

TDMS

DATA EVALUATION RECORD

PAGE 1 OF

CASE GS

Glyphosate

PM 25

CHEM 103601

Isopropylamine glyphosate (N-(phosphon

BRANCH EEB DISC

FORMULATION 31% & MON0818

FICHE/MASTER ID MCOGLY03

CITATION: Forbis, ^{Boudreau & Schofield} (1982) Dynamic 48-hour acute toxicity of
Roundup to Gammarus pseudolimnaeus, Project No: 28747, Study
No: AB-82-035, Accession No. 249159

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:

Dennis J. McLane

DATE: 9-9-85

APPROVED BY: Raymond W. Matheny
TITLE: Head, Section 1
ORG: EEB/HED
LOC/TEL:

SIGNATURE:

Raymond W. Matheny

DATE: 9-9-85



2009161

Suppl.

DATA EVALUATION RECORD

1. Chemical: Glyphosate
2. Test Material: Roundup formulation 31.01% glyphosate
(41.83% IPA salt of glyphosate)
3. Study Type: Forty-eight hour Gammarus pseudolimnaeus LC50
4. Study ID: Forbis, A.D., Boudreau, P., Schofield, M.,
(1982) Dynamic 48-Hour Acute Toxicity of Roundup
to Gammarus pseudolimnaeus, Project No. 28747
Study No. AB-82-035 Analytical Bio-Chemistry
Laboratories. Submitted by Monsanto Agricultural
Products Co., St. Louis, Missouri, for EPA
Registration 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. However, it does not meet the guideline requirements. Using the toxicity categories of Brooks et al. (1973) the acute LC50 of 42 mg/L would place Roundup into the category of slightly toxic.
8. Recommendation:

N/A
9. Background:

This study was first received by RD on December 27, 1982, and reviewed by W. Faatz of EEB on March 4, 1983. The present review was initiated by the Registration Standard for the chemical, glyphosate.
10. Discussion of Individual Tests:

N/A

11. Materials and Methods: (excerpted from the citation)

- a. Test Gammarids: The test lot of Gammarus were collected from a wild population, moved to the laboratory and held in a controlled temperature area on a 16-hour daylight photoperiod at the same temperature used for testing (17 ± 2 °C). During the holding period, they were maintained in an aquarium with a substrate of deciduous leaves and gravel. The gammarids were fed a standard Tetra-Min® fish food. Late juvenile to mature adults (at least 6th instar) were chosen for exposure to the toxicant and were not fed during the test. The taxonomic keys were used to identify the gammarids as Gammarus pseudolimnaeus.
- b. Test System: A proportional diluter system described by Mount and Brungs was used for the intermittent introduction of Roundup® and diluent water into the test aquaria. The test was conducted in 1-liter glass beakers with notched over-flows to provide 900 ml of test solution per beaker. The test chambers were placed in a water bath with temperature maintained at 17 ± 2 °C with the use of a thermostatically controlled submersible heating element. Each test concentration was replicated four times by means of a flow splitting device incorporated into the diluter system. Aerated well water was delivered to the test chambers at a minimum rate of 12 ml/minute/chamber, an amount which replaced the 900 ml test volume 19.2 times per day.
- 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).
- d. Test Procedure - Biological: The test was initiated on April 2, 1982, by random assignment of 5 Gammarus pseudolimnaeus to each of the liter test aquaria after test solutions had been flowing through the aquaria for 24 hours. The gammarids were exposed to the following nominal concentrations of Roundup®: 100 mg/L, 47 mg/L, 20 mg/L, 11 mg/L, and 5.4 mg/L. The gammarids were observed for mortality and abnormal behavior initially and once ever 24 hours for the 48-hour test period. Immobile individuals were removed at each observation. A computerized LC₅₀ program developed by Stephan et al. (8) was used to calculate the EC₅₀ values and their 95 percent confidence limits.

- e. 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.

12. Reported Results: (excerpted from the citation)

<u>Nominal</u> <u>Concentration mg/L</u>	<u>Measured</u> <u>Concentration mg/L</u>	<u>Immobility</u>	
		<u>24-hr</u>	<u>48-hr</u>
0	0	0	0
5.4	4.4	0	0
11.0	9.1	0	2(10%)
20.0	20.0	0	4(20%)
47.0	39.0	0	9(45%)
100.0	110.0	1(5%)	17(85%)

13. Study Author's Conclusions/QA Measures:

(excerpted from citation)

Based on the measured exposure concentrations [glyphosate] and the resulting "immobilities", the 24- and 48-hour EC₅₀ values for the dynamic exposure of Gammarus pseudolimnaeus to ROUNDUP formulation were > 110 and 42 mg per liter of water, respectively. The 48-hour no-effect-level in this study was 4.4 mg/L.

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 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 indicated they were in good health and should not bias the observed mortality in the study. A final inspection of all data and records on April 23, 1982, indicated that the report submitted to you is an accurate reflection of the study as it was conducted by ABC Laboratories.

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

- a. Test Procedures: The following items did not follow the guidelines:

1. The gammarids were described as late juvenile to mature adults > 6th instar; the guidelines requires < 2nd instar gammarids.

2. The dosage levels are not 60 percent apart or consistently separated. ($5.4/11 \text{ mg/L} = 45\%$, $11/20 \text{ mg/L} = 55\%$, $20/47 \text{ mg/L} = 42\%$, $47/100 \text{ mg/L} = 47\%$).

- b. Statistical Analysis: The methods and calculations of the LC_{50} are in keeping with the guidelines. The LC_{50} from the nominal concentrations and the estimated Roundup by glyphosate measurements were very similar, $LC_{50} = 42 \text{ mg/L}$ versus 44.3 mg/L respectively. The statistical analysis meets the guidelines requirements.
- c. Discussion/Results: The use of older gammarids than $< 2\text{nd instar}$ is critical and would be expected to change the animals resistance to the toxic effects to any chemical. Based on this, the study does not adequately meet the guideline requirements.
- d. Adequacy of Study:
1. Classification: Supplemental for formulation of Roundup.
 2. Rationale: The gammarids were $> 6\text{th instar}$ rather than the required $< 2\text{nd instar}$.
 3. Repairability: Only a study with the correct age invertebrate will be sufficient.

15. Completion of One-Liner for Study:

Completed July 25, 1985.

16. CBI Appendix:

N/A

DATA EVALUATION RECORD

1. Chemical: Glyphosate; Roundup
2. Formulation: Formulated Product 31% active ingredient
Surfactant [REDACTED]
3. Citation

Dynamic 48-Hour Acute Toxicity of Roundup to Grammarus pseudolimnaeus.

Conducted by: Analytical Biochemistry
Laboratories, Inc.
Columbia, MO 65205

Project No: 28747
Study No: AB-82-035
Report Date: April 27, 1982
Study Director: Allan D. Forbis

Accession Number 249159

4. Reviewed by: Wayne C. Faatz, Ph.D.
 5. Date Reviewed: July 1983
 6. Test Type: Aquatic Invertebrate
- Species: Grammarus pseudolimnaeus

Reported Results

Nominal Concentration mg/l	Measured Concentration mg/l	Immobility	
		24-hr	48-hr
0	0	0	0
5.4	4.4	0	0
11.0	9.1	0	2(10%)
20.0	20.0	0	4(20%)
47.0	39.0	0	9(45%)
100.0	110.0	1(5%)	17(85%)
EC ₅₀	C.I.	HRS	
>110	-	24	
42	31-62	48	

The 48 hour no effect level is 4.4 mg/l.

Reviewers Conclusions

The test is scientifically sound and allows comparisons be made between the technical and formulated material based on active ingredient. By comparing the technical and formulated material an assessment on the toxicity of the surfactant can be made.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Material/Methods

The follow description of the material/methods was taken directly from the report.

I. Test Gammarids

The test lot of Gammarus were collected from a wild population, moved to the laboratory and held in a controlled temperature area on a 16-hour daylight photoperiod at the same temperature used for testing ($17 \pm 2^\circ\text{C}$). During the holding period, they were maintained in an aquarium with a substrate of deciduous leaves and gravel. The gammarids were fed a standard Tetra-Min® fish food. Late juvenile to mature adults (at least 6th instar) were chosen for exposure to the toxicant and were not fed during the test. The taxonomic keys were used to identify the gammarids as Gammarus pseudolimnaeus.

II. Test System

A proportional diluter system described by Mount and Brungs was used for the intermittent introduction of Roundup® and diluent water into the test aquaria. The test was conducted in 1-liter glass beakers with notched over-flows to provide 900 ml of test solution per beaker. The test chambers were placed in a water bath with temperature maintained at $17^\circ\text{C} \pm 2^\circ\text{C}$ with the use of a thermostatically controlled submersible heating element. Each test concentration was replicated four times by means of a flow splitting device incorporated into the diluter system. Aerated well water was delivered to the test chambers at a minimum rate of 12 ml/minute/chamber, an amount which replaced the 900 ml test volume 19.2 times per day.

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).

IV. Test Procedure - Biological

The test was initiated on April 2, 1982, by random assignment of 5 Gammarus pseudolimnaeus to each of the liter test aquaria after test solutions had been flowing through the aquaria for 24 hours. The gammarids were exposed to the following nominal concentrations of Roundup®: 100 mg/l, 47 mg/l, 20 mg/l, 11 mg/l and 5.4 mg/l. The gammarids were observed for mortality and abnormal behavior initially and once ever 24 hours for the 48-hour test period. Immobile individuals were removed at each observation. A computerized LC_{50} program developed by Stephan et al. (8) was used to calculate the EC_{50} 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

Test Procedure

The test procedure is appropriate.

Statistical Analysis

The data was analysed by EEB using a One way Analysis of Variance. The test results are essentially the same as those submitted by the registrant.

Discussion/Results

The calculated LC_{50} is supported by the data.

Conclusion

1. Category: Supplemental
2. Rationale: There is no specific requirement for a test with the formulated product. This test allows a comparison between the technical and formulated product because the surfactant is known to be toxic to aquatics.
3. Repairability: None

ROUNDUP FORMULATED WITH SURFACTANT
AQUATIC INVERTEBRATE GRAMMARUS

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
110	20	17	85	0.1288414
39	20	9	45	41.19015
20	20	4	20	0.5908966
9.1	20	2	10	0.02012253
4.4	20	0	0	

[New Message-of-the-Day available]
9.536743E-05

THE BINOMIAL TEST SHOWS THAT 20 AND 110 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 43.96019

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	0.2084193	43.71594	30.09886	64.22929

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	0.1224789	1	0.8226711

SLOPE = 2.335601
95 PERCENT CONFIDENCE LIMITS = 1.518211 AND 3.152991

LC50 = 41.92301
95 PERCENT CONFIDENCE LIMITS = 30.71292 AND 62.06019

LC10 = 11.98658
95 PERCENT CONFIDENCE LIMITS = 6.311342 AND 17.40642

June
5-492

01008
01108 ROUNDUP FORMULATED WITH SURFACTANT
AQUA-VERTEBRATE GRAMMARUS

*estimated Roundup
concentrations from
measured levels of glyphosate*

CONC. NUMBER NUMBER PERCENT BINOMIAL
 EXPOSED DEAD DEAD PROB. (PERCENT)
110 20 17 85 0.1288414
39 20 9 45 41.19015
20 20 4 20 0.5908966
9.1 20 2 10 0.02012253
4.4 20 0 0

[New Message-of-the-Day available]
9.536743E-05

THE BINOMIAL TEST SHOWS THAT 20 AND 110 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 43.96019

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	0.2084193	43.71594	30.09886	64.22929

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	0.1224789	1	0.8226711

X SLOPE = 2.335601
95 PERCENT CONFIDENCE LIMITS = 1.518211 AND 3.152991

X LC50 = 41.92301
95 PERCENT CONFIDENCE LIMITS = 30.71292 AND 62.06019

LC10 = 11.98658
95 PERCENT CONFIDENCE LIMITS = 6.311342 AND 17.40642

MCLANE ROUNDUP GAMMERIDS LC50

Nominal Concentrations

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
100	20	17	85	.128841
47	20	9	45	41.1901
20	20	4	20	.590897
11	20	2	10	.0201225
5.4	20	0	0	9.53674E-05

THE BINOMIAL TEST SHOWS THAT 20 AND 100 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 51.2811

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	.142525	44.6579	32.5761 69.1623

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABIL
3	.119903	1	.733866

SLOPE = 2.50166
95 PERCENT CONFIDENCE LIMITS = 1.63541 AND 3.36791

LC50 = 44.2807
95 PERCENT CONFIDENCE LIMITS = 33.1307 AND 63.4101

LC10 = 13.7579
95 PERCENT CONFIDENCE LIMITS = 7.55531 AND 19.5202
