

9-6-88

Accession Number 406386-26

DATA EVALUATION RECORD

1. CHEMICAL: MON 7200/MON 15100.
2. TEST MATERIAL: MON 7200, 91.5% active ingredient, a yellowish crystalline solid.
3. STUDY TYPE: Honey Bee Acute Contact LD50.
Species Tested: Apis mellifera.
4. CITATION: Hoxter K.A. and Jaber, M. 1987. MON 7200: An Acute Contact Toxicity Study with the Honey Bee. Prepared by Wildlife International Ltd., Easton, Maryland. Submitted by Monsanto Company, St. Louis, Missouri. Study Number 139-236/WL-87-84. Accession Number 406386-26.

5. REVIEWED BY:

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Signature: P. Kosalwat
Date: 7/26/88

6. APPROVED BY:

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Signature: James R. Newman
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for Henry T. Craven
Supervisor, EEB/HED
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Signature: Richard M. Lee
Date: 9/6/88

7. CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for a honey bee acute contact test. With an LD50 value of 81 micrograms a.i./bee, MON 7200 is considered relatively non-toxic to honey bee (Apis mellifera).
8. RECOMMENDATIONS: N/A.

9. BACKGROUND:
10. DISCUSSION OF INDIVIDUAL TESTS: N/A.
11. MATERIALS AND METHODS:

- A. Test Animals: The apparently healthy, worker honey bees (*Apis mellifera*) were obtained from the Wildlife International Ltd. bee colony. All test bees were 1 to 7 days of age at initiation of the study. Seven days prior to the initiation of the study, one frame containing pupae was selected from the hives and placed in a Marsh Roll-X automatic incubator. On the day of study initiation, all bees that had emerged were immobilized with N₂ and at least 50 bees were placed into each chamber.
- B. Test System: The test chambers were disposable one-pint rolled paper containers measuring approximately 87 mm in diameter and 85 mm high. Each container was covered with a disposable (approximately 90 mm) plastic Petri dish through which a 20 ml glass vial containing an approximately 50% sugar/water solution was inserted. The opening of the vial was covered with cheese cloth to prevent leakage. This food source was available ad libitum to the test bees throughout the study.

Each test solution was prepared by weighing out calculated amounts of MON 7200. The test substance was then mixed with sufficient pesticide-grade acetone to prepare a 10 ml volume. All dosages were adjusted to 100% active ingredient (a.i.), based on the reported purity of the test substance. Therefore, all dosages and the LD50 value were reported as micrograms a.i./bee. Test solutions were used on the same day of preparation.

The test bees were maintained in the dark except during dosing and during daily observations. Test temperatures at the time of observations ranged from 22°C to 24°C. Average relative humidity was 78%.

- C. Dosage: 48-hour acute contact LD50 test. Nominal dosages were 13, 22, 36, 60, and 100 micrograms a.i./bee, applied to thorax and/or abdomen with a micropipette.
- D. Design: Five treatment groups were tested along with a solvent control and a negative control. Two replicates were tested at each dosage with 50 bees per replicate.

The solvent control bees received a volume of acetone equal to the largest volume used during the test (i.e., 2 microliters/bee).

Test chambers containing 50 or more bees were selected by random draw for dosing. The bees in each replicate were again immobilized with N₂ and laid out on paper. Fifty bees were individually dosed on the thorax and/or abdomen with 2 microliters of the appropriated test solution using an Eppendorf Digital Pipette. Dosed bees were returned to the test chamber, and any surplus bees discarded. Negative control bees were treated identically to all other bees with the exception of dosing.

Immediately following dosing, the bees were observed for mortality and signs of toxicity. Observations were made twice on the day of initiation and once on Day 1 and Day 2 after dosing. Mean body weights for the bees were determined at the initiation of the study by weighing each group of 50 bees. The mean individual weight and standard deviation among groups was 0.073 g $\pm$ 0.008 g (SD).

E. Statistics: An LD50 value along with 95% confidence limits was calculated using Stephan computer program. The LD50 value was used to classify the test material according to the toxicity categories of Atkins et al. (1976).

12. REPORTED RESULTS: Mortalities observed in all treatment and control groups are presented in Table 2 (attached). The honey bee 48-hour contact LD50 value for MON 7200 was determined to be 81 ug a.i./bee with 95% confidence limits of 53 and 256 ug a.i./bee.
13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: MON 7200 was classified as relatively non-toxic according to the toxicity categories of Atkins (1976).

The study was examined for conformance with Good Laboratory Practices as published by the OPP/USEPA (Federal Register, Volume 48, No. 230, November 29, 1983) and the final report was reviewed and signed by the Quality Assurance Unit of Wildlife International Ltd.

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

- A. Test Procedure: The test procedures are in accordance with the protocols recommended in the SEP. However, in Addendum I, the preparation of the first dosage of test solution should be 0.0709 grams MON/Q.S. 10 ml acetone because the 0.709 g reported as being used in the preparation would yield the dosage of 130 ug a.i./bee instead of 13 ug a.i./bee.
- B. Statistical Analysis: The reviewer recalculated the LD50 value using EPA's TOXANAL program and obtained the same result (attached).
- C. Discussion/Results: This study is scientifically sound and appears to be well conducted. Compare to other pesticides (Table 1, attached), an LD50 value of 81 micrograms a.i./bee classifies MON 7200 as relatively non-toxic to Apis mellifera when administered through direct contact as topical solution.
- D. Adequacy of the Study:
 - (1) Classification: Core.
 - (2) Rationale: N/A.
 - (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: N/A.

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NOBALMAT MON 7100 APIS MELLIFERA 7-13-88

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. PERCENT
100	100	58	58	0
50	100	39	39	0
25	100	29	29	0
12	100	5	5	0
10	100	10	10	0

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 80.68575

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	S	LC50	95 PERCENT CONFIDENCE LIMITS
1	.5331973	80.68575	63.67125 111.9813

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	S	GOODNESS OF FIT PROBABILITY
7	.0736271	2.799812 3.843916E-02

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

SLOPE = 1.924030
95 PERCENT CONFIDENCE LIMITS = .7476364 AND 3.100408

LC50 = 80.67525
95 PERCENT CONFIDENCE LIMITS = 57.06883 AND 125.7416

LC10 = 17.71274
95 PERCENT CONFIDENCE LIMITS = 3.692337 AND 26.72465

Table 1. *Acute contact toxicity of some pesticides to worker honeybees determined in the laboratory during 1964, 1965 and 1966*

Mean median lethal doses (LD 50) expressed as μg compound per insect and mean slopes of regression lines, n is number of regression lines used to obtain each mean. LD 90 values derived from these means are also given.

	1964				1965				1966			
	n	Mean LD ₅₀ (μg)	LD ₉₀ (μg)	Mean slope	n	Mean LD ₅₀ (μg)	LD ₉₀ (μg)	Mean slope	n	Mean LD ₅₀ (μg)	LD ₉₀ (μg)	Mean slope
Azinphos-methyl	—	—	—	—	—	—	—	—	2	0.063	0.13	4.0
Mevinphos	6	0.070	0.10	7.3	—	—	—	—	—	—	—	—
Bidrin'	1	0.076	0.10	9.6	—	—	—	—	—	—	—	—
Dimethoate	3	0.12	0.17	8.4	9	0.11	0.14	11	7	0.098	0.15	7.4
Dieldrin	8	0.16	0.23	7.6	6	0.16	0.26	6.0	—	—	—	—
Diazinon	2	0.22	0.30	9.4	—	—	—	—	—	—	—	—
Malathion	2	0.27	0.38	8.5	3	0.22	0.32	8.1	—	—	—	—
Pyrethrins	4	0.29	0.45	6.6	4	0.13	0.20	4.4	—	—	—	—
Phorate	3	0.32	0.42	11	—	—	—	—	2	0.33	0.57	5.3
MHC	3	0.46	0.68	7.4	6	0.20	0.33	5.8	—	—	—	—
Demeton-methyl	3	0.74	0.90	15	1	0.41	0.52	12	3	0.54	0.95	5.2
Endrin	—	—	—	—	3	1.2	2.1	4.9	2	0.65	1.5	3.6
Carbaryl	2	1.3	7.4	1.7	1	1.1	1.5	0.96	—	—	—	—
Chlordane	3	1.4	1.9	10	—	—	—	—	—	—	—	—
Carbophenothion	—	—	—	—	—	—	—	—	2	1.4	6.6	1.9
Allethrin	4	3.4	4.6	9.7	—	—	—	—	—	—	—	—
DDT	3	3.9	6.2	6.4	—	—	—	—	—	—	—	—
Disulfoton	3	4.1	5.9	8.1	4	4.3	8.0	4.7	2	5.0	6.8	9.4
Menazon	3	4.3	8.1	3.0	—	—	—	—	—	—	—	—
Endosulfan	4	7.1	13	4.6	—	—	—	—	—	—	—	—
Ethyl mercury chloride	2	22	43	4.4	—	—	—	—	—	—	—	—
Standard deviation (%)	27	—	—	23	21	—	—	24	20	—	—	20

From: Stevenson, J. H. 1968. Laboratory studies on the acute contact and oral toxicities of insecticides to honey bees. *Ann. Appl. Biol.* 61(3): 467-472.