DP Barcode : D188505
PC Code No : 121701
EEB Out : FEB/23 1994

To: Phillip Hutton
Product Manager 18
Registration Division (H7505C)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 062552-00001
Chemical Name : Azadirachtin
Type Product : biochemical (IGR)
Product Name : Azatin EC
Company Name : Agridyne Tech Inc.
Purpose : Review follow-up data for conditional registration.

Action Code: 320 Date Due: 5/6/93
Reviewer: Regina Hirsch

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

DATA EVALUATION RECORD

1. **CHEMICAL:** Azadirachtin.
Shaughnessey No. 121701.

2. **TEST MATERIAL:** NPI-720; Lot No. 21380; Sublot No. 1088-44C; 10% active ingredient; a tannish powder.

3. **STUDY TYPE:** 141-1. Acute Contact LD_{50} Test. Species Tested: Honey Bee (*Apis mellifera*).

4. **CITATION:** Lynn, S.P. and K.A. Hoxter. 1992. NPI-720: An Acute Contact Toxicity Study with the Honey Bee. Laboratory Project No. 279-103. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Agridyne Technologies, Inc., Salt Lake City, UT. EPA MRID No. 424636-02.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]
Date: 4/6/93

[signature] 12/6/93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 4/6/93

~~Henry T. Craven~~, M.S.
Supervisor, EEB/EFED
USEPA

Signature: [signature]
Date: 2 22 94

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for an acute contact study using a formulated product, but not a technical material. A 48-hour LD_{50} of >2.5 µg ai/bee classifies the test material as moderately toxic to honey bees (*Apis mellifera*). The NOEL was 2.5 µg ai/bee. (adjusted LD_{50})

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Seven days before test initiation, one frame of bee (*Apis mellifera*) pupae was placed in an environmental chamber. The bees were allowed to emerge as adults and were 1 to 7 days old at the initiation of the test. The bees appeared to be in good health at test initiation.

B. Test System: Bees were contained in one pint rolled paper containers (87 mm in diameter and 85 mm high). Each container was covered with a plastic petri plate in which a 20-ml glass vial containing 50% sugar/water was inserted. The vial opening was covered with cheesecloth to prevent leakage. This food source was available *ad libitum* throughout the test. A sponge affixed to the chamber was misted daily to increase humidity.

Bees were kept in a room that was supplied with 8 hours of light/day. The temperature was maintained at 24-25°C, and the mean relative humidity was 59%.

C. Dosage: Forty-eight-hour acute contact test. The doses were not corrected for the purity of the test material (10%). Five treatment levels representing 1.6, 3.1, 6.3, 12.5, and 25 µg/bee were tested along with a solvent control (2 µl acetone/bee) and a negative control.

An appropriate amount of the test material was diluted to the final volume of 10 ml in acetone to prepare the highest concentration dosing solution. Lower concentration dosing solutions were prepared by serial dilutions.

D. Design: Two replicates of 25 bees each were indiscriminately selected for both treatments and controls. The bees were immobilized with nitrogen and laid out on paper. They were then dosed individually on the thorax and/or abdomen with 2 µl of test solution. Negative control bees were handled identically to treated bees, but were not dosed with any material. Solvent control bees received only acetone. Observations of mortality and toxic symptoms were recorded twice on day 0 and once on day 1 and day 2.

E. **Statistics:** An LD_{50} value was determined by visual inspection due to the pattern of mortality in this study. The LD_{50} value was used to classify the test substance according to Atkins' toxicity categories. The categories were: highly toxic (less than 2 µg/bee), moderately toxic (greater than or equal to 2 µg/bee but less than 11 µg/bee), and relatively nontoxic (greater than or equal to 11 µg/bee).

12. **REPORTED RESULTS:** Cumulative mortalities of the test bees during the 48-hour exposure period are presented in Table 1 (attached). At test termination, mortality in the negative control and solvent control was 10 and 8%, respectively. A small number of bees were noted as immobile or lethargic in both control groups one hour after dosing. All other control bees were normal in appearance and behavior throughout the test.

Mortality in the test groups ranged between 10 and 18%. A small number of bees were noted as immobile or lethargic in all treatment groups one hour after dosing. All other bees were normal in appearance and behavior throughout the test. Mortality and immobility were not believed to be treatment related due to the lack of a dose response.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The test material, NPI-720, was classified as relatively non-toxic according to the toxicity categories of Atkins. The honey bee 48-hour contact LD_{50} for this material was determined to be greater than 25 µg/bee. The no-observed-effect level (NOEL) was 25 µg/bee.

The study director confirmed that this study was conducted in compliance with Good Laboratory Practice (GLP) standards (40 CFR Part 160) with the exception that test substance characterization was the responsibility of the sponsor. Additionally, samples of the dosing solutions were not collected to confirm test concentrations. Quality Assurance and GLP statements were included in the report.

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedures generally followed the protocols recommended by the SEP and Subdivision L guidelines.

B. **Statistical Analysis:** Upon review of the mortality data, the reviewer concurs that the LD_{50} was greater than 25 µg/bee. Analysis of variance and Dunnett's

test were conducted to verify the NOEL. The results were the same as the authors' (see attached printout).

C. Discussion/Results: It is unclear whether the test material is technical or a formulated product. This test fulfills the guidelines for a formulated product, but not a technical material. The material was only tested to a dosage level of 2.5 µg active ingredient (ai)/bee.

This study is scientifically sound and fulfills the requirements for an acute contact study using a formulated product, but not a technical material. A 48-hour LD_{50} of >2.5 µg ai/bee classifies the test material as moderately toxic to honey bees (*Apis mellifera*). The NOEL was 2.5 µg ai/bee. (adjusted LD_{50})

D. Adequacy of the Study:

(1) **Classification:** Core for a formulated product only.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. COMPLETION OF ONE-LINER: Yes, 3-30-93.

TABLE 1
CUMULATIVE MORTALITY OF HONEY BEES
EXPOSED TO NPI-720 FOR 48 HOURS

Experimental Group	Concentration (µg/bee)	Day 0 First Observation Replicate* A	Day 0 First Observation Replicate* B	Day 0 Second Observation Replicate A	Day 0 Second Observation Replicate B	Day 1 Replicate A	Day 1 Replicate B	Day 2 Replicate A	Day 2 Replicate B	Replicates Combined	% Mortality
Negative Control	0	0(1/0)	0	0(1/1)	0(3/0)	2	3	2	3	5/50	10
Solvent Control	0	0(0/1)	0	0(2/0)	0	4	0	4	0	4/50	8
Treatment	1.6	0(0/2)	1(0/3)	0(2/0)	2(3/0)	2	5	3	6	9/50	18
	3.1	0(1/3)	0(3/0)	0(2/0)	4	5	4	5	4	9/50	18
	6.3	0(1/0)	0(1/0)	0(2/0)	1	2	2	2	3	5/50	10
	12.5	0(0/1)	0(0/2)	1(1/0)	0(4/0)	2	6	2	6	8/50	16
	25	0(2/0)	0(0/2)	1(2/0)	1(4/0)	5	2	5	2	7/50	14

*Each replicate contained 25 bees.

( ) Indicates bees found immobile/lethargic.

The LD50 value was determined to be greater than 25 µg/bee.

bee mortality - NPI-720

Summary Statistics and ANOVA

Transformation = None

Group dosage (µg/bee)	n	Mean	s.d.	cv%
1 = control	2	23.0000	2.8284	12.3
2 1.6	2	20.5000	2.1213	10.3
3 3.1	2	20.5000	.7071	3.4
4 6.3	2	22.5000	.7071	3.1
5 12.5	2	21.0000	2.8284	13.5
6 25.0	2	21.5000	2.1213	9.9

1 = solvent control

NOEL = 25 µg/bee
(2.5 mg ai/bee)

*) the mean for this group is significantly less than
the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -5.891115
This difference corresponds to -25.61 percent of control

Between groups sum of squares = 11.000000 with 5 degrees of freedom.

Error mean square = 4.333333 with 6 degrees of freedom.

Bartlett's test p-value for equality of variances = .809

Ecological Effects Branch One-Liner Data Entry Form

Chemical Azadirachtin Shaughnessy No. 121701 Pesticide Use Insecticide

INVERTEBRATE ACUTE TOXICITY	% AI	EC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Apis mellifera	10	>2.5 µg ai/bee (NA[a]) NA[a]	48 hrs/ acute Contact	2.5 µg ai/bee	1992/1993	424636-02 Core[b]	WIL	MM
2.								
3.								
4.								
5.								

COMMENTS: a = not applicable

b = core for a formulated product.