Data Evaluation Report on the acute toxicity of MON 77360 (Glyphosate) to Rainbow Trout (*Oncorhynchus mykiss*)

PMRA Submission Number {.....} EPA MRID Number 45365003

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D294119
	OECD Data Point	
	EPA MRID	45365003
	EPA Guideline	72-1(c)

Test material: MON 77360 (formulation)
Common name: Glyphosate
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: Not reported

Purity: 30.0% w/w glyphosate acid equivalents

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan
Date: 3/4/2004

QC Reviewer: Greg Hess
Staff Scientist, Dynamac Corporation

Signature:
Date: 3/4/2004

Primary Reviewer: ~~Stephanie Syslo.~~
OPP/EFED/ERB III Stephen Carey, Biologist

Signature: ~~Stephanie Syslo~~
Date: 6/28/04

Secondary Reviewer(s): Anita Pease
{EPA/OECD/PMRA}

Date: Anita Pease
9/23/04

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 103601

Date Evaluation Completed:

CITATION: Drottar, K. and Krueger, H. 2000. MON 77360: A 96-Hour Static Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 139A-207. Study submitted by Monsanto Company, Ceregen Business Unit, St. Louis, MO. Study initiated April 1, 1997 and completed November 7, 2000.

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Rainbow Trout (*Oncorhynchus mykiss*) were exposed to Glyphosate at mean-measured concentrations of <0.833 (LOQ; negative control), 2.6, 4.2, 6.9, 12, and 17 mg MON 77360/L (a formulation that is 41% isopropylamine glyphosate by weight, corresponding to 360 g glyphosate acid per liter) under static conditions. Nominal concentrations were 0 (negative control), 2.6, 4.3, 7.2, 12, and 20 mg MON 77360/L. After 96 hours of exposure, mortality was 100% in the 6.9, 12, and 17 mg MON 77360/L treatment groups. There were no mortalities in the control, 2.6, or 4.2 mg MON 77360/L treatment groups. The LC_{50} (with 95% C.I.) was 5.4 (4.2-6.9) mg MON 77360/L, which categorizes MON 77360 as moderately toxic to juvenile Rainbow Trout (*Oncorhynchus mykiss*) on an acute toxicity basis. Sub-lethal effects included erratic swimming and lying on bottom with little motion in surviving fish from the 12 mg MON 77360/L treatment group after 5 hours. No other sub-lethal effects were observed. The NOAEC and LOAEC observed for mortality and sub-lethal effects were 4.2 and 6.9 mg MON 77360/L, respectively.

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater fish (§72-1) because the mean weight of the fish at study termination was 0.18 g, which is less than half the recommended initial weight range of 0.5 to 5 g. Consequently, this study provides useful information, but is classified SUPPLEMENTAL. Based on the results of this study, MON 77360 is categorized as moderately toxic to juvenile Rainbow Trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

Results Synopsis

Test Organism Size/Age (mean Weight or Length):	0.18 g (wet), 24 mm (mean of 10 control fish at study termination)
Test Type (Flow-through, Static, Static Renewal):	Static

96-Hour
LC_{50}: 5.4 mg MON 77360/L 95% C.I.: 4.2-6.9 mg MON 77360/L
NOAEC: 4.2 mg MON 77360/L
LOAEC: 6.9 mg MON 77360/L
Endpoints affected: Mortality and sub-lethal effects

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the Series 72 of Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms; U.S. Environmental Protection Agency, Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Fish; ASTM Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians; and OECD Guideline for Testing of Chemicals 203: Fish, Acute Toxicity Test. Deviation from §72-1c included:

1. The hardness (132 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH (8.4-8.7) was slightly greater than recommended (7.2-7.6).

2. Mean fish weight (0.18 g), determined from 10 control fish at test termination, was less than the recommended initial range of 0.5-5g.

These deviations do not affect the validity of the study. However, this study does not fulfill guideline requirements.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with GLP standards of the U.S. EPA (40 CFR Part 160), OECD, and Japan MAFF (p. 3).

A. MATERIALS:

1. Test Material MON 77360 (formulation containing 30% glyphosate)

Description: Yellow liquid

Lot No./Batch No. : GLP-9703-7576-F

Purity: 30.0%

Stability of Compound Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination at 0 hour (81-101% of nominal, 48 hours (88-107% of nominal), and 96 hours (98-106% of nominal).

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. OECD requirements were not reported.

Storage conditions of test chemicals: Stored at room temperature.

2. Test organism:

Species: Rainbow Trout (*Oncorhynchus mykiss*)

Age at test initiation: Juvenile

Weight at study initiation: Not provided; the wet weight of 10 control fish measured at test termination averaged 0.18 g (range of 0.15 to 0.22 g).

Length at study initiation: Not provided; the length of the 10 control fish measured at test termination averaged 24 mm (range of 22 to 25 mm).

Source: Mt. Lassen Trout, Red Bluff, CA.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: The definitive nominal test concentrations were based on results of range finding toxicity tests. The range-finding study results were not reported.

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation period:	Fish were held for 33 days prior to testing (including 14 day holding period and 49 hour acclimation).	
Conditions: (same as test or not)	Same as test	
Feeding:	Commercially-prepared diet (Ziegler Brothers) was provided except during the 48 hours prior to and during testing.	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Health: (any mortality observed)	During acclimation, fish showed no signs of disease or stress (mortality not reported).	
Duration of the test	96-hour	*EPA/OECD requires: 96 hour*
Test condition static/flow through	Static	
Type of dilution system- for flow through method. Renewal rate for static renewal	N/A N/A	*EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	Test water was not aerated during the definitive test.	*EPA requires: no aeration; OECD permits aeration*

Parameter	Details	Remarks Criteria
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass aquaria 19 L 15 L	 *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water	The dilution water was filtered, laboratory well water.	*EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water;* *OECD permits dechlorinated tap water.*

Parameter	Details	Remarks Criteria
Water parameters: Hardness	132 mg $CaCO_3$ /L	The hardness (132 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH (8.4-8.7) was slightly greater than recommended (7.2-7.6).
pH	8.4-8.7	
Dissolved oxygen	9.4-10.2 mg/L (≥87% saturation)	
Total Organic Carbon	<1.0 mg/L (August 21, 1996 sample)	
Particulate Matter	Not reported, TDS was 261 ppm	
Metals	Not contaminated	
Pesticides	Not detected	
Chlorine	Not reported, well water was used for dilution water prep.	
Temperature	12.0-12.7°C	
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	The DO and pH were measured in alternate replicates at test initiation and every 24 hours thereafter. Temperature was measured in each replicate at the beginning and end of the test. Also, the temperature in one negative control replicate was continuously measured. Hardness was measured in dilution water at test initiation.	

Parameter	Details	Remarks Criteria
Concentration of test material: nominal: measured:	0 (negative control), 2.6, 4.3, 7.2, 12, and 20 mg MON 77360/L <0.833 (LOQ; negative control), 2.6, 4.2, 6.9, 12, and 17 mg MON 77360/L	*EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series*
Solvent (type, percentage, if used)	N/A	*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.*
Number of fish/replicates: negative control: solvent control: treated:	20 fish, divided into two replicates containing 10 fish each N/A 20 fish, divided into two replicates containing 10 fish each	*EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration*
Biomass loading rate	0.12 g fish/L	*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*
Lighting	16-hours light/8-hours dark, with a 30-minute transition period.	Light intensity of 380 lux (test initiation). *EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Animals were not fed during testing.	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	84.0-112% of nominal 0.833 mg MON 77360/L Not reported.	Based on matrix spikes (at 1.00, 7.50, and 30.0 mg MON 77360/L) analyzed concurrently with the samples.

Parameter	Details	Remarks Criteria
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sub-lethal effects/toxicity symptoms	Mortality and sub-lethal effects	
Observation intervals	5, 24, 48, 72 and 96 hours of exposure	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, mortality was 100% in the mean measured 6.9, 12, and 17 mg MON 77360/L treatment groups. There were no mortalities in the control, 2.6, or 4.2 mg MON 77360/L treatment groups.

Table 3: Effect of MON 77360 on mortality of Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, mg MON 77360/L, measured and (nominal conc.)	No. of fish at start of study	Observation period					
		0-24 Hours		48-72 Hours		96 Hours	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative control	20	0	0	0	0	0	0
2.6 (2.6)	20	0	0	0	0	0	0
4.2 (4.3)	20	0	0	0	0	0	0
6.9 (7.2)	20	20	100	20	100	20	100
12 (12)	20	20	100	20	100	20	100
17 (20)	20	20	100	20	100	20	100
NOAEC (mortality)	4.2						
LC_{50} (95% C.I.)	5.4 (4.2-10)						
Positive control, if used mortality: LC_{50}:	N/A*	N/A	N/A	N/A	N/A	N/A	N/A

* N/A = Not Applicable

B. NON-LETHAL TOXICITY ENDPOINTS:

Sub-lethal effects included erratic swimming and lying on bottom with little motion in surviving fish from the mean measured 12 mg MON 77360/L treatment group after 5 hours. No other sub-lethal effects were observed.

Table 4. Sub-lethal effect of MON 77360 on Rainbow Trout (*Oncorhynchus mykiss*).

Treatment, mg MON 77360/L, measured and (nominal conc.)	**endpoint at 24 Hours**	**endpoint at 48 Hours**	**endpoint at 72 Hours**	**endpoint at 96 Hours**
	% affected[1]	**% affected**	**% affected**	**% affected**
Negative control	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
2.6 (2.6)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
4.2 (4.3)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
6.9 (7.2)	---	---	---	---
12 (12)	---	---	---	---
17 (20)	---	---	---	---
NOAEC (sub-lethal)	4.2			
LOAEC (sub-lethal)	6.9			
EC_{50}	Not determined			
Positive control, if used % sub-lethal effect: EC_{50}:	N/A*	N/A	N/A	N/A

[1] % Affected is the number of fish exhibiting symptoms/number of surviving fish x 100.
--- 100% mortality
* N/A = Not Applicable

C. REPORTED STATISTICS:

Statistical Method: The 96-hour LC_{50} value (with 95% confidence limits) was calculated by binomial probability with non-linear interpolation (C.E. Stephan computer program). The NOAEC and LOAEC were visually determined, based on observed treatment-related mortality and sub-lethal effects.

96-Hour
LC_{50}: 5.4 mg MON 77360/L 95% C.I.: 4.2-6.9 mg MON 77360/L
NOAEC: 4.2 mg MON 77360/L
LOAEC: 6.9 mg MON 77360/L
Endpoints affected: Mortality and sub-lethal effects

D. VERIFICATION OF STATISTICAL RESULTS:

The 96-hour LC_{50} was determined using the binomial method via TOXANAL statistical software, as this method provided a sound 95% confidence interval, while the probit method did not. The NOAEC was visually determined as the highest concentration which exhibited no significant mortality (<10%) or sub-lethal effects. All toxicity values were determined in terms of the reported mean measured treatment concentrations.

96-Hour
LC_{50}: 5.4 mg MON 77360/L 95% C.I.: 4.2-6.9 mg MON 77360/L
Probit slope: N/A
NOAEC: 4.2 mg MON 77360/L
LOAEC: 6.9 mg MON 77360/L
Endpoints affected: Mortality and sub-lethal effects

E. STUDY DEFICIENCIES:

This study is scientifically valid. However, the mean fish weight of 0.18 g was determined from 10 control fish at study termination and was less than half the recommended initial weight range of 0.5-5 g. According to US EPA Pesticide Reregistration Rejection Rate Analysis document (EPA 738-R-94-035, December 1994):

> "The range of preferred weights for test fish is rather wide, thereby giving the individual registrants flexibility in obtaining appropriate sized fish for the test. The Agency requires consistency in size of test fish to allow for comparison of test results among chemicals."

Consequently, this study does not fulfill guideline requirements for an acute toxicity study with the Rainbow Trout (*Oncorhynchus mykiss*) and is classified SUPPLEMENTAL.

F. REVIEWER'S COMMENTS:

Results of the reviewer's statistical verification were identical to those of the study authors.

The registrant provided the following information in an email to EFED (attn: Vickie Walters) dated 10/29/03: MON 77360 is 41% isopropylamine glyphosate by weight, corresponding to 360 g glyphosate acid per liter. It contains a surfactant blend as specified on the CSF for 524-475, and is a complete product for application

without the need of added surfactant.

Mean measured values reported for the 6.9, 12 and 17 mg MON 77360/L treatment levels were determined using the arithmetic mean of the 0- and 48-hour measured values because 100% mortality was observed by 48 hours and thus, no further results could be gleaned by continuing the test for 96 hours at the 6.9, 12 and 17 mg MON 77360/L treatment levels. Mean measured values reported for the negative control, 2.6 and 4.2 mg MON 77360/L treatment levels were determined using the arithmetic mean of the 0-, 48- and 96-hour measured values.

G. CONCLUSIONS:

This study is scientifically sound, but does not satisfy the guideline requirements for an acute toxicity study with freshwater fish (§72-1) because the mean weight of the fish at study termination was 0.18 g, which is less than half the recommended initial weight range of 0.5 to 5 g. Consequently, this study provides useful information, but is classified SUPPLEMENTAL. Based on the results of this study, MON 77360 is categorized as moderately toxic to juvenile Rainbow Trout (*Oncorhynchus mykiss*) on an acute toxicity basis.

96-Hour
LC_{50}: 5.4 mg MON 77360/L 95% C.I.: 4.2-6.9 mg MON 77360/L
NOAEC: 4.2 mg MON 77360/L
LOAEC: 6.9 mg MON 77360/L
Endpoints affected: Mortality and sub-lethal effects

III. REFERENCES:

U.S. Environmental Protection Agency. 1982. *Pesticide Assessment Guidelines, FIFRA Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms*. EPA 540/9-82-024.

U.S. Environmental Protection Agency. 1985. *Standard Evaluation Procedure, Acute Toxicity Test for Freshwater Fish*. Hazard Evaluation Division. Office of Pesticide Programs, EPA 540/9-85-006. Washington, D.C.

ASTM Standard E729-88a. 1994. *Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians*. American Society for Testing and Materials.

OECD. 1993. OECD Guidelines for Testing of Chemicals. *Guideline 203: Fish, Acute Toxicity Test*. Adopted by the Council on 12 July 1992.

APHA, AWWA, WPCF. 1995. *Standard Methods for the Examination of Water and Wastewater*. 16th Edition, American Public Health Association. American Water Works Association. Water Pollution Control Federation, New York.

Stephan, C.E. 1977. " Methods for Calculating and LC_{50}", *Aquatic Toxicology and Hazard Evaluation*. American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

Stephan, C.E. 1978. U.S. EPA. Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

TOXANAL RESULTS: Calculated using the reported mean measured concentrations (Table 3, p. 19).

LC50:

	EXPOSED	DEAD	DEAD	PROB.(PERCENT)
17	20	20	100	9.536742E-05
12	20	20	100	9.536742E-05
6.9	20	20	100	9.536742E-05
4.2	20	0	0	9.536742E-05
2.6	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 4.2 AND 6.9 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 5.383308

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.