2-2-94

MRID No. 416138-12

DATA EVALUATION RECORD

1. **CHEMICAL:** Benefin.
Shaughnessey No. 084301.

2. **TEST MATERIAL:** Benefin (Compound 054521); N-(n-butyl)-N-ethyl-2,6-dinitro-ααα-trifluoro-p-toluidine; Lot No. 231EF4; 96.15% purity; a bright yellow powder.

3. **STUDY TYPE:** Acute Contact LD_{50} Test. Species Tested: Honey Bee (*Apis mellifera*).

4. **CITATION:** Hoxter, K.A. and M. Jaber. 1990. The Acute Contact Toxicity of Benefin to the Honey Bee. Laboratory Project No. 151-115. Conducted by Wildlife International Ltd., Easton, MD. Submitted by DowElanco. EPA MRID No. 416138-12.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: M. Mossler
Date: 6/15/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: P. Kosalwat
Date: 6/18/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
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Signature: 7/2/94 2-2-94
Date:

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for an acute contact study with the honey bee. A 48-hour LD_{50} of >101 µg ai/bee classifies benefin as relatively non-toxic to honey bees (*Apis mellifera*). The NOEL was 101 µg ai/bee.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

A. **Test Animals:** Two frames of honey bee (*Apis mellifera*) pupae from in-house hives were placed in an incubator for 4 days to allow pupae to emerge as adults. The bees were apparently healthy and were 1 to 4 days old at the initiation of the test. The mean weight of the test bees was 0.104 ±0.007 g/bee.

B. **Test System:** Bees were contained in one pint rolled paper containers (87 mm in diameter and 85 mm high). Each container was covered with a plastic petri plate in which a 20-ml glass vial containing 50% sugar/water was inserted. Cheese cloth covered the opening of the vial to prevent leakage. This food source was available *ad libitum* throughout the test. Bees were kept in continual darkness except during dosing and observation. The temperature was maintained at 18-22°C, and the relative humidity was 79%.

C. **Dosage:** Forty-eight-hour acute contact test. Five treatment levels representing 14, 23, 38, 63, and 105 µg benefin/bee were tested along with a solvent control (2 µl acetone/bee) and a negative control.

The test material was dissolved in acetone to a volume of 10 ml. The doses were corrected for the percentage active ingredient (ai) of the test substance.

D. **Design:** Two replicates of 25 bees each were used for each treatment and the controls. Twenty-five randomly selected bees were immobilized with nitrogen and laid out on paper. The bees were dosed individually on the thorax and/or abdomen with 2 µl of the test solution. Negative control bees were handled identically to treated bees, but were not dosed with any material. Solvent control bees received only acetone. Observations were recorded twice on day 0 and once on days 1 and 2.

E. **Statistics:** An LD_{50} value was estimated by visual inspection of the mortality data due to the lack of a true dose-response. The LD_{50} value was used to classify the test substance according to Atkins' toxicity categories. The categories were: highly toxic (less than 2 µg/bee), moderately toxic (greater than or equal to 2 µg/bee but less than 11 µg/bee), and relatively nontoxic (greater than or equal to 11 µg/bee).

12. **REPORTED RESULTS:** At test termination, negative and solvent control mortalities were 8 and 14%, respectively. Mortalities at the 14, 23, 38, 63 and 105 µg ai/bee doses were 8, 6, 10, 10, and 14%, respectively. Mortality in the highest dosage group was the same as the solvent control and was not considered to be treatment-related. No overt signs of toxicity were observed in any test group.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The acute contact LD_{50} of benefin for honey bee was determined to be greater than 105 µg ai/bee, which classifies the compound as relatively non-toxic according to the toxicity categories of Atkins. The no-observed-effect dosage was 105 µg ai/bee.

The study director confirmed that this study was conducted in compliance with Good Laboratory Practice Standards (40 CFR Part 160) with the exception that samples of the dosing solutions were not taken for confirmation of test concentration. A Quality Assurance statement was included in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures generally follow Subdivision L and SEP guidelines.

B. **Statistical Analysis:** Because of the lack of a dose-response, the reviewer estimated the LD_{50} to be greater than 105 µg ai/bee. Since the solvent control and highest dosage group mortality were the same, it is apparent that the no-observed-effect level (NOEL) is 105 µg ai/bee.

C. **Discussion/Results:** Upon review of the dosage calculations (Addendum III, attached), it was determined that the highest dosage was actually 101 µg ai/bee, rather than 105 µg ai/bee.

This study is scientifically sound and fulfills the requirements for an acute contact study with the honey bee. A 48-hour LD_{50} of >101 µg ai/bee classifies benefin as relatively non-toxic to honey bees. The NOEL was determined to be 101 µg ai/bee.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER:** Yes, 5-21-92.

ADDENDUM III

TEST SOLUTION PREPARATION

The test solutions were prepared as follows:

14 µg a.i./bee:
0.0686 g Benefin/Q.S. 10 ml acetone. = 13 µg ai/bee

23 µg a.i./bee:
0.1158 g Benefin/Q.S. 10 ml acetone. = 22 "

38 µg a.i./bee:
0.1890 g Benefin/Q.S. 10 ml acetone. = 36 "

63 µg a.i./bee:
0.3151 g Benefin/Q.S. 10 ml acetone. = 61 "

105 µg a.i./bee:
0.5252 g Benefin/Q.S. 10 ml acetone. = 101 "

For each dosage level, a calculated amount of test substance was weighed into a tared premarked scintillation vial using Scale #8. Sufficient pesticide grade acetone was added to bring the final volume to 10 ml. Each test solution was shaken by hand to mix after dissolving in acetone and just prior to dosing. The test substance completely dissolved in acetone. Test bees were dosed using an Eppendorf digital pipette at 2 µl volume.

Shaughnessey # 084301 Chemical Name Benefin Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour ~~EC~~ LD$_{50}$	96.15	LD$_{50}$ = >101 ~~pp~~ µg ai/bee* (95% C.L. n/a) Control Mortality (%) = 8 Solvent Control Mortality (%) = 14 Species: Apis mellifera Slope = n/a # Animals/Level = 100/control/50/trt Temperature = 18-22°C Lab: Wildl. Int. 48-Hour Dose Level ~~pp~~ µg ai/bee* /(% Effect) 13 (8), 22 (6), 36 (10), 61 (10), 101 (14) MRID # 416138-12 Comments: * - ~~diet~~ based on nominal concentrations NOEL = 101 µg ai/bee*	M. Mossler 5/21/92	Core
96-Hour LC$_{50}$	_____	LC$_{50}$ = pp (95% C.L.) Control Mortality (%) = Solvent Control Mortality (%) = Species: Slope = # Animals/Level = Temperature = Lab: 96-Hour Dose Level pp /(% Mortality) ( ), ( ), ( ), ( ), ( ) MRID # Comments:	_____	_____