8/30-99

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM
DP BARCODE No: D252188
PC Code No: 108800 and 108801

SUBJECT: Review of Honeybee Toxicity Studies with Metolachlor and *s*-Metolachlor isomer

FROM: Brian Montague, Fisheries Biologist
Environmental Risk Branch I
Environmental Fate and Effects Division
Brian Montague 8/30/99

THRU: Arnet Jones, Chief
Environmental Risk Branch I
Environmental Fate and Effects Division (7507C)
Jim Costello for AJ

TO: Joanne Miller, Product Manager 23
Registration Division (7505C)

The Environmental Fate and Effects Division has completed review of honey bee acute contact and oral acute toxicity studies conducted with metolachlor and the s isomer of metolachlor. These studies were required to fulfill FIFRA guideline testing requirements for non-target pollinator insect toxicity determination for products containing these two active ingredients.

Both studies (MRIDs 44718401 and 44718402) have demonstrated that metolachlor and s-metolachlor display low toxicity to honeybees at dosage levels well above the level of 25 ug ai/bee considered nearly non-toxic by the Agency. All oral LD50 values were above 85 ug ai/bee and all acute contact LD50 values were above 110 ug ai/bee. These studies satisfy the oral and acute contact non-target insect testing requirements for metolachlor and s-metolachlor. Further questions regarding these studies may be directed to Brian Montague at 305-6438 or Arnet Jones at 305-7416.

# DATA EVALUATION RECORD
## § 141-1 - HONEY BEE ACUTE CONTACT AND ORAL $LD_{50}$ TEST

1. **CHEMICAL:** Metolachlor PC Code No.: 108801

2. **TEST MATERIAL:** CGA 24705 technical Purity: 97%

3. **CITATION:**

| | |
|---|---|
| Author: | M.H. Bew |
| Title: | Acute Contact and Oral Toxicity of CGA-24705 to Honeybees |
| Study Completion Date: | October 30, 1992 |
| Laboratory: | MAFF Central Science Laboratory, National Bee Unit, Warwickshire, England |
| Laboratory Study ID: | CC06092 |
| Sponsor: | Novartis Crop Protection, Inc., Greensboro, NC |
| DP Barcode: | D252188 |
| MRID No.: | 447184-01 |

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist, Golder Associates Inc.

**Signature:** Mark Mossler **Date:** 7/15/99

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

**Signature:** P. Kosalwat **Date:** 7/15/99

5. **APPROVED BY:**

**Signature:** Brian Montague **Date:** 8/23/99

6. **STUDY PARAMETERS:**

**Scientific Name of Test Organism:** *Apis mellifera*
**Definitive Study Duration:** 48 hours

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for honey bee acute contact and oral toxicity tests. The acute contact and oral $LD_{50}$ were both >110 µg/bee, which classifies the test material as relatively non-toxic to honey bees. The NOEL was determined to be 110 µg/bee for both the contact and oral tests.

8. **ADEQUACY OF THE STUDY:**

**A. Classification:** Core

B. **Rationale:** N/A

C. **Repairability:** N/A

9. **GUIDELINE DEVIATIONS:**

1. Bee age, cage size, and application volume were unspecified.

2. The environmental conditions (lighting, temperature, and humidity) during the test were not reported.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. **Test Organisms**

| Guideline Criteria | Reported Information |
|---|---|
| **Species:** Honey bee (*Apis mellifera*) | *Apis mellifera* |
| **Age at beginning of test:** Worker bees of uniform age. | Reported as worker honeybees with a low incidence of disease |
| **Supplier** | In-house hives |
| **All bees from the same source?** | Yes |

B. **Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Cage size adequate?** | Cage size not specified |
| **Lighting:** Bees should be maintained in the dark. | Lighting not specified |
| **Temperature:** 27°C (80°F). | Not reported |
| **Relative humidity:** Approx. 65% | Not reported |



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C. Test Design

| Guideline Criteria | Reported Information |
|---|---|
| **Range finding test?** | Yes, ≤3% mortality at dosages up to 110 µg/bee both orally and topically |
| **Reference toxicant tested?** | No |
| **Method of administration:** Whole body exposure in a nontoxic dust diluent; or topical exposure via microapplicator. | No specific information on exposure route other than "topical" or "oral." Each bee consumed 20 µL of 50% sucrose solution in the oral test |
| **Nominal doses:** Sufficient number of dosage levels to yield statistically sound data unless it can be determined that the $LD_{50}$ will be greater than 25 µg/bee. | Nominal concentrations of 0.11, 1.1, 11, and 110 µg/bee for both the contact and oral tests |
| **Controls:** Negative control and/or diluent/solvent control | Diluent (acetone) control for the contact test, and negative control for the oral test |
| **Number of bees per cage:** 25 (recommended) | 10 bees per cage (both tests) |
| **Number of cages per group:** 3 replicate cages per group is recommended. | 4 cages per treatment or control group (both tests) |
| **Carrier:** Non-toxic dust (e.g, Pyrolite). | N/A |
| **Solvent:** Distilled water or the following solvents: dimethyl-formamide, triethylene glycol, methanol, acetone, ethanol. | Acetone for the contact test |
| **Volume of test solution:** ≤2 µL/bee (for contact tests). | Contact test: not reported Oral test: 20 µL of treatment solution/bee |
| **Observations period:** At least 48 hours. | 48 hours for both tests |

**12. REPORTED RESULTS:**

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes |
| **Controls:** Mortality not more than 15% | No control mortality |
| **Raw data included?** | Yes |
| **Signs of toxicity (if any) were described?** | No signs of toxicity were reported |

Mortality - Contact Test

| Nominal Dosage (µg/bee) | No. of Bees | Cumulative Number of Dead Bees | | |
|---|---|---|---|---|
| | | Hour of Study | | |
| | | 4 | 24 | 48 |
| Control | 40 | 0 | 0 | 0 |
| 0.11 | 40 | 0 | 0 | 0 |
| 1.1 | 40 | 0 | 0 | 2 |
| 11 | 40 | 0 | 0 | 0 |
| 110 | 40 | 0 | 0 | 0 |

Mortality - Oral Test

| Nominal Dosage (µg/bee) | No. of Bees | Cumulative Number of Dead Bees | | |
|---|---|---|---|---|
| | | Hour of Study | | |
| | | 4 | 24 | 48 |
| Control | 40 | 0 | 0 | 0 |
| 0.11 | 40 | 0 | 3 | 3 |
| 1.1 | 40 | 0 | 0 | 0 |
| 11 | 40 | 0 | 1 | 1 |
| 110 | 40 | 0 | 0 | 0 |

Reported Statistical Results - Contact Test

Statistical Method: Visual inspection

| | |
|---|---|
| $LD_{50}$: >110 µg/bee | 95% C.I.: N/A |
| NOEL: not reported | Probit Slope: N/A |

Reported Statistical Results - Oral Test

Statistical Method: Visual inspection

| | |
|---|---|
| $LD_{50}$: >110 µg/bee | 95% C.I.: N/A |
| NOEL: not reported | Probit Slope: N/A |

13. **VERIFICATION OF STATISTICAL RESULTS:** The pattern and magnitude of mortality precluded the use of statistical analysis.

14. **REVIEWER'S COMMENTS:** It was noted that several items of information pertaining to environmental conditions (lighting, temperature, humidity) and study design (contact test application volume, bee age, and cage size) were omitted from the report. The test was apparently conducted under GLPs, which require information of this type to be included in submitted data. The lack of control mortality supports the assumption that the bees were held in an appropriate test system. However, future reports should contain the aforementioned information.

This study is scientifically sound, fulfills the guideline requirements for honey bee acute contact and oral toxicity tests, and can be classified as **Core**. The acute contact and oral $LD_{50}$ were both >110 µg/bee. This value classifies the test material as relatively non-toxic to *Apis mellifera*. The NOEL was determined to be 110 µg/bee for both the contact and oral tests.

# DATA EVALUATION RECORD
## § 141-1 - HONEY BEE ACUTE CONTACT AND ORAL $LD_{50}$ TEST

1. **CHEMICAL:** s-Metolachlor PC Code No.: 108800

2. **TEST MATERIAL:** CGA 77102 Purity: 98.5%

3. **CITATION:**

Author: M.P. Candolfi
Title: CGA-77102: Laboratory Oral and Contact Test with the Honeybee, *Apis mellifera*, Based on the EPPO Guideline 170 (1992)
Study Completion Date: February 3, 1997
Laboratory: Springborn Laboratories (Europe) AG, Horn, Switzerland
Laboratory Study ID: 97-137-1008
Sponsor: Novartis Crop Protection, Inc., Greensboro, NC
DP Barcode: D252188
MRID No.: 447184-02

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist, Golder Associates Inc.

**Signature:** [signature] **Date:** 7/15/99

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates Inc.

**Signature:** P. Kosalwat **Date:** 7/15/99

5. **APPROVED BY:**

**Signature:** [signature] **Date:** 8/23/99

6. **STUDY PARAMETERS:**

**Scientific Name of Test Organism:** *Apis mellifera*
**Definitive Study Duration:** 72 hours

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for honey bee acute contact and oral toxicity tests. The acute contact and oral $LD_{50}$ were >200 and >85 µg ai/bee, respectively, which classify the test material as relatively non-toxic to honey bees. The NOEL values were determined to be 200 and 85 µg ai/bee, respectively, for the contact and oral tests.

8. ADEQUACY OF THE STUDY:

A. **Classification:** Core

B. **Rationale:** N/A

C. **Repairability:** N/A

9. GUIDELINE DEVIATIONS:

1. In the oral test, the number of replicates was either one, two, or three.

2. Raw data were not submitted.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. **Test Organisms**

| Guideline Criteria | Reported Information |
|---|---|
| **Species:** Honey bee (*Apis mellifera*) | *Apis mellifera* |
| **Age at beginning of test:** Worker bees of uniform age. | 5-12 day old worker bees |
| **Supplier** | O. Keller, Mörschwil, Switzerland |
| **All bees from the same source?** | Yes |

B. **Test System**

| Guideline Criteria | Reported Information |
|---|---|
| **Cage size adequate?** | 12.5-cm cubes |
| **Lighting:** Bees should be maintained in the dark. | 16 hours light/8 hours dark |
| **Temperature:** 27°C (80°F). | 23.5-25.5°C |
| **Relative humidity:** Approx. 65% | 60-73% |

C. Test Design

| Guideline Criteria | Reported Information |
|---|---|
| **Range finding test?** | Yes, 30% mortality at the highest oral dosage (160 µg ai/bee) and 20% mortality at the highest contact dosage (200 µg ai/bee) |
| **Reference toxicant tested?** | Yes, dimethoate |
| **Method of administration:** Whole body exposure in a nontoxic dust diluent; or topical exposure via microapplicator. | Contact: 1 µL/bee<br>Oral: up to 10 µL/bee of treatment solution |
| **Nominal doses:** Sufficient number of dosage levels to yield statistically sound data unless it can be determined that the $LD_{50}$ will be greater than 25 µg/bee. | Contact: only tested at 200 µg ai/bee<br><br>Oral: Based on amount ingested, 9 dosages up to 85 µg ai/bee |
| **Controls:** Negative control and/or diluent/solvent control | Carrier (acetone) and blank (water) controls for the contact test, and a negative control (20% honey water) for the oral test |
| **Number of bees per cage:** 25 (recommended) | 10 bees per cage (both tests) |
| **Number of cages per group:** 3 replicate cages per group is recommended. | Contact: 3 cages per group<br>Oral: 1, 2, or 3 cages per group |
| **Carrier:** Non-toxic dust (e.g, Pyrolite). | N/A |
| **Solvent:** Distilled water or the following solvents: dimethyl-formamide, triethylene glycol, methanol, acetone, ethanol. | Acetone for the contact test |
| **Volume of test solution:** ≤2 µL/bee (for contact tests). | Contact test: 1 µL<br>Oral test: up to 10 µL of treatment solution/bee |



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| Guideline Criteria | Reported Information |
|---|---|
| **Observations period:** At least 48 hours. | 72 hours for both tests |

**12. REPORTED RESULTS:**

| Guideline Criteria | Reported Information |
|---|---|
| **Quality assurance and GLP compliance statements were included in the report?** | Yes, compliance was to Swiss GLPs |
| **Controls:** Mortality not more than 15% | Contact: no mortality<br>Oral: 3% mortality |
| **Raw data included?** | No, only mean data |
| **Signs of toxicity (if any) were described?** | Yes |

Mortality - Contact Test

| Nominal Dosage (µg ai/bee) | No. of Bees | Cumulative Number of Dead Bees | | |
|---|---|---|---|---|
| | | Hour of Study | | |
| | | 24 | 48 | 72 |
| Blank Con. | 30 | 0 | 0 | 0 |
| Carrier Con. | 30 | 0 | 0 | 0 |
| 200 | 30 | 1 | 1 | 3 |

Other Observations: At the 48 hour time period, 3% of the 200 µg-treated bees were noted as affected in addition to the 3% found dead.

Mortality - Oral Test

| Nominal Dosage (µg ai/bee) | No. of Bees | Percentage Dead Bees | | |
|---|---|---|---|---|
| | | Hour of Study | | |
| | | 24 | 48 | 72 |
| Control | 30 | 0 | 0 | 3 |
| 3.1 | ?* | 0 | 0 | 3 |
| 5.2 | ? | 0 | 3 | 3 |
| 12.1 | ? | 0 | 0 | 0 |
| 15.9 | ? | 5 | 10 | 10 |
| 25.0 | ? | 0 | 0 | 0 |
| 37.5 | ? | 0 | 10 | 10 |
| 50.0 | ? | 0 | 0 | 0 |
| 65.0 | ? | 5 | 5 | 5 |
| 85.0 | ? | 0 | 0 | 0 |

*The total number of bees in the treatment groups was either 10, 20, or 30.

Reported Statistical Results - Contact Test

Statistical Method: Visual inspection

$LD_{50}$: >200 µg ai/bee 95% C.I.: N/A
NOEL: 200 µg ai/bee Probit Slope: N/A

Reported Statistical Results - Oral Test

Statistical Method: Visual inspection

$LD_{50}$: >85 µg ai/bee 95% C.I.: N/A
NOEL: 85 µg ai/bee Probit Slope: N/A

13. **VERIFICATION OF STATISTICAL RESULTS:** The pattern and magnitude of mortality precluded the use of statistical analysis.

14. **REVIEWER'S COMMENTS:** Although a fasting period (2 hours) was used prior to the introduction of the treated solutions, it did not appear that the bees were very hungry during the dosing of the oral test. The vials should have been left in the cages longer or the bees should have been starved for a longer period in order to fulfill the study design. However, since the mortality observed at the highest dosage level was actually less than the control, it is apparent that the test material did not affect the bees when consumed.

This study is scientifically sound, fulfills the guideline requirements for honey bee acute contact and oral toxicity tests, and can be classified as **Core.** The acute contact and oral $LD_{50}$ were >200 and >85 µg ai/bee, respectively. These values classify the test material as relatively non-toxic to *Apis mellifera*. The NOEL values were determined to be 200 and 85 µg ai/bee, respectively, for the contact and oral tests.



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