

**Data Evaluation Report on the Acute Toxicity of MON 77360 (Glyphosate) to Freshwater Invertebrates - *Daphnia magna***

PMRA Submission Number {.....}

EPA MRID Number 45365004

**Data Requirement:** PMRA DATA CODE {.....}  
EPA DP Barcode D294119  
OECD Data Point  
EPA MRID 45365004  
EPA Guideline §72-2

**Test material:** MON 77360 (formulation) **Purity:** 30.0% w/w glyphosate acid equivalents  
**Common name:** Glyphosate  
**Chemical name:** IUPAC: Not reported  
CAS name: Not reported  
CAS No.: Not reported  
Synonyms: Not reported

**Primary Reviewer:** Rebecca Bryan  
Staff Scientist, Dynamac Corporation

**Signature:** Rebecca Bryan  
**Date:** 3/4/2004

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**Primary Reviewer:** ~~Stephanie Syslo~~ Stephen Carey  
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**Date:** June 10, 2004

**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:** Drotter, K.R. and H.O Kreuger. 2000. MON 77360: A 48-Hour Static Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Unpublished study performed by Wildlife International, Ltd., Easton, MD. Laboratory Project No. 139A-205. Study sponsored by Monsanto Company, Ceregen Business Unit, St. Louis, MO. Study initiated April 1, 1997 and completed November 7, 2000.



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## EXECUTIVE SUMMARY:

The 48-hour acute toxicity of MON 77360 to the water flea, *Daphnia magna*, was studied under static conditions. Neonate daphnids were exposed to the test material at nominal concentrations of 0 (negative control), 1.6, 2.6, 4.3, 7.2, and 12 mg MON 77360/L. Mean measured concentrations were <0.833 (LOQ, negative control), 1.6, 2.6, 4.5, 7.4, and 12 mg MON 77360/L.

By 48 hours, mortality was 0% in the control group and in the 2.6 mg MON 77360/L test group, and 5, 5, 5, and 70% in the 1.6, 4.5, 7.4, and 12 mg MON 77360/L treatment groups, respectively. At 48 hours, one lethargic daphnid was observed in both the 7.4 and 12 mg MON 77360/L levels. No other sub-lethal effects were observed. The calculated 48-hour  $EC_{50}$  was 10.5 mg MON 77360/L, which categorizes MON 77360 as slightly toxic to the water flea (*Daphnia magna*) on an acute toxicity basis. The 48-hour NOEC and LOEC values were 2.6 mg MON 77360/L and 4.5 mg MON 77360/L, respectively, for immobilization.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrates (§72-2). This study is classified as CORE.

## Results Synopsis

Test Organism Age (eg. 1<sup>st</sup> instar): Neonates, <24 hours old  
Test Type (Flow-through, Static, Static Renewal): Static

### 48-Hour

$EC_{50}$ : 10.5 mg MON 77360/L      95% C.I.: 9.5 - 12.2 mg MON 77360/L  
NOEC: 2.6 mg MON 77360/L  
LOEC: 4.5 mg MON 77360/L

## 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 Invertebrates; ASTM Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians; and OECD Guideline for Testing of Chemicals, 202: *Daphnia* sp. Acute Immobilization Test and Reproduction Test. The following deviations from §72-2 were noted:

1. Pre-test mortality of the laboratory culture and/or brood was not described.
2. The loading rate was not specified.
3. The water hardness (140 mg/L as  $CaCO_3$ ) was approx. three times higher than recommended (40-48 mg/L as  $CaCO_3$ ).
4. The pH (8.3-8.6) was higher than recommended (7.2-7.6).

These deviations did not affect the acceptability or validity of the study.

**COMPLIANCE:** Signed and dated GLP, Quality Assurance, and Data Confidentiality 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.

**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% glyphosate acid equivalents (a.e.)

**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 and 48 hours. Recoveries were 94-103% of nominal concentrations on Day 0 and 102-113% of nominal on Day 2.

**Storage conditions of test chemicals:**

Stored at room temperature.

*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.*

**2. Test organism:**

**Species:** *Daphnia magna*

**Age at test initiation:** Neonates, <24 hours old

**Source:** In-house laboratory cultures; neonates were obtained from six individual adult daphnids.

**B. STUDY DESIGN:**

**1. Experimental Conditions**

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

b. Definitive Study

Table 1: Experimental Parameters

| Parameter                                         | Details                                                                                                                                                          | Remarks                                                                                                                                               |
|---------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                   |                                                                                                                                                                  | Criteria                                                                                                                                              |
| Acclimation period:                               | Continuous laboratory cultures were maintained (at least 19 days).                                                                                               | <i>EPA requires 7 day minimum acclimation period.</i>                                                                                                 |
| Conditions: (same as test or not)                 | Same as test                                                                                                                                                     |                                                                                                                                                       |
| Feeding:                                          | <i>Daphnia</i> cultures were fed a mixture of yeast, Cerophyll and trout chow with a suspension of the freshwater green alga, <i>Selenastrum capricornutum</i> . |                                                                                                                                                       |
| Health: (any mortality observed)                  | No signs of disease or stress.                                                                                                                                   |                                                                                                                                                       |
| Duration of the test                              | 48 hours                                                                                                                                                         | <i>EPA requires 48 hours</i>                                                                                                                          |
| Test condition - static/flow through              | Static                                                                                                                                                           | <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> |
| Type of dilution system (for flow through method) | N/A                                                                                                                                                              |                                                                                                                                                       |
| Renewal rate (for static renewal)                 | N/A                                                                                                                                                              |                                                                                                                                                       |
| Aeration, if any                                  | No aeration during testing.                                                                                                                                      |                                                                                                                                                       |

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| Parameter                                                                                                                                                                                       | Details                                                                                                                                                                                                            | Remarks                                                                                                                                                                                                                                                                                                                                |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                                 |                                                                                                                                                                                                                    | Criteria                                                                                                                                                                                                                                                                                                                               |
| <u>Test vessel</u><br>Material: (glass/stainless steel)<br><br>Size:<br>Fill volume:                                                                                                            | Glass beakers<br><br>250 mL<br>220 mL                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                                                                 |                                                                                                                                                                                                                    | <i>EPA requires: size 250 ml or 3.9 L<br/>fill 200 ml</i>                                                                                                                                                                                                                                                                              |
| Source of dilution water                                                                                                                                                                        | The dilution water was freshwater obtained from an on-site laboratory well (40-m deep). The well water was filtered and aerated prior to use.                                                                      |                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                                                                 |                                                                                                                                                                                                                    | <i>EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water.</i>                                                                                                                                                                                                                              |
| <u>Water parameters:</u><br>Hardness<br><br>pH<br><br>Dissolved oxygen<br><br>Temperature<br><br>Total Organic Carbon<br><br>Particulate matter<br><br>Metals<br><br>Pesticides<br><br>Chlorine | 140 mg/L as CaCO <sub>3</sub><br><br>8.3-8.6<br><br>8.7-9.2 mg/L (≥96% saturation)<br><br>19.8-20.1°C<br><br><1.0 mg/L<br><br>Not reported, TDS = 261 mg/L<br><br>Not contaminated<br><br><LOD<br><br>Not reported | The hardness and pH were higher than recommended.                                                                                                                                                                                                                                                                                      |
|                                                                                                                                                                                                 |                                                                                                                                                                                                                    | Results of the analysis of the well water on August 14, 1996 for pesticides, organics, and metals were provided.                                                                                                                                                                                                                       |
|                                                                                                                                                                                                 |                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                        |
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|                                                                                                                                                                                                 |                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                        |
|                                                                                                                                                                                                 |                                                                                                                                                                                                                    | <i>EPA requires:<br/>hardness: 40 - 48 mg/L as CaCO<sub>3</sub><br/>pH: 7.2 - 7.6<br/>-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary &gt; 1°C<br/>Dissolved oxygen:<br/>Static: ≥ 60% during 1<sup>st</sup> 24 hr and<br/>≥ 40% during 2<sup>nd</sup> 24 hr<br/>Flow-through: ≥60%</i> |

| Parameter                                                                                        | Details                                                                                                                                          | Remarks                                                                                                                                                                                                                                                                                                                                                             |
|--------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                                                                  |                                                                                                                                                  | Criteria                                                                                                                                                                                                                                                                                                                                                            |
| <u>Number of organisms per replicate</u><br>Solvent control:<br>Negative control:<br>Treatments: | N/A<br>10<br>10                                                                                                                                  | The biomass loading rate was not specified.<br><br><i>EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static <math>\leq 0.8</math> g/L at <math>\leq 17^{\circ}\text{C}</math>, <math>\leq 0.5</math> g/L at <math>&gt; 17^{\circ}\text{C}</math>; flow-through: <math>\leq 1</math> g/L/day.</i> |
| <u>Number of replicates</u><br>Solvent control:<br>Negative control:<br>Treatments:              | N/A<br>2<br>2                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                     |
| Treatment concentrations<br>nominal:<br><br><br>measured:                                        | 0 (negative control), 1.6, 2.6, 4.3, 7.2, and 12 mg MON 77360/L<br><br><0.833 (LOQ, negative control), 1.6, 2.6, 4.5, 7.4, and 12 mg MON 77360/L | <i>EPA requires a geometric series with each concentration being at least 60% of the next higher one.</i>                                                                                                                                                                                                                                                           |
| Solvent (type, percentage, if used)                                                              | N/A                                                                                                                                              | <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 a 30-minute transition period.                                                                                  | Light intensity was approximately 408 lux at test initiation.<br><br><i>EPA requires 16 hours light, 8 hours dark.</i>                                                                                                                                                                                                                                              |
| Feeding                                                                                          | Animals were not fed during testing.                                                                                                             | <i>EPA/OECD requires: No feeding during the study</i>                                                                                                                                                                                                                                                                                                               |
| Stability of chemical in the test system                                                         | Recoveries were 94-103% of nominal concentrations on Day 0 and 102-113% of nominal on Day 2.                                                     |                                                                                                                                                                                                                                                                                                                                                                     |

| Parameter                                                            | Details              | Remarks                                                                                                 |
|----------------------------------------------------------------------|----------------------|---------------------------------------------------------------------------------------------------------|
|                                                                      |                      | Criteria                                                                                                |
| Recovery of chemical                                                 | 84.3-107% of nominal | Based on matrix spikes (at 1.00, 7.50, and 20.0 mg MON 77360/L) analyzed concurrently with the samples. |
| Level of Quantitation                                                | 0.833 mg MON 77360/L |                                                                                                         |
| Level of Detection                                                   | Not reported.        |                                                                                                         |
| 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 | immobility<br>lethargy    |          |
| Observation intervals                                | After 1, 24, and 48 hours |          |
| Were raw data included?                              | Yes, sufficient           |          |
| Other observations, if any                           | N/A                       |          |

## II. RESULTS AND DISCUSSION

### A. MORTALITY

By 48 hours, mortality was 0% in the control and the 2.6 mg MON 77360/L test group, and 5, 5, 5, and 70% in the 1.6, 4.5, 7.4, and 12 mg MON 77360/L groups, respectively. The NOEAC based on immobility data was 2.6 mg MON 77360/L

Table 3: Effect of MON 77360 on immobilization of *Daphnia magna*.

| Treatment,<br>mg MON 77360/L<br>Measured and<br>(nominal) concn. | No. of<br>organisms | Observation period |   |          |   |                |    |
|------------------------------------------------------------------|---------------------|--------------------|---|----------|---|----------------|----|
|                                                                  |                     | 1 Hour             |   | 24 Hours |   | 48 Hours       |    |
|                                                                  |                     | No.                | % | No       | % | No             | %  |
| Negative Control                                                 | 20                  | 0                  | 0 | 0        | 0 | 0              | 0  |
| 1.6 (1.6)                                                        | 20                  | 0                  | 0 | 0        | 0 | 1              | 5  |
| 2.6 (2.6)                                                        | 20                  | 0                  | 0 | 0        | 0 | 0              | 0  |
| 4.5 (4.3)                                                        | 20                  | 0                  | 0 | 0        | 0 | 1              | 5  |
| 7.4 (7.2)                                                        | 20                  | 0                  | 0 | 0        | 0 | 1              | 5  |
| 12 (12)                                                          | 20                  | 0                  | 0 | 0        | 0 | 14             | 70 |
| NOEAC                                                            |                     | 12                 |   | 12       |   | 2.6            |    |
| EC <sub>50</sub> (95% C.I.)                                      |                     | ND                 |   | >12      |   | 11 (7.4 - >12) |    |

ND - Not determined

### B. SUB-LETHAL TOXICITY ENDPOINTS:

At 48 hours, one lethargic daphnid was observed at both the 7.4 and 12 mg MON 77360/L treatment levels. No other sub-lethal effects were observed. The EC<sub>50</sub> value of 11 mg MON 77360/L (95% C.I.: 7.4 - > 12) was based on immobility and other sub-lethal effects.

Table 4: Sub-lethal Effects of MON 77360 on *Daphnia magna*.

| Treatment,<br>mg MON 77360/L<br>measured and<br>(nominal) conc. | Observation period |            |                              |            |
|-----------------------------------------------------------------|--------------------|------------|------------------------------|------------|
|                                                                 | 24 hours           |            | 48 hours                     |            |
|                                                                 | endpoint           | % affected | endpoint                     | % affected |
| Negative Control                                                | Lethargic          | 0          | Lethargic                    | 0          |
| 1.6 (1.6)                                                       | Lethargic          | 0          | Lethargic                    | 0          |
| 2.6 (2.6)                                                       | Lethargic          | 0          | Lethargic                    | 0          |
| 4.5 (4.3)                                                       | Lethargic          | 0          | Lethargic                    | 0          |
| 7.4 (7.2)                                                       | Lethargic          | 0          | Lethargic                    | 5          |
| 12 (12)                                                         | Lethargic          | 0          | Lethargic                    | 5          |
| NOEAC                                                           | 12                 |            | 2.6                          |            |
| LOAEC                                                           | >12                |            | 4.5                          |            |
| EC <sub>50</sub> (with 95% C.I.),                               | ND                 |            | 11 (95% C.I. not calculable) |            |

ND - Not determined

### C. REPORTED STATISTICS:

The EC<sub>50</sub> values were calculated using the binomial probability method (C.E. Stephan computer program). The EC<sub>50</sub> and NOAEC were determined from mortality and immobility, and sub-lethal effects data, respectively. The results were determined using mean-measured concentrations.

#### 48-Hour

LC<sub>50</sub>/EC<sub>50</sub>: 11 mg MON 77360/L

95% C.I.: 7.4 - >12 mg MON 77360/L

NOAEC: 2.6 mg MON 77360/L

LOEC: 4.5 mg MON 77360/L

### D. VERIFICATION OF STATISTICAL RESULTS:

The 48-hour EC<sub>50</sub> was determined for immobile daphnids using the moving average method via TOXANAL statistical software, as this method provided a more sound 95% confidence interval, compared to the probit and binomial methods. The NOAEC value was visually determined as the highest concentration which exhibited no sub-lethal effects. All toxicity values were determined in terms of the reported mean measured treatment concentrations.

#### 48-Hour

EC<sub>50</sub>: 10.5 mg MON 77360/L

95% C.I.: 9.5 - 12.2 mg MON 77360/L

Slope: 3.25 (probit)

NOAEC: 4.5 mg MON 77360/L

LOAEC: 7.4 mg MON 77360/L

**E. STUDY DEFICIENCIES:**

There were no significant deviations from U.S. EPA guideline §72-2 that affected the acceptability or validity of this study.

**F. REVIEWER'S COMMENTS:**

The reviewer's conclusions differed slightly from those reported by the study authors. The reviewer determined the EC<sub>50</sub> value to be 10.5 mg MON 77360/L (95% C.I.: 9.5 - 12.2 mg MON 77360/L), which was slightly lower and narrower 95% confidence interval compared to the study authors' reported value, 11 mg MON 77360/L (95% C.I.: 7.4 - >12 mg MON 77360/L), presumably due to the different statistical methods used. The study authors' reported NOAEC and LOAEC values were one treatment level lower than reported reviewer values, presumably due to the different endpoints used to visually estimate the values. Consequently, the reviewer determined EC<sub>50</sub> value and the study authors determined NOAEC and LOAEC values are reported in the Conclusion and Executive Summary sections of this DER because they are more conservative estimates of the acute toxicity of MON 77360 to the Cladoceran (*Daphnia magna*).

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.

**G. CONCLUSIONS:**

This study is scientifically sound, fulfills U.S. EPA guideline §72-2, and is classified as CORE. Based on the results of this study, MON 77360 is categorized as slightly toxic to the Cladoceran, *Daphnia magna*, on an acute toxicity basis. The 48-hour NOAEC and LOAEC values were 2.6 mg MON 77360/L and 4.5 mg MON 77360/L, respectively, for immobilization.

**48-Hour**

EC<sub>50</sub>: 10.5 mg MON 77360/L      95% C.I.: 9.5 - 12.2 mg MON 77360/L  
NOAEC: 2.6 mg MON 77360/L  
LOAEC: 4.5 mg MON 77360/L

### 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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**APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:**

**TOXANAL RESULTS:** Calculated using the reported mean measured concentrations.

**LC50:**

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

| SPAN | G        | LC50     | 95 PERCENT CONFIDENCE LIMITS |          |
|------|----------|----------|------------------------------|----------|
| 1    | .1836774 | 10.51135 | 9.476684                     | 12.24656 |

RESULTS CALCULATED USING THE PROBIT METHOD

| ITERATIONS | G        | H        | GOODNESS OF FIT PROBABILITY |
|------------|----------|----------|-----------------------------|
| 7          | 5.867098 | 9.035244 | 0                           |

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED  
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.245207  
95 PERCENT CONFIDENCE LIMITS = -4.615364 AND 11.10578

LC50 = 11.85546  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 4.814729  
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY