DATA EVALUATION RECORD
ACUTE CONTACT LD_{50} TOXICITY TEST WITH THE HONEY BEE
§ 141-1

1. **CHEMICAL:** spinosad PC Code No.: 110003

2. **TEST MATERIAL:** XDE-105 Purity: 88%

3. **CITATION**

Authors: Hoxter, K.A., W. L. Bernard and J. B. Beavers
Title: XDE-105: An Acute Contact Toxicity with the Honey Bee

Study Completion Date: December 18, 1992

Laboratory: Wildlife International Ltd.
8598 Commerce Drive
Easton, Maryland 21601

Sponsor: DowElanco
Indianapolis, IN

Laboratory Report ID: 103-384

MRID No.: 43414547

DP Barcode: D218791

4. **REVIEWED BY:** Joanne Edwards, Entomologist, EEB, EFED

Signature: Joanne L Edwards **Date:** 4/27/96

5. **APPROVED BY:** Leslie Touart, Head, Section 1, EEB, EFED

Signature: L. W. T. **Date:** 3/25/96

6. **STUDY PARAMETERS**

Age of Test Organisms at Test Initiation: 1 to 4 days
Exposure Duration: 48 hours

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for an acute contact study with the honey bee. Based on the results of this 48-hour acute contact study, the LD_{50} was determined to be 0.0029 micrograms of active ingredient per bee (µg ai/bee). This classifies XDE-105 as highly toxic to honey bees.

8. **ADEQUACY OF THE STUDY**

A. Classification: Core

B. Rationale: The study is scientifically sound and meets guideline requirements.

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS** None.

10. **SUBMISSION PURPOSE:** Submitted to support new chemical registration.

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species: Honey Bee (*Apis mellifera L.*)	Honey Bee (*Apis mellifera L.*)
Age at beginning of test: Worker bees of uniform age.	1 to 4 days old
Source	Wildlife International Apiary Easton, Maryland 21601
Were bees from diseased-free colonies?	Yes
Were bees kept in conditions conforming to proper cultural practices?	Hives were maintained according to honey bee husbandry practices recommended by the State of Maryland.

B. Test System

Guideline Criteria	Reported Information
Test Chambers	The test chambers were disposable one pint rolled paper containers measuring approximately 87 mm in diameter and 85 mm high.
Photoperiod	8 hrs light/16 hours darkness
Temperature during exposure	Range: 21.6 to 22.5 °C
Relative humidity during exposure	80 % ± 13 %
Feeding	Each container was covered with a disposable plastic petri dish through which an inverted 20 ml glass vial was inserted. The vial contained a sugar/water solution (approx. 1 to 1 ratio). A sponge also was inserted through the top of the container cover and moistened with water at least once daily to increase humidity within the test chamber and provide an additional source of water for the bees.

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	Yes
Definitive Test **Nominal concentrations:** At least five, in a geometric scale, unless LD_{50} > 25 µg ai/bee	Geometric Series: 0.0008, 0.0016, 0.0031, 0.0063 and 0.0125 µg ai/bee
Controls: Water control or vehicle control (if vehicle is used)	Solvent (acetone) and negative controls were used.
Number of bees per chamber:	A minimum of 25 bees were placed in each test chamber and two replicate tests were performed per dosing regime; 50 bees per treatment, including controls
Vehicle:	Acetone
Amount of vehicle per bee:	2 µl of acetone was applied to the thorax and/or abdomen of each bee.
Were bees immobilized prior to testing? (describe)	Bees were immobilized with nitrogen twice: first, prior to removal from the acrylic holding boxes just before being placed in the holding containers, and then again, in the holding containers just prior to dosing.
How were doses administered? (describe)	The five test doses were administered topically in a droplet to the abdomen and/or thorax of each nitrogen immobilized bee.
Were bees randomly or impartially assigned to test groups?	Yes
Control(s)	Solvent (acetone) and negative controls were maintained concurrently.

Guideline Criteria	Reported Information
Preparation of Dosing Solutions (describe)	A calculated amount of XDE-105 was mixed with sufficient pesticide grade acetone to represent the highest dosage, 0.0125 µg ai/bee. Lower concentration dosing suspensions were then prepared by serial dilution.
Observations period 48 hours	Observations were recorded at the following intervals: @ times on day on initiation and then approx. 24 and 48 hrs after initiation

12. **REPORTED RESULTS**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Were there no observed adverse effects on bees at the greatest aging interval?	No
Control Mortality	2% (negative control) 1% (solvent control)
Were raw data included?	Excerpted
Were signs of toxicity (if any) described?	Yes

Mortality and Observations

Dosage (μg ai/bee)	Number of Bees Exposed	Mean % Dead at 48 hrs
Negative Control	100	2%
Solvent Control (acetone)	100	1%
0.0008	100	1%
0.0016	100	6%
0.0031	100	73%
0.0063	100	92%
0.0125	100	99%

Reported Statistical Results

Statistical Method: Stephan Computer Program (binomial test was used). NOEC was determined by visual inspection of the mortality data.

LD_{50}: 0.0025 μg ai/bee (95% C.I.: 0.0016 - 0.0031 μg ai/bee)
NOEC: 0.0016 μg ai/bee based on treatment related mortality and signs of toxicity at doses $\geq$ 0.0031 μg ai/bee

14. VERIFICATION OF STATISTICAL RESULTS

The reviewer used EPA's Toxanal Program to determine the LD_{50} (see attached printout). The moving average method was used.
LD_{50}: 0.0029 μg ai/bee (95% C.I.: 0.0026 - 0.0031 μg ai/bee)
NOEC: 0.0016 μg ai/bee based on visual observation of the data

The LD_{50} of 0.0029 μg ai/bee classifies XDE-105 as highly toxic to honey bees.

15. **REVIEWER'S COMMENTS:** No major study deviations were noted. The authors reported that the photoperiod was longer than 8 hours of light on two dates, the extensions ranging from 5 to 7 minutes. The authors also reported that the relative humidity of the study room exceeded the specified range of 20% -90% when the readings ranged from 94-100%. These deviations were not found to affect the overall quality of the study.

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

jedwards spinosad - acute bee

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.0125	100	99	99	0
.0063	100	92	92	0
.0031	100	73	73	0
.0016	100	6	6	0
.0008	100	1	1	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 2.527212E-03

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.018381E-02	2.857961E-03	2.599385E-03	3.136565E-03

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.441073	6.872742	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 4.57362
95 PERCENT CONFIDENCE LIMITS = 1.536127 AND 7.611113

LC50 = 2.768018E-03
95 PERCENT CONFIDENCE LIMITS = 1.710099E-03 AND 4.536778E-03

LC10 = 1.46046E-03
95 PERCENT CONFIDENCE LIMITS = 3.625951E-04 AND 2.171359E-03

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DER dated 4/27/96 (MRID 43414547) Spinosad

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