DATA EVALUATION RECORD
HONEY BEE - ACUTE ORAL LC_{50} 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: [signature] **Date:** 3/5/04

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

Signature: Stephen Carey **Date:** 6/28/04

Secondary Review: Anita 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_{50} 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_{50}: >100 µg MON 77360/bee
NOEL: 50 µg MON 77360/bee
95% C.I.: N/A
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
Species: Species of concern (*Apis mellifera, Megachile rotundata,* or *Nomia melanderi*)	*Apis mellifera*
Age at beginning of test:	1 to 5 days old
Supplier:	Apiary at Wildlife International, Ltd., Easton, Maryland.
All bees from the same source?	Yes

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	Stainless steel cylinders (9 cm diameter and 9 cm high) with plastic petri dish (10 cm diameter) covering each end.

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

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	No range finding test was reported.
Reference toxicant test?	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.
Method of administration:	The test solutions were mixed with a 50% sucrose solution and all dosages were consumed within a five-hour period.
Nominal doses:	6.25, 12.5, 25.0, 50.0, and 100 µg MON 77360/bee.
Controls: Negative control and/or diluent/solvent control	Negative control
Number of colonies per group:	3 replicates; 20 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	N/A
Feeding:	The test solutions were provided for approximately 5 hours. Then, the bees were supplied with untreated 50% sucrose solution, *ad libitum*.
Observations period:	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 µg*/bee	No. of bees	Percent Mortality (%)[1]		
		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.

[1] 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_{50} values were estimated due to less than 50% mortality. The LD_{50} 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_{50}: >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_{50} values were estimated due to less than 50% mortality. The LD_{50} was based on the nominal concentrations. The NOEC was visually based on a slight increase in mortalities in the highest treatment group.

Results:
LD_{50}: >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_{50} 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_{50} 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_{50} 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.* 2nd Ed. Griffin Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews.* Vol. II, 2:115-145.

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.

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.