Data Evaluation Report on the acute toxicity of MON 77360 (Glyphosate) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....} EPA MRID Number 45365002

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D294119
	OECD Data Point	
	EPA MRID	45365002
	EPA Guideline	72-1(a)

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

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Date: 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 Bluegill Sunfish (*Lepomis macrochirus*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 139A-206. 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, Bluegill Sunfish (*Lepomis macrochirus*) were exposed to MON 77360 (a formulation that is 41% isopropylamine glyphosate by weight, corresponding to 360 g glyphosate acid per liter) at mean-measured concentrations of <0.833 (LOQ; negative control), 2.5, 4.4, 6.8, 10, and 18 mg MON 77360/L 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 35, 100, and 100% in the 6.8, 10, and 18 mg MON 77360/L treatment groups, respectively. There were no mortalities in the control, 2.5, or 4.4 mg MON 77360/L treatment groups. The LC_{50} (with 95% C.I.) was 7.3 (4.4-10) mg MON 77360/L, which categorizes MON 77360 as moderately toxic to juvenile Bluegill Sunfish (*Lepomis macrochirus*) on an acute toxicity basis. Sub-lethal effects included erratic swimming, lying on bottom with little motion and lethargy during the test and in surviving fish from the 6.8 mg MON 77360/L treatment group. The NOAEC and LOAEC observed for mortality and sub-lethal effects were 4.4 and 6.8 mg MON 77360/L, respectively.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with Bluegill Sunfish [§72-1(a)]. This study is classified as CORE.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): 0.76 g (wet), 33 mm (mean of 10 control fish at study termination)
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour
LC_{50}: 7.3 mg MON 77360/L 95% C.I.: 4.4-10 mg MON 77360/L
NOAEC: 4.4 mg MON 77360/L
LOAEC: 6.8 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 §72-1a included:

1. The hardness (136 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH (7.9-8.6) was slightly greater than recommended (7.2-7.6).

This deviation does not affect the validity or acceptability of the study.

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 (a.e.)

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 (82-99% of nominal, 48 hours (84-105% of nominal), and 96 hours (94-111% of nominal). Results were reviewer-calculated.

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: Bluegill Sunfish (*Lepomis macrochirus*)

Age at test initiation: Juvenile

Weight at study initiation: Not provided; the wet weight of 10 control fish measured at test termination averaged 0.76 g (range of 0.60 to 1.4 g).

Length at study initiation: Not provided; the length of the 10 control fish measured at test termination averaged 33 mm (range of 29 to 40 mm).

Source: Northern Aquatics, Rhinebeck, New York.

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: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	Fish were held for 244 days prior to testing (including 14 day holding period and 50 hour acclimation). Same as test Commercially-prepared diet (Ziegler Brothers) and live brine shrimp nauplii (Artemia sp) were provided except during the 48 hours prior to and during testing. During acclimation, fish showed no signs of disease or stress (mortality not reported).	*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*
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Static 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*
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*

Parameter	Details	Remarks Criteria
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.*
Water parameters: Hardness	136 mg $CaCO_3$ /L	The hardness (136 mg/L as $CaCO_3$) was higher than recommended (40-48 mg/L as $CaCO_3$). The pH (7.9-8.6) was slightly greater than recommended (7.2-7.6).
pH	7.9-8.6	
Dissolved oxygen	5.4-8.4 mg/L (≥62% saturation)	
Total Organic Carbon	<1.0 mg/L (August 21, 1996 sample)	
Particulate Matter	Not reported	
Metals	Acceptable levels	
Pesticides	Not detected	
Chlorine	Not reported	
Temperature	21.5-22.1°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 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), 2.5, 4.4, 6.8, 10, and 18 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	0.50 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 385 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*

Parameter	Details	Remarks Criteria
Recovery of chemical Level of Quantitation Level of Detection	88.8-119% 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.
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	2, 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 35, 100 and 100% in the 6.8, 10, and 18 mg MON 77360/L treatment groups, respectively. There were no mortalities in the control, 2.5 or 4.4 mg MON 77360/L treatment groups.

Table 3: Effect of MON 77360 on mortality of Bluegill Sunfish (*Lepomis macrochirus*).

Treatment, mg MON 77360/L, measured and (nominal conc.)	No. of fish at start of study	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
2.5 (2.6)	20	0	0	0	0	0	0
4.4 (4.3)	20	0	0	0	0	0	0
6.8 (7.2)	20	0	0	4	20	7	35
10 (12)	20	20	100	20	100	20	100
18 (20)	20	20	100	20	100	20	100
NOAEC (mortality)	4.4 mg MON 77360/L						
LC_{50} (95% C.I.)	7.3 (4.4-10) mg MON 77360/L						
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, lying on bottom with little motion and lethargy in surviving fish from the 6.8 mg MON 77360/L treatment group during the test.

Table 4. Sub-lethal effect of MON 77360 on Bluegill Sunfish (*Lepomis macrochirus*).

Treatment, mg MON 77360/L, measured and (nominal conc.)	Observation period			
	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
2.5 (2.6)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
4.4 (4.3)	No abnormalities detected	No abnormalities detected	No abnormalities detected	No abnormalities detected
6.8 (7.2)	No abnormalities detected	Erratic swimming-12%; Lying on bottom-12%.	Lethargic-31%; Lying on bottom-13%.	Lethargic-46%.
10 (12)	---	---	---	---
18 (20)	---	---	---	---
NOAEC (sub-lethal)	4.4 mg MON 77360/L			
LOAEC (sub-lethal)	6.8 mg MON 77360/L			
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 NOEC and LOEC were visually determined, based on observed treatment-related mortality and sub-lethal effects.

96-Hour
LC_{50}: 7.3 mg MON 77360/L 95% C.I.: 4.4-10 mg MON 77360/L
NOEC: 4.4 mg MON 77360/L
LOEC: 6.8 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}: 7.3 mg MON 77360/L 95% C.I.: 4.4-10 mg MON 77360/L
Probit slope: N/A
NOAEC: 4.4 mg MON 77360/L
LOAEC: 6.8 mg MON 77360/L
Endpoints affected: Mortality and sub-lethal effects

E. STUDY DEFICIENCIES:

There were no significant deviations from U.S. EPA guideline §72-1a that affected the acceptability or validity of this study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions 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 10 and 18 mg MON 77360/L treatment levels were determined using the average of the 0- and 48-hour measured values because 100% mortality was observed by 24 hours and thus, no further results could be gleaned by continuing the test for 96 hours at the 10 and 18 mg MON 77360/L treatment levels. Mean measured values reported for the negative control, 2.5, 4.4 and 6.8 mg MON 77360/L treatment levels were determined using the average of the 0-, 48- and 96-hour measured values.

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-1a, and is classified as CORE. Based on the results of this study, MON 77360 is categorized as moderately toxic to juvenile Bluegill Sunfish (*Lepomis macrochirus*) on an acute toxicity basis.

96-Hour
LC_{50}: 7.3 mg MON 77360/L 95% C.I.: 4.4-10 mg MON 77360/L
NOAEC: 4.4 mg MON 77360/L
LOAEC: 6.8 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.

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

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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.

LC50:

	EXPOSED	DEAD	DEAD	PROB.(PERCENT)
18	20	20	100	9.536742E-05
10	20	20	100	9.536742E-05
6.8	20	7	35	13.1588
4.4	20	0	0	9.536742E-05
2.5	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 4.4 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 7.279839

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.