7-20-94

MRID No. 426614-21

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:** 72-1. Freshwater Fish Acute Toxicity Test. Species Tested: Rainbow Trout (*Oncorhynchus mykiss*).

4. **CITATION:** Holmes, C.M. and J.P. Swigert. 1993. MON 12000: A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*). Wildlife International Ltd. Project No. 139A-148. Performed by Wildlife International Ltd., Easton, MD. Submitted by Monsanto Agricultural Company, St. Louis, MO. EPA MRID No. 426614-21.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 4/13/93

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 4/13/93

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

Signature: [illegible] 6/24/94
Date: [illegible] 7-20-94 Norman J. Cook 07.20.94

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater fish. The 96-hour LC_{50} of rainbow trout exposed to MON 12000 was >131 mg a.i./l mean measured concentration (the only concentration tested). Therefore, MON 12000 is considered practically non-toxic to *Oncorhynchus mykiss*. The NOEC was 131 mg a.i./l.

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

9. **BACKGROUND:**

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

1

11. MATERIALS AND METHODS:

A. Test Animals: Rainbow trout (*Oncorhynchus mykiss*) were obtained as eyed eggs from Mt. Lassen Trout Farm, Red Bluff, CA. The fish were hatched and maintained at the laboratory for 94 days prior to test initiation. The water in which the fish were held had a temperature of 11.3-12.3°C, a pH of 7.9-8.2, and a hardness and alkalinity of 188 and 194 mg/l as $CaCO_3$, respectively. The fish were fed a salmon mash and/or salmon starter during holding except for 48 hours prior to the study. The fish were acclimated to the test conditions for approximately 50 hours prior to the test.

At test termination, a representative group of control fish (n=10) had a mean weight of 3.59 g (range of 2.75-4.28 g) and a mean length of 56 mm (range of 50-61 mm).

B. Test System: A diluter apparatus was used to deliver test solutions to the test vessels. The test material stock solution and the solvent stock solution were each directed to mixing chambers via a peristaltic pump, and diluted with a flow of dilution water (controlled by a rotameter). The mixed solutions were distributed to the replicate chambers. The test system was started 24 hours prior to test initiation in order to establish the test concentration.

The test vessels were 25-l polyethylene aquaria filled with 15 l of test solution at a depth of 17.5 cm. The test chambers received 6 volume additions of test solution per day. The aquaria were arbitrarily positioned in a temperature-controlled water bath (12 ±1°C). The water bath was enclosed in a ventilation hood to minimize the potential for cross-contamination.

Fluorescent tubes (e.g. Chroma 50), controlled by an automatic timer, provided 16 hours of light at a light intensity of 323 lux. Thirty-minute dawn/dusk simulations were provided.

The dilution water was fresh, medium-hard well water obtained on site from a 45-meter deep well. The dilution water was filtered and spray-aerated prior to use in the study. In the 4-week period preceding the study, the dilution water had a pH of 8.0-8.3, a conductivity of 320 µmhos/cm, and a hardness and alkalinity of 136-148 and 174-186 mg/l as $CaCO_3$, respectively.

2

One stock solution (0.1 g MON 12000/ml) was prepared by dissolving the test substance in dimethyl formamide (DMF).

C. Dosage: Ninety-six-hour, flow-through test. One nominal test concentration (120 mg/l) was selected for this study. A well water control and a solvent control (1.2 ml DMF/l) were also included.

D. Design: Ten fish were impartially removed from the holding tank and distributed to each test vessel. Three replicates were used for the exposure concentration and two replicates for each control. The fish were not fed during the study. The instantaneous biomass loading rate was 2.39 g/l.

Observations of mortality and clinical signs of toxicity were made at 6.5, 24, 48, 72, and 96 hours. The dissolved oxygen concentration (DO) and pH were measured in alternate replicates of each control and every replicate of the test concentration every day of the study. The temperature was measured at test initiation and termination in each test chamber, and was monitored continuously in one control vessel.

Samples from each replicate were collected at 0, 48, and 96 hours for chemical analysis using high performance liquid chromatography.

E. Statistics: No statistical analyses were necessary.

12. REPORTED RESULTS: The mean measured concentration was 131 mg a.i./l (Table 1, attached). "There was a white precipitate observed in the test treatment mixing chambers throughout the study. No precipitate was observed in the test chambers during the test."

No mortality or adverse effects were observed in the controls or the exposure concentration (Table 3, attached). The 96-hour LC_{50} was >131 mg a.i./l, based on the mean measured concentration. The 96-hour no mortality concentration and NOEC were $\geq$131 mg a.i./l.

During the study, the test solutions had a DO of 8.0-9.2 mg/l, a pH of 7.6-8.2, and a temperature of 11.3-12.8°C.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES: "Based on the results of this study, MON 12000 is considered to be practically non-toxic to rainbow trout."

GLP compliance and quality assurance statements were included in the report indicating that this study was conducted in accordance with EPA Good Laboratory Practices (40 CFR Part 160), except that the test substance characterization was performed by the Sponsor and not audited by Wildlife International Ltd.

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

A. Test Procedure: The test procedures were generally in accordance with the SEP, but deviated as follows:

The solvent concentration was 1.2 ml/l; the SEP recommends a solvent concentration not greater than 0.1 ml/l in a flow-through study.

The number of organisms and replicates used in the controls (n=20) was not the same as that used in the exposure concentration (n=30). The number of organisms should have been the same in all treatments.

A 50-hour period of acclimation of the test organism to test conditions was reported. A two-week acclimation period to test conditions is recommended.

B. Statistical Analysis: No statistical analyses were required.

C. Discussion/Results: Though the solvent concentration was much greater than recommended, the solvent did not appear to affect the study results. The study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater fish. Since no mortality occurred in the only exposure concentration tested, the 96-hour LC_{50} was >131 mg a.i./l which indicates that MON 12000 is practically non-toxic to *Oncorhynchus mykiss*. The NOEC 131 mg a.i./l.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 1 April 1993.

4

MoN 12000

Page_____ is not included in this copy.

Pages 5 through 6 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 MON 12000 Shaughnessy No. 128721 Pesticide Use

AQUATIC VERTEBRATE TOX.	% AI	LC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. *Oncorhynchus mykiss*	98.5	>131 mg/l (NA) NA	96 h flow-thru	131 mg/l	1993/1993	426614-21 Core	WIL	RGM
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC LC_{50}	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								

COMMENTS: WIL=Wildlife International Limited; NA=not applicable; results based on the mean measured concentration.

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:** 72-1(a) Freshwater Fish Acute Toxicity Test. Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).

4. **CITATION:** Holmes, C.M. and J.P. Swigert. 1993. MON 12000: A 96-Hour Flow-Through Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*). Wildlife International Ltd. Project No. 139A-147. Performed by Wildlife International Ltd., Easton, MD. Submitted by Monsanto Agricultural Company, St. Louis, MO. EPA MRID No. 426614-22.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 4/13/93

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 4/13/93

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

Signature: Henry T. Craven 6/24/94
Date: 6 24 93 07.20.94

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater fish. The 96-hour LC_{50} of bluegill exposed to MON 12000 was >118 mg a.i./l mean measured concentration (the only concentration tested). Therefore, MON 12000 is considered practically non-toxic to *Lepomis macrochirus*. The NOEC was 118 mg a.i./l.

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

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. Test Animals: Juvenile bluegill (*Lepomis macrochirus*) were obtained from Delmarva Ecological Laboratory, Inc., Middletown, DE. The fish were maintained at the laboratory for at least 14 days prior to test initiation. The water in which the fish were held had a temperature of 19.8-21.5°C, a pH of 8.1-8.4, and a hardness and alkalinity of 172-180 and 156-180 mg/l as $CaCO_3$, respectively. The fish were fed a flaked food during holding except for 48 hours prior to the study. The fish were acclimated to the test conditions for approximately 26 hours prior to the test.

At test termination, a representative group of control fish (n=10) had a mean weight of 0.61 g (range of 0.29-1.07 g) and a mean length of 31 mm (range of 27-34 mm).

B. Test System: A proportional diluter apparatus was used to deliver test solutions to the test vessels. The test material stock solution and the solvent stock solution were each directed to mixing chambers via a peristaltic pump, and diluted with a flow of dilution water (controlled by a rotameter). The mixed solutions were distributed to the replicate chambers. The test system was started 22 hours prior to test initiation in order to establish concentrations.

The test vessels were 25-l polyethylene aquaria filled with 15 l of test solution at a depth of 17.5 cm. The test chambers received 6 volume additions of test solution per day. The aquaria were arbitrarily positioned in a temperature-controlled water bath (22 ±1°C). The water bath was enclosed in a ventilation hood to minimize the potential for cross-contamination.

Fluorescent tubes (e.g. Chroma 50), controlled by an automatic timer, provided 16 hours of light at a light intensity of 366 lux. Thirty-minute dawn/dusk simulations were provided.

The dilution water was fresh, medium-hard well water obtained on site from a 45-meter deep well. The dilution water was filtered and spray-aerated prior to use in the study. In the 4-week period preceding the study, the dilution water had a pH of 8.0-8.3, a conductivity of 320 µmhos/cm, and a hardness and alkalinity of 136-144 and 174-190 mg/l as $CaCO_3$, respectively.

9

One stock solution (0.1 g MON 12000/ml) was prepared by dissolving the test substance in dimethyl formamide (DMF).

C. Dosage: Ninety-six-hour, flow-through test. One nominal test concentration (120 mg/l) was selected for this study. A well water control and a solvent control (1.2 ml DMF/l) were also included.

D. Design: Ten bluegill were impartially removed from the holding tank and distributed to each test vessel. Three replicates were used for the exposure concentration and two replicates for each control. The fish were not fed during the study. The instantaneous biomass loading rate was 0.40 g/l.

Observations of mortality and clinical signs of toxicity were made at 7, 24, 48, 72, and 96 hours. The dissolved oxygen concentration (DO) and pH were measured in alternate replicates of each control and every replicate of the test concentration every day of the study. The temperature was measured at test initiation and termination in each test chamber, and was monitored continuously in one control vessel.

Samples from each replicate were collected at 0, 48, and 96 hours for chemical analysis using high performance liquid chromatography.

E. Statistics: No statistical analyses were necessary.

12. REPORTED RESULTS: The mean measured concentration was 118 mg a.i./l (Table 1, attached). "There was a white precipitate observed in the test treatment mixing chambers throughout the study. No precipitate was observed in the test chambers."

No mortality or adverse effects were observed in the controls or the exposure concentration (Table 3, attached). The 96-hour LC_{50} was >118 mg a.i./l, based on the mean measured concentration. The 96-hour no mortality concentration and NOEC were $\geq$118 mg a.i./l.

During the study, the test solutions had a DO of 8.0-8.7 mg/l, a pH of 7.8-8.5, and a temperature of 21.1-22.3°C.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES: MON 12000 is considered to be practically non-toxic to bluegill sunfish.

10

GLP compliance and quality assurance statements were included in the report indicating that this study was conducted in accordance with EPA Good Laboratory Practices (40 CFR Part 160), except that the test substance characterization was performed by the Sponsor and not audited by Wildlife International Ltd.

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

A. Test Procedure: The test procedures were generally in accordance with the SEP, but deviated as follows:

The solvent concentration was 1.2 ml/l; the SEP recommends a solvent concentration not greater than 0.1 ml/l in a flow-through study.

The number of organisms and replicates used in the controls (n=20) was not the same as that used in the exposure concentration (n=30). The number of organisms should have been the same in all treatments.

A 26-hour period of acclimation of the test organism to test conditions was reported. A two-week acclimation period to test conditions is recommended.

B. Statistical Analysis: No statistical analyses were required.

C. Discussion/Results: Though the solvent concentration was much greater than recommended, the solvent did not appear to affect the study results. This study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater fish. Since no mortality occurred in the only exposure concentration tested, the 96-hour LC_{50} was >118 mg a.i./l which indicates that MON 12000 is practically non-toxic to *Lepomis macrochirus*. The NOEC 118 mg a.i./l.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 1 April 1993.

MoN 12000

Page_____ is not included in this copy.

Pages 12 through 13 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 MON 12000 Shaughnessy No. 128721 Pesticide Use _____

AQUATIC VERTEBRATE TOX.	% AI	LC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. *Lepomis macrochirus*	98.5	>118 mg/l (NA) NA	96 h flow-thru	118 mg/l	1993/1993	426614-22 Core	WIL	RGM
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	**% AI**	**MATC LC_{50}**	**DAYS**	**AFFECTED PARA.**	**STUDY/REVIEW DATES**	**MRID/ CATEGORY**	**LAB**	**RC**
1.								
2.								

COMMENTS: WIL=Wildlife International Limited; NA=not applicable; results based on the mean measured concentration.

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:** 72-2. Freshwater Invertebrate Acute Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Holmes, C.M. and J.P. Swigert. 1993. MON 12000: A 48-Hour Flow-Through Acute Toxicity Test with the Cladoceran (*Daphnia magna*). Project No. 139A-146. Performed by Wildlife International Ltd., Easton, MD. Submitted by Monsanto Agricultural Company, St. Louis, MO. EPA MRID No. 426614-23.

5. **REVIEWED BY:**

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

Signature: Rosemary Graham Mora
Date: 4/12/93

6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 4/12/93

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

Signature: 6/24/94
Date: 7-20-94 07.20.94

7. **CONCLUSIONS:** The study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater invertebrates. The 48-hour EC_{50} of *Daphnia magna* exposed to MON 12000 was >107 mg a.i./l mean measured concentration (the only concentration tested). Therefore, MON 12000 is considered practically non-toxic to *Daphnia magna*. The NOEC was 107 mg a.i./l.

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

9. **BACKGROUND:**

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

11. MATERIALS AND METHODS:

A. Test Animals: *Daphnia magna* neonates (<24 hours old) were obtained from in-house cultures. The neonates used in this study were obtained from three adult daphnids which were held for at least 14 days prior to collection of the neonates. The adult daphnids were held in dilution water at a temperature of 19.7-20.9°C, at a pH of 8.2-8.4, and a hardness and alkalinity of 104-116 and 158-162 mg/l as $CaCO_3$, respectively. The adults were fed an algal suspension and a mixture of yeast, Cerophyll®, and trout chow.

B. Test System: A continuous-flow proportional diluter was used to deliver test solutions to the test vessels. The test material stock solution and the solvent stock solution were each directed to a mixing chamber via a peristaltic pump, and diluted with a flow of dilution water (controlled by a rotameter). The mixed solutions were distributed to the replicate chambers. The test system was started 22 hours prior to test initiation in order to establish equilibrium of the test concentration.

The test compartments were 300-ml glass beakers with screen-covered holes on opposite sides. The test solution depth was 4 cm. The beakers were suspended in Teflon®-lined, 8-l polyethylene aquaria filled with 6.5 l of solution. The aquaria received 13.8 volume additions of test solutions per day and were arbitrarily positioned in a temperature-controlled water bath (20 ±1°C). The water bath was enclosed in a ventilation hood to minimize the potential for cross-contamination.

Fluorescent tubes (e.g. Chroma 50), controlled by an automatic timer, provided 16 hours of light at a light intensity of 538 lux. Thirty-minute dawn/dusk simulations were provided.

The dilution water was fresh, medium-hard well water obtained on site from a 45-meter deep well. The dilution water was filtered and spray-aerated prior to use in the study. In the 4-week period preceding the study, the dilution water had a pH of 8.2-8.3, a conductivity of 320 µmhos/cm, and a hardness and alkalinity of 136-152 and 174-194 mg/l as $CaCO_3$, respectively.

16

One stock solution (0.1 g MON 12000/ml) was prepared by dissolving the test substance in dimethyl formamide (DMF).

C. **Dosage:** Forty-eight-hour, flow-through test. One nominal test concentration (120 mg/l) was selected for this study. A well water control and a solvent control (1.2 ml DMF/l) were also included.

D. **Design:** Ten daphnids were impartially removed from the holding tanks and distributed to 25-ml plastic cups. The daphnids were then pipetted into each test vessel. Three replicates were used for the exposure concentration and two replicates for each control. The daphnids were not fed during the test.

Observations of mortality/immobility and clinical signs of toxicity were made at 6.5, 24, and 48 hours. The dissolved oxygen concentration (DO) and pH were measured in alternate replicates of each control and every replicate of the test concentration every day of the study. The temperature was measured at test initiation and termination in each test chamber, and was monitored continuously in one control vessel.

Samples from each replicate were collected at 0, 24, and 48 hours for chemical analysis using high performance liquid chromatography.

E. **Statistics:** No statistical analyses were necessary.

12. **REPORTED RESULTS:** The mean measured concentration was 107 mg a.i./l (Table 1, attached). "There was a white precipitate observed in the test treatment mixing chambers throughout the study. No precipitate was observed in the test chambers during the test."

No mortality or adverse effects were observed in the controls or at the exposure concentration (Table 3, attached). "However, at 6.5 hours, six organisms in one replicate of the 107 mg MON 12000/l treatment were observed floating on the surface. The organisms were re-submerged and appeared normal throughout the remainder of the test. This did not appear to be a treatment related effect." The 48-hour EC_{50} was >107 mg a.i./l, based on the mean measured concentrations. The no mortality/immobilization concentration and NOEC were $\geq$107 mg a.i./l.

During the study, the test solutions had a DO of 7.9-8.7 mg/l, a pH of 7.8-8.4, and a temperature of 19.8-20.4°C.

17

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES: MON 12000 is considered to be practically non-toxic to *Daphnia magna*.

GLP compliance and quality assurance statements were included in the report indicating that this study was conducted in accordance with EPA Good Laboratory Practices (40 CFR Part 160), except that the test substance characterization was performed by the Sponsor and not audited by Wildlife International Ltd.

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

A. Test Procedure: The test procedures were generally in accordance with the SEP, but deviated as follows:

The number of organisms and replicates used in the controls (n=20) was not the same as that used in the exposure concentration (n=30). The number of organisms should have been the same in all treatments.

The report does not clearly present the test system, since it cannot be determined whether the test solution was able to flow between test beakers. Two or more replicate vessels per treatment is recommended.

First instar test organisms should be from the fourth or later broods of a given parent. The author did not indicate which brood was the source of the test animals.

B. Statistical Analysis: No statistical analyses were required.

C. Discussion/Results: The study is scientifically sound and fulfills the guideline requirements for an acute toxicity test using freshwater invertebrates. The 48-hour EC_{50} was >107 mg a.i./l which indicates that MON 12000 is practically non-toxic to *Daphnia magna*. The NOEC 107 mg a.i./l.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 1 April 1993.

18

MoN 12000

Page_____ is not included in this copy.

Pages 19 through 20 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 MON 12000 Shaughnessy No. 128721 Pesticide Use _____

INVERTEBRATE ACUTE TOXICITY	% AI	EC_{50} (95%CL) SLOPE	HRS/ TYPE	NOEC	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1. *Daphnia magna*	98.5	107 mg/l (NA) NA	48 h flow-thru	107 mg/l	1993/1993	426614-23 CORE	WIL	RGM
2.								
3.								
4.								
5.								
6.								
7.								
CHRONIC TOX.	% AI	MATC LC_{50}	DAYS	AFFECTED PARA.	STUDY/REVIEW DATES	MRID/ CATEGORY	LAB	RC
1.								
2.								

COMMENTS: WIL=Wildlife International Ltd.; NA=not applicable; results based on the mean measured concentration.

UPGRADE TO DATA EVALUATION RECORD
(MRID 426614-24 and Addendum MRID 431955-01 of 3/14/93)

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

2. **TEST MATERIAL:** MON 12000; Batch No. SIB-9104-3106-T; 99.3% purity; a white powder.

3. **STUDY TYPE:** 123-1. Non-Target Plants: Seed Germination/ Seedling Emergence Phytotoxicity Test - Tier 2. Species Tested: Soybean, Lettuce, Radish, Tomato, Cucumber, Cabbage, Oat, Ryegrass, Corn, and Onion.

4. **CITATION:** Chetram, R.S. 1992. Tier 2 Seed Germination/ Seedling Emergence Nontarget Phytotoxicity Study Using MON 12000. Laboratory Study No. BL91-464. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by The Agricultural Group of Monsanto Company, St. Louis, MO. EPA MRID No. 426614-24.

5. **REVIEWED BY:**

Alvaro A. Yamhure, Aquatic Biologist (7507-C) **Signature:**
Section 2, Ecological Effects Branch,
Environmental Fate and Effects Division **Date:** 6/25/94

6. **APPROVED BY:**

Norm Cook, Head Section 2, (7507-C) **Signature:** 07-29-94
Ecological Effects Branch,
Environmental Fate and Effects Division **Date:**

7. **CONCLUSIONS:** These studies are scientifically sound and meet the requirements for Tier-2 seed germination and seedling emergence tests using non-target plants and is **upgraded to core** . For the emergence study, the solvent system negatively impacted only the growth of the control cabbage plants by 7%.

The most sensitive species for seedling emergence was lettuce with a dry weight EC_{25} of $2X^{10-5}$ lb ai/A. Because the **registrant clarified the most important data gaps with** Addendum MRID **431955-01** about the effects of the solvent mixture for the germination study and the composition of said mixture, these sudies are upgraded to core.

Project Officer
6 24 94

22

Seed Germination: The most sensitive species was cabbage. The 6-day NOEL, LOEL, EC_{25}, and EC_{50} for cabbage germination were 0.015, 0.030, 0.011, and 0.040 lb ai/A, respectively.

Seedling Emergence:
Seedling Emergence and Survival: Ryegrass appeared to be the most sensitive valid species with respect to seedling emergence. The NOEL, LOEL, EC_{25}, and EC_{50} were 0.07, 0.21, 0.20, and >1.90 lb ai/A, respectively.

By 21 days after treatment, radish appeared to be the most sensitive species with respect to survival (based on overall values). The NOEL, LOEL, EC_{25}, and EC_{50} were 0.023, 0.070, 0.090, and 1.50 lb ai/A, respectively.

Plant Phytotoxicity: The most sensitive species with regard to plant phytotoxicity was lettuce. The NOEL and LOEL for this species were 0.000094 and 0.00028 lb ai/A, respectively.

Plant Height: The most sensitive species was lettuce, with NOEL, LOEL, EC_{25}, and EC_{50} values of 0.000012, 0.000024, 0.000099, and 0.00024 lb ai/A, respectively.

Plant Dry Weight: Lettuce was again the most sensitive species with respect to dry weight. The NOEL, LOEL, EC_{25}, and EC_{50} were 0.000012, 0.000024, **0.00002**, and 0.0001 lb ai/A, respectively.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Plants: Dicotyledon plants were represented by six species from five families (i.e., soybean, lettuce, radish, tomato, cucumber, and cabbage). Monocotyledon plants were represented by four species from two families (i.e., corn, oat, ryegrass, and onion). Cultivars, lot numbers, seed sources, and germination ratings were provided in the report.

B. Test System:
Seed Germination: Two circles of blue blotter were placed in the bottom of a glass petri plate (100 x 15 mm). The highest concentration test solution for the

base study was prepared in a 1% acetonitrile/deionized water solution and then diluted serially with this same solvent mixture to achieve the lower concentration solutions. The highest concentration test solution for the continuation study was prepared in a 0.25% acetonitrile/deionized water solution and subsequently serially diluted. Twelve milliliters of the test solution were added to each plate of soybean, cucumber, oat, and corn. Ten milliliters were added to plates of lettuce, radish, tomato, cabbage, ryegrass, and onion.

Ten seeds of each crop were added to each petri plate within 169 minutes of test solution preparation. The plates containing crops with the same concentration were then impartially placed in plastic boxes (31 x 23 x 10 cm) with raised mesh bottoms and tight-fitting lids to prevent moisture loss. Water was added to the bottom of each box to increase humidity. The petri plates were incubated in the dark at 24-27°C, except lettuce, which was incubated at 18-20°C. All crops were incubated for six days.

Seedling Emergence: Ten seeds of each crop were planted in plastic pots (7.5 x 7.5 x 6.0 cm), filled with sterilized sandy loam soil (pH 7.5, 0.7% organic matter) and perlite obtained from the laboratory facility. A plexiglass template was used to create planting holes in the soil, allowing for uniform planting depth and seed distribution. Soybean, cucumber, oat, and corn were planted at a depth of 2.5 cm, while the remaining six species were planted at a depth of 1.3 cm. Each treatment replicate was placed on an aluminum tray which was placed in the spray plot. The spray plot was 45.5 x 15.5 in. (i.e., 4.9 ft^2).

All applications were performed in a spray booth equipped with a single nozzle. A nozzle height of 10.5 inches and a nozzle pressure of 35 psi were used. The test spray solutions were prepared by dissolving the material in a 10% tetrahydrofuran/90% acetonitrile solution. The plants were sprayed at the equivalent of 468 l/ha (50 gpa) within 31 to 60 minutes after solution preparation.

The pots were hand watered (10-14 ml/pot) during the first 48 hours to facilitate movement of the test material to the seed zone. Lettuce plants were kept in an area maintained at 19-22°C. After 48 hours, the

pots were watered four times a day and a total of 11-22 ml of water was used to irrigate each pot per day.

C. **Dosage:** In both seed germination and seedling emergence tests, Mon 12000 was applied at the rates of 0.023, 0.070, 0.21, 0.63, and 1.9 lb active ingredient (ai)/acre (A) to all plant species. For the germination test, one continuation study was performed. Two continuation studies were conducted for the emergence test. Rates ranging from 0.0000059 to 0.030 lb ai/A were applied to selected test species. The test solutions were corrected for the percent purity of the test material (99.3%).

D. **Design:**

Seed Germination: Each treatment/crop combination was replicated four times (i.e., 10 seeds/plate, 4 plates/ treatment). After 6 days of incubation, the percentage of germinated seeds was determined by counting the number of seeds which had radicle lengths of 5 mm or greater.

Seedling Emergence: Each crop/treatment combination was replicated four times (i.e., 10 seeds/pot, 4 pots/ treatment level). After treatment, the pots were randomized in a greenhouse. Trays were rotated 180° twice weekly to reduce phototropism.

Temperature, relative humidity, photoperiod, and illuminance during the period of growth were provided in the report.

The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment at 10 and 14 days after treatment. Seedling height and survival were measured 21 days after treatment and phytotoxicity ratings were recorded 10, 14, and 21 days after treatment for all species. Twenty-one days after treatment, the plants within treatment replicates (pots) were cut at the soil level and dried in pre-weighed foil sheets at 100°C for a minimum of 48 hours.

The phytotoxicity ratings evaluated five observable toxic effects: 0-indicates no effect; 1-indicates slight plant effect; 2-indicates a moderate effect (e.g., mild stunting or chlorosis); 3-indicates a severe effect with recovery possible; 4-indicates a total effect (very poor vigor); and 5-moribund or plant death.

E. Statistics: All calculations are based on nominal rates. All data were entered into a Lotus 1-2-3 spreadsheet. The spreadsheet calculated replicate means, treatment means, percent effect, standard deviations, and analysis of variance tables. Treatment means were used to calculate the percent effect resulting from the treatment. The percent effect was calculated using the following equation:

$$\% \text{ effect} = \frac{(\text{treatment mean} - \text{control mean})}{\text{control mean}} \times 100$$

A randomized complete block analysis of variance (ANOVA) was performed on treatment level x replicate means. Prior to analysis, phytotoxicity data were converted to the proportion of the maximum rating. When the ANOVA indicated a significant difference from the control, treatment means were subjected to a one-tailed comparison test (Dunnett's) to determine which treatments were significantly ($p < 0.05$) different from the control. The no-effect-level (NOEL) was determined as the highest treatment rate not statistically different from the control or the rate below which 25% inhibition was witnessed.

The percent detrimental effect values were input into a computer program which fit the data to various mathematical equations. The least squares error of fit and F-value were used as criteria to judge which equation provided the best representation of the response. The selected equation was used to determine the EC_{25} and EC_{50} values.

12. REPORTED RESULTS: The author based all reported results on nominal concentrations. The percent recoveries of MON 12000 in the base and continuation studies ranged between 81 and 136% (Tables I-V, attached).

Seed Germination: No significant difference in percent germination existed between controls and each treatment level for soybean, cucumber, oat, ryegrass, corn, and onion. The NOELs for radish and tomato, lettuce, and cabbage were 0.21, 0.023, and 0.015 lb ai/A, respectively. The NOEL for the remaining six species was 1.9 lb ai/A. No EC_{25} or EC_{50} values were computed due to the lack of definite dose-response relationships except for lettuce and cabbage. The EC_{50} values for these two species are 0.11 and 0.030 lb ai/A, respectively.

Seedling Emergence:
Percent Emergence and Survival: By the end of 14 days, three of the species tested demonstrated significant reductions in emergence. The NOELs for percent emergence (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean = lettuce = radish = tomato = cucumber = cabbage = oat (1.9) < corn (0.63) < ryegrass (0.070) < onion (0.0077).

Due to lack of definite dose-response relationships, regression analysis was only conducted for ryegrass, corn, and onion. The EC values for these species are presented in Table XX (attached).

By the end of 21 days, corn demonstrated increased emergence, and seedling survival was not significantly reduced at any of the treatment rates, as was the case with the seven species that were unaffected at 14 days after application (except for radish and lettuce). Four species demonstrated significant reductions in survival at some rate of MON 12000. The NOELs for percent survival (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean = tomato = cucumber = cabbage = oat = corn (1.9) < ryegrass (0.63) < lettuce (0.21) < radish (0.023) < onion (0.0077).

Due to lack of significant rate effects, regression analysis was only conducted for lettuce and radish. The EC values for these species are presented in Table XX.

Plant Phytotoxicity: *By the end of the 21 day test period, every species demonstrated significant signs of phytotoxicity at some rate of MON 12000 tested.* The NOELs for phytotoxicity (in lb ai/A) for the test species, in increasing sensitivity, are:

oat (0.023) < tomato = cucumber (0.0077) < soybean = ryegrass = corn (0.0026) < radish = cabbage = onion (0.00028) < lettuce (0.000094).

No EC values were computed from the phytotoxicity data.

Plant Height: By the end of 21 days, all of the test species demonstrated significant reductions in height at some rate of MON 12000. The NOELs for plant height (in lb ai/A) for the test species, in increasing sensitivity, are:

oat (0.0077) < soybean = tomato = cucumber = corn (0.0026) < radish = ryegrass (0.00085) < cabbage = onion (0.00028) < lettuce (0.000012).

Regression analysis was conducted for all test species. The EC values are presented in Table XX.

Plant Dry Weight: By the end of 21 days, all of the test species demonstrated significant reductions in weight at some rate of Mon 12000. The **NOELs** for plant weight (in lb ai/A) for the test species, in increasing sensitivity, are:

soybean = cucumber = oat = corn (0.0026) < radish = cabbage = ryegrass (0.00028) < onion (0.000094) < tomato (0.000047) < lettuce (0.000012).

Regression analysis was conducted for all test species. The EC values are presented in Table XX.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: A no-effect concentration was reached for each parameter measured for all crops tested. The lowest NOEL for each parameter were as follows: percent germination - cabbage (0.015 lb ai/A), percent emergence - onion (0.0077), phytotoxicity - lettuce (0.000094 lb ai/A), percent survival - onion (0.0077 lb ai/A), plant height - lettuce (0.000012 lb ai/A), plant dry weight - lettuce (0.000012 lb ai/A).

The Quality Assurance Unit of Pan-Agricultural Laboratories, Inc. was responsible for the assurance of compliance with Good Laboratory Practice (GLP) Standards as outlined in 40 CFR Part 160. GLP and QA statements were enclosed in the report and the analytical appendix.

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

A. Test Procedure: The test procedures generally adhered to the SEP and Subdivision J guidelines, except for the following:

Only one parameter (germination) was measured or recorded for the germination study.

The protocol submitted with the study indicated that control plants would be sprayed with deionized water, with an appropriate solvent if necessary. Consequently, a negative control was not included in the test design.

The rate dilution progression for the base study for both the emergence and germination studies and the first emergence continuation study was 3x, rather than the recommended 2x.

B. **Statistical Analysis:** Probit and mean comparison (Dunnett's test) analyses were conducted on cabbage germination and lettuce dry weight data for the germination and emergence studies, respectively (see attached printouts). The reviewer obtained the same or similar results as the author for the NOEL and EC values.

C. **Discussion/Results:** Results of the chemical analyses indicated that the actual concentrations were near nominal concentrations (attached). The reviewer therefore believes that the nominal concentrations are representative of actual rates applied and accepts the results in terms of nominal concentrations.

Seed Germination: The most sensitive species was cabbage. The 6-day NOEL, lowest-observed-effect level (LOEL), EC_{25}, and EC_{50} for cabbage germination were 0.015, 0.030, 0.011, and 0.040 lb ai/A, respectively. A rate response was observed for lettuce, and the EC_{25} should have been determined for this species.

Seedling Emergence: The solvent system used in this study detrimentally affected the development of onion plants (>25% phytotoxicity in the control). The results for this species are therefore invalid.

Seedling Emergence and Survival: Ryegrass appeared to be the most sensitive valid species with respect to seedling emergence. The NOEL, LOEL, EC_{25}, and EC_{50} were 0.07, 0.21, 0.20, and >1.90 lb ai/A, respectively.

By 21 days after treatment, radish appeared to be the most sensitive species with respect to survival (based on overall values). The NOEL, LOEL, EC_{25}, and EC_{50} were 0.023, 0.070, 0.090, and 1.50 lb ai/A, respectively.

Plant Phytotoxicity: The most sensitive species with regard to plant phytotoxicity was lettuce. The NOEL and LOEL for this species were 0.000094 and 0.00028 lb ai/A, respectively.

Plant Height: The most sensitive species was lettuce, with NOEL, LOEL, EC_{25}, and EC_{50} values of 0.000012,

0.000024, 0.000099, and 0.00024 lb ai/A, respectively. Although not computed by the author, reasonable estimates of the EC_{50} for tomato and oat are 0.023 and 1.9 lb ai/A, respectively.

Plant Dry Weight: Lettuce was again the most sensitive species with respect to dry weight. The NOEL, LOEL, EC_{25}, and EC_{50} were 0.000012, 0.000024, 0.00002, and 0.0001 lb ai/A, respectively. A rate response was observed for tomato and cabbage, and the EC_{25} should have been determined for these species (the reviewer had to).

These studies are scientifically sound and meet the requirements for Tier-2 seed germination and seedling emergence tests using non-target plants. The results for onion from the emergence test are not scientifically sound and do not meet the requirements.

D. Adequacy of the Study:

(1) **Classification:** Core. According to the upgrade for this study, MRID431955-01, non of the tested plants showed adverse effects to the solvent treatment in the emergence study. Only cabbage showed a dry weight of 7%.

(2) **Repairability:** Study has been repaired and upgraded to core. The author confirmed that the solvent controls were treated with the appropriate mixtures and the appropriate EC values were determined with only dry weight for cabbage seedling emergence showing a detrimental effect (7%) at 21 day when treated with 90% acetonitrile and 10% THF so the results for all species except onion for the emergence test are upgraded to the "core" category.

15. COMPLETION OF ONE-LINER: Yes, 3-26-93. Updated 6/25/94

UPGRADE TO DATA EVALUATION RECORD
(MRID 426614-24 and Addendum MRID 431955-01 of 3/14/93)

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

2. **TEST MATERIAL:** MON 12000; Batch No. SIB-9104-3106-T; 99.3% purity; a white powder.

3. **STUDY TYPE:** 123-1. Non-Target Plants: Seed Germination/ Seedling Emergence Phytotoxicity Test - Tier 2. Species Tested: Soybean, Lettuce, Radish, Tomato, Cucumber, Cabbage, Oat, Ryegrass, Corn, and Onion.

4. **CITATION:** Chetram, R.S. 1992. Tier 2 Seed Germination/ Seedling Emergence Nontarget Phytotoxicity Study Using MON 12000. Laboratory Study No. BL91-464. Conducted by Pan-Agricultural Laboratories, Inc., Madera, CA. Submitted by The Agricultural Group of Monsanto Company, St. Louis, MO. EPA MRID No. 426614-24.

5. **REVIEWED BY:**

Alvaro A. Yamhure, Aquatic Biologist (7507-C), Section 2, Ecological Effects Branch, Environmental Fate and Effects Division — **Signature:** **Date:**

6. **APPROVED BY:**

Norm Cook, Head Section 2, (7507-C) Ecological Effects Branch, Environmental Fate and Effects Division — **Signature:** **Date:**

7. **CONCLUSIONS:** These studies are scientifically sound and meet the requirements for Tier-2 seed germination and seedling emergence tests using non-target plants and is **upgraded to core** . For the emergence study, the solvent system negatively impacted only the growth of the control cabbage plants by 7%.

The most sensitive species for seedling emergence was lettuce with a dry weight EC_{25} of $2X^{10-5}$ lb ai/A. Because the **registrant clarified the most important data gaps with Addendum MRID 431955-01** about the effects of the solvent mixture for the germination study and the composition of said mixture, these sudies are upgraded to core.

31