

Data Evaluation Report on the Acute Toxicity of a Glyphosate SL formulation to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number {.....}

EPA MRID Number 45374003

Data Requirement: PMRA DATA CODE {.....}
EPA DP Barcode D275559
OECD Data Point Immobilization
EPA MRID 45374003
EPA Guideline 72-2

Test material:

Purity: 27.25%

Common name: Glyphosate 360 g/L SL formulation
Chemical name: *N*-(phosphonomethyl)glycine
CAS name: *N*-(phosphonomethyl)glycine
CAS No.: 1071-83-6
Synonyms: YF11357

Primary Reviewer: Mary Thomas, M.S.
Staff Scientist, Dynamac Corporation

Signature: *Mary Thomas*
Date: 10/11/01

QC Reviewer: Teri Myers, Ph.D.
Staff Scientist, Dynamac Corporation

Signature: *Teri S Myers*
Date: 10/11/01

Primary Reviewer: Stephen Carey, Biologist
EPA/OPPTS/OPP/EFED/ERB3

Date: 02/13/02
Signature: *Stephen Carey*

Reference/Submission No. {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 417300

Date Evaluation Completed: February 13, 2002

CITATION: Swarbrick, R.H., and N. Shillabeer. 1999. GLYPHOSATE Acute toxicity to *Daphnia magna* of a 360 g l⁻¹ SL formulation. Unpublished study performed by Brixham Environmental Laboratory, AstraZeneca, Brixham Devon TQ5 8BA, UK and sponsored by ZENECA Agrochemicals, Fernhurst Haslemere, Surrey GU27 3JE, UK. Brixham study number: AG0360/C. Study initiated October 12, 1999 and completed October 14, 1999.



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EXECUTIVE SUMMARY:

The 48-hr-acute toxicity of an SL formulation of Glyphosate (27.25% a.i. N-(phosphonomethyl)glycine) to *Daphnia magna* was studied under static conditions. Dilution water control served as a comparison to five nominal concentrations of glyphosate SL formulation of 100, 180, 320, 560 and 1000 mg/L. Mean measured concentrations of glyphosate a.i. were 0 (control), 26, 50, 87, 160, and 280 mg a.i./L. Mean measured concentrations of the SL formulation were 95.4, 183.4, 319.3, 587.2, and 1027.5 mg/L. The 48-hour glyphosate formulation EC₅₀ was 164.3 mg/L (44.8 mg a.i./L), based on mean measured concentrations. As a result, this SL formulation of Glyphosate is classified as practically non-toxic to *Daphnia magna* on an acute toxicity basis. The 48-hr- NOEC based on toxic effects was 95.4 mg/L. Toxicity effects included immobilization at treatment levels greater than 95.4 mg/L.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates. This study is classified as Core for a formulated product.

Results Synopsis

Test Organism Age (eg. 1st instar): ≤24 hrs. old

Test Type (Flow-through, Static, Static Renewal): Static

LC50: NA

95% C.I.: NA

EC50: 164.3 mg/L (44.8 mg a.i./L)

95% C.I.: 139.5 to 190.8 mg/L

NOEC: 95.4 mg/L

Probit Slope: 7.60

95% C.I.: Not reported

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OECD guideline 202, Part I - 48h EC50 Acute Immobilisation Test, also satisfies requirements of the relevant US EPA and EU guidelines.

Deviations included:

1. Water hardness (221 mg/L CaCO₃) was significantly higher than recommended (40-48 mg/L as CaCO₃) and th pH (7.89) was slightly higher than recommended (7.2-7.6).
2. The study author failed to report the OECD test chemical physical characteristics (i.e., vapor pressure, and specific activity).
3. The study author failed to indicate the level of analytical detection.

None of these deviations affected the acceptability or the validity of the study.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

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A. MATERIALS:

1. Test Material Glyphosate 360 g/L⁻¹ SL formulation (*N*-(phosphonomethyl)glycine)

Description: Clear brown mobile liquid

Lot No./Batch No. : J2290/006

Purity: 27.25%

Stability of Compound

Under Test Conditions: During the course of the study, mean measured concentrations ranged from 96 to 104% and averaged $101.8 \pm 4.1\%$ of nominal concentrations for test solutions from 27 to 270 mg a.i./L. Measured glyphosate a.i. concentrations at test initiation were virtually identical to measured concentrations at 48 hours, showing that the test material was stable under test conditions. OECD requirements were not reported.

Water solubility: 9.1 G/L @ 20°C

Vapor pressure: Not reported

Specific activity: Not reported

Molecular weight: Not reported

Storage conditions of

test chemicals: The test material was stored in the dark at ambient temperature.

2. Test organism:

Species: *Daphnia magna*

Age at test initiation: <24 hrs. old

Source: Instars from a single culture vessel.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: Not reported

b) Definitive Study

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Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period: Conditions: (same as test or not) Feeding:	NA Same as test <i>Daphnia</i> cultures were fed a defined diet of <i>Chlorella vulgaris</i> and microencapsulated "Frippak Booster". During the course of the study <i>Daphnia</i> were not fed.	Dilution water was same as culture water
Health: (any mortality observed)	Prior to the test period no disease was reported.	<i>EPA requires 7 day minimum acclimation period</i> <i>No feeding during study</i>
Duration of the test	48 hours	Shorter test duration than required. <i>EPA requires 96 hours</i>
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	Static NA NA	<i>EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period</i>
Aeration, if any	Prior to use, the dilution water was aerated for >2 hours. The test solutions were not aerated.	
<u>Test vessel</u>		
Material: (glass/stainless steel) Size: Fill volume:	Glass 250 mL 200 mL	<i>EPA requires: size 250 ml or 3.9 L fill 200 ml</i>
Source of dilution water	Reconstituted water	<i>EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.</i>

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Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u> Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	221 mg/L CaCO ₃ 7.89 ≥ 95 % saturation 20 ± 1°C 1.4 mg/L Not reported	Water hardness was higher than required by EPA. The pH was slightly higher than recommended. <hr/> EPA requires: hardness: 40 - 48 mg/L as CaCO ₃ pH: 7.2 - 7.6 -Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C Dissolved oxygen: Static: ≥ 60% during 1 st 48 hr and ≥ 40% during 2 nd 48 hr Flow-through: ≥60%
Number of replicates Solvent control: Negative control: Treatments:	NA 1 4	
Number of organisms per replicate Solvent control: Negative control: Treatments:	NA 20 20	Five treatment levels plus water control with 20 <i>Daphnia</i> per treatment. Biomass loading rate was equivalent to 25 <i>Daphnia</i> /L. <hr/> EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day.

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Parameter	Details	Remarks
		Criteria
Treatment concentrations nominal:	Water control, 100, 180, 320, 560, and 1000 mg/L (formulation); 27, 49, 87, 150, and 270 mg a.i./L (glyphosate)	Mean measured concentrations are the average of samples analyzed on days 0, and 2. Formulation contained 27.25% a.i.
measured:	control, 95.4, 183.4, 319.3, 587.2, and 1027.5 mg/L (formulation); <0.0020, 26, 50, 87, 160, and 280 mg a.i./L (glyphosate)	<i>EPA requires a geometric series with each concentration being at least 60% of the next higher one.</i>
Solvent (type, percentage, if used)	NA	<i>EPA requires solvents not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests.</i>
Lighting	16 hours light, 8 hours dark with 20 minute dawn and dusk transition periods	<i>EPA requires 16 hours light, 8 hours dark.</i>
Stability of chemical in the test system	Not reported	
Recovery of chemical	96 - 104%	
Level of Quantitation	0.0020 mg/L	
Level of Detection	Not reported	
Positive control {if used, indicate the chemical and concentrations}	NA	
Other parameters, if any	NA	

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2. Observations:

Table 2: Observations

Criteria	Details	Remarks
		Criteria
Parameters measured including the sublethal effects	Toxicity effects (immobilization)	
Observation intervals	Daily	
Were raw data included?	Yes	
Other observations, if any	NA	

II. RESULTS AND DISCUSSION

A. TOXICITY ENDPOINTS:

Immobilization was observed at all treatment levels by 48 hours. In the 95.4, 183.4, 319.3, 587.2, and 1027.5 treatment levels, 5, 60, 100, 100, and 100% of daphnids were immobilized, respectively.

Table 3: Effect of glyphosate SL formulation on the immobilization of *Daphnia magna*.

Treatment (mg formulation/L) [record measured and nominal conc. used] ¹	Observation period			
	Day 24		Day 48	
	endpoint	% affected	endpoint	% affected
Dilution water control	Immobilization	0	Immobilization	0
Solvent control	NA	NA	NA	NA
Positive control, if used	NA	NA	NA	NA
95.4 (100)	Immobilization	0	Immobilization	5
183.4 (180)	Immobilization	0	Immobilization	60
319.3 (320)	Immobilization	0	Immobilization	100
587.2 (560)	Immobilization	0	Immobilization	100
1027.5 (1000)	Immobilization	5	Immobilization	100
NOEC mg/L	95.4			

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Treatment (mg formulation/L) [record measured and nominal conc. used] ¹	Observation period			
	Day 24		Day 48	
	endpoint	% affected	endpoint	% affected
LOEC	183.4			
EC ₅₀ mg a.i./L	164.3 mg/L (44.8 mg a.i./L)			

* Nominal concentrations are presented in parentheses as mg./L glyphosate.

NR = Not reported.

C. REPORTED STATISTICS:

The study authors indicated that moving average method was used to calculate the EC₅₀ value and its 95% confidence interval.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Statistical verification of the NOAEC was performed using a Bonferroni t-test via TOXSTAT software. The EC₅₀ was determined using the probit method via TOXANAL software. The reviewer calculated these values using the mean measured concentrations of the formulation.

EC₅₀: 164.3 mg/L (44.8 mg a.i./L) 95% C.I.: 139.5 to 190.8 mg/L

NOEC: 95.4 mg/L

Probit Slope: 7.60

95% C.I.: Not reported

E. STUDY DEFICIENCIES:

The water hardness in this study was substantially higher (221 mg/L CaCO₃) than that recommended by US EPA (40-48 mg/L CaCO₃), but this deviation did not cause the pH of the test solution to vary greatly outside the range considered acceptable by EPA. The high water hardness also did not appear to largely impact the solubility of the test material, as stability of the test substance over the test period was >80% of nominal concentrations, with the exception of the two highest test concentrations.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions are similar to those reported by the study authors. Differences between the reviewer's estimates and the study authors' are attributed to the fact that the reviewer calculated NOAEC and EC₅₀ values using the mean measured concentrations of the formulation, whereas the study authors used the nominal concentrations of the formulation. The mean measured concentrations more accurately reflect test substance exposure during the study, and so these values are reported for risk assessment purposes.

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G. CONCLUSIONS:

This study is scientifically sound and fulfills EPA guidelines 72-2. This study is classified as Core for a formulated product. The 48 hour EC₅₀ was determined to be 164.3 mg/L (44.8 mg a.i./L), which classifies this SL formulation of glyphosate as practically non-toxic to daphnids on an acute toxicity basis. The NOEC was determined to be 95.4 mg/L.

EC50: 164.3 mg/L (44.8 mg a.i./L)	95% C.I.: 139.5 to 190.8 mg/L
NOEC: 95.4 mg/L	
Probit Slope: 7.60	95% C.I.: Not reported

III. REFERENCES:

- 1) OECD (1984). OECD Guidelines for Testing of Chemicals, Method 202, Part I (Acute Immobilisation Test). Adopted 4 April 1984.
- 2) Environmental Protection Agency (1985). Hazard Evaluation Division Standard Evaluation Procedure 540/9-85-005. Acute toxicity test for freshwater invertebrates.
- 3) Official Journal of the European Communities, L 383 A, Part C.2, Acute toxicity for *Daphnia*. 29 December 1992.
- 4) Elendt B P and Bias W R (1990). Trace Nutrient Deficiency in *Daphnia magna* Cultured in Standard Medium for Toxicity Testing. Effects of the Optimisation of Culture Conditions on Life History Parameters of *Daphnia magna*. Water Research, 24, 1157-1167.
- 5) Stephan C E (1977). Methods for calculating an LC50. Proceedings first annual symposium on aquatic toxicology. Aquatic Toxicology and Hazard Evaluation. Ed: Mayer F L Hamelink J L, ASTM STP 634 65-84.

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APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

immobilization

File: 4003i

Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	5.000	5.000		
2	95.4	4.750	4.750	0.391	
3	183.4	2.000	2.000	4.688	*
4	319.3	0.000	0.000	7.813	*
5	587.2	0.000	0.000	7.813	*
6	1027.5	0.000	0.000	7.813	*

Bonferroni T table value = 2.55 (1 Tailed Value, P=0.05, df=18,5)

immobilization

File: 4003i

Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2		Ho:Control<Treatment			
GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	95.4	4	1.634	32.7	0.250
3	183.4	4	1.634	32.7	3.000
4	319.3	4	1.634	32.7	5.000
5	587.2	4	1.634	32.7	5.000
6	1027.5	4	1.634	32.7	5.000