2.2, and 1.2 mg/L. These values ranged from 65 to 90% of the expected nominal values.

In addition to the above LC_{50} values, the results indicated a 96-hour, no-observed-effect concentration of 2.2 mg/L. Mortality data is presented in Table 4. No adverse behavior was observed in the test solutions preceding mortality.

Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured in the control, low concentration and high concentration throughout the test (Table 3). The dissolved oxygen concentrations which stayed between 95 and 100 percent saturation were considered adequate for testing (2) and ammonia concentrations were below the toxic level (9). The pH values were consistent with the control throughout the study.

The study was conducted following the intent of the Good Laboratory Practice Regulations (10) and the final report was reviewed by Analytical Bio-Chemistry Laboratories Quality Assurance Unit.

All original raw data was provided to Monsanto Company, with a copy retained at Analytical Bio-Chemistry Laboratories.

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

a. Test Procedures: The following items did not meet the guideline requirements:

1. The mean weight of the test fish should be between 0.5 and 5 g rather than 0.25 g.

2. The length of the pretest fasting period was not reported, though fasting was indicated.

3. The nominal dose levels were irregularly spaced, (1.6 to 3.4 = 47%, 3.4 to 5.8 = 59%, 5.8 to 10 = 58%, 10 to 20 = 50%).

b. Statistical Analysis: The previous review by W. Faatz provided an EEB computer printout which is identical to the study's printout. Both agreed the binomial method is the only applicable method, and the 96-hour LD_{50} is 5.8 (4.4 and 8.3) mg/L.

c. Discussion/Results: The items mentioned under Test Procedures are not expected to appreciably change the study results. Studies by Folmar indicate smaller fish are more susceptible to Roundup. However, it should also be mentioned that the LC_{50} was derived from figures calculated from measured concentrations of glyphosate. The Roundup concentration was calculated by adjusting the measured glyphosate value by the 31 percent. The following table compares the nominal Roundup levels to estimated levels from measured values of glyphosate:

Nominal	Estimated
1.6	1.29
3.4	2.43
5.8	4.97
10	9.26
20	17.8

To compare the differences in the LC_{50} values derived with the estimated versus the nominal concentration of Roundup, nominal LC_{50} value was calculated. It is 7.4 (5.8 and 10) mg/L compared to 5.8 (4.4 and 8.3) mg/L. In this case, the reported LC_{50} is adequate for both hazard assessment and meeting the guideline criteria for formulated product, such as, Roundup.

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DATA EVALUATION RECORD

1. Chemical: Roundup® Glyphosate

2. Formulation: Roundup Formulated product 31% ai.

3. Citation:

"Dynamic 96-HOur Acute Toxicity of Roundup® to Bluegill Sunfish

Analytical Biochemical Laboratories, Inc.
Columbia, Mo. 65205

Project No. 28746 Study No.: AB-82-33
March 31, 1982
Study Director: Allan D. Forbis

4. Reviewed by: Wayne C. Faatz, Ph.D.
Wildlife Biologist

5. Date Reviewed: 4 March 1983

6. Test Type: Acute Freshwater Fish
LC_{50}

Test Species: Bluegill Sunfish
Lepomis macrochirus

7. Reported Results

The LC_{50} is based on measured concentrations of the active ingredient though the formulated product was used as the test material.

	Roundup® mg/l		
Exposure (hr.)	LC50 (formulated Products)	95%	C.I.
24	10	8.3 - 18	
48	7.0	4.4 - 8.3	
72	6.3	4.4 - 8.3	
96	5.8	4.4 - 8.3	

8. Reviewers Conclusions:

This study is scientifically sound. The test was submitted to support Monsanto's contention that the present surfactant is less toxic than the one previously used.

Material/Methods

Test Procedure

Test Fish: The bluegill sunfish used in the test were obtained from Osage catfisheries, in Osage Beach, Missouri. They were identified to species using taxonomic keys. All fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. During the holding period the fish received a standard commercial fish food (Rangen's®) daily in an amount equivalent to 3% of their body weight. The test fish were not fed during the acclimation and test periods. The bluegill sunfish used for this experiment had a mean weight of 0.25g and a mean standard length of 21 mm. Weight and length measurements were made on the control group of fish at termination of the test. As a quality check the bluegill sunfish were challenged with a reference compound, Antimycin A. The observed 96 hour LC_{50} and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating the fish were in good condition.

Test System: A proportional diluter system was used for intermittent introduction of Roundup® and diluent water into the test aquaria. Aerated well water was delivered to glass aquaria at a rate of 150 ml/min./aquarium, an amount sufficient to replace the 30 liter test volume at least 7 times in a 24 hour period. The test aquaria were immersed in a circulating water bath held at 22°C ($\pm$ 1°) b submerged heating elements.

Test Compound: The Roundup® standard was received on Feb. 24, 1982, in good condition. The sample upon receipt was observed to be a yellow liquid and was refrigerated at 4°C. The stock solution was prepared in deionized water on a weight/volume basis (mg/l.).

Test Procedure: The test was initiated on March 22, 1982, by random assignment of 20 bluegill sunfish to each of the 30 liter test aquaria after test solutions had been flowing through, the aquaria for 24 hours. The fish were exposed to the following nominal concentrations of Roundup®: 20 mg/l, 10 mg/l, 5.8 mg/l, 3.4 mg/l and 1.6 mg/l. The fish were observed once every 24 hours for the 96 hour test period. Dead individuals were removed at each observation. A computerized LC_{50} program by Stephan was used to calculate the LC_{50} values and their 95% confidence limits.

Water quality parameters of temperature, dissolved oxygen, pH and ammonia were measured throughout the test and were within acceptable limits.

The acutual Roundup® concentrations were verified by analysis of its active ingredient Glyphosate

Results:

In addition to the calculated LC_{50} values, the results indicated a 96-hour, no observed effect concentration of 2.2 mg/l. No adverse behavior was observed in the test solutions preceeding mortality.

Reviewers Evaluation

A. Test Procedure

The test procedure is acceptable.

B. Statistical Analysis

The analysis done by EEB agress closely to the one submitted by the registrant. (See attached calculation).

C. Discussion/Results

An LC_{50} 5.8 (4.4 - 8.3) is supported by the data. The LC_{50} is the measured amount of ai in the formulated product.

D. Conclusions

1. Category: Supplemental

2. Rationale: A specific test with the formulated producted is not required. This test was done because the surfactant in the formulated product is toxic to aquatics. The test allows a comparison between the technical product on the formulated product with surfactant

3. Repairability: None

Nominal Roundup Values

MCLANE (GLYPHOSATE) BLUEGILL

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
20	20	20	100	9.53674E-05
10	20	20	100	9.53674E-05
5.8	20	1	5	2.00272E-03
3.4	20	0	0	9.53674E-05
1.6	20	0	0	9.53674E-05

THE BINOMIAL TEST SHOWS THAT 5.8 AND 10 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 7.34807

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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WAYNE C. FAATZ ROUNDUP FORMULATED WITH SURFACTANT
BLUE GILL SUNFISH 96 HR LC 50

Estimated from measured concentration of glyphosate

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
18	20	20	100	9.536743E-05
8.3	20	20	100	9.536743E-05
4.4	20	1	5	0.002002716
2.2	20	0	0	9.536743E-05
1.2	20	0	0	9.536743E-05

THE BINOMIAL TEST SHOWS THAT 4.4 AND 8.3 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 5.796411

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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