10-27-93

MRID No. 427730-01

DATA EVALUATION RECORD

1. **CHEMICAL:** MON 12000.
Shaughnessey No. 128721.

2. **TEST MATERIAL:** MON 12000; Batch/Lot/NBR No. NIS-9012-2631T; 99.3% active ingredient; a white powder.

3. **STUDY TYPE:** 72-3(a) Estuarine Fish Flow-Through Acute Toxicity Test. Species Tested: Sheepshead Minnow (*Cyprinodon variegatus*).

4. **CITATION:** Swigert, J.P. and G.J. Smith. 1993. MON 12000: A 96-Hour Flow-Through Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). Project No. 139A-142. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Monsanto Agricultural Company, St. Louis, MO. EPA MRID No. 427730-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]
Date: 10/27/93

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 10/27/93

~~Henry T. Craven, M.S.~~
Supervisor, EEB/EFED
USEPA

Signature: [signature] 6/24/94
Date: /Goodyear 7.20.94 Norman J. Cook 07.20.94

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an estuarine fish acute toxicity test using sheepshead minnows. The sampling methodology did not indicate whether observed precipitates were excluded from the analytical samples. Based on mean measured concentration, the 96-hour LC_{50} was >125 mg/l which classifies MON 12000 as practically non-toxic to sheepshead minnows. The NOEC was 125 mg/l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile sheepshead minnows (*Cyprinodon variegatus*) were obtained from in-house cultures. The fish were fed a commercially-available flaked food and live brine shrimp except for the final 54 hours before the test. During the 14-day period prior to test initiation, the temperature, salinity, and pH of the culture water were 21.1-23.0°C, 22-26 parts per thousand (ppt), and 7.8-8.0, respectively. The fish were acclimated to the test conditions for approximately 54 hours before the test. During holding and acclimation, the fish showed no signs of disease or stress.

All fish used in the test were from the same year class. At test termination, 10 control fish had an average length of 22 mm (18-25 mm) with an average weight of 0.37 g (0.17-0.64 g).

B. Test System: A continuous-flow diluter system was used to prepare and deliver the test solutions. The test chambers were Teflon®-lined, 25-l polyethylene aquaria filled with 15 l of test solution. The solution depth was approximately 16.5 cm. Approximately 6 volume additions were delivered to the chambers every 24 hours. The diluter was preconditioned with the test material for approximately 29 hours prior to testing.

The aquaria were arbitrarily positioned under a ventilation hood in a temperature-controlled water bath (22 ±1°C) under a 16-hour light photoperiod with 30-minute dawn and dusk simulations. Light intensity at the test solution surface was approximately 430 lux.

Natural seawater, collected at Indian River Inlet, DE, was diluted with well water, aerated, and filtered before use as test dilution water. During the 28-day period prior to test initiation, the mean pH and salinity of the dilution water were 8.0-8.2 and 25 ppt, respectively.

One stock solution was prepared. The stock (100 mg/ml) was prepared by dissolving the test material in dimethylformamide (DMF). The stock was injected into the diluter mixing chambers.

C. **Dosage:** Ninety-six-hour, flow-through test. Based on preliminary testing, one nominal concentration (120 mg/l), a solvent control, and a dilution water control were tested. The nominal test concentration was mg/l of whole material (i.e., not adjusted for the percentage active ingredient). The solvent control contained 1.2 ml of DMF/l of solution.

D. **Design:** Sheepshead minnows were impartially removed from holding tanks in groups of two and distributed to the test chambers until each contained 10 fish. Two replicates were used for the controls and three replicates were used for the treatment. Biomass loading during the test was 0.04 g/l/day or 0.25 g/l at any given time.

Observations of mortality and treatment-related effects were made at 18, 24, 48, 72, and 96 hours. The dissolved oxygen concentration (DO) and pH were measured in alternating replicates of each control and in all replicates of the treatment at test initiation and at each 24-hour observation. The temperature of one of the control chambers was monitored continuously, and the temperature in each replicate vessel was measured at the beginning and end of the test. The salinity of the dilution water control was measured at test initiation.

Test solution samples were collected from each test chamber at 0, 48, and 96 hours. The samples were analyzed for MON 12000 using high performance liquid chromatography.

E. **Statistics:** The median lethal concentration (LC_{50}) values were calculated, if necessary, using a computer program developed by C.E. Stephan.

12. **REPORTED RESULTS:** The measured concentrations ranged between 99 and 108% of nominal, and averaged 125 mg/l over the course of the study (Table 1, attached). A white precipitate was observed in the mixing chambers and treatment chambers throughout the study.

No treatment-related mortality or sublethal effects were noted in the dilution water control, solvent control, or treatment solutions during the study (Table 3, attached).

During the test, the DO ranged from 6.0 to 6.8 mg/l (>60% of saturation). The pH values ranged from 7.6 to 8.2 and the

3

temperature was 21.7-22.4°C. The salinity of the dilution water control at test initiation was 25 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour LC_{50} value for sheepshead minnows exposed to MON 12000 was >125 mg/l. The no mortality concentration was 125 mg/l.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. Characterization of the test material was the responsibility of the sponsor.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

The salinity of the dilution water in the study was 25 ppt. The recommended testing salinity for sheepshead minnows is 10-17 ppt.

Acclimation of the sheepshead minnows to the test conditions (54 hours) was shorter than recommended (two weeks).

The controls should have been replicated three times, rather than two times.

The amount of solvent used (1.2 ml/l) was greater than recommended (0.5 ml/l).

B. **Statistical Analysis:** Upon review of the mortality and response data, the reviewer concurs that the 96-hour LC_{50} was >125 mg/l.

C. **Discussion/Results:** Precipitates were observed in the treatment solution. Since the sampling methodology in neither the report nor analytical appendix clarified whether these precipitates were excluded from the analytical samples (i.e., filtering, centrifuging), the amount of material in solution is in question.

This study is scientifically sound but does not meet the guideline requirements for an estuarine fish acute toxicity test using sheepshead minnows. Based on mean measured concentration, the 96-hour LC_{50} was >125 mg/l

which classifies MON 12000 as practically non-toxic to sheepshead minnows. The NOEC was 125 mg/l.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The sampling methodology did not indicate whether precipitates were excluded from the analytical samples.

(3) **Repairability:** Yes. If the authors can clarify the sampling methodology, and the precipitates were excluded from the analytical samples, then this study can be upgraded to the "core" category.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 9-28-93.

MoN 12000

Page_____ is not included in this copy.

Pages 6 through 7 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Ecological Effects Branch One-Liner Data Entry Form

Chemical HOE 12000 Shaughnessy No. 128721 Pesticide Use Herbicide

AQUATIC VERTEBRATE TOX.	% AI	LC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. Cyprinodon variegatus	99.3	>125 mg/l*	96 hr	125 mg/l*	1993/1993	427730-01 Supplemental	WIL	HM
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC LC_{50}	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								
3.								

COMMENTS: * Mean measured concentration

8

DATA EVALUATION RECORD

1. **CHEMICAL:** MON 12000.
Shaughnessey No. 128721.

2. **TEST MATERIAL:** MON 12000; Batch/Lot/NBR No. SIB-9112-3533-T; 98.5% active ingredient; a white powder.

3. **STUDY TYPE:** 72-3(c) Estuarine Shrimp Flow-Through Acute Toxicity Test. Species Tested: Mysid (*Mysidopsis bahia*).

4. **CITATION:** Swigert, J.P. and G.J. Smith. 1993. MON 12000: A 96-Hour Flow-Through Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*). Project No. 139A-141B. Prepared by Wildlife International Ltd., Easton, MD. Submitted by Monsanto Agricultural Company, St. Louis, MO. EPA MRID No. 427730-02.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: [signature]
Date: 10/27/93

6. **APPROVED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 10/27/93

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

Signature: [signature]
Date: 6/24/94

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute estuarine shrimp toxicity study. Based on mean measured concentrations, the 96-hour LC_{50} value for mysids was 109 mg/l. Therefore, MON 12000 is classified as practically non-toxic to mysid shrimp. The NOEC was 16 mg/l.

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

9. **BACKGROUND:**

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

5.25

9

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile mysids (<24 hours old) were obtained from in-house cultures. Brooding adults were held under conditions similar to those during testing for at least 14 days before the juveniles were collected. During this period, water temperature was 24.8-25.3°C, salinity was 24-28 parts per thousand (ppt), and the pH was 8.0-8.1.

B. **Test System:** A continuous-flow diluter system was used to prepare and deliver the test solutions. The test compartments were 500-ml glass beakers with two screen-covered holes on each side. The compartments were suspended in Teflon®-lined, 8-l polyethylene aquaria filled with 6.5 l of test solution. The solution depth was approximately 18 cm. Approximately 6 volume additions were delivered to the chambers every 24 hours. The diluter was preconditioned with the test material for 24 hours prior to testing. The aquaria were impartially positioned under a ventilation hood in a temperature-controlled water bath (25 ±1°C) under a 16-hour light photoperiod with 30-minute dawn and dusk simulations. Light intensity at the test solution surface was 861 lux.

One stock solution was prepared for each of the five concentrations. The primary stock (100 mg/ml) was prepared by dissolving the test material in dimethylformamide (DMF). Aliquots of this stock were diluted with DMF to prepare four additional stocks. The stocks were injected into the diluter mixing chambers.

Natural seawater, collected at Indian River Inlet, DE, was diluted with well water, aerated, and filtered before use as test dilution water. The mean salinity of the dilution water was 25 ppt and the pH was 8.1 during the 4-week period immediately preceding the test.

C. **Dosage:** Ninety-six-hour, flow-through test. Based on the results of preliminary testing, five nominal concentrations (15.6, 25.9, 43.2, 72.0, and 120 mg/l), a solvent control, and a dilution water control were tested. The nominal test concentrations were mg/l of whole material (i.e., not adjusted for the percentage active ingredient). The concentration of solvent in the solvent control and exposures was 1.2 ml/l.

D. Design: Mysids were impartially removed from holding tanks using wide-bore, disposable pipettes and distributed to 25-ml plastic containers until each contained 10 individuals (15 individuals at the highest treatment level). The containers were dipped into the test chambers to release the mysids. Two replicates were used, for a total of 20 individuals per concentration (30 at the highest treatment level). The mysids were fed live brine shrimp nauplii daily during the test.

Observations of mortality and treatment-related effects were made at 2, 24, 48, 72, and 96 hours. The dissolved oxygen concentration (DO) and pH were measured in alternate replicates of each test level at the beginning of the test and at each 24-hour observation. The temperature was monitored continuously in one of the control chambers and measured at test initiation and termination in each replicate vessel. Salinity of the dilution water control was measured at the beginning of the test.

Test solution samples were collected from each test chamber at 0, 48, and 96 hours. The samples were analyzed for MON 12000 using high performance liquid chromatography.

E. Statistics: The median lethal concentration (LC_{50}) values were calculated using a computer program developed by C.E. Stephan. Binomial probability was used to determine the 96-hour LC_{50}.

12. REPORTED RESULTS: The mean measured concentrations were 16, 25, 43, 72, and 127 mg/l (Table 1, attached). Although white precipitate was noted in the mixing chambers of the three highest concentration solutions, precipitate was not noted in the test solutions.

No mortality or sublethal effects were noted in the dilution water or solvent control solutions (Table 3, attached). Similarly, no mortality or sublethal effects were noted in the 16 mg/l treatment group. Mortality at test termination in the 25, 43, 72, and 127 mg/l groups was 5, 5, 5, and 70%, respectively.

During the test, the DO was >60% of saturation (5.9-6.7 mg/l). The pH values ranged from 7.5 to 8.2 and the temperature was 24.4-25.0°C. The salinity of the dilution water control was 24 ppt.

11

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour LC_{50} value for mysids was 109 mg/l with a 95% confidence interval of 72-127 mg/l. The no mortality concentration and no-observed-effect concentration (NOEC) were 16 and 72 mg/l, respectively.

Quality Assurance and Good Laboratory Practice Statements were included in the report, indicating that the study was conducted in accordance with U.S. EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. Characterization of the test material was the responsibility of the sponsor.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

The recommended temperature for mysid toxicity tests is 22 ±1°C. The temperature used in the study was approximately 25°C.

The salinity of the dilution water in the study was 24 ppt. The recommended salinity for estuarine shrimp is 10-17 ppt.

The amount of solvent used (1.2 ml/l) was greater than recommended (0.5 ml/l).

B. **Statistical Analysis:** The reviewer used mean measured concentrations and EPA's Toxanal program to calculate the 96-hour LC_{50} value. The results are the same as the authors' (see attached printout).

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for an acute estuarine shrimp toxicity study. Based on mean measured concentrations, the 96-hour LC_{50} value for mysids was 109 mg/l. Therefore, MON 12000 is classified as practically non-toxic to mysid shrimp. The NOEC was 16 mg/l.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

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

12

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 9-29-93.

MON 12000

Page_____ is not included in this copy.

Pages 14 through 15 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.