DATA EVALUATION RECORD

1. **CHEMICAL:** Profenofos.
Shaughnessey No. 111401.

2. **TEST MATERIAL:** Profenofos technical (Curacron); O-(4-bromo-2-chlorophenyl)-O-ethyl-s-propyl phosphorothioate; ID No. FL-881610, ARS9312; Batch Code P707240; 90.4% active ingredient, a cloudy yellow liquid.

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

4. **CITATION:** Winter, P.A. 1990. Profenofos: An Acute Contact Study with the Honey Bee. Laboratory Report No. 108-321. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 416273-08.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
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Signature: Mark A. Mossler
Date: 4/9/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
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Signature: P. Kosalwat
Date: 4/9/91

Henry T. Craven, M.S.
Supervisor, EEB/HED
USEPA

Signature: Henry T. Craven
Date: 6/11/91

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute contact LD_{50} test using honey bees. With a 48-hour LD_{50} value of 0.0953 µg ai/bee, profenofos technical is considered highly toxic to Apis mellifera, when administered as a solution. The no-effect concentration for this study was 0.0469 µg ai/bee.

6 hrs

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

9. **BACKGROUND:** N/A.

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Two frames of honey bee (Apis mellifera) pupae were placed in an incubator for 2 days to allow pupae to emerge as adults. All test bees were 1 to 2 days old at the initiation of the test and were apparently healthy.

B. **Test System:** Bees were placed in one-pint rolled paper containers (87 mm in diameter, 85 mm high). Each container was covered with a plastic petri plate in which a 20-ml glass vial containing 50% sugar/water solution was inserted. This food source was available ad libitum throughout the test. A sponge affixed to the test chamber was misted daily to increase the humidity within the test chamber. The test chamber received 8 hours of light per day. Temperature ranged between 21°C to 22°C with a mean relative humidity of 88%.

C. **Dosage:** The appropriate amount of test material was dissolved in acetone. Serial dilutions were then made for the lower concentrations tested. Ten treatment levels representing 0.01175, 0.0235, 0.0469, 0.0938, 0.1875, 0.375, 0.75, 1.5, 3.0, and 6.0 µg active ingredient (ai)/bee were tested along with a solvent control and a negative control.

D. **Design:** The test consisted of 10 treatment levels, a control, and a solvent control. Two replicates of 25 bees each were used for the treatment and controls. Twenty-five randomly selected bees were immobilized with nitrogen and laid out on paper. The bees were dosed individually on the thorax and/or abdomen with 2 µl of the appropriate test solution. Negative control bees were handled identically to treated bees. Solvent control bees received only acetone. Observations of mortality and signs of toxicity were recorded twice on day 0, and once on days 1 and 2.

E. **Statistics:** An LD_{50} value and 95% confidence limits

2

were calculated by binomial probability. The LD_{50} value was used to classify the test substance according to 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, negative control and solvent control mortalities were 4% and 10%, respectively. Mortality at the 0.01175, 0.0235, and 0.0469 µg ai/bee doses were 10%, 0%, and 14%, respectively. These mortalities were not dose responsive and were not considered treatment related. Mortalities at 0.0938 and 0.1875 µg ai/bee were 44% and 90%, respectively. Total mortality was apparent in the higher doses.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "In conclusion, profenofos was classified as highly toxic according to the toxicity categories. The honey bee acute contact LD_{50} value for profenofos was determined to be approximately 0.102 µg ai/bee with 95% confidence limits of 0.0469 to 0.1875 µg ai/bee. The no observed effect level was 0.0469 µg ai/bee, based on treatment related mortality and signs of toxicity at higher doses."

The Quality Assurance Unit of Wildlife International Ltd., was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Standards. Both statements of compliance with GLPs and QA were enclosed.

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

A. **Test Procedure:** The test procedures generally follow the protocols recommended by the SEP.

B. **Statistical Analysis:** One-way analysis of variance (Dunnett's), was performed on the 48-hour data to determine the no-effect concentration. Probit analysis was conducted on these same data to determine the LD_{50}. Both computer printouts are attached. The results are in near agreement with the author's. However, the reviewer's LD_{50} is 0.0953 µg ai/bee, in comparison to 0.102 µg ai/bee. Since the reviewer's LD_{50} is more

3

conservative, and will better protect non-target insects, it will be taken to be the correct value.

C. **Discussion/Results:** With a 48-hour LD_{50} of 0.0953 µg ai/bee, profenofos technical is considered highly toxic to honey bees (Apis mellifera). The no-effect concentration was determined to be 0.0469 µg ai/bee.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** The test follows previously approved protocols.

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

15. **COMPLETION OF ONE-LINER:** Yes, March 25, 1991.

TABLE [illegible]

CUMULATIVE MORTALITY OF HONEY BEES EXPOSED TO PROFENOFOS FOR 48 HOURS BY REPLICATE*

Experimental Group	Concentration (µg a.i./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	1	0	1	0	1	1	1	1	2/50	4%
Solvent Control	0	1	2	1	2	2	3	2	3	5/50	10%
Treatment											
	0.01175	0	0	0	0	0	0	5	0	5/50	10%
	0.0235	0	0	0	0	0	0	0	0	0/50	0%
	0.0469	4(1)	1(2)	5	1	5	1	5	2	7/50	14%
	0.0938	4(1)	3(3)	4(1)	7	9	11	9	13	22/50	44%
	0.1875	5(6)	11(1)	9(3)	17(2)	20	24	21	24	45/50	90%
	0.375	24(1)	22(3)	24(1)	24(1)	25	24	25	25	50/50	100%
	0.75	24(1)	23(2)	25	25	25	25	25	25	50/50	100%
	1.5	23(2)	24(1)	24(1)	25	25	25	25	25	50/50	100%
	3	24(1)	25	25	25	25	25	25	25	50/50	100%
	6	25	25	25	25	25	25	25	25	50/50	100%

*Each replicate contained 25 bees.

( ) Indicates bees found immobile.

The LD50 value was determined to be 0.102 µg a.i./bee with a 95% confidence interval of 0.0469 and 0.1875 µg a.i./bee.

5

bee mortality

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	2	2.5000	.7071	28.3
2 .0175	2	2.5000	3.5355	141.4
3 .0235	2	.0000	.0000	.0
4 .0469	2	5.5000	2.1213	60.6
5* .0938	2	11.0000	2.8284	25.7
6* .1875	2	22.5000	2.1213	9.4
7* .375	2	25.0000	.0000	.0

NOEC = 0.0469 mg a.i./bee

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

Minimum detectable difference for Dunnett's test = 5.837955
This difference corresponds to 233.52 percent of control

Between groups sum of squares = 1271.428571 with 6 degrees of freedom.

Error mean square = 4.285714 with 7 degrees of freedom.

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* *
* Warning - the test for equality of variances *
* could not be computed as 1 or more of the *
* variances is zero. *
* *
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6

Dea mortality

Estimated EC Values and Confidence Limits

Point	Conc.	95% Confidence Limits Lower	Upper
EC 1.00	0.0255	0.0169	0.0334
EC 5.00	0.0376	0.0276	0.0461
EC10.00	0.0461	0.0358	0.0550
EC15.00	0.0530	0.0425	0.0621
EC50.00	0.0953	0.0834	0.1096
EC85.00	0.1712	0.1441	0.2193
EC90.00	0.1967	0.1624	0.2610
EC95.00	0.2416	0.1933	0.3388
EC99.00	0.3552	0.2665	0.5557

$y = 9.16 + 4.07x$

y = probit % inhibited

$x = \log(\text{risk})$

Shaughnessy No. [illegible] | Chemical Name Profenofos | Chemical Class | Page 1 of 1

Study/Species/Lab/ Accession	Chemical % a.i.	Results	Reviewer/ Date	Validation Status

14-Day Single Dose Oral LD_{50}

Species

Lab

Acc.

LD_{50} = mg/kg (95% C.L.) Contr. Mort. (%) =

Slope = # Animals/Level = Age (Days) = Sex =

14-Day Dose Level mg/kg/(% Mortality)

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Comments:

14-Day Single Dose Oral LD_{50}

Species

Lab

Acc.

LD_{50} = mg/kg (95% C.L.) Contr. Mort. (%) =

Slope = # Animals/Level = Age (Days) = Sex =

14-Day Dose Level mg/kg/(% Mortality)

( ), ( ), ( ), ( ), ( )

Comments:

8-Day Dietary LC_{50}

Species

Lab

Acc.

LC_{50} = ppm (95% C.L.) Contr. Mort. (%) =

Slope = # Animals/Level = Age (Days) = Sex =

8-Day Dose Level ppm/(% Mortality)

( ), ( ), ( ), ( ), ( )

Comments:

8-Day Dietary LC_{50}

Species

Lab

Acc.

LC_{50} = ppm (95% C.L.) Contr. Mort. (%) =

Slope = # Animals/Level = Age (Days) = Sex =

8-Day Dose Level ppm/(% Mortality)

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Comments:

48-Hour ~~LC_{50}~~ LD_{50}

Species Bee - Apis Mellifera

Lab Wildlife International 90.4%

Acc. MRID No. 416273-08

LC_{50} = 0.0953 µg/Bee ~~pp~~ (95% C.L. 0.0834 - 0.1096 µg)

Contr. Mort. (%) = 4%

Sol. Contr. Mort. (%) = 10%

Slope = 4.07 # Animals/Level = 50

Temperature = 22°C

48-Hour Dose Level (µg/Bee) ~~pp~~ /(% Mortality)

0.01175 (10%), 0.0235 (0%), 0.0469 (14%), 0.0938 (44%), 0.1875 (90%), 0.375 (100%), 0.75 (100%), 1.5 (100%), 3.0 (100%), 6.0 (100%)

Comments: Acute Contact

M. Mossler 3/25/91 Core

96-Hour LC_{50}

Species

Lab

Acc.

LC_{50} = pp (95% C.L.) Con. Mort. (%) = Sol. Con. Mort. (%) =

Slope = # Animals/Level = Temp. =

96-Hour Dose Level pp /(% Mortality)

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Comments:

96-Hour LC_{50}

Species

Lab

Acc.

LC_{50} = pp (95% C.L.) Con. Mort. (%) = Sol. Con. Mort. (%) =

Slope = # Animals/Level = Temp. =

96-Hour Dose Level pp /(% Mortality)

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Comments: