

MRID No. 420641-03

DATA EVALUATION RECORD

1. **CHEMICAL:** Mon 13900, Shaughnessey Number: 999999 911596
2. **TEST MATERIAL:** Mon 13900, 96.4% active ingredient, brown granules.
3. **STUDY TYPE:** A 48-hour static acute toxicity test with the cladoceran (Daphnia magna)
4. **CITATION:** Burgess, David, Stephen Hicks, and John Bucksath. 1991. Acute toxicity of Mon 13900 to Daphnia magna. Guideline 72-2. Project ID 39217 Submitted by Monsanto Agricultural Company. Performed by ABC Laboratories, Inc. Environmental Biology Division. MRID No. 420641-03.

5. **REVIEWED BY:**

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Signature: 

Date: 6/17/93

6. **APPROVED BY:**

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Date: 6/30/93

7. **CONCLUSIONS:** This study appears to be scientifically sound but does not fulfill the data requirements for a 48 hour static acute toxicity test with the cladoceran Daphnia magna. The 48 hour NOEC was 17 mg/L. A 48 hour EC₅₀ value could not be calculated due to the low mortality at the highest test concentration.
8. **RECOMMENDATIONS:** A new study is needed.
9. **BACKGROUND:** N/A
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A
11. **MATERIALS AND METHODS:**
 - A. **TEST ANIMALS:** First instar Daphnia magna, less than 24 hours old, were used in this test. Daphnids were cultured in an in-house daphnid culture which has been maintained by ABC since 1977. The cultures were maintained in a constant environment with a temperature of 20 ± 2°C. The photoperiod was maintained at 16 hours light and 8 hours dark with a 30 minute dawn to



dusk transition periods. Daphnids were fed during the holding period.

- B. **TEST SYSTEM:** The test chambers were 250 ml glass beakers containing 200 mls of daphnid culture/test water. Total loading was 20 mls of water per daphnid. All test vessels were covered with loose-fitting petri dish covers to minimize evaporation. The study was carried out in a temperature controlled water bath ($20 \pm 1^\circ\text{C}$). The photoperiod was maintained with artificial light, 37-74 foot-candles, at 16 hours light and 8 hours dark.

The dilution water was the same as that in the culture area, hard blended water (a combination of well water and reverse osmosis water blended to a hardness of 160-180 mg/L).

- C. **DOSAGE:** The daphnids were exposed to 5 test concentrations (in duplicate) and a control. The test concentrations (nominal) used were 3.3, 6.5, 13, 25, and 50 mg of active ingredient per liter. These concentrations were chosen based on range finding toxicity tests. Mean measured concentrations were 2.3, 4.2, 8.4, 17, and 31 mg/L.
- D. **DESIGN:** Ten first instar daphnids were randomly distributed in the test beakers within 30 minutes of test solution preparation. All vessels were observed once at 2, 24, and 48 hours for signs of toxicosis. Water chemistry parameters were measured in replicates A and B of all test concentrations at 0 and 48 hours (Table 6).
- E. **STATISTICS:** The computer program by Stephan et al was used to analyze the data. Due to the low mortality, linear regression of log probit transformation of the data was also used.

12. **REPORTED RESULTS:** Preliminary testing in hard blended water showed that Mon 13900 is not completely soluble, due to this, the recovery of Mon 13900 was expected to be low. However, the test material appeared to be stable in the system based on information supplied by the sponsor and the consistent measurements at 0 and 48 hours. The measured concentration stock solution was 69 mg/L which was 69% of the nominal concentration at 0 hour.

Mortality results and behavioral observations, based on mean measured concentrations, are summarized in Table 4. For water quality measurements, see Table 6.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The 48 hour EC_{50} for Daphnia magna exposed to Mon 13900 was 26 mg/L based on total adverse effects. The 48 hour NOEC was 17 mg/L.

The study has a quality assurance statement signed by a quality assurance officer.

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

- A. **TEST PROCEDURE:** This test is in accordance with EPA's SEP protocol, except there was no solvent control.
- B. **STATISTICAL ANALYSIS:** Due to low mortality, no statistics were performed. Therefore, the report results could not be verified.
- C. **DISCUSSION/RESULTS:** An EC_{50} could not be calculated as mortality at the highest test concentration was only 25%. The 48 hour NOEC was 17 mg/L.
- D. **ADEQUACY OF STUDY:**
 - (1) **CLASSIFICATION:** Supplemental
 - (2) **RATIONALE:** Failure to test at a concentration that produced $\geq 67\%$ mortality.
 - (3) **REPARABILITY:** N/A

TABLE 2

Measured Concentrations of MON 13900 During the 48-Hour
Static Acute Toxicity Study with *Daphnia magna*

Sample	Nominal Concentration (mg/L)	Mean Measured Concentration (mg/L)				Percent Nominal
		0-Hour	48-Hour	48-Hour Mean	Mean	
Reagent Blank	---	<0.80	<0.80	<0.80	<0.80	---
Control	---	<0.80	<0.80	<0.80	<0.80	---
Level 1	3.3	2.4	2.0 2.1	2.1	2.3	70
Level 2	6.5	4.2	4.3 4.0	4.2	4.2	65
Level 3	13	8.3	8.6 8.2	8.4	8.4	65
Level 4	25	17	16 17	17	17	68
Level 5	50	29	32 33	33	31	62
Diluter ^a Stock	100	69	---	---	---	69

^aMean \pm S.D. = 66 \pm 3.1%

^a Stock not included in this calculation.

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TABLE 4
Acute Toxicity of MON 13900 to *Daphnia magna*

Test Material	EC ₅₀ (mg/L)	
	24-Hour ^a (95 % C. I.)	48-Hour ^c (95 % C. I.)
MON 13900	> 31 b	26 (17 and 31)

^a Estimated EC₅₀ value obtained by using the binomial method.

^b 95 % Confidence Intervals could not be determined.

^c EC₅₀ and 95 % Confidence Intervals obtained by using the binomial method.

NOTE: Bioassay conducted at 20 (±1)°C.

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TABLE 5

Individual Mortality and Behavioral Observations Obtained
During the Static Acute Toxicity Test of MON 13900 to
Daphnia magna

Mean Measured Concentration mg/L	Rep.	Number Placed in Test Vessel	24-Hour		48-Hour	
			Cum. Mort.	Obs. ^a	Cum. Mort.	Obs. ^a
Control	A	10	0	----	0	----
	B	10	0	----	0	----
2.3	A	10	0	----	0	----
	B	10	0	----	0	----
4.2	A	10	0	----	0	----
	B	10	0	----	0	----
8.4	A	10	0	----	0	----
	B	10	0	----	0	----
17	A	10	0	----	0	----
	B	10	0	----	0	----
31	A	10	0	9N;1SUR	3	4N;3OB
	B	10	0	10N	2	2Q;5OB; 1CT,OB

^a Unless otherwise indicated, all daphnids were normal in appearance and behavior and all treated solutions were clear without a precipitate.

The following abbreviations were used for observations: N = Normal; OB = On Bottom; SUR = Surfacing; Q = Quiescent; CT = Coated with Extraneous Material.

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TABLE 6

Water Quality Measurements During the Acute Toxicity
Test of MON 13900 to *Daphnia magna*

Mean Measured Test Conc. mg/L	0-Hours (Replicate A)						48-Hours (Replicate B)					
	Temp. ^a °C	D.O. ^b mg/L	pH ^c	Temp. ^a °C	D.O. ^b mg/L	pH ^c	Temp. ^a °C	D.O. ^b mg/L	pH ^c	Temp. ^a °C	D.O. ^b mg/L	pH ^c
Control	20	8.3	8.3	20	8.3	8.3	19	8.5	8.4	19	8.5	8.4
2.3	21	8.1	8.1	21	8.3	8.1	19	8.4	8.3	19	8.4	8.3
4.2	21	8.3	8.1	21	8.3	8.1	19	8.3	8.3	19	8.4	8.3
8.4	21	8.3	8.1	21	8.3	8.1	19	8.3	8.4	19	8.4	8.4
17	21	8.3	8.2	21	8.3	8.2	19	8.2	8.3	19	8.3	8.4
31	21	8.5	8.1	21	8.5	8.1	19	8.3	8.3	19	8.3	8.4

^a Temperature - Digital thermometer.

^b Dissolved oxygen concentrations - YSI Model 54 ARC Dissolved Oxygen Probe.

^c pH - Corning Model 140 pH/mV meter with Sensorex Model S200C electrode.

Note: Dissolved oxygen saturation at the test temperatures of 19 and 21°C are 8.9 and 8.5 mg/L (corrected for altitudinal pressure at ABC Laboratories), respectively.

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