DP Barcode: 393680 MRID No.: 485786-01

DATA EVALUATION RECORD
HONEY BEE - ACUTE CONTACT LD_{50} DEFINITIVE 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 Definitive Test
Study Completion Date:	August 9, 2011
Laboratory:	Stillmeadow, Inc.
Sponsor:	Iron Salts Work Group, c/o Lilly Miller Brands, Atlanta, GA
Laboratory Report ID:	15336-11
MRID No.:	485786-01
DP Barcode:	393680

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

Signature: **Date:** 10/28/11

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:	48 hours

7. **CONCLUSIONS:**

LD_{50}: >100 µg a.i./bee
NOAEL: 100 µg a.i./bee

8. ADEQUACY OF THE STUDY:

A. Classification: Acceptable

B. Rationale: N/A

C. Reparability: N/A

9. GUIDELINE DEVIATIONS:

1. Relative humidity in this study was reported as ranging from 56 to 93%; 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 definitive test was submitted to establish an LD_{50} for 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
Cage size adequate?	Disposable cardboard containers with screen; size not specified

Guideline Criteria	Reported Information
Lighting:	Constant darkness (except during dosing and observations)
Temperature:	27-29°C
Relative humidity:	56-93%

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 doses:	6.25, 12.5, 25, 50, 100 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.	Not used
Feeding:	50% aqueous sucrose solutions, *ad libitum*
Observations period:	4, 24, and 48 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: 98.3% survival 24 hours: 98.3% survival 48 hours: 85% 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
Test Substance				
Negative Control (Untreated)	60	1.7	1.7	15
Solvent (Water) Control	60	0	1.7	13.3
6.25	60	0	0	10
12.5	60	0	0	16.7
25	60	0	0	5
50	60	0	1.7	10
100	60	0	0	10
Toxic Standard				
0.01	60	1.7	3.3	8.3
0.10	60	0	0	15
1.0	60	15	83.3	91.7

Observations:
In the negative control group and the solvent (water) control group, all surviving bees had no observable abnormalities. In the test substance groups, all surviving bees had no observable abnormalities, except for one bee in the first cup in the 50 µg a.i./bee treatment group at 48 hours, which showed unspecified signs of toxicity.

Statistical method:
Results were evaluated by comparing mortality between treated and control groups. An LD_{50} with slope function and 95% confidence limits was to be calculated. However, because test group mortality at the lower levels was greater than at the highest dose level and because there was no mortality over 50% in the test substance groups, the slope function and 95% confidence limits could not be calculated. The LD_{50} determination was

based on mortality at the highest dose level at 48 hours. A one-way parametric analysis of variance (ANOVA) with Tukey's Multiple Comparisons Test was performed to compare mortality among treatment groups at each observation period.

Reported Statistical Results:
There were no significant differences between the test substance groups and the control groups.

13. VERIFICATION OF STATISTICAL RESULTS:

The highest mortality was 16.7%; therefore, the LD_{50} is considered to be >100 µg a.i./bee. Fisher's Exact Test was used to determine if there were any significant differences between the test substance groups and the control group; there were none.

Results - Contact Test:

48-hour LD_{50}: >100 µg a.i./bee
NOAEL: 100 µg a.i./bee

14. REVIEWER'S COMMENTS:

Relative humidity in this study was reported as ranging from 56 to 93%; 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.

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 DEAD	NUMBER OF ALIVE	TOTAL ANIMALS
CONTROL	9	51	60
6.25	6	54	60
TOTAL	15	105	120

Critical Fisher's value (60,60,9) (alpha=0.05) is 2.0. b value is 6.
Since b is greater than 2.0 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	51	9	60
12.5	50	10	60
TOTAL	101	19	120

Critical Fisher's value (60,60,51) (alpha=0.05) is 42.0. b value is 50.
Since b is greater than 42.0 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test

IDENTIFICATION	NUMBER OF DEAD	NUMBER OF ALIVE	TOTAL ANIMALS
CONTROL	9	51	60
25	3	57	60

TOTAL	12	108	120

Critical Fisher's value (60,60,9) (alpha=0.05) is 2.0. b value is 3.
Since b is greater than 2.0 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test

	NUMBER OF		
IDENTIFICATION	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	9	51	60
50	6	54	60
TOTAL	15	105	120

Critical Fisher's value (60,60,9) (alpha=0.05) is 2.0. b value is 6.
Since b is greater than 2.0 there is no significant difference
between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test

	NUMBER OF		
IDENTIFICATION	DEAD	ALIVE	TOTAL ANIMALS
CONTROL	9	51	60
100	6	54	60
TOTAL	15	105	120

Critical Fisher's value (60,60,9) (alpha=0.05) is 2.0. b value is 6.
Since b is greater than 2.0 there is no 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	6.25	60	6	
2	12.5	60	10	
3	25	60	3	
4	50	60	6	
5	100	60	6	