

DP Barcode: D355006

MRID No.: 474712-03

DATA EVALUATION RECORD
ACUTE CONTACT TOXICITY TEST WITH THE HONEY BEE
850.3020

1. **CHEMICAL**: Dimethyl Disulfide PC Code No.: 029088

2. **TEST MATERIAL**: Dimethyl Disulfide Purity: 99.1%

3. **CITATION**

Authors: Porch, J.R. and H.O. Krueger

Title: Dimethyl Disulfide: An Acute Contact Toxicity Study with the Honey Bee

Study Completion Date: July 1, 2008

Laboratory: Wildlife International, Ltd.
Easton, Maryland 21601

Sponsor: Arkema Inc.
Philadelphia, PA 19103-3222

Laboratory Report ID: 524-105

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4. **REVIEWED BY**: John Marton, Staff Scientist, Cambridge Environmental, Inc.

Signature: 

Date: 10/30/08

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental, Inc.

Signature: 

Date: 11/04/08

5. **APPROVED BY**: Valerie Woodard, OPP/EFED/ERB5

Signature: 

Date: 3/20/09

6. **DISCLAIMER**: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to honey bees following contact exposure. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be



accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

7. STUDY PARAMETERS

Test Species: Honey Bee (*Apis mellifera*)

Age of Test Organisms at Test Initiation: Young adult worker bees

Exposure Duration: 48 Hrs

8. CONCLUSIONS: LD₅₀ >100 ug ai/bee

Toxicity category: Practically non-toxic

Slope of Response N/A

NOAEL 100 µg ai/bee

9. ADEQUACY OF THE STUDY

A. Classification: Acceptable

B. Rationale:

C. Repairability:

10. GUIDELINE DEVIATIONS: This study was conducted following guidelines outlined in the U.S. Environmental Protection Agency Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.3020: *Honey Bee Acute Contact Toxicity*; OECD Guidelines for the Testing of Chemicals, 214: *Honeybees, Acute Contact Toxicity Test*; EPPO Guideline 170, *Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honey Bees*; and Atkins, et al., 1975. The following deviations from OPPTS 850.3020 were noted:

1. It was not reported if test organisms were maintained under proper culturing practices.
2. Analytical verification of the concentrations in the feed solution was not conducted.
3. Results from a periodic screening analysis of dilution water were not provided.

These deviations do not impact the acceptability of the study.

11. SUBMISSION PURPOSE: This study was submitted to provide information on the effects of dimethyl disulfide on honey bees (*Apis mellifera*) following acute contact exposure for the purpose of new chemical registration.

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species	Honey Bee (<i>Apis mellifera L.</i>)
Age at beginning of test Worker bees of uniform age.	Young adult worker bees
Source	Obtained from an apparently healthy hive maintained by the University of Maryland.
Were bees from disease-free colonies?	Yes
Were bees kept in conditions conforming to proper cultural practices?	Not reported

B. Test System

Guideline Criteria	Reported Information
Test Chambers	Perforated stainless steel cylinders (9 cm in diameter and 9 cm high). Each end was covered with a disposable plastic Petri dish (~10 cm diameter). An inverted 20 mL glass vial containing 50% sucrose solution was inserted through the lid of the chamber. Vials were covered with gauze to prevent leakage while allowing bees to feed. Food was provided <i>ad libitum</i> throughout the test.
Temperature during exposure	25-27°C
Relative humidity during exposure	44-62%
Lighting	Continuous darkness except during dosing and observations
Feeding	Bees were provided with a 50% sucrose solution <i>ad libitum</i> .

C. Test Design

Guideline Criteria	Reported Information
Nominal dosage levels tested	6.25, 12.5, 25.0, 50.0 and 100 ug ai/bee
Number of bees exposed per dosage level	60, equally divided among 3 replicates
Other experimental design information	Bees were anaesthetized with nitrogen and individually dosed with the appropriate solution. Doses were administered as a 2 µL droplet on the abdomen and/or thorax with an adjustable micropipette.
Bees randomly or impartially assigned to test groups	Yes
Control	Negative control bees were handled identically as treated bees, but did not receive any treatment.
Solvent control	Solvent control bees were dosed with acetone. Bees in the positive control were dosed with 0.05, 0.10 and 0.30 µg ai/bee of dimethoate. As with bees in the dimethyl disulfide treatments, 60 bees, equally divided among 3 replicates, were treated with the appropriate doses of the positive control.
Total observation period and frequency of interim observations	The test was conducted for 48 hours, with observations being made twice during the first four hours, and then at 24 and 48 hours after dosing.

13. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with GLP Standards as published by the U.S. Environmental Protection Agency (40 CFR, Parts 160 and 792, 17 August 1989); OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF (11 NohSan, Notification No. 6283, Agricultural Production Bureau 1, October 1999) with the following exceptions: the identity, strength, purity and composition of the test substance were not determined before use in the study; the dosing solutions were not analyzed to verify concentration, homogeneity or stability of the test and reference substances in the carrier; and periodic screening analyses of well water for potential contaminants were not performed according to GLP, but were performed using a certified laboratory and standard US EPA analytical methods.
Observed adverse effects on bees at respective dosages	One bee in the 12.5 µg ai/bee treatment level was observed with a loss of equilibrium 1.75 hours after dosing, but appeared normal and healthy throughout the remainder of the test. All surviving bees in the treatment groups and controls appeared normal and healthy throughout the test with the exception of one negative control replicate. All bees in replicate C of the negative control died during the test, and it was not known why. Therefore, this replicate was excluded from analyses.
Control and Solvent Control Mortality	7.5 and 5.0% in the negative and solvent

Guideline Criteria	Reported Information
	controls, respectively.
Were raw data included?	No; however, adequate replicate data were provided.

Mortality and Observations

Experimental Group (µg ai/bee)	Number Exposed	Number (Percent) Dead	Observations
Negative Control	40	3 (7.5%)	All surviving bees appeared normal and healthy.
Solvent Control	60	3 (5.0%)	All surviving bees appeared normal and healthy.
6.25	60	4 (6.7%)	All surviving bees appeared normal and healthy.
12.5	60	3 (5.0%)	All surviving bees appeared normal and healthy.
25.0	60	2 (3.3%)	All surviving bees appeared normal and healthy.
50.0	60	3 (5.0%)	All surviving bees appeared normal and healthy.
100	60	3 (5.0%)	All surviving bees appeared normal and healthy.

Agency Statistical Analysis

Method Used: The LD₅₀ value was visually determined based on a lack of ≥50% mortality. The NOAEC value was visually determined to be the highest nominal treatment level as the highest mortality (7.5%) was observed in the negative control.

Results: **LD₅₀** >100 ug ai/bee
Toxicity category: Practically non-toxic
Slope of Response N/A
NOAEL 100 µg ai/bee

14. REVIEWER'S COMMENTS

The reviewer's results were identical to those of the study authors.

Replicate C in the negative control experienced total mortality and there was no explanation given for its occurrence. According to OPPTS 850.3020 guidelines, if there is more than 20% mortality in the negative control, a study should be unacceptable. However, because mortality was low and comparable in the remaining two control replicates and all treated replicates, the study author's dropped replicate C from the analysis. Without replicate C, there was still adequate sample size to meet guideline requirements (i.e., at least 25 bees per level). Therefore, it is the reviewer's opinion that this should not impact the study acceptability.

Following 48 hours of exposure to the positive control, mortality was 15, 42 and 97% in the nominal 0.05, 0.10 and 0.30 $\mu\text{g ai/bee}$ treatment levels, respectively. The 24-hour LD_{50} for dimethoate was 0.127 $\mu\text{g ai/bee}$ (95% C.I. of 0.111-0.146 $\mu\text{g ai/bee}$ with a slope of 4.358 with a 95% confidence interval of 3.377 and 5.339; Chi-square goodness of fit 2.593, with a probability of 0.107). This LD_{50} was within the desired range (0.10-0.30 $\mu\text{g ai/bee}$).

The in-life portion of the definitive toxicity test was conducted from April 17 to April 19, 2008.

15. REFERENCES

U.S. Environmental Protection Agency. 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.3020: *Honey Bees Acute Contact Toxicity*.

Organization for Economic Cooperation and Development. 1998. OECD Guidelines for Testing of Chemicals. Guideline 214, *Honeybees, Acute Contact Toxicity Test*. (Original Guideline adopted 21st September 1998).

European and Mediterranean Plant Protection Organization. 1992. *Guideline on Test Methods for Evaluating the Side-Effects of Plant Protection Products on Honey Bees* (No. 170). OEPP/EPPA Bulletin, 22, 203-215.

Atkins, E.L., E.A. Greywood, and R.L. Macdonald. 1975. Toxicity of pesticides and other agricultural chemicals to honey bees: Laboratory studies. *Univ. of Calif. Div. of Agric. Sci Leaflet* 2287. 38 pp.

Finney, D.J. 1971. *Statistical Methods in Biological Assay*. Second Edition. Griffin Press, London.

Thompson, W.R. 1947. *Bacteriological Reviews*. Vol II, No. 2 (June): 115-145.