DATA EVALUATION RECORD

1. Chemical: PP321 (Karate)

2. Test Material: Technical, 96% ai; EC formulation, 5.04% ai

3. Study Type: Honey bee acute contact and oral LD50

Species Tested: Apis mellifera

4. Study ID: Gough, H.J., I.G. Collins, C.J. Everett, and W. Wilkinson. 1984. PP321: Acute contact and oral toxicity to honey bees (Apis mellifera). Submitted by ICI Americas Inc., Wilmington, DE. Reg. No. 10182-OA. Acc. No. 400524-09.

5. Reviewed By:

Allen W. Vaughan Entomologist EEB/HED

Signature: Allen W. Vaughan

Date: 7.1.87

Signature: Allen W. Vaughan

6. Approved By:

Norman J. Cook Supervisory Biologist EEB/HED

Date: 7.1.87

7. Conclusions:

This study is scientifically sound. Toxicity values for the technical material were as follows:

24-hr contact $LD_{50} = 0.051$ ug/bee 48-hr contact $LD_{50} = 0.038$ ug/bee

24-hr oral LD₅₀ = 0.965 ug/bee

48-hr oral LD₅₀ = 0.909 ug/bee /

Toxicity values for the formulation were as follows:

24-hr contact $LD_{50} = 0.095$ ug/bee

48-hr contact $LD_{50} = 0.098$ ug/bee

24-hr oral LD₅₀ = 0.570 ug/bee

48-hr oral $LD_{50} = 0.483$ ug/bee

On the basis of these figures, PP321 is considered highly toxic to honey bees. This study fulfills the guideline requirement for an acute contact toxicity determination on honey bees with the technical material.

8. Recommendations: N/A

- 9. <u>Background</u>: This study was submitted by ICI Americas in support of registration.
- 10. Discussion of Individual Tests: N/A
- 11. Materials and Methods
 - A. <u>Test Animals</u> were worker bees obtained from research colonies.
 - B. Test System: General: Bees were collected by sweeping them from the combs into a plastic bucket. Bees were anesthetized by feeding carbon dioxide gas into the bucket. They were then placed in cages and allowed to recover. During the test period, bees were kept in a controlled temperature room at 25-26.5°C and 67-71% R.H. Mortality was evaluated at 1,2,4,24, and 48 hrs after treatment.

Oral Tests: Stock solutions and dilutions were prepared in aqueous 50% sucrose solution, containing 5% acetone for technical PP321. Each group of 10 bees was fed 0.2 ml of a given concentration. Control bees were fed 0.2 ml per cage of 50% sucrose solution alone, or 50% sucrose with 5% acetone in the case of technical PP321. When all the test material had been taken, bees were fed sucrose solution.

Contact Tests: The diluent for technical PP321 was acetone. The diluent for formulated PP321 was deionized water containing 660 mg/l Agral. After recovery from initial anesthetization, bees were reanesthetized, and a l ul drop of a given concentration was applied to the thorax of each bee with a microsyringe. Control bees were treated with the appropriate solvent only.

- C. <u>Dose</u>: Oral application through feeding tube in sucrose solution; acetone diluent for technical. Contact application using microapplicator; acetone solvent (technical) or water/Agral solvent (formulation).
- D. Design: 30 bees per dose level and control, divided into 3 reps.; replicated two times over time. Six dose levels for contact tests (0.2, 0.1, 0.05, 0.02, 0.01, and 0.005 ug ai per bee). Seven dose levels for oral test with technical (0.25, 0.1, 0.05, 0.025, 0.01, 0.005, and 0.0025 ug ai per bee). Six dose levels for oral test with formulation (same as for technical but delete 0.0025 ug dose level).

- E. Statistics: Data were adjusted for control mortality using Abbott's formula. Results were analyzed by plotting logit transformation of percentage kill against the log dose in ug ai per bee.
- 12. Reported Results: Reported LD₅₀ values are listed above under #7, "Conclusions."

13. Study Author's Conclusions/O.A. Measures:

Reported LD_{50} values are listed above. The values obtained indicate that PP321 is highly toxic to honey bees under the conditions of these tests.

Protocol and final report audits were conducted by ICI's Ouality Assurance Unit.

14. Reviewer's Discussion and Interpretation of the Study

A. Test Procedures:

Procedures were in accordance with protocols recommended in the guidelines. There were no problems in this regard.

B. Statistical Analysis:

Results of the analyses were validated using Stephan's LD_{50} program on the IBM-PC. Validation supports the results of the study authors' analyses. Computer printouts are attached for data from contact tests.

C. Discussion/Results:

With acute LD50 values in all cases (contact and oral, 24-hr and 48-hr values) less than 1 ug ai per bee, PP321 is highly toxic to honey bees.

D. Adequacy of Study:

- 1. Classification: Core
- 2. Rationale: Guidelines protocol
- 3. Reparability: N/A
- 15. Completion of One-Liner: N/A
- 16. CBI Appendix: N/A

vaughan	PP321 hone	y bee 06-30-	87 Acute contact	, technical , 24-hr. *********
CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
•2	60	49	81.66666	0
• 1	60	43	71.66666	0
• 05	60	41	68.33334	0
•02	60	10	16.66667	0
.01	60	1	1.666667	0
• 005	60	1	1.666667	O

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 3.660556E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN G LC50 95 PERCENT CONFIDENCE LIMITS

4 .0339873 4.968683E-02 4.134872E-02

6.003242E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS G H GOODNESS OF FIT PROBABILITY

4 .2285148 3.842075 3.99524E-03

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

SLOPE = 2.100021 95 PERCENT CONFIDENCE LIMITS = 1.096144 AND 3.103899

LC50 = 5.167326E-02 95 PERCENT CONFIDENCE LIMITS = 3.036115E-02 AND 9.497541E-02

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

vaughan	PP321 H	oney bee 06-30-8	37 Acute contact	t, technical, 48-hr.
*****	******	***********	********	********
CONC.	NUMBER	NUMBER	PERCENT	BINOMIAL
	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
•2	59	58	98.3051	0
• 1	59	48	81 • 3559	0
• 05	59	42	71.1864	Ô
.02	59	10	16.9492	Ō
.01	59	2	3.3898	Ŏ
.005	59	2	3.3898	Õ

THE BINOMIAL TEST SHOWS THAT .02 AND .05 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .035368

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN G LC50 95 PERCENT CONFIDENCE LIMITS

5 2.206006E-02 3.947009E-02

3.348027E-02 4.699921E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS G H GOODNESS OF FIT PROBABILITY

4 2.732832E-02 1 5.283547E-02

SLOPE = 2.649374 95 PERCENT CONFIDENCE LIMITS = 2.211398 AND 3.087349

LC50 = 3.849998E-02 95 PERCENT CONFIDENCE LIMITS = 3.286567E-02 AND 4.511907E-02

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

vaughan	PP321 Hone	y bee 06-30-	87 Acute contact	, EC , 24-hr.
CONC.	NUMBER	NUMBER	PERCENT	**************************************
.2	EXPOSED 58	DEAD 52	DEAD 89.6552	PROB.(PERCENT) O
•1 •05	58 58	33 4	56.8965 6.8966	0
.02 .01	60 60	1	1.666667	Ŏ
.005	60	1	1.666667	0

THE BINOMIAL TEST SHOWS THAT .05 AND .1 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.211501E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN G LC50 95 PERCENT CONFIDENCE LIMITS

3 2.809963E-02 9.933721E-02

8.553101E-02 .1179608

RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS G H GOODNESS OF FIT PROBABILITY
6 3.181901 35.53753 O
A PROBABILITY OF O 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 = 2.877876 95 PERCENT CONFIDENCE LIMITS =-2.255646 AND 8.011398

LC50 = .0931382 95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY, THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

vaughan		Honey bee 06-3	0-87 Acute contact	t, Ec, 48-hr.
*****	******	**********	******	*******
CONC.	NUMBER EXPOSED	NUMBER	PERCENT DEAD	BINOMIAL PROB (PERCENT)
•2	55	49	89.09091	O O
•1 •05	55 55	32 5	58.1818 9.090899	0
•02 •01	60	1	1.666667	ŏ
.005	60 60	1	5 1.666667	0

THE BINOMIAL TEST SHOWS THAT .05 AND .1 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.028816E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
SPAN G LC50 95 PERCENT CONFIDENCE LIMITS
3 2.978541E-02 .0963262 8.258962E-02
.1148825

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS G H GOODNESS OF FIT PROBABILITY

6 .811498 9.646499 0

A PROBABILITY OF O 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 = 2.468375 95 PERCENT CONFIDENCE LIMITS = .2447841 AND 4.691965

LC50 = 8.948258E-02 95 PERCENT CONFIDENCE LIMITS = 2.981667E-02 AND 1.812841