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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)
Kim 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

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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

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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.

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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: 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: 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%

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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 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 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 Oral: 1, 2, or 3 cages per group
Carrier: Non-toxic dust (e.g, Pyrolite).	N/A
Solvent: Distilled water or the following solvents: dimethylformamide, triethylene glycol, methanol, acetone, ethanol.	Acetone for the contact test
Volume of test solution: ≤2 µL/bee (for contact tests).	Contact test: 1 µL 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 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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