DATA EVALUATION RECORD
HONEY BEE - ACUTE ORAL LC_{50} TEST
Non-Guideline (OECD 213)

1. **CHEMICAL:** Penoxsulam PC Code No.: ~~199031~~ 119031

2. **TEST MATERIAL:** XDE-638 Purity: 97.7%

3. **CITATION:**

Author: J. A. Kranzfelder

Title: XDE-638: Acute Oral Toxicity Test with the Honeybee (*Apis mellifera*)

Study Completion Date: July 3, 2000

Laboratory: ABC Laboratories
7200 E. ABC Lane
Columbia, Missouri 65202

Sponsor: The Dow Chemical Company
Midland, MI
for
Dow AgroSciences LLC
Indianapolis, IN 46268

Laboratory Report ID: ABC Study No. 45474/Dow Study No. 990123

DP Barcode: D288160

MRID No.: 45831125

4. **REVIEWED BY:** Rebecca Bryan, Staff Scientist, Dynamac Corporation

Signature: Rebecca Bryan **Date:** 12/29/03

APPROVED BY: Dana Worcester, Staff Scientist, Dynamac Corporation

Signature: Dana Worcester **Date:** 12/29/03

5. **APPROVED BY:** ~~Bill Erickson~~
James J. Goodyear, Ph.D.
Ecological Effects Biologist
Office of Pesticide Programs
703-305-7726

Signature: JGoodyear **Date:**

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1

6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Apis mellifera*

Age or Size of Test Organism at Test Initiation: Not reported

Type of Concentrations: Nominal and actual intake

Definitive Study Duration: 48 hours

7. CONCLUSIONS:

The honey bee, *Apis mellifera* L., was exposed to XDE-638 for 48 hours, at two vehicle concentrations (1% and 1.7%) due to solubility constraints.

The 1% vehicle concentration test had nominal concentrations of 3.8, 7.6, 15, 30, and 60 µg a.i./bee (Actual ingested doses were 4.3, 8.3, 15.3, 29.7, and 64.3 µg a.i./bee, respectively). By 48 hours, there was 0, 0, 10, 0, and 0% mortality observed in the 4.3, 8.3, 15.3, 29.7, and 64.3 µg a.i./bee treatment groups, respectively, compared to 0% mortality in the negative and 1% vehicle control groups.

The 1.7% vehicle concentration test had nominal concentrations of 6.5, 13, 25, 50, and 100 µg a.i./bee (Actual ingested doses were 5.2, 13.7, 29, 33.3, and 85.3 µg a.i./bee, respectively). By 48 hours, there was 10, 3, 17, 0 and 0% mortality observed in the 5.2, 13.7, 29, 33.3, and 85.3 µg a.i./bee treatment groups, respectively, compared to 0% mortality in the 1.7% vehicle control group.

The LD_{50} value was >100 µg a.i./bee. As a result, XDE-638 is categorized as practically nontoxic to honeybees on an acute oral basis.

This acute contact study is classified as Supplemental. This acute oral study is scientifically sound, but it is not a guideline study and does not fulfill an OPP guideline requirement.

Reported Statistical Results:
LD_{50}: >100 µg a.i./bee 95% C.I.: N/A
NOAEL: Not determined Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: The acute oral study is scientifically sound but is classified as Supplemental.

B. Rationale: The study is scientifically sound but is classified as Supplemental because it is a non-guideline study and does not fulfill an OPP guideline requirement.

C. Repairability: N/A

(2)

9. GUIDELINE DEVIATIONS:

The age of honey bees at study initiation was not reported.

10. SUBMISSION PURPOSE: This study was submitted to provide data on the acute oral toxicity of XDE-638 to honeybees for the purpose of chemical registration.

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
Supplier:	Gibbons Honey Farm, Rocheport, Missouri
All bees from the same source?	Yes, from a single, disease-free colony.

B. Test System

Guideline Criteria	Reported Information
Cage size adequate?	The cages were plastic and screened. Cages are 14-cm wide x 20-cm long x 10-cm high.
Lighting:	Continuous darkness except at observation periods.
Temperature:	24.8-25.6°C
Relative humidity:	36.8-53.2%

C. Test Design

Guideline Criteria	Reported Information
Range finding test?	The definitive test concentrations were based on a previous acute contact toxicity study. The contact LD_{50} was >100 µg a.i./bee.
Reference toxicant test?	The reference toxicant, dimethoate, was tested for 24 hours. The test concentrations were 0.020, 0.20, and 0.40 µg/bee (assuming 100% consumption).

Guideline Criteria	Reported Information
Method of administration:	The test substance was mixed with a 500 g/L (w/v) sucrose solution. The 1% vehicle concentration test had 1.0 mL of each stock added to separate 100 mL volumes of the sucrose solutions. The 1.7% vehicle concentration test had 1.7 mL of each stock added to separate 100 mL volumes of the sucrose solutions.
Nominal doses:	1% vehicle concentration test: 3.8, 7.5, 15, 30, and 60 µg a.i./bee (Actual ingested doses were 4.3, 8.3, 15.3, 29.7, and 64.3 µg a.i./bee, respectively). 1.7% vehicle concentration test:6.5, 13, 25, 50, and 100 µg a.i./bee (Actual ingested doses were 5.2, 13.7, 29, 33.3, and 85.3 µg a.i./bee, respectively).
Controls: Negative control and/or diluent/solvent control	Negative control and Vehicle control
Number of colonies per group:	Negative Control: 7 replicates; 10 bees/replicate Vehicle Controls and Treatment Groups: 3 replicates; 10 bees/replicate
Solvent: The following solvents: acetone, dimethylformamide, triethylene glycol, methanol, ethanol.	DMSO
Feeding:	The test solutions were provided for 1.25 hours. Then, the bees were supplied with untreated 500 g/L sucrose solution, *ad libitum*.
Observations period:	48 hours

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control performance:	0% negative and vehicle control mortality by 48 hours.
Raw data included:	Replicate data were provided.

Guideline Criteria	Reported Information
Signs of toxicity (if any) were described?	If a bee was on its "back."

Mortality

Dosage µg a.i./bee (Measured dosage µg a.i./bee)[1]	No. of bees	Percent Mortality (%)[2]		
		4 Hours	24 hours	48 hours
Test Substance (XR-225)				
Control Group	70	0	0	0
1% Vehicle Control	30	0	0	0
3.8 (4.3)	30	0	0	0
7.5 (8.3)	30	0	0	0
15 (15.3)	30	0	0	10
30 (29.7)	30	0	0	0
60 (64.3)	30	0	0	0
1.7% Vehicle Control	30	0	0	0
6.5 (5.2)	30	0	0	10
13 (13.7)	30	0	3	3
25 (29)	30	3	7	17
50 (33.3)	30	0	0	0
100 (85.3)	30	0	0	0
Toxic Standard (dimethoate, µg/bee):				
Control	30	0	0	N/A
0.020 (0.020)	30	0	0	N/A
0.20 (0.20)	30	100	100	N/A
0.40 (0.38)	30	100	100	N/A

[1] Actual intake concentrations were reviewer-calculated averages from replicate calculated dosages.
[2] Percent mortalities were reviewer-calculated based on replicate data (Table 5-7, pp. 20-22).

Observations: By 48 hours, there was 0, 0, 10, 0, and 0% mortality observed in the 4.4, 8.3, 15.3, 29.7, and 64.3 µg a.i./bee treatment groups, respectively, compared to 0% mortality in the negative and 1% vehicle control groups. By 48 hours, there was 10, 3, 17, 0 and 0% mortality observed in the 5.2, 13.7, 29, 33.3, and 85.3 µg a.i./bee treatment groups, respectively, compared to 0% mortality in the 1.7% vehicle control group.

Statistical method: The LD_{50} values were estimated due to less than 50% mortality. The LD_{50} was based on the measured concentrations.

Reported Statistical Results:

LD_{50}: >85.3 µg a.i./bee 95% C.I.: N/A
NOAEL: Not reported Probit Slope: N/A

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical method: The LD_{50} could be visually determined, as mortality did not exceed 50%. The NOAEC could not be determined for the 1.7% vehicle control group, but was 64.3 µg a.i./bee for the 1% vehicle if it is assumed that the single death was do to causes other than penoxsulam.

Results:

LD_{50}: >85.3 µg a.i./bee 95% C.I.: N/A
NOAEL: 64.3 µg a.i./bee Probit Slope: N/A

14. REVIEWER'S COMMENTS:

The LD_{50} value was >85.3 µg a.i./bee. As a result, XDE-638 is categorized as practically nontoxic to honeybees on an acute oral basis. The study is scientifically sound but is classified as Supplemental because it is a non-guideline study and does not fulfill an OPP guideline requirement.

The bees were starved for approximately 2 hours prior to introduction of the definitive test solution feeders.

The 24-hour LD_{50} of the toxic standard, dimethoate, was 0.062 µg/bee. This value was determined by the SAS Spearman-Karber method. The LD_{50} for dimethoate was outside the published range of toxicity to honeybees (0.10-0.35 µg a.i./bee). However, the LD_{50} was consistent with the historical laboratory values.

The mean actual consumed dosages were reviewer-calculated from replicate calculated dosages. The consumption of the 1% vehicle concentration diets ranged from 74 to 100% and the consumption of the 1.7% vehicle concentration diets ranged from 22 to 100%. The consumption of the reference substance diets ranged from 83 to 100%.

15. REFERENCES:

Organization for Economic Cooperation and Development. 1997. Decision of the Council, Revised Principles of GLP [C(97)186/Final].

The SAS System for Windows, Release 6.12. Copyright 1989-96 by SAS Institute, Cary, North Carolina, 27512-8000 USA.

6

Gough, H.J., McIndoe, E.C., Lewis, G.B. (1994). The use of dimethoate as a reference compound in laboratory acute toxicity tests on honey bees (*Apis mellifera* L.). 1981-1992. Journal of Apicultural Research 22, 119-125.

ICBPR. Validation Exercise on the Use of Dimethoate as a Toxic Reference Substance in Toxicity Tests on Honeybees (in preparation).

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

survival (1.7% vehicle control)
File: 1125s Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	vehicle control	100.000	100.000	34.500
2	5.2	90.000	90.000	24.500
3	13.7	96.667	96.667	27.000
4	29.0	83.333	83.333	16.000
5	33.3	100.000	100.000	34.500
6	85.3	100.000	100.000	34.500

Calculated H Value = 6.241 Critical H Value Table = 11.070
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

survival (1.7% vehicle control)
File: 1125s Transform: NO TRANSFORM

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 4 2 3 1 5 6
4	29.0	83.333	83.333	\
2	5.2	90.000	90.000	. \
3	13.7	96.667	96.667	. . \
1	vehicle control	100.000	100.000	. . . \
5	33.3	100.000	100.000	. . . . \
6	85.3	100.000	100.000	. . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 SE = 3.172

8