Data Evaluation Report on the acute toxicity of a Glyphosate SL formulation to Rainbow trout (*Oncorhyncus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 45374001

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D275559
OECD Data Point	Mortality
EPA MRID	45374001
EPA Guideline	72-1

Test material: **Purity:** 27.25%

Common name: Glyphosate 360 g/L SL formulation

Chemical name: IUPAC: N-(Phosphonomethyl)glycine

CAS name: N-(Phosphonomethyl)glycine

CAS No.: 1071-83-6

Synonyms: YF11357

Primary Reviewer: Dana Worcester, M.S.
Staff Scientist, Dynamac Corporation

Signature: Dana Worcester
Date: 10/11/01

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Date: 02/13/02
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Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 417300

Date Evaluation Completed: February 13, 2002

CITATION: Swarbrick R.H. and Shillabeer N. 1999. Glyphosate: Acute Toxicity to Rainbow Trout (*Oncorhyncus mykiss*) of a 360 g/L SL Formulation. Unpublished study performed by Brixham Environmental Laboratory, Surrey, UK. Laboratory Project ID AG0360/B and sponsored by Zeneca Agrochemicals, Berkshire, UK. Sponsor Project ID 43919. Completed October 15, 1999.

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhyncus mykiss*) were exposed to an SL formulation of Glyphosate (27.25% a.i. N-(phosphonomethyl)glycine) under static conditions. Mean measured concentrations of glyphosate a.i. were 0 (control), 27, 50, 85, 160, and 270 mg a.i./L. Mean measured concentrations of the glyphosate SL formulation were 0, 99, 183.5, 319.3, 587.2, and 1027.5 mg/L. The 96-h glyphosate formulation LC_{50} was 824 mg/L (224.5 mg a.i./L), based on mean measured concentrations. As a result, this SL formulation of Glyphosate is classified as practically nontoxic to rainbow trout *(Oncorhyncus mykiss*) according to the classification system of the U.S. EPA. The NOEC was 587.2 mg/L based on mortality and 183.5 mg./L based on sublethal toxic symptoms.

This toxicity study is classified supplemental for a formulated product because it does not satisfy the guideline requirements for an acute freshwater fish toxicity study. There were several procedural deviations including the use of dechlorinated tap water and aeration of the test vessels, which may have impacted water quality and test substance exposure (EPA-540/9-85-006, 72-1). However, EPA is not requiring that the study be repeated at this time.

Results Synopsis

Test Organism Size/Age(mean Weight or Length): 1.65 g and 48 mm
Test Type (Flowthrough, Static, Static Renewal): Static
LC_{50}: 824 mg/L (224.5 mg a.i./L) 95% C.I.: 587.2-1027.5 mg/L
NOEC: 587.2 mg/L (based on mortality); 183.5 mg/L (based on sublethal effects)
Probit Slope: NA
EC_{50}: Not reported
Endpoint(s) Affected: Mortality and sublethal effects such as weak swimming, loss of balance and dark discoloration.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was conducted in accordance with procedures formulated by the EPA Acute Toxicity Test for Freshwater Fish (EPA-540/9-85-006); Data requirements followed OECD Guideline No. 203 (OPP Guideline No. 72-1, 3)

Deviations included the following:

1. Dechlorinated dilution water was used. US EPA advises against the use of dechlorinated tap water as dilution water.
2. Control and test vessels were exposed to gentle aeration. US EPA prohibits aeration of the test solutions.
3. The biomass loading rate (1.1 g/L) under static conditions was higher than required by EPA guidelines (≤ 0.8 g/L at ≤ 17°C).
4. Acclimation period (7 days) was shorter than EPA guidelines (14 days).
5. Test organism weight and length at study initiation were not reported.
6. The pH (6.6-7.6) low end of the pH range was lower than required by EPA guidelines (7.2-7.6).
7. Precipitation was observed at the highest concentration (1000 mg/L). Didn't state if filtration or centrifugation was conducted before chemical analysis.
8. Total organic carbon was not reported.

These deviations, together, impacted the acceptability of this study.

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

A. MATERIALS:

1. Test Material Glyphosate SL formulation

Description: Brown liquid

Lot No./Batch No. : AG0462

Purity: 27.25%

Stability of Compound Under Test Conditions: Test concentrations were measured at 0 and 96 hours. Mean recovery of Glyphosate ranged from 100 to 107% of nominal.

Storage conditions of test chemicals: The test substance was stored at room temperature in the dark.

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss*)
Age at test initiation: Not reported
Weight at study initiation: Not reported; at conclusion mean = 1.65 g
Length at study initiation: Not reported; at conclusion 48 mm
Source: Houghton Springs Fish Farm, Winterborne, Blandford Forum, Dorset

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study

No range finding study was conducted.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation: period: conditions: (same as test or not) Feeding: Health: (any mortality observed)	 7 days Same as test, except for temperature (12±2°C) Not fed 48-hours prior to test initiation; not fed during exposure No mortality 7 days prior to the test	 *EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96-hour	*(EPA/OECD requires: 96 hour)*

Parameter	Details	Remarks Criteria
Test condition static/flow through	 Static	
Type of dilution system- for flow through method. Renewal rate for static renewal	NA NA	*(EPA requires: Must provide reproducible supply of toxicant)* *(EPA requires: Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Gentle aeration	*(EPA requires: no aeration; OECD permits aeration)*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass aquaria 40 x 28 x 28 cm 15 L	*EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution)*

Parameter	Details	Remarks Criteria
Source of dilution water Quality:	Tap water passed through activated carbon, filtered, dechlorinated with sodium thisulfate, filtered (25 & 10 µm), and UV-irradiated.	Dechlorinated tap water is prohibited for use as dilution water by US EPA. *(EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water); OECD permits dechlorinated tap water.*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	 48.3 mg/L $CaCO_3$ 6.6-7.6 ≥ 60% saturation Not reported Not reported Not reported Not reported <2 µg/L 14.2-15.2°C NA DO, pH, and temperature were measured daily. Temperature measured continuously	 *(EPA hardness: 40 - 48 mg as $CaCO_3$/L; OECD allows 10 -250 mg as $CaCO_3$/L)* *(EPA pH: 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8);* *OECD allows pH 6.0 - 8.5* (EPA Dissolved Oxygen: *Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%); OECD requires at least 80% saturation value.* *(EPA temperture: estuarine/marine: 22 ± 1 °C* *OECD requires 21 - 25°C for bluegill and 13 - 17°C for rainbow trout* *(EPA salinity: 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰)* *(EPA water quality: measured at beginning of test and every 48 hours)*

Parameter	Details	Remarks Criteria
Number of replicates/groups: control: solvent control: treated ones:	 1 NA 1	*(EPA/OECD requires: Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geo-metric series)*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 10 10	*(EPA: ≥ 10/concentration); OECD requires at least 7 fish/concentration*
Biomass loading rate	1.1 g fish/L/day	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Test concentrations: nominal: measured:	Formulation - 100, 180, 320, 560, 1000 mg/L (27, 49, 87, 150, 270 mg/L a.i.) Formulation - 99, 183.5, 319.3, 587.2, and 1027.5 mg/L (27, 50, 85, 160, 270 mg/L a.i.)	Formulation contained 27.25% a.i.
Solvent (type, percentage, if used)	NA	*EPA requires: Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD requires solvent , exceed 100 mg/L.*
Lighting	16:8	*(EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*

Parameter	Details	Remarks Criteria
Feeding	Not fed 48-hour prior to study or during exposure	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	100-107% 0.0020 mg/L Not reported	
Positive control {if used, indicate the chemical and concentrations}	NA	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and symptoms of toxicity	
Observation intervals	24, 48, 72 and 96 hours of exposure	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes	
Other observations, if any	NA	

II. RESULTS and DISCUSSION:

A. <u>MORTALITY</u>:
By 96-hours 90% mortality was observed at the 1027.5 mg/L test concentration and greater than 30% of the population in the 587.2 mg/L test concentration were dead or exhibited symptoms of toxicity.

Table 3: Effect of Glyphosate SL formulation on mortality of Rainbow trout (*Oncorhyncus mykiss*).

Treatment (mg formulation/L) [record measured and nominal conc. used][1]	No. of fish at start of study	Observation period							
		Day 1		Day 2		Day 3		Day 4	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Control (dilution water only)	10	0	0	0	0	0	0	0	0
Solvent control	NA	NA	NA	NA	NA	NA	NA	NA	NA
100 (99)	10	0	0	0	0	0	0	0	0
180 (183.5)	10	0	0	0	0	0	0	0	0
320 (319.3)	10	0	0	0	0	0	0	0	0
560 (587.2)	10	0	0	0	0	0	0	0	0
1000 (1027.5)	10	0	0	Not reported	30	Not reported	80	Not reported	90
NOEC[2]	180 mg/L								
LC_{50}[2]	800 mg/L (700-920 mg/L)								
Positive control, if used mortality: LC_{50}:	NA	NA	NA	NA	NA	NA	NA	NA	NA

[1] Measured values are presented in parentheses.
[2] Study authors based NOEC and LC_{50} estimates on nominal concentrations of the formulation. Determination of the NOAEC was based on mortality and symptoms of toxicity.

B. NON-LETHAL TOXICITY ENDPOINTS:

The Sponsor stated that the observed sublethal effects were loss of balance, weak swimming and dark discoloration.

Table 4. Sub-lethal effect of Glyphosate SL formulation on Rainbow trout (*Oncorhyncus mykiss*).

Treatment (mg formulation/L) [record measured and nominal conc. used][1]	Observation period			
	endpoint at Day 1	endpoint at Day 2	endpoint at Day 3	endpoint at Day 4
	% affected	% affected	% affected	% affected
Control (dilution water only)	<10%	<10%	<10%	<10%
Solvent control	NA	NA	NA	NA
100 (99)	<10%	<10%	<10%	<10%
180 (183.5)	<10%	<10%	<10%	<10%
320 (319.3)	<10%	<10%	<10%	11-30%
560 (587.2)	<10%	11-30%	<10%	>30%
1000 (1027.5)	>30%	>30%	>30%	>30%
NOEC	183.5 mg/L			
LOEC	319.3 mg/L			
EC_{50}	Not reported			
Positive control, if used % sublethal effect: EC_{50}:	NA	NA	NA	NA

[1] Measured values are presented in parentheses.
[2] Study authors based NOEC and LC_{50} estimates on nominal concentrations of the formulation.

C. REPORTED STATISTICS:

The study authors reported that the LC_{50} value and 95% confidence limits were calculated by the moving average angle method and were based on nominal concentrations of the formulation. The study authors did not report the method used to determine the no observed effect level, but this estimate was based on both mortalities and symptoms of toxicity.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The reviewer determined the NOAEC visually because mortality was only shown at the highest test concentration, 1027.5 mg/L. The LC_{50} was determined using the binomial method via the TOXANAL software program.

LC_{50}: 824 mg/L (224.5 mg a.i./L) (based on mortality); 95% C.I.: 587.2 to 1027.5 mg/L
NOEC: 587.2 mg/L (based on mortality); 183.5 (based on toxic symptoms) Slope: N/A

E. STUDY DEFICIENCIES:

There were several deviations from US EPA experimental procedure (EPA-540/9-85-006, 72-1). Major deviations included the use of dechlorinated dilution water and aeration of test vessels during the study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with those of the study authors. However, the reviewer's LC_{50} estimate was slightly higher than the study authors' because the reviewer based this estimate on the mean measured concentrations, while the authors based the estimate on the nominal concentrations. The reviewer recommends using the estimate based on the mean measured concentrations, because this more accurately reflects test substance exposure during the study.

The glyphosate formulation contained 27.25% a.i. NOEC and LC_{50} estimates were based on the formulation concentrations rather than the active ingredient (N-(phosphonomethyl)glycine) concentrations. The formulation's limit of solubility in water is quoted to be 9.1 g/L which the reviewer agreed when precipitation occurred at the highest concentration (10 g/L). The reviewer recommends centrifuging the highest concentration before chemical analysis, the amount that was bio-available may have been considerable less than the study authors' 104% nominal.

There were several deviations from US EPA experimental procedure (EPA-540/9-85-006, 72-1). Major deviations included the use of dechlorinated dilution water and aeration of test vessels during the study. As a result, this study is classified as Supplemental for a formulated product.

G. CONCLUSIONS:

This study is classified as Supplemental due to several deviations from US EPA protocol, including the use of dechlorinated tap water and aeration of the test vessels. This study does not fulfills EPA guidelines 72-1, however, EPA is not requiring that the study to be repeated at this time because it contains useful information. The LC_{50} was determined to be 824 mg/L (224.5 mg a.i./L), based on mean measured concentrations. As a result, this SL formulation of Glyphosate is classified as practically nontoxic to rainbow trout on an acute basis *(Oncorhyncus mykiss)* according to the classification system of the U.S. EPA.

LC_{50}: 824 mg./L (224.5 mg a.i./L) 95% C.I.: 587.2 to 1027.5 mg/L
NOEC: 587.2 mg/L (based on mortality); 183.5 mg/L (based on toxic symptoms)

III. REFERENCES:

U.S. Environmental Protection Agency. 1985. Hazard Evaluation Division. Standard Evaluation Procedure. EPA-540/9-85-006. Acute Toxicity Test for Freshwater Fish.

OECD. 1992. OECD Guidelines for Testing Chemicals. Method 203. Fish, Acute Toxicity Test.

Official Journal of the European Communities, L 383 A, Part C.1, Acute Toxicity for Fish. December 29, 1992.