

**DATA EVALUATION RECORD**  
**HONEY BEE - ACUTE ORAL LC<sub>50</sub> TEST**  
**Non-Guideline (OECD 213)**

1. **CHEMICAL**: Glyphosate

PC Code No.: 103601

2. **TEST MATERIAL**: MON 77360

Purity: 30.0% w/w glyphosate acid equivalents

3. **CITATION**:

Author: Palmer, S. and Krueger, H.

Title: MON 77360: An Acute Oral Toxicity Study with the Honey Bee

Study Completion Date: February 16, 2001

Laboratory: Wildlife International Ltd.  
8598 Commerce Drive  
Easton, MD 21601

Sponsor: Monsanto Company  
800 North Lindbergh Boulevard  
St. Louis, Missouri 63167

Laboratory Report ID: 139-432

DP Barcode: D294119

MRID No.: 45370302

4. **REVIEWED BY**: Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: *Rebecca Bryan*

**Date**: 3/5/04

**APPROVED BY**: Teri S. Myers, Ph.D., Staff Scientist, Dynamac Corporation

Signature: *Teri S. Myers*

**Date**: 3/5/04

5. **APPROVED BY**: Stephanie Syslo, Environmental Scientist, OPP/EFED/ERB III

Signature: *Stephen Casey*

**Date**: *6/28/04*

Secondary Review : *Amie Pease*

*9/23/04*



**6. STUDY PARAMETERS:**

**Scientific Name of Test Organism:** *Apis mellifera*

**Age or Size of Test Organism at Test Initiation:** 1 to 5 days old

**Type of Concentrations:** Nominal

**Definitive Study Duration:** 48 hours

**7. CONCLUSIONS:**

The honey bee, *Apis mellifera* L., was exposed to MON 77360 (glyphosate) for 48 hours in an oral toxicity test, at concentrations of 6.25, 12.5, 25.0, 50.0, and 100 µg MON 77360/bee. By 48 hours, there was 3, 0, 43, 2, 5, and 12% mortality observed in the control, 6.25, 12.5, 25, 50, and 100 µg MON 77360/bee treatment groups, respectively. The mortalities in the 12.5 µg MON 77360/bee treatment group were uncharacteristic and, given bee response in the remaining test groups, considered to be aberrant and not treatment-related. The NOEC was visually determined to be 50 µg MON 77360/bee because mortality at the highest concentration exceeded 10%.

**The LD<sub>50</sub> value was >100 µg MON 77360/bee. As a result, MON 77360 is categorized as practically nontoxic to honeybees on an oral basis.**

**This acute contact study is classified as Supplemental.** This acute oral study is scientifically sound, but it is a non-guideline study and does not fulfill an OPP guideline requirement.

**Reported Statistical Results:**

LD<sub>50</sub>: >100 µg MON 77360/bee

95% C.I.: N/A

NOEL: 50 µg MON 77360/bee

Probit Slope: N/A

**8. ADEQUACY OF THE STUDY:**

**A. Classification:** Supplemental.

**B. Rationale:** This acute oral study is scientifically sound however, it is a non-guideline study and does not fulfill an OPP guideline requirement.

**C. Repairability:** N/A

**9. GUIDELINE DEVIATIONS:**

None.

**10. SUBMISSION PURPOSE:** This study was submitted to provide data on the acute oral toxicity of MON 77360 to honeybees for the purpose of chemical registration.

**11. MATERIALS AND METHODS:****A. Test Organisms**

| Guideline Criteria                                                                                                          | Reported Information                                         |
|-----------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------|
| <b>Species:</b><br>Species of concern ( <i>Apis mellifera</i> ,<br><i>Megachile rotundata</i> , or <i>Nomia melanderi</i> ) | <i>Apis mellifera</i>                                        |
| <b>Age at beginning of test:</b>                                                                                            | 1 to 5 days old                                              |
| <b>Supplier:</b>                                                                                                            | Apiary at Wildlife International, Ltd.,<br>Easton, Maryland. |
| <b>All bees from the same source?</b>                                                                                       | Yes                                                          |

**B. Test System**

| Guideline Criteria         | Reported Information                                                                                                      |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------|
| <b>Cage size adequate?</b> | Stainless steel cylinders (9 cm diameter<br>and 9 cm high) with plastic petri dish (10<br>cm diameter) covering each end. |

| Guideline Criteria        | Reported Information                               |
|---------------------------|----------------------------------------------------|
| <b>Lighting:</b>          | Continuous darkness except at observation periods. |
| <b>Temperature:</b>       | 27.2-27.3°C                                        |
| <b>Relative humidity:</b> | 63-86%                                             |

**C. Test Design**

| Guideline Criteria                                                                                            | Reported Information                                                                                                                                                                |
|---------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Range finding test?</b>                                                                                    | No range finding test was reported.                                                                                                                                                 |
| <b>Reference toxicant test?</b>                                                                               | The reference toxicant, dimethoate, was tested concurrently with the definitive test under the same test conditions. The test concentrations were 0.05, 0.15, and 0.45 µg a.i./bee. |
| <b>Method of administration:</b>                                                                              | The test solutions were mixed with a 50% sucrose solution and all dosages were consumed within a five-hour period.                                                                  |
| <b>Nominal doses:</b>                                                                                         | 6.25, 12.5, 25.0, 50.0, and 100 µg MON 77360/bee.                                                                                                                                   |
| <b>Controls:</b><br>Negative control and/or diluent/solvent control                                           | Negative control                                                                                                                                                                    |
| <b>Number of colonies per group:</b>                                                                          | 3 replicates; 20 bees/replicate                                                                                                                                                     |
| <b>Solvent:</b><br>The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol. | N/A                                                                                                                                                                                 |
| <b>Feeding:</b>                                                                                               | The test solutions were provided for approximately 5 hours. Then, the bees were supplied with untreated 50% sucrose solution, <i>ad libitum</i> .                                   |
| <b>Observations period:</b>                                                                                   | 48 hours                                                                                                                                                                            |

**12. REPORTED RESULTS:**

| Guideline Criteria                                                           | Reported Information                                 |
|------------------------------------------------------------------------------|------------------------------------------------------|
| Quality assurance and GLP compliance statements were included in the report? | Yes                                                  |
| Control performance:                                                         | 3% negative control mortality by 48 hours.           |
| Raw data included:                                                           | Replicate data were provided.                        |
| Signs of toxicity (if any) were described?                                   | Yes (immobility, lethargy, and loss of equilibrium). |

**Mortality**

| Dosage<br>µg*/bee            | No. of bees | Percent Mortality (%) <sup>1</sup> |    |    |
|------------------------------|-------------|------------------------------------|----|----|
|                              |             | Hour of Study                      |    |    |
|                              |             | 3.75                               | 24 | 48 |
| Test Substance (MON 77360)   |             |                                    |    |    |
| Control Group                | 60          | 0                                  | 0  | 3  |
| 6.25                         | 60          | 0                                  | 0  | 0  |
| 12.5                         | 60          | 0                                  | 20 | 43 |
| 25                           | 60          | 0                                  | 0  | 2  |
| 50                           | 60          | 0                                  | 0  | 5  |
| 100                          | 60          | 0                                  | 0  | 12 |
| Toxic Standard (dimethoate): |             |                                    |    |    |
| 0.05                         | 60          | 0                                  | 2  | 3  |
| 0.15                         | 60          | 0                                  | 70 | 83 |
| 0.45                         | 60          | 2                                  | 98 | 98 |

\* Test substance concentrations are expressed as µg MON 77360/bee, while toxic standard concentrations are expressed as µg a.i./bee.

<sup>1</sup> Percent mortalities were reviewer-calculated based on replicate mortality and immobilization data

(Tables 1-2, pp. 16-17).

Observations: By 48 hours, there was 3, 0, 43, 2, 5, and 12% mortality observed in the control, 6.25, 12.5, 25, 50, and 100 µg MON 77360/bee treatment groups, respectively. The mortalities in the 12.5 µg MON 77360/bee treatment group were not considered treatment-related. Immobile bees were observed after 48 hours in the 12.5, 50, and 100 µg MON 77360/bee treatment groups. The other sublethal effects included one lethargic bee in both the 12.5 and 25 µg MON 77360/bee treatment groups, and one bee with loss of equilibrium in the 100 µg MON 77360/bee treatment group. These effects were considered incidental to treatment.

Statistical method: The LD<sub>50</sub> values were estimated due to less than 50% mortality. The LD<sub>50</sub> was based on the nominal concentrations. The NOEC was based on a slight increase in mortalities in the highest treatment group.

**Reported Statistical Results:**

|                                          |                   |
|------------------------------------------|-------------------|
| LD <sub>50</sub> : >100 µg MON 77360/bee | 95% C.I.: N/A     |
| NOEL: 50 µg MON 77360/bee                | Probit Slope: N/A |

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical method: The LD<sub>50</sub> values were estimated due to less than 50% mortality. The LD<sub>50</sub> was based on the nominal concentrations. The NOEC was visually based on a slight increase in mortalities in the highest treatment group.

**Results:**

|                                          |                   |
|------------------------------------------|-------------------|
| LD <sub>50</sub> : >100 µg MON 77360/bee | 95% C.I.: N/A     |
| NOEL: 50 µg MON 77360/bee                | Probit Slope: N/A |

**14. REVIEWER'S COMMENTS:**

The reviewer's conclusions were identical to the study authors'; **The LD<sub>50</sub> value was >100 µg MON 77360/bee. As a result, MON 77360 is categorized as practically nontoxic to honeybees on an oral basis.**

The LD<sub>50</sub> of the toxic standard, dimethoate, was 0.11 µg a.i./bee with 95% confidence interval of 0.09 to 0.12 µg a.i./bee. This value was determined by the moving average method. The LD<sub>50</sub> was consistent with the published data.

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.

## **15. REFERENCES:**

- European and Mediterranean Plant Protection Organization. 1992. *Guideline on Test Methods for Evaluating the Side Effects of Plant Protection Products on Honey Bees*, Guideline 170. EPPO Bulletin. 22, 203-215.
- University of Maryland. 1977. *Bee Keeping in Maryland*. University of Maryland Cooperative Extension Bulletin No. 223.
- Stephan, C.E. 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota  
Personal Communication.
- Finney, D.J. 1971. *Statistical Methods in Biological Assay*. 2<sup>nd</sup> Ed. Griffin Press, London.
- Thompson, W.R. 1947. *Bacteriological Reviews*. Vol. II, 2:115-145.
- Stephan, C.E. 1977. "Methods for Calculating and LC<sub>50</sub>", *Aquatic Toxicology and Hazard Evaluation*. American Society for Testing and Materials. Publication Number STP 634, pp 65-84.
- Gough, H.J., McIndoe, E.C., Lewis, G.B. 1994. *The Use of Dimethoate as a Reference Compound in Laboratory Acute Toxicity Tests on Honey Bees (Apis mellifera L.)*. 1981-1992. Journal of Apicultural Research 33(2): 119-125.