

US EPA ARCHIVE DOCUMENT

DATA EVALUATION RECORD

1. Chemical: Glyphosate
2. Test Material: Roundup - 31% glyphosate (41.8% IPA salt of glyphosate)
3. Study Type: Ninety-six hour Salmo gairdneri LC50
4. Study ID: Forbis, A.D., Boudreau, P. and Schofield, M. (1982) Dynamic 96-Hour Acute Toxicity of Roundup (AB-82-34) to Rainbow Trout (Salmo gairdneri) Project No. 28745, Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO 65205, submitted by Monsanto Company for EPA Reg. No. 524-308 on December 27, 1982, 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 LC50 of 8.2 (6.4-9.9) mg/L would place Roundup - 31% glyphosate 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. Materials and Methods: (excerpted from citation)

- a. Test Fish: The rainbow trout used in the test were obtained from Spring Creek Trout Hatchery in Lewistown, Montana. The fish were identified to species using taxonomic keys. All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, is included in the Appendix. 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 rainbow trout used for this experiment had a mean weight of 2.4 g and a mean standard length of 54 mm. Weight and length measurements were made on the control group of fish at termination of the test and are included in the Appendix. As a quality check, the rainbow trout were challenged with a reference compound, Antimycin A. The observed 96-hour LC₅₀ and 95 percent confidence limits were within the 95 percent confidence limits reported in the literature, indicating that the fish were in good condition. Results of this test are included in the Appendix.
- b. Test System: A proportional diluter system described by Mount and Brungs, with the modifications of McAllister et al. was used for the intermittent introduction of Roundup® and diluent water into the test aquaria. Aerated well water (Table 2) was delivered to the glass aquaria at a rate of 150 ml/minute/aquarium, an amount which was sufficient to replace the 30 liter test volume at least 7 times in a 24 hour period. The test aquaria were immersed in a temperature controlled water bath held at 12 °C (+1°).
- c. 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). Standard weights and dilution volumes can be found in the Appendix. The Antimycin A standard used for the reference test was obtained from Sigma Chemical Company, Type III, crystalline, Lot 125C-0152.
- d. Biological: The test was initiated on March 26, 1982, by random assignment of 20 rainbow trout 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 for mortality and abnormal behavior initially and once every 24 hours for the 96-hour test period. Dead individuals were removed at each observation. A computerized LC50 program developed by Stephan was used to calculate the LC50 values and their 95 percent confidence limits.

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

12. Reported Results: (excerpted from study)

The calculated LC50 values for Roundup® formulation in rainbow trout based on measured water concentrations of the active ingredient glyphosate are:

Hour Exposure	Roundup® LC50	mg/L 95% C.I
24	15.0	9.9-23.0
48	12.0	6.4-23.0
72	9.0	6.4-23.0
96	8.2	6.4-9.0

(The mortality results are shown below.)

Nominal Concentration (mg/L)	Mean Measured Concentration (mg/L)	Mortality			
		24 hr	48 hr	72 hr	96 hr
0	0	0	0	0	0
1.6	1.9	0	0	0	0
3.4	3.6	0	0	0	0
5.8	6.4	0	0	0	0
10.0	9.9	0	6 (30%)	14 (70%)	19 (95%)
20.0	23.0	20(100%)	20(100%)	20(100%)	20(100%)

13. Study Author's Conclusions/QA Measures:

(excerpted from citation)

In accordance with ABC Laboratories intent that all studies conducted at our facilities are designed and function in conformance with Good Laboratory Practice Regulations and the protocols for individual laboratory studies, an inspection of the final report for Roundup was conducted and found to be in an acceptable form by a member of our Quality Assurance Unit. An inspection of the daily mortality rate of the test organisms prior to the initiation of the study indicates they

were in good health and should not bias the observed mortality rate in the study. An in-progress study inspection was conducted on March 26, 1982. The final inspection of all data and records on April 12, 1982, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories. (Signed by the Quality Assurance Officer).

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

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

1. The length of the pretest fasting period was not reported.
2. 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 LC₅₀ is 8.2 (6.4 to 9.0) mg/L.

c. Discussion/Results: The items mentioned under Test Procedures are not expected to appreciably change the study results:

However, it should be mentioned that the LC₅₀ 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. (See 12, Reported Results of this review).

To compare the differences in the LC₅₀ values derived with the estimated versus the nominal concentration of Roundup, the nominal LC₅₀ value was calculated. It is 7.9 (5.8 and 10) mg/L when the binomial test is used. In this case, the 8.2 (6.4-9.0) mg/L the reported value and the LC₅₀ value calculated on the nominal level are not significantly different for the purpose of this test. Hence, the reported LC₅₀ is adequate.

d. Adequacy of Study:

1. Classification: Core for the Roundup formulation - 41.8 percent IPA salt of glyphosate.

2. Rationale: The guidelines have been met for formulated product testing.

3. Repairability: N/A

15. Completion of One-Liner for Study:

Completed July 25, 1985.

16. CBI Appendix:

N/A

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DATA EVALUATION RECORD

1. CHEMICAL: Roundup®, Glyphosate

2. FORMULATION:

Roundup Formulated Product
31% glyphosate

3. CITATION:

"Dynamic 96-Hour Acute Toxicity of Roundup® to Rainbow Trout."

Analytical Biochemistry Laboratories, Inc.
Columbia, MO 65205

Project No. 28745
Study No. AB-82-34
Date of Report: April 16, 1982
Study Director: Allan D. Forbis

4. REVIEWED BY: Wayne C. Faatz, Ph.D
Wildlife Biologist

5. DATE REVIEWED: 11 March 1983

6. TEST TYPE: Acute Freshwater Fish LC50

Test Species: Rainbow Trout
Salmo gairdneri

7. REPORTED RESULTS

The calculated LC50 values for Roundup® formulation in rainbow trout based on measured water concentrations of the active ingredient glyphosate are:

Hour Exposure	Roundup®	mg/l
	LC50	95% C.I
24	15.0	9.9-23.0
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72	9.0	6.4-23.0
96	8.2	6.4-9.0

Mortality

Nominal Concentration (mg/l)	Mean Measured Concentration (mg/l)	Mortality			
		24 hr	48 hr.	72 hr.	96 hr.
0	0	0	0	0	0
1.6	1.9	0	0	0	0
3.4	3.6	0	0	0	0
5.8	6.4	0	0	0	0
10.0	9.9	0	6/20	7/20	19/20
20.0	23.0	0	20/20	20/20	20/20

The results indicated a 96-hour, no observed effect concentration of 6.4 mg/l.

8. Reviewer's Conclusion

This study is scientifically sound. The test was submitted to test the toxicity of the surfactant in conjunction with the active ingredient glyphosate. The surfactant is [REDACTED] For aquatic uses the product is known as Rodeo and has no surfactant.

Material/Methods

The Material/Methods were taken directly from the text.

I. Test Fish

The rainbow trout used in the test were obtained from Spring Creek Trout Hatchery in Lewistown, Montana. The fish were identified to species using taxonomic keys. All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing. A daily record of fish observations during the holding period, along with prophylactic and therapeutic disease treatments, is included in the Appendix. 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 rainbow trout used for this experiment had a mean weight of 2.4 g and a mean standard length of 54 mm. Weight and length measurements were made on the control group of fish at termination of the test and are included in the Appendix. As a quality check, the rainbow trout were challenged with a reference compound, Antimycin A. The observed 96-hour LC50 and 95% confidence limits were within the 95% confidence limits reported in the literature, indicating that the fish were in good condition. Results of this test are included in the Appendix.

II. Test System

A proportional diluter system described by Mount and Brungs, with the modifications of McAllister et al. was used for the intermittent introduction of Roundup® and diluent water into the test aquaria. Aerated well water (Table 2) was delivered to the glass aquaria at a rate of 150 ml/minute/aquarium, an amount which was sufficient to replace the 30 liter test volume at least 7 times in a 24 hour period. The test aquaria were immersed in a temperature controlled water bath held at 12°C (+1°).

III. 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). Standard weights and dilution volumes can be found in the Appendix. The Antimycin A standard used for the reference test was obtained from Sigma Chemical Company, Type III, crystalline, Lot 125C-0152.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

IV. Test Procedure - Biological

The test was initiated on March 26, 1982, by random assignment of 20 rainbow trout 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 for mortality and abnormal behavior initially and once every 24 hours for the 96-hour test period. Dead individuals were removed at each observation. A computerized LC50 program developed by Stephan was used to calculate the LC50 values and their 95% confidence limits.

V. Test Procedure - Chemical and Physical

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

Reviewers Evaluation

A. Test Procedure

The test procedure is appropriate for this type of test.

B. Statistical Analysis

The data was checked using a one way ANOVA with Duncan's Multiple Range Test. The results were essentially the same as submitted by the registrant. The 96-hr LC50 is 8.2 mg/l (6.4-10.0) (See attached data)

C. Results

The analysis confirms an 96-hr. LC50 of 8.2 mg/l (6.4-10.0). The LC50 is the measured amount of ai in the formulated product.

D. Conclusions

1. Category: Supplemental

2. Rationale: A specific test with the formulated product is not required. The test was done because the surfactant in the formulated product, [REDACTED] is toxic to aquatics. The test allows a comparison between the technical product and the formulated product with surfactant.

3. Repairability: None

INERT INGREDIENT INFORMATION IS NOT INCLUDED

McGill

WAYNE C. FAATZ ROUNDUP FORMULATED WITH SURFACTANT
RAINBOW TROUT 96 HR. LC50

estimated measured

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
23	20	20	100	9.536743E-05
9.9	20	19	95	0.002002716
6.4	20	0	0	9.536743E-05
3.6	20	0	0	9.536743E-05
1.9	20	0	0	9.536743E-05

THE BINOMIAL TEST SHOWS THAT 6.4 AND 9.9 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 8.191306

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.

MCLANE ROUNDUP RAINBOW

Nominal concentration

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
20	20	20	100	9.53674E-05
10	20	19	95	2.00272E-03
5.8	20	0	0	9.53674E-05
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 7.89323

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.
