10-12-84

1. CHEMICAL: INM6316

2. FORMULATION: Technical - 93.4 - 95%

3. CITATION: Meade, A.B. 1984. Acute contact LD_{50} study in honey bees (Apis mellifera L.) with INM6316. In EPA Acc. No. 072846. Subm. by E.I. Du Pont de Nemours and Co., June, 1984.

4. REVIEWER: Allen W. Vaughan
Entomologist
EEB/HED

5. DATE REVIEWED: October 12, 1984

6. TEST TYPE: Honey bee acute contact LD_{50}

7. REPORTED RESULTS: In two separate tests, mortality at 12.5 micrograms per bee, the highest rate tested, did not exceed 15%. Based on these findings, the acute contact LD_{50} was estimated to be greater than 12.5 micrograms per bee (relatively non-toxic.)

8. REVIEWER'S CONCLUSIONS: This study is scientifically sound, and shows INM6316 to be relatively non-toxic to honey bees. The study fulfills the guideline requirements for the honey bee acute contact LD_{50} study.

Materials and Methods (Author's text)

The methods described in this report are consistent with the procedures specified in Subdivision L - Hazard Evaluation: Nontarget Insects, of the FIFRA Registration Guidelines US EPA, October 15, 1982.

Test Bees

One hundred and eighty bees (Apis mellifera L.) were used in Test A, and two hundred and forty in Test B. All were collected from healthy hives maintained at Du Pont's Stine Farm for at least four years.

The colonies are housed and maintained in accordance with standard bee keeping practices.

Du Pont's bee colonies are allowed to forage freely. Once the bees are brought into the laboratory, they are fed a 50 percent honey/water solution. During the test this honey/water solution is contained in petri dishes, with one petri dish allocated to each test chamber.

Bee Collection

On the day that a test started, enough bees were collected to complete that test. Bees were obtained by applying smoke to the entrance of the hive. When calm, the test bees were transferred to the laboratory.

Holding Environment

Once taken to the laboratory, the test bees were maintained at a 16:8 (L:D) photocycle and temperature of 78°F.

Test Chamber

The test chambers in which treated bees were placed consisted of 8 oz wax squat "Sweetheart" ice cream containers. The bees were confined, ten per chamber, with 1/14 inch mesh copper screen held in place by two size 12 rubber bands, crossing the screen at right angles. In test A, food was provided by placing the screen end of the chamber on a sponge cube that rested in a petri dish (9 cm diameter x 1 cm deep) containing 50 percent honey/water solution. For Test B, a petri dish (3.5 cm diameter x 1 cm deep) containing two one-inch lengths of dental cotton and 50 percent honey/water solution was placed in the chamber before the bees were confined. Both feeding methods were efficient, but the latter made for easier handling and observation.

Treatment Levels

Because of solubility problems, INM6316 was not tested at rates exceeding 12.5 ug/ul. It was applied at 12.5 and 6.25 ug/bee in Test A and 12.5, 6.25, 3.125, and 1.5625 ug/bee in Test B.

In both tests, carbaryl was applied at 4, 2, 1, 0.5, 0.25, and 0.125 ug/bee.

A positive control in which twenty bees were treated with the solvent was included in each test. A negative control of 20 untreated bees was included in Test B.

At each treatment level there were two replications of ten bees each.

Dose Preparation/Administration

Fifty milligrams of INM6316 was dissolved in 4 ml of acetone, resulting in a concentration of 12.5 ug/ul. From this stock solution lower doses were derived. The same principle was followed in preparing the carbaryl formulations.

Test bees were immobilized by confining them in 8 oz cylindrical plastic containers (11 cm diameter) in groups of approximately 100, and placing them in a freezer for four minutes.

Once immobilized, each bee received the appropriate dose in one milliliter of acetone, applied with a micropipette. Each bee was held by a wing with feather weight forceps, and the dose applied dorsally on the bee's thorax. The bee was then transferred to the appropriate test chamber for observation.

Observations

Mortality responses were noted at the end of 48 hours.

Statistical Analysis

LD_{50} and LD_{90} values were derived by a Computer Probit Analysis Procedure.

Discussion/Results

Table 1 - Test A

RATES (UG/BEE)	PERCENT MORTALITY[1] (48 HOURS) INM6316-11	CARBARYL[2]
12.5	0	—[3]
6.25	0	--
4	—	100
3.125	—	--
2	—	94
1	—	50
0.5	—	38
0.25	—	0
0.125	—	6

[1]VALUES CORRECTED USING ABBOTT'S FORMULA (MORTALITY IN POSITIVE CONTROL UNITS = 20%).

[2]CARBARYL (PROBIT ANALYSIS)
LD_{50}= 0.7860 UG/BEE
LD_{90} = 1.9124 UG/BEE
SLOPE = 3.3191

[3]DASH INDICATES COMPOUND NOT TESTED AT THAT RATE.

Table 2 - Test B

RATES (UG/BEE)	PERCENT MORTALITY[1] (48 HOURS) INM6316-7	CARBARYL[2]
12.5	0	—[3]
6.25	15	—
4	—	100
3.125	0	—
2	—	95
1.5625	0	—
1	—	35
0.5	—	10
0.25	—	0
0.125	—	5

[1]THERE WAS NO MORTALITY IN CONTROL UNITS.
[2]CARBARYL (PROBIT ANALYSIS)
LD_{50} = 1.0537 UG/BEE
LD_{90} = 1.9256 UG/BEE
SLOPE = 4.8944
[3]DASH INDICATES COMPOUNDS NOT TESTED AT THAT RATE.

Reviewer's Evaluation

A. Test Procedures

Procedures followed standard EPA guidelines and appeared to be sound.

B. Statistical Analysis

Analysis as performed by the author was assumed to be valid. Information provided in the report was insufficient to allow independent validation.

C. Discussion/Results

Study fulfills guideline requirements and shows INM-6316 to be relatively non-toxic to honey bees.

D. Conclusions

1. Category - Core

2. Rationale - Study fulfills guideline requirements

3. Repairability - N/A