DP Barcode: 392679 MRID No.: 48549601

DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT LD_{50} LIMIT TEST

1. **CHEMICAL:** Ferrous sulfate monohydrate PC Code No.: 050507

2. **TEST MATERIAL:** Ferrous sulfate monohydrate Purity: 92.16%

3. **CITATION**

Authors:	Younger, Cole
Title:	Ferrous sulfate monohydrate – Final Report – Honey Bee, *Apis mellifera*, Acute Contact Toxicity Limit Test
Study Completion Date:	July 8, 2011
Laboratory:	Stillmeadow, Inc.
Sponsor:	Iron Salts Work Group, c/o Lilly Miller Brands, Atlanta, GA
Laboratory Report ID:	14938-11
MRID No.:	48549601
DP Barcode:	392679

4. **REVIEWED BY:** Anita Ullagaddi, Biologist, USEPA/OPP/EFED/ERB1

Signature: **Date:** 10/21/11

5. **REVIEWED BY:** Michael Lowit, Ecologist, USEPA/OPP/EFED/ERB1

Signature: Michael Lowit **Date:** 11/4/11

6. **STUDY PARAMETERS:**

Age of Test Organism at Test Initiation:	Young adult workers
Type of Dosage:	Nominal (Contact)
Test Duration:	96 hours

7. **CONCLUSIONS:**

LD_{50}: Not determined
NOAEL: <25 µg a.i./bee based on 48.3% mortality at 48 hours.

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: This study does not meet the data requirement for a honeybee acute contact toxicity test because mortality was observed at the limit dose (25 µg ai/bee) at 48 hrs, the standard test duration of a definitive study. Therefore, a definitive study is required to establish an LD_{50}. Mortality in the control group did not exceed 20% up to 48 hours, but mortality exceeded 20% in the control group at 72 and 96 hours. Therefore, data at 72 and 96 hours are unacceptable.

C. Reparability: N/A

9. GUIDELINE DEVIATIONS:

1. Relative humidity in this study was reported as ranging from 34 to 97%; OCSPP guidelines suggest that humidity should be maintained between 50 and 80%.
2. The age and pre-test health of the bees were not reported.

10. SUBMISSION PURPOSE: This limit test was submitted to provide the effects on honey bees (*Apis mellifera*) following acute contact exposure to ferrous sulfate monohydrate.

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:	Not reported (young adults)
Supplier:	Stillmeadow, Inc. bee colony
All bees from the same source?	Not reported

B. Test System

Guideline Criteria	Reported Information

Guideline Criteria	Reported Information
Cage size adequate?	Disposable cardboard containers with screen; size not specified
Lighting:	Constant darkness (except during dosing and observations)
Temperature:	26-29°C
Relative humidity:	34-97%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	None reported.
Reference toxicant test?	Dimethoate (0.01 µg a.i./bee, 0.1 µg a.i./bee, 1.0 µg a.i./bee)
Method of administration:	Contact test: Dissolved in deionized water; after anesthetization with CO_2, 2 µL applied per bee
Nominal dose:	25 µg a.i./bee
Controls: Negative control and/or diluent/solvent control	negative control – deionized water
Number of bees per group:	3 cups of 20 bees each
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	N/A, the test substance was not soluble in acetone
Feeding:	50% aqueous sucrose solutions, *ad libitum*
Observations period:	1,2, 4, 24, 48, 72, and 96 hours after application

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	4 hours: 100% survival 24 hours: 100% survival 48 hours: 85% survival 72 hours: 73.3% survival 96 hours: 48.3% survival
Raw data included:	Yes
Signs of toxicity (if any) were described?	No

Mortality

Dosage µg a.i./bee	No. of Bees	Percent Mortality (%)				
		Hour of Study				
		4	24	48	72	96
Test Substance						
Negative Control	60	0	0	15	26.7	51.7
25	60	0	1.7	48.3	73.3	88.3
Toxic Standard						
0.01	60	1.7	1.7	10.0	13.3	21.7
0.10	60	0	0	13.3	45	58.3
1.0	60	5	25	45	63.3	75

Observations:
In the negative control group, all surviving bees had no observable abnormalities. In the test substance group, all surviving bees had no observable abnormalities, except for one bee in the third cup at 4 hours, which was moribund.

Statistical method:
Only one dose was used for this limit test; therefore, an LD_{50} could not be statistically calculated. In the test substance group, 48.3% mortality was observed at 48 hours, which increased to 73.3% and 88.3% at 72 and 96 hours, respectively.

Reported Statistical Results:
The study author performed a one-way parametric analysis of variance (ANOVA) with Tukey's Multiple Comparison Test to compare mortality among treatment groups at each observation period. The study author found that at 72 and 96 hours, the mortality in the treatment group was significantly different than the control group. However, the 48.3% mortality observed at 48 hours was not significant.

13. VERIFICATION OF STATISTICAL RESULTS:

The use of only one treatment level precludes the calculation of an LD_{50} value. In contrast to the study author, the reviewer determined that the 48.3% mortality observed at 48 hours was statistically significant using Fisher's Exact Test. Furthermore, the

reviewer considers 48.3% mortality a biologically significant effect.

According to the study author, observations were extended to 72 and 96 hours per protocol because mortality for the test substance group increased by more than 10% from 24 to 48 hours and control mortality was less than 20%. Mortality at 72 and 96 hours was not assessed by the reviewer because the control mortality was unacceptable (>20%) at those time points.

Results - Contact Test:
48-hour LD_{50}: Not determined
NOAEL: <25 µg a.i./bee

14. REVIEWER'S COMMENTS:

Relative humidity in this study was reported as ranging from 34 to 97%; OCSPP guidelines suggest that humidity should be maintained between 50 and 80%.

The age of the bees was not reported. In addition, no observations of pre-test health were reported. It is not known whether the bees were suffering from any of the common viral, fungal, or bacterial diseases.

Although the 850.3020 guideline only recommends the test be carried out to 48 hours, the increased mortality observed in the control group at 72 and 96 hours complicates the results. Because greater than 20% control mortality was observed, the data at 72 and 96 hours should not be used for risk assessment purposes.

This limit test does not meet the data requirement for a honeybee acute contact toxicity test because mortality was observed at the limit dose (25 µg ai/bee) at 48 hrs, the standard test duration of a definitive study. Therefore, a definitive study is required to establish an LD_{50}.

15. REFERENCES:

U.S. Environmental Protection Agency, Ecological Effects Test Guidelines, OPPTS 850.3020 Honey Bee Acute Contact Toxicity Public Draft. EPA 712–C–96–147 (1996).

Fisher's Exact Test

IDENTIFICATION	NUMBER OF ALIVE	DEAD	TOTAL ANIMALS
CONTROL	51	9	60
25	31	29	60
TOTAL	82	38	120

Critical Fisher's value (60,60,51) (alpha=0.05) is 42.0. b value is 31.
Since b is less than or equal to 42.0 there is a significant difference between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Tests

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG 0.05
	CONTROL	60	9	
1	25	60	29	*