JAN 12 1993

MEMORANDUM

Subject: Phase IV Response for Reregistration D184069, D166717, D172925,and D170796. Thidiazuron.

From: Anthony Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division
H7507C

To: Linda Deluise, PM 52
(Tom Myers)
Reregistration Branch
Special Review and Reregistration Division
H7508W

EEB had completed the Phase IV review for the chemical thidiazuron. **The following study was reviewed in 1981 and rated as invalid** and must be repeated (see also attached Phase IV Requirements Table):

(71-2(a) Acute Avian Dietary,Quail (MRID No. 81629)) 12/16/93 Probably never effective

The following study was reviewed with this submission and rated **supplemental:**

72-4(b) Life-Cycle Aquatic Invertebrate **[MRID No. 421320-02. For this study see corresponding conclusions below].**

Summaries of the conclusions arrived at by EEB about the most recently reviewed Thidiazuron studies evaluated as part of this Phase IV are as follows:

CONCURRENCES

SYMBOL	EEB	H7507C						
SURNAME	Yamhuak	AJM						
DATE	1/10/93	1-12-93						

EPA Form 1320-1 (12-70) OFFICIAL FILE COPY

FISH STUDIES

MRID No. 422703-01 72-4(a)
CITATION: Cohle, P. and M. Muckerman. 1992. Early Life-Stage Toxicity of Thidiazuron to Fathead Minnows (*Pimephales promelas*) in a Flow-Through System. ABC Report No. 39113. Nor-Am Study No. 506 AW. Performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by Nor-Am Chemical Company, Pikeville, NC. EPA MRID No. 422703-01

CONCLUSIONS: This study is scientifically sound and meets the requirements for an early life-stage toxicity test using freshwater fish. The test concentrations were too low to determine the MATC. Based on mean measured concentrations, the NOEC was 5.7 mg/l, the highest concentration tested. Given the low solubility encountered in the testing of the toxicant (Thidiazuron) in water and that the testing was conducted at the highest water solubility possible under testing conditions, **this study is rated as core;** however, in the future we expect the registrant to follow our recommendations on alternative ways of increasing the solubility of the test compound.

MRID No. 418461-01 72-3(a)
CITATION: Schupner, J.K. and B.J. Stachura. 1991. The Static Acute Toxicity of Thidiazuron to the Sheepshead Minnow, *Cyprinodon variegatus*. Performed by NOR-AM Chemical Company, NOR-AM Research Center, Pikeville, North Carolina. Study No. 504AW. Submitted by NOR-AM Chemical Company. EPA MRID No. 418461-01.

CONCLUSIONS: This study is scientifically sound and meets the requirements for a 96-hour static acute toxicity test using marine fish. Given the low solubility of the toxicant (Thidiazuron) in sea water and the low toxicity to the test species - LC_{50} >36 mg/l- (Cyprinodon variegatus) **this study is rated as core;** however, in the future, we expect the registrant to follow our recommendations on alternative ways of increasing the solubility of test compounds [see Page 4;Section 14(C) of the corresponding attached DER]. The NOEL is therefore established as 36 mg/l, the highest measured test concentration used.

MRID No.420692-01 72-1(a)
CITATION: Schupner, J.K. and B.J. Stachura. 1991. The Acute Toxicity of Thidiazuron Technical to the Bluegill Sunfish, Lepomis macrochirus, in a Static System. Laboratory Project ID No. 511AW. Prepared and submitted by NOR-AM Chemical Company, Pikeville, NC. EPA MRID No. 420692-01.

CONCLUSIONS: This study is scientifically sound and meets the requirements for a 96-hour static acute toxicity test using fish. Given the low solubility of the toxicant (Thidiazuron) in freshwater and the low toxicity to the test species - LC_{50} >32 mg/(Lepomis macrochirus) **this study is rated as core;** however, in the future we expect the registrant to follow our recommendations on alternative ways of increasing the solubility of test compounds [see Page 4;Section 14(C) of the corresponding attached DER]. The NOEC to the bluegill sunfish is therefore established at 32 mg/l.

MRID No.420692-02 72-1(c)
CITATION: Schupner, J.K. and B.J. Stachura. 1991. The Acute Toxicity of Thidiazuron Technical to the Rainbow Trout, Oncorhynchus mykiss, in a Static System. Laboratory Project ID No. 512AW. Prepared and submitted by NOR-AM Chemical Company, Pikeville, NC. EPA MRID No. 420692-02.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements for a static freshwater fish toxicity study. Given the low solubility of the toxicant (Thidiazuron) in water and the low toxicity to the test species - 96-hour LC_{50}>19 mg ai/l (mean measured concentration) this study is rated as **core.** This LC_{50} value classifies thidiazuron technical as slightly toxic to rainbow trout. The NOEC was 19 mg ai/l. The dosage level tested was less than 100 mg/l and not high enough to produce a precise LC_{50}. In the future we expect the registrant to follow our recommendations on alternative ways of increasing the solubility of test compounds.

AQUATIC INVERTEBRATE STUDIES

MRID No. 418461-02 72-3(c)
CITATION: Schupner, J.K. 1991. The Static Acute Toxicity of Thidiazuron to the Mysid Shrimp, *Mysidopsis bahia*. Performed by NOR-AM Chemical Company, NOR-AM Research Center, Pikeville, North Carolina. Study No. 503AW. Submitted by NOR-AM Chemical Company. EPA MRID No. 418461-02.

CONCLUSIONS: This study is scientifically sound and fulfills the requirements **(core)** for an acute static toxicity test using marine shrimp. Two important environmental testing values (pH and salinity)were significantly above the maximum recommended and test mysids (96 hours old) used were older than the advisable age (24 hours old); however, we at EEB recognize that the best age at which to test shrimp issue has not been sufficiently emphasized to the registrants and therefore must not hold the registrants responsible until appropiate official communication has been made. With a 96-hour LC_{50} of 3.2 mg/l mean measured concentration, thidiazuron is classified as moderately toxic to mysid shrimp. The NOEC was 1.4 mg/l mean measured concentration.

MRID No. 421320-01 72-3(b)
CITATION: Ward, G.S. 1991. Thidiazuron Technical: Acute Effect on New Shell Growth of, the Eastern Oyster, Crassostrea virginica. Laboratory Project ID. J9101006b. Nor-Am Study No. 505 AW. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Nor-Am Chemical Company, Pikeville, NC. EPA MRID No. 421320-01.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements (**Core**) for a shell deposition test using the eastern oyster. The 96-hour EC_{50}, based on the percent difference in shell deposition compared to the control, was 5.4 mg/l mean measured concentration. The 96-hour LC_{50} was 4.3 mg/l mean measured concentration. Therefore, thidiazuron technical is classified as moderately toxic to eastern oysters. The NOEC was reported as 1.06 mg/l (mean measured concentration).

MRID No. 421320-02 72-2(a)
CITATION: Blakemore, G.C. and M. Muckerman. 1991. Chronic Toxicity of Thidiazuron to Daphnia magna Under Flow-Through Test Conditions. Final Report No. 39114. Nor-Am Study No. 507-AW. Prepared by ABC Laboratories, Inc., Columbia, MO. Submitted by Nor-Am Chemical Company, Pikeville, NC. EPA MRID No. 421320-02.

CONCLUSIONS: This study is scientifically sound but **does not meet the requirements** for a chronic, flow-through toxicity test using the freshwater invertebrate, Daphnia magna. Further, statistically significant effects on daphnid length which is considered to be an important endpoint in this type of test, was observed at all tested concentrations (NOEL was not established and in this case the problem is not solubility related since we are talking about the low end of the solubility range), therefore an MATC value for this statistic could not be established. The 21-day EC_{50} based on immobilization was 0.68 mg/l mean measured concentration. The LOEL was 0.1 mg/l. **This study is rated as supplemental.**

PLANT STUDIES

MRID No. 418191-01 122-1(b)
CITATION: Downey, S.S 1990. Investigation into the Phytotoxic Effects of Thidiazuron on Vegetative Vigor (Tier 1). Laboratory Report ID. No. 502 AW. Conducted by NOR-AM Chemical Company, Environmental Sciences Department, Pikeville, NC, Submitted by NOR-AM Chemical Company, Wilmington, DE. EPA MRID No. 418191-01.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements (**core**) for a Tier 1 vegetative vigor non-target plant phytotoxicity test. Onion, wheat, carrot, cucumber, lettuce, soybean, and tomato were all scientifically affected in some parameter observed. The NOEC for these species was therefore <0.2 lb ai/A. The NOEC for the remaining three species (corn, oat, and radish) was 0.2 lb ai/A. A summary of the species and parameters affected by greater than 25% is presented in the corresponding DER.

MRID No. 417611-04 122-2
CITATION: Hughes, J.S. 1990. The Toxicity of Thidiazuron Technical to Selenastrum capricornutum. Laboratory Project No. B643-01-03. Conducted by Malcomn Pirnie, Inc., Tarrytown, NY. Submitted by NOR-AM Chemical Company, Pineville, NC. EPZ MRID No. 417611-04.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements (**core**) for a Tier 1 non-target aquatic plant study. Exposure of S. capricornutum to 0.15 mg ai/1 thidiazuron (nominal concentration) for 5 days resulted in a 17% reduction in growth. The NOEC was 0.15 mg ai/l.

MRID NO. 418213-01 122-1(a)
CITATION: Downey, D.S. 1991. Investigation into the Phytotoxic Effects of Thidiazuron on Seedling Emergence (Tier 1). Laboratory Report ID. No. 501 AW. Conducted by NOR-AM Chemical Company, Environmental Sciences Department, Pikeville, NC. Submitted to NOR-AM Chemical Company, Wilmington, DE. EPA MRID No. 418213-01.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements (**core**) for a Tier 1 seedling emergence non-target plant phytotoxicity test. The NOEC of thidiazuron for all species, except oat and tomato, was 0.2 lb ai/A. The NOEC for oat and tomato was <0.2 lb ai/A. All effects were less than 25%.

MRID No. 417862-03 122-1(a)
CITATION: Downey, S.S. 1991. Investigation into the Phytotoxic Effects of Thidiazuron on Seed Germination (Tier 1). Laboratory Report ID. No. 500 AW. Conducted by NOR-AM Chemical Company, Environmental Sciences Department, Pikeville, NC. Submitted by NOR-AM Chemical Company, Wilmington, DE. EPA MRID No. 417862-03.

CONCLUSIONS: This study is scientifically sound and meets the guideline requirements (**core**) for a Tier 1 seed germination non-target plant phytotoxicity test. The NOEC for all test species was 0.2 lb ai/A.

The results obtained from still-pending ecological effects studies as well as any relevant environmental fate data will determine whether additional testing may be needed for hazard assessment purposes.

If you have any questions please contact Alvaro Yamhure (305-6179).

Date:12/14/92
Case No: 4092
Chemical No:120301 (Thidiazuron)

PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition[1]	Use Pattern[2] [1]	Does EPA Have Data To Satisfy This Requirement? (Yes, No)	Bibliographic Citation	Must Additional Data Be Submitted under FIFRA3(c)(2)(B)?
6 Basic Studies in Bold					
71-1(a) Acute Avian Oral, Quail/Duck	TGAI	A	YES	66186	NO
71-1(b) Acute Avian Oral, Quail/Duck	(TEP)				
71-2(a) Acute Avian Diet, Quail	TGAI	A	NO	81629	YES
71-2(b) Acute Avian Diet, Duck	TGAI	A	YES	66169	NO
71-3 Wild Mammal Toxicity					
71-4(a) Avian Reproduction Quail					
71-4(b) Avian Reproduction Duck					
71-5(a) Simulated Terrestrial Field Study					
71-5(b) Actual Terrestrial Field Study					
72-1(a) Acute Fish Toxicity Bluegill	TGAI	A	YES	420692-01	NO
72-1(b) Acute Fish Toxicity Bluegill	(TEP)				
72-1(c) Acute Fish Toxicity Rainbow Trout	TGAI	A	YES	420692-02	NO
72-1(d) Acute Fish Toxicity Rainbow Trout	(TEP)				
72-2(a) Acute Aquatic Invertebrate Toxicity	TGAI	A	YES	66167	NO
72-2(b) Acute Aquatic Invertebrate Toxicity	(TEP)				
72-3(a) Acute Estu/Mari Tox Fish	TGAI	A	YES	418461-01	NO
72-3(b) Acute Estu/Mari Tox Mollusk	TGAI	A	YES	421320-01	NO
72-3(c) Acute Estu.Mari Tox Shrimp	TGAI	A	YES	418461-02	NO

* In Bibliographic Citation column indicates study may be upgradeable

Date:12/14/92
Case No: 4092
Chemical No:120301 (Thidiazuron)

PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition[1]	Use Pattern[2] [1]	Does EPA Have Data To Satisfy This Requirement? (Yes, No)	Bibliographic Citation	Must Additional Data Be Submitted under FIFRA3(c)(2)(B)?
72-3(d) Acute Estu/Mari Tox Fish	(TEP)				
72-3(e) Acute Estu/Mari Tox Mollusk	(TEP)				
72-3(f) Acute Estu/Mari Tox Shrimp	(TEP)				
72-4(a) Early Life-Stage Fish	TGAI	A	YES	422703-01	NO
72-4(b) Live-Cycle Aquatic Invertebrate	TGAI	A	NO	421320-02	YES
72-5 Life-Cycle Fish					
72-6 Aquatic Org. Accumulation					
72-7(a) Simulated Aquatic Field Study					
72-7(b) Actual Aquatic Field Study					
122-1(a) Seed Germ./Seedling Emerg.	TGAI	A	YES	417862-03	NO
122-1(b) Vegetative Vigor	TGAI	A	YES	418231-01 & 418191-01	NO
122-2 Aquatic Plant Growth	TGAI	A	YES	417611-04	NO
123-1(a) Seed Germ./Seedling Emerg.					
123-1(b) Vegetative Vigor					
123-2 Aquatic Plant Growth					
124-1 Terrestrial Field Study					
124-2 Aquatic Field Study					
141-1 Honey Bee Acute Contact					
141-2 Honey Bee Residue on Foliage					
141-5 Field Test for Pollinators					

1. **Composition: TGAI = Technical grade of the active ingredient.**
2. **Uses: A = Terrestrial food crop.**

* In Bibliographic Citation column indicates study may be upgradeable

DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron.
Shaughnessey No. 120301.

2. **TEST MATERIAL:** Thidiazuron Technical; CAS No. 51707-55-2; Batch No. 7/9.82; 99.3% active ingredient; a tan granule.

3. **STUDY TYPE:** 72-4. Freshwater Fish Early Life-Stage Toxicity Test. Species Tested: Fathead Minnow (*Pimephales promelas*).

4. **CITATION:** Cohle, P. and M. Muckerman. 1992. Early Life-Stage Toxicity of Thidiazuron to Fathead Minnows (*Pimephales promelas*) in a Flow-Through System. ABC Report No. 39113. Nor-Am Study No. 506 AW. Performed by Analytical Bio-Chemistry Laboratories, Inc., Columbia, MO. Submitted by Nor-Am Chemical Company, Pikeville, NC. EPA MRID No. 422703-01.

5. **REVIEWED BY:**

Alvaro A. Yamhure,
Aquatic Biologist
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature:

Date: 12/21/92

6. **APPROVED BY:**

Dan Rieder,
Head Section 3
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature: Daniel Rieder

Date: 1.6 93

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for an early life-stage toxicity test using freshwater fish. The test concentrations were too low to determine the MATC. Based on mean measured concentrations, the NOEC was 5.7 mg/l, the highest concentration tested. Given the low solubility encountered in the testing of the toxicant (Thidiazuron) in water and that the testing was conducted at the highest water solubility possible under testing conditions, **this study is rated as core;** however, in the future we expect the registrant to follow our recommendations on alternative ways of increasing the solubility of test compounds.

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8. **RECOMMENDATIONS:** Alternative methods of increasing the water solubility/accommodation of pesticides in water for testing purposes may be desirable.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Newly fertilized eggs (<24 hours post-spawn) of fathead minnow (*Pimephales promelas*) were obtained from the spawning culture at the testing facility.

B. **Test System:** The test system was a 2-l proportional diluter which intermittently delivered test solutions to replicate test chambers. Duplicate glass aquaria were divided into two replicate chambers, each measuring 30.7 x 15.7 cm with a water depth of 24.5 cm, yielding an 11.8-liter chamber volume. All chamber drains were covered with stainless steel screen. The diluter delivered an average of 88 l of solution per day to each replicate chamber which was equivalent to 7.5 volume additions per day.

Embryo incubation cups were clear glass jars (9-cm diameter) with Nitex screen bottoms. One cup was suspended in each test chamber. A rocker arm apparatus was used to gently oscillate the incubation cups in the test chambers.

Sixteen hours of light at an intensity of 484-613 lux at the water surface were provided each day. Thirty-minute dawn and dusk simulation periods were provided from day 3 post-hatch. Developing embryos and newly hatched fry were shielded from excess light exposure. Test temperature was maintained at 25 ±2°C by a thermostatically-controlled water bath.

The dilution water was a mixture of untreated and treated (reverse osmosis) well water. A representative sample of water had a pH of 8.0-8.3, a specific conductivity of 411-439 µmhos/cm, and a hardness and alkalinity of 168-178 and 182-190 mg/l as $CaCO_3$, respectively.

A stock solution was prepared in dimethylformamide (DMF). This solution was mixed with 5 l of dilution

water then transferred to a rectangular tank with 50 ml of DMF and diluted to 1000 l with dilution water (final concentration 24 mg/l). The solution was mixed for approximately 48 hours. The diluter stock was delivered to the diluter where it was combined with dilution water to a concentration of 6.0 mg/l, the highest test concentration. The remaining test concentrations were prepared by 50% dilution. The test material flowed through the test system for 5 days prior to test initiation

C. **Dosage:** Thirty-five-day, flow-through test. Nominal test concentrations selected based on results of preliminary testing were 0.38, 0.75, 1.5, 3.0, and 6.0 mg/l. A dilution water control and solvent control were also included. The solvent control contained a DMF concentration of 0.014 ml/l which was slightly higher than the DMF concentration used in exposure levels 1-5.

D. **Design:** Four replicates of each treatment and control were included in the test. Duplicate test aquaria per concentration were arranged in one row on one tier using a random number table to assign specific test concentrations, providing a nested experimental design.

Embryos were selected and impartially distributed to the incubation cups, by twos, until each cup contained 20 eggs. Hatching was complete on test day 5 at which time the fry were released into their respective chambers. Biomass loading was 0.027-0.041 g/l/day at study termination.

Larvae were fed live brine shrimp nauplii and rotifers daily beginning on day 4. Beginning on day 11, salmon starter mash was added to the diet. From study day 21 on, only brine shrimp and mash were fed. Food was added three times daily. Feeding was discontinued 24 hours prior to test termination. The aquaria were cleaned as needed.

Behavior, appearance, and survival of embryos and larvae were observed and recorded daily. Dead individuals were removed at each observation. At test termination (test day 35), the larvae were counted and standard length and blotted wet weight of each individual were measured.

Dissolved oxygen concentration (DO), pH, conductivity, hardness, alkalinity, and temperature were measured on

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days 0, 1, 7, 14, 21, 28, and 35. Temperature and DO were measured in one replicate of each treatment and control. Hardness, alkalinity, pH, and conductivity were measured in one replicate of the control, lowest and highest test concentrations. Temperature was also measured twice daily and continuously in a centrally-located chamber throughout the test period.

Water samples were collected from each test concentration for the determination of thidiazuron using high performance liquid chromatography. These samples were collected on test days 0, 1, 7, 14, 21, 28, and 35.

E. **Statistics:** A t-test or two-tailed Fisher's exact test demonstrated no significant difference between the dilution water control data and solvent control data. Therefore, the pooled control data were used to assess the response of the treatment groups. Shapiro-Wilk normality test statistics were computed within each test concentration to assess departures from normality. When the data suggested departures from normality, the data were examined for indications of central tendency. If the variability between replicates within aquaria was not significant or there was no strong evidence of biological significance, then the aquaria within concentrations and replicate within aquaria error sources were combined.

Egg hatchability and survival data were analyzed using frequency analysis comparing the pooled control responses to each exposure level. A one-way analysis of variance (ANOVA) procedure for nested design experiments, coupled with Dunnett's one-tailed means comparison procedure, was used to analyze the growth data. Statistical differences were concluded when $p \leq 0.05$.

12. **REPORTED RESULTS:** Mean measured concentrations were 0.38, 0.65, 1.4, 2.9, and 5.7 mg/l (Table III, attached). These values ranged from 87 to 100% of nominal concentrations.

Hatch began on day 4 and was completed on day 5. There was no concentration-related delay in hatch. Hatching success was not significantly affected at any concentration (Table IX, attached). Total survival in the dilution water control and solvent control was 80 and 75%, respectively (Table IX, attached). One fish in replicate D of the dilution water control was inadvertently killed while the chamber was being cleaned and was not counted as test-related mortality. Fry

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survival (30-days post-hatch) at the 0.65 and 1.4 mg/l concentrations were significantly reduced when compared to the pooled control data. Since a significant reduction was not indicated at either of the next two higher test levels, it was concluded that the statistical reductions were not biologically significant. Total survival (survival of embryos and larvae combined) was not significantly affected by the concentration of thidiazuron technical.

Fish length and weight were not significantly affected by the concentration of thidiazuron technical when compared to the pooled control results (Table X, attached).

Continuous temperature data for study days 7 to 15 were lost due to equipment malfunction. During the study, pH was 8.0-8.3, specific conductance was 392-439 µmhos/cm, and hardness and alkalinity were 164-178 and 174-192 mg/l as $CaCO_3$, respectively. The dissolved oxygen concentration was 5.8-8.5 mg/l (73-105% of saturation at 25 and 24°C, respectively). Based on daily temperature readings, the temperature range was 23.8-24.9°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Based on the lack of biologically significant effects on fathead minnow hatchability, survival, and growth, a lowest-observed-effect concentration (LOEC) could not be determined. Data from range finding tests indicated that the LOEC was within the range of 5.7 to 8.9 mg/l. The no-observed-effect concentration (NOEC) was 5.7 mg/l mean measured concentration, the highest concentration tested. The NOEC exceeds the estimated environmental concentration for thidiazuron (0.004 mg/l) by more than 1000 fold. Chronic fish toxicity is not expected to be a concern.

A GLP compliance statement was included in the report indicating that the study was conducted in accordance with EPA Good Laboratory Practice Regulations (40 CFR, Part 160). A quality assurance statement was also included.

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

A. **Test Procedure:** The test procedure is generally in accordance with the SEP guidelines, except for the following deviations:

No LOEC was determined; the SEP requires that one concentration adversely affect a life stage and one concentration not adversely affect a life stage.

The relative standard deviation (RSD) for the dilution water control replicate A was 44.7% and higher than that considered acceptable (40%) in the SEP.

A malfunction in the data log recorder for continuous temperature from day 7 to 15 was reported; however, the continuous temperature measurement was not reported for the period in which the data recorder was functioning properly.

Embryos were "impartially" selected and distributed; the SEP requires random distribution of test organisms to the test vessels (ofcourse, the registrant may mean random when stating "impartial").

B. **Statistical Analysis**: Hatchability, fry survival, and total survival data were analyzed using one-way ANOVA and Dunnett's test (see attached printouts 1-3). Fish length and weight were analyzed using two-way ANOVA and Bonferroni's test (see attached printouts 4-9). The reviewer's NOEC, 5.7 mg/l mean measured concentration, was the same as the authors'.

C. **Discussion/Results**: The relative standard deviation (RSD) for the dilution water control replicate A was 44.9% (Table X and printout 4, attached) and higher than that considered acceptable (40%) in the SEP. This deviation may weaken the study, but in the reviewer's opinion, is not significant enough to cause the study to be classified as invalid. The NOEC value (5.7 mg a.i./l, the highest concentration tested) would normally indicate the need for further testing to obtain an maximum acceptable toxicant concentration for thidiazuron technical on fathead minnow early life-stages; however, until the issue of how to improve the testing of materials with low water solubility is decided by EPA we accept the fact that this test represent a good faith testing effort by the registrant and the NOEC of 5.7 mg/l is accepted as the best possible estimate of toxicity to the test organism given solubility problems.

This study is scientifically sound and meets the requirements for an early life-stage toxicity test using freshwater fish. Based on mean measured concentrations, the NOEC was 5.7 mg/l, the highest concentration tested. An MATC was not determined.

D. **Adequacy of the Study**:

(1) **Classification:** Core

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 12-7-92.(Amended by EEB on 12/21/92).

THIDIAZURON

Page_____ is not included in this copy.

Pages 16 through 18 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.

DATABASE ENTRY FORM
FOR ACUTE OR CHRONIC TOXICITY STUDIES

1. **Chemical** Thidiazuron **Shaughnessy** 120301
2. **Common Name Of Organism Tested** Fathead minnow
3. **Scientific Name** Pimephales promelas
4. **Age Of Organisms** <24 old embryos
5. **Guideline No.** 72-4
6. **Type Of Dosing Method (Circle One) Or Study**

   1. Oral 2. Dietary ~~3. Reproduction~~ 4. Static
   5. Static Renewal (6. Flowthrough) 7. Acute Contact
   8. Other _____

7. **% AI Of Test Substance** 99.3
8. **Study Duration (Hrs Or Days)** 35-day
9. **Dose Type (Circle One)** A. LD50 B. LC50 C. EC50 (D. MATC)
10. **Toxicity Level** A. mg/kg B. ppm (C. mg/l) D. µg/l E. ng/l F. µg/bee G. Other
11. **95% C.L.s** N/A
12. **Curve Slope** N/A
13. **NOEL** 5.7 mg/l no MATC generated
14. **Study Date (YEAR)** 1992
15. **Study Review Date (YEAR)** 1992
16. **Category (Circle One)** CORE (SUPPLEMENTAL) INVALID
17. **MRID of Accession Number** 422703-01
18. **Laboratory** Analytical Bio-Chemistry Inc.
19. **Reviewer** Lon Rifici
20. **For Reproductive Studies (avian or aquatic) Indicate Which Parameter Affected At What Toxicity Level.**

    Eggs Layed _____ % Cracked _____ % Viable _____
    % Live Embryos _____ % Egghatch 14D Survivors _____;
    Growth Effected at none Other Effects none

422703-01, thidiazuron, fathead minnow hatchability
File: a:42270301.dt1 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.1301	CALCULATED t VALUE =	-0.1920
GRP2 (BLANK CRTL) MEAN =	1.1426	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-0.0125		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.007	0.001	0.089
Within (Error)	21	0.283	0.013	
Total	27	0.290		

Critical F value = 2.57 (0.05,6,21)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.130	0.813		
2	dilution contrl	1.143	0.825	-0.153	
3	0.38	1.129	0.813	0.012	
4	0.65	1.109	0.800	0.262	
5	1.4	1.148	0.825	-0.220	
6	2.9	1.130	0.813	0.000	
7	5.7	1.163	0.825	-0.406	

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	4			
2	dilution contrl	4	0.177	21.8	-0.012
3	0.38	4	0.177	21.8	0.000
4	0.65	4	0.177	21.8	0.013
5	1.4	4	0.177	21.8	-0.012
6	2.9	4	0.177	21.8	0.000
7	5.7	4	0.177	21.8	-0.012

422703-01, thidiazuron, fathead minnow fry survival
File: a:42270301.dt2 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.2917	CALCULATED t VALUE =	-2.1328
GRP2 (BLANK CRTL) MEAN =	1.3910	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-0.0994		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.182	0.030	3.969
Within (Error)	21	0.161	0.008	
Total	27	0.343		

Critical F value = 2.57 (0.05,6,21)
Since F > Critical F REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.292	0.922		
2	dilution contrl	1.391	0.970	-1.605	
3	0.38	1.212	0.873	1.281	
4	0.65	1.196	0.860	-1.545	
5	1.4	1.134	0.820	2.542	*
6	2.9	1.308	0.928	-0.263	
7	5.7	1.316	0.932	-0.392	

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	4			
2	dilution contrl	4	0.099	10.7	-0.048
3	0.38	4	0.099	10.7	0.050
4	0.65	4	0.099	10.7	0.062
5	1.4	4	0.099	10.7	0.102
6	2.9	4	0.099	10.7	-0.005
7	5.7	4	0.099	10.7	-0.010

422703-01, thidiazuron, fathead minnow total survival
File: a:42270301.dt3 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.0523	CALCULATED t VALUE =	-0.8945
GRP2 (BLANK CRTL) MEAN =	1.1056	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	-0.0533		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.060	0.010	1.177
Within (Error)	21	0.180	0.009	
Total	27	0.240		

Critical F value = 2.57 (0.05,6,21)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	1.052	0.750		
2	dilution contrl	1.106	0.798	-0.814	
3	0.38	1.011	0.713	0.631	
4	0.65	0.979	0.688	1.122	
5	1.4	0.966	0.675	1.326	
6	2.9	1.049	0.750	0.048	
7	5.7	1.068	0.763	-0.246	

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	4			
2	dilution contrl	4	0.149	19.9	-0.047
3	0.38	4	0.149	19.9	0.038
4	0.65	4	0.149	19.9	0.063
5	1.4	4	0.149	19.9	0.075
6	2.9	4	0.149	19.9	0.000
7	5.7	4	0.149	19.9	-0.012

thidiazuron technical : fathead minnow early life stage

TRT 1 = Solvent control

TRT 2 = Dilution water control

TRT 3 = 0.38 mg/l

TRT 4 = 0.65 mg/l

TRT 5 = 1.4 mg/l

TRT 6 = 2.9 mg/l

TRT 7 = 5.7 mg/l

ANOVA on Weights
LEVELS ENCOUNTERED DURING PROCESSING ARE:
TRT
1.0000 2.0000 3.0000 4.0000 5.0000 6.0000
7.0000
REP
1.0000 2.0000 3.0000 4.0000

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	0.0158	6	0.0026	0.6555	0.6857
REP	0.0168	3	0.0056	1.3877	0.2461
TRT*REP	0.0536	18	0.0030	0.7386	0.7710
ERROR	1.5354	381	0.0040		

LEAST SQUARES MEANS.

			MEAN	SD	(N)	RSD (%)
TRT	=	1.0000	0.2051	0.0601	60	29.3
TRT	=	2.0000	0.2053	0.0738	62	35.9
TRT	=	3.0000	0.2055	0.0598	57	
TRT	=	4.0000	0.2059	0.0784	55	
TRT	=	5.0000	0.2199	0.0614	54	
TRT	=	6.0000	0.2059	0.0540	60	
TRT	=	7.0000	0.1965	0.0510	61	
TRT	=	1.0000				
REP	=	1.0000	0.2209	0.0815	14	36.9
TRT	=	1.0000				
REP	=	2.0000	0.2015	0.0625	13	31.0
TRT	=	1.0000				
REP	=	3.0000	0.1974	0.0486	16	24.6
TRT	=	1.0000				
REP	=	4.0000	0.2005	0.0498	17	24.8
TRT	=	2.0000				
REP	=	1.0000	0.1958	0.0876	17	44.7 **
TRT	=	2.0000				
REP	=	2.0000	0.2177	0.0671	16	30.8

TRT	=	2.0000				
REP	=	3.0000	0.2186	0.0719	15	32.9
TRT	=	2.0000				
REP	=	4.0000	0.1892	0.0675	14	35.7
TRT	=	3.0000				
REP	=	1.0000	0.2066	0.0690	12	
TRT	=	3.0000				
REP	=	2.0000	0.2108	0.0773	13	
TRT	=	3.0000				
REP	=	3.0000	0.1941	0.0459	17	
TRT	=	3.0000				
REP	=	4.0000	0.2105	0.0531	15	
TRT	=	4.0000				
REP	=	1.0000	0.1891	0.0688	14	
TRT	=	4.0000				
REP	=	2.0000	0.2047	0.0526	15	
TRT	=	4.0000				
REP	=	3.0000	0.2062	0.0933	12	
TRT	=	4.0000				
REP	=	4.0000	0.2236	0.0991	14	
TRT	=	5.0000				
REP	=	1.0000	0.2454	0.0731	15	
TRT	=	5.0000				
REP	=	2.0000	0.2327	0.0431	13	
TRT	=	5.0000				
REP	=	3.0000	0.1879	0.0632	14	
TRT	=	5.0000				
REP	=	4.0000	0.2136	0.0467	12	
TRT	=	6.0000				
REP	=	1.0000	0.2156	0.0523	16	
TRT	=	6.0000				
REP	=	2.0000	0.2063	0.0564	14	
TRT	=	6.0000				
REP	=	3.0000	0.1927	0.0630	16	
TRT	=	6.0000				
REP	=	4.0000	0.2089	0.0443	14	
TRT	=	7.0000				
REP	=	1.0000	0.2041	0.0635	16	
TRT	=	7.0000				
REP	=	2.0000	0.2175	0.0469	12	
TRT	=	7.0000				
REP	=	3.0000	0.1799	0.0337	17	
TRT	=	7.0000				
REP	=	4.0000	0.1844	0.0519	16	

BONFERRONI ADJUSTMENT.
MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	1.0000	1.0000			
3	1.0000	1.0000	1.0000		
4	1.0000	1.0000	1.0000	1.0000	
5	1.0000	1.0000	1.0000	1.0000	1.0000
6	1.0000	1.0000	1.0000	1.0000	1.0000
7	1.0000	1.0000	1.0000	1.0000	1.0000

	6	7
6	1.0000	
7	1.0000	1.0000

thidiazuron technical : fathead minnow early life stage

ANOVA on Lengths
LEVELS ENCOUNTERED DURING PROCESSING ARE:
TRT

1.0000	2.0000	3.0000	4.0000	5.0000	6.0000
7.0000					

REP

1.0000	2.0000	3.0000	4.0000

ANALYSIS OF VARIANCE

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
TRT	38.1412	6	6.3569	1.1628	0.3255
REP	20.6280	3	6.8760	1.2577	0.2886
TRT*REP	58.1869	18	3.2326	0.5913	0.9060
ERROR	2082.8932	381	5.4669		

LEAST SQUARES MEANS.

			MEAN	SD	(N)
TRT	=	1.0000	22.4587	2.2885	60
TRT	=	2.0000	22.6160	2.6258	62
TRT	=	3.0000	22.7879	2.1054	57
TRT	=	4.0000	22.6496	2.7595	55
TRT	=	5.0000	23.0441	2.2946	54
TRT	=	6.0000	22.5931	2.0676	60
TRT	=	7.0000	21.9611	2.0050	61
TRT	=	1.0000			
REP	=	1.0000	22.5298	3.0576	14
TRT	=	1.0000			
REP	=	2.0000	22.6075	2.2426	13
TRT	=	1.0000			
REP	=	3.0000	22.5088	1.8187	16
TRT	=	1.0000			
REP	=	4.0000	22.1887	2.1776	17
TRT	=	2.0000			
REP	=	1.0000	22.0648	3.0042	17
TRT	=	2.0000			
REP	=	2.0000	22.9918	2.6000	16
TRT	=	2.0000			
REP	=	3.0000	22.9643	2.7933	15
TRT	=	2.0000			
REP	=	4.0000	22.4432	2.0732	14
TRT	=	3.0000			
REP	=	1.0000	22.8907	2.5379	12
TRT	=	3.0000			
REP	=	2.0000	22.8405	2.5025	13
TRT	=	3.0000			
REP	=	3.0000	22.5923	1.7668	17

TRT	=	3.0000			
REP	=	4.0000	22.8280	1.9170	15
TRT	=	4.0000			
REP	=	1.0000	22.0370	2.7325	14
TRT	=	4.0000			
REP	=	2.0000	22.8794	1.7745	15
TRT	=	4.0000			
REP	=	3.0000	22.6062	3.0665	12
TRT	=	4.0000			
REP	=	4.0000	23.0760	3.4862	14
TRT	=	5.0000			
REP	=	1.0000	23.8779	2.9338	15
TRT	=	5.0000			
REP	=	2.0000	23.4065	1.4435	13
TRT	=	5.0000			
REP	=	3.0000	22.0116	2.2824	14
TRT	=	5.0000			
REP	=	4.0000	22.8805	1.8698	12
TRT	=	6.0000			
REP	=	1.0000	22.8492	1.6731	16
TRT	=	6.0000			
REP	=	2.0000	22.7218	2.2544	14
TRT	=	6.0000			
REP	=	3.0000	22.0999	2.7108	16
TRT	=	6.0000			
REP	=	4.0000	22.7014	1.4833	14
TRT	=	7.0000			
REP	=	1.0000	22.0665	2.0729	16
TRT	=	7.0000			
REP	=	2.0000	23.0216	1.7945	12
TRT	=	7.0000			
REP	=	3.0000	21.2545	1.6058	17
TRT	=	7.0000			
REP	=	4.0000	21.5016	2.2348	16

BONFERRONI ADJUSTMENT.
MATRIX OF PAIRWISE COMPARISON PROBABILITIES:

	1	2	3	4	5
1	1.0000				
2	1.0000	1.0000			
3	1.0000	1.0000	1.0000		
4	1.0000	1.0000	1.0000	1.0000	
5	1.0000	1.0000	1.0000	1.0000	1.0000
6	1.0000	1.0000	1.0000	1.0000	1.0000
7	1.0000	1.0000	1.0000	1.0000	0.2978

	6	7

6	1.0000	
7	1.0000	1.0000

Raw data

PRINTOUT # 10

TITLE: 422703-01, thidiazuron, fathead minnow hatchability
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.8000	1.1071
1	solvent control	2	0.7000	0.9912
1	solvent control	3	0.8500	1.1731
1	solvent control	4	0.9000	1.2490
2	dilution contrl	1	0.9000	1.2490
2	dilution contrl	2	0.8000	1.1071
2	dilution contrl	3	0.8000	1.1071
2	dilution contrl	4	0.8000	1.1071
3	0.38	1	0.7500	1.0472
3	0.38	2	0.7500	1.0472
3	0.38	3	0.9000	1.2490
3	0.38	4	0.8500	1.1731
4	0.65	1	0.8000	1.1071
4	0.65	2	0.8500	1.1731
4	0.65	3	0.8000	1.1071
4	0.65	4	0.7500	1.0472
5	1.4	1	0.9000	1.2490
5	1.4	2	0.7500	1.0472
5	1.4	3	0.9000	1.2490
5	1.4	4	0.7500	1.0472
6	2.9	1	0.9000	1.2490
6	2.9	2	0.7000	0.9912
6	2.9	3	0.8500	1.1731
6	2.9	4	0.8000	1.1071
7	5.7	1	0.9000	1.2490
7	5.7	2	0.6000	0.8861
7	5.7	3	0.9500	1.3453
7	5.7	4	0.8500	1.1731

TITLE: 422703-01, thidiazuron, fathead minnow fry survival
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.8800	1.2171
1	solvent control	2	0.9300	1.3030
1	solvent control	3	0.9400	1.3233
1	solvent control	4	0.9400	1.3233
2	dilution contrl	1	0.9400	1.3233
2	dilution contrl	2	1.0000	1.4588
2	dilution contrl	3	0.9400	1.3233
2	dilution contrl	4	1.0000	1.4588
3	0.38	1	0.8000	1.1071
3	0.38	2	0.8700	1.2019
3	0.38	3	0.9400	1.3233
3	0.38	4	0.8800	1.2171
4	0.65	1	0.8800	1.2171
4	0.65	2	0.8800	1.2171
4	0.65	3	0.7500	1.0472
4	0.65	4	0.9300	1.3030
5	1.4	1	0.8300	1.1458
5	1.4	2	0.8700	1.2019
5	1.4	3	0.7800	1.0826
5	1.4	4	0.8000	1.1071
6	2.9	1	0.8900	1.2327
6	2.9	2	1.0000	1.4588
6	2.9	3	0.9400	1.3233
6	2.9	4	0.8800	1.2171
7	5.7	1	0.8900	1.2327
7	5.7	2	1.0000	1.4588
7	5.7	3	0.9000	1.2490
7	5.7	4	0.9400	1.3233

TITLE: 422703-01, thidiazuron, fathead minnow total survival
FILE: a:42270301.dt3
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.7000	0.9912
1	solvent control	2	0.6500	0.9377
1	solvent control	3	0.8000	1.1071
1	solvent control	4	0.8500	1.1731
2	dilution contrl	1	0.8500	1.1731
2	dilution contrl	2	0.8000	1.1071
2	dilution contrl	3	0.7500	1.0472
2	dilution contrl	4	0.7900	1.0948
3	0.38	1	0.6000	0.8861
3	0.38	2	0.6500	0.9377
3	0.38	3	0.8500	1.1731
3	0.38	4	0.7500	1.0472
4	0.65	1	0.7000	0.9912
4	0.65	2	0.7500	1.0472
4	0.65	3	0.6000	0.8861
4	0.65	4	0.7000	0.9912
5	1.4	1	0.7500	1.0472
5	1.4	2	0.6500	0.9377
5	1.4	3	0.7000	0.9912
5	1.4	4	0.6000	0.8861
6	2.9	1	0.8000	1.1071
6	2.9	2	0.7000	0.9912
6	2.9	3	0.8000	1.1071
6	2.9	4	0.7000	0.9912
7	5.7	1	0.8000	1.1071
7	5.7	2	0.6000	0.8861
7	5.7	3	0.8500	1.1731
7	5.7	4	0.8000	1.1071

		TRT	REP	LENGTH	WEIGHT
CASE	1	1.0000	1.0000	26.1518	0.2410
CASE	2	1.0000	1.0000	27.3945	0.1940
CASE	3	1.0000	1.0000	23.4904	0.3150
CASE	4	1.0000	1.0000	26.2492	0.2370
CASE	5	1.0000	1.0000	21.6107	0.3510
CASE	6	1.0000	1.0000	23.9261	0.3160
CASE	7	1.0000	1.0000	20.9816	0.2280
CASE	8	1.0000	1.0000	23.6467	0.2120
CASE	9	1.0000	1.0000	23.5577	0.1160
CASE	10	1.0000	1.0000	18.6194	0.3100
CASE	11	1.0000	1.0000	19.5862	0.1710
CASE	12	1.0000	1.0000	20.9816	0.1360
CASE	13	1.0000	1.0000	16.5198	0.1870
CASE	14	1.0000	1.0000	22.7016	0.0790
CASE	15	1.0000	2.0000	21.3748	0.2130
CASE	16	1.0000	2.0000	23.0619	0.1890
CASE	17	1.0000	2.0000	27.1600	0.1990
CASE	18	1.0000	2.0000	25.9335	0.2030
CASE	19	1.0000	2.0000	23.9787	0.3350
CASE	20	1.0000	2.0000	21.7352	0.1840
CASE	21	1.0000	2.0000	23.5165	0.1770
CASE	22	1.0000	2.0000	21.5017	0.3070
CASE	23	1.0000	2.0000	23.2690	0.1520
CASE	24	1.0000	2.0000	22.3676	0.1780
CASE	25	1.0000	2.0000	20.8426	0.2350
CASE	26	1.0000	2.0000	19.2724	0.1390
CASE	27	1.0000	2.0000	19.8841	0.1080
CASE	28	1.0000	3.0000	22.3217	0.1480
CASE	29	1.0000	3.0000	24.1478	0.2230
CASE	30	1.0000	3.0000	23.2690	0.2750
CASE	31	1.0000	3.0000	21.9334	0.2620

CASE	32	1.0000	3.0000	19.8942	0.2680
CASE	33	1.0000	3.0000	24.0315	0.1940
CASE	34	1.0000	3.0000	24.9637	0.2250
CASE	35	1.0000	3.0000	25.1185	0.1900
CASE	36	1.0000	3.0000	22.4192	0.1340
CASE	37	1.0000	3.0000	21.4359	0.2190
CASE	38	1.0000	3.0000	21.4355	0.2090
CASE	39	1.0000	3.0000	23.1301	0.1970
CASE	40	1.0000	3.0000	23.2124	0.1710
CASE	41	1.0000	3.0000	23.3456	0.1750
CASE	42	1.0000	3.0000	21.2877	0.1710
CASE	43	1.0000	3.0000	18.1952	0.0980
CASE	44	1.0000	4.0000	24.4010	0.1490
CASE	45	1.0000	4.0000	25.2481	0.1850
CASE	46	1.0000	4.0000	24.4961	0.2340
CASE	47	1.0000	4.0000	22.4570	0.1940
CASE	48	1.0000	4.0000	22.6617	0.2460
CASE	49	1.0000	4.0000	24.1749	0.2380
CASE	50	1.0000	4.0000	24.5439	0.2420
CASE	51	1.0000	4.0000	22.8770	0.1710
CASE	52	1.0000	4.0000	21.3566	0.1130
CASE	53	1.0000	4.0000	21.2063	0.3000
CASE	54	1.0000	4.0000	20.1888	0.1680
CASE	55	1.0000	4.0000	23.5196	0.2520
CASE	56	1.0000	4.0000	21.8622	0.2010
CASE	57	1.0000	4.0000	21.2296	0.2160
CASE	58	1.0000	4.0000	17.2857	0.1560
CASE	59	1.0000	4.0000	20.9882	0.1250
CASE	60	1.0000	4.0000	18.7111	0.2190
CASE	61	2.0000	1.0000	23.5165	0.1480
CASE	62	2.0000	1.0000	24.7848	0.2180
CASE	63	2.0000	1.0000	27.3381	0.1150
CASE	64	2.0000	1.0000	27.6489	0.1570
CASE	65	2.0000	1.0000	22.1426	0.1960
CASE	66	2.0000	1.0000	23.1354	0.1120
CASE	67	2.0000	1.0000	23.6794	0.1470
CASE	68	2.0000	1.0000	22.3705	0.2210
CASE	69	2.0000	1.0000	21.7556	0.4190
CASE	70	2.0000	1.0000	24.6017	0.2350
CASE	71	2.0000	1.0000	20.0833	0.1760
CASE	72	2.0000	1.0000	19.6212	0.1050
CASE	73	2.0000	1.0000	18.7094	0.3370
CASE	74	2.0000	1.0000	17.6669	0.1330
CASE	75	2.0000	1.0000	20.4613	0.2640
CASE	76	2.0000	1.0000	19.5185	0.0940
CASE	77	2.0000	1.0000	18.0682	0.2520
CASE	78	2.0000	2.0000	18.3995	0.2460
CASE	79	2.0000	2.0000	27.3035	0.3080
CASE	80	2.0000	2.0000	24.5675	0.3200
CASE	81	2.0000	2.0000	20.5873	0.2390
CASE	82	2.0000	2.0000	21.1713	0.1470
CASE	83	2.0000	2.0000	23.4422	0.1020
CASE	84	2.0000	2.0000	24.6578	0.2540
CASE	85	2.0000	2.0000	26.3155	0.3280
CASE	86	2.0000	2.0000	23.3871	0.1560
CASE	87	2.0000	2.0000	26.1224	0.1600
CASE	88	2.0000	2.0000	23.1391	0.1860
CASE	89	2.0000	2.0000	25.0364	0.2120
CASE	90	2.0000	2.0000	21.7333	0.2600
CASE	91	2.0000	2.0000	22.1951	0.1800
CASE	92	2.0000	2.0000	20.4921	0.2320
CASE	93	2.0000	2.0000	19.3188	0.1530
CASE	94	2.0000	3.0000	24.4852	0.3070
CASE	95	2.0000	3.0000	23.2850	0.2580
CASE	96	2.0000	3.0000	25.9073	0.2960
CASE	97	2.0000	3.0000	22.5460	0.2240
CASE	98	2.0000	3.0000	25.1635	0.2230
CASE	99	2.0000	3.0000	24.1548	0.2210
CASE	100	2.0000	3.0000	22.0040	0.3360

CASE	101	2.0000	3.0000	18.3446	0.2150
CASE	102	2.0000	3.0000	24.6233	0.2730
CASE	103	2.0000	3.0000	27.1145	0.1130
CASE	104	2.0000	3.0000	25.0567	0.2070
CASE	105	2.0000	3.0000	18.8363	0.1910
CASE	106	2.0000	3.0000	22.6343	0.2100
CASE	107	2.0000	3.0000	22.6214	0.1260
CASE	108	2.0000	3.0000	17.6874	0.0790
CASE	109	2.0000	4.0000	26.3441	0.2060
CASE	110	2.0000	4.0000	22.8812	0.1780
CASE	111	2.0000	4.0000	25.1185	0.3060
CASE	112	2.0000	4.0000	24.4218	0.2180
CASE	113	2.0000	4.0000	22.6443	0.1240
CASE	114	2.0000	4.0000	23.7744	0.1750
CASE	115	2.0000	4.0000	22.3849	0.2870
CASE	116	2.0000	4.0000	22.6246	0.2860
CASE	117	2.0000	4.0000	20.7945	0.1960
CASE	118	2.0000	4.0000	19.3510	0.1820
CASE	119	2.0000	4.0000	22.5033	0.0940
CASE	120	2.0000	4.0000	21.9419	0.1030
CASE	121	2.0000	4.0000	19.8942	0.1680
CASE	122	2.0000	4.0000	19.5255	0.1260
CASE	123	3.0000	1.0000	22.7840	0.1730
CASE	124	3.0000	1.0000	27.3532	0.2210
CASE	125	3.0000	1.0000	20.8484	0.3550
CASE	126	3.0000	1.0000	27.0742	0.2170
CASE	127	3.0000	1.0000	21.1346	0.2820
CASE	128	3.0000	1.0000	26.0788	0.1660
CASE	129	3.0000	1.0000	21.6107	0.1460
CASE	130	3.0000	1.0000	22.1572	0.2930
CASE	131	3.0000	1.0000	22.9658	0.1470
CASE	132	3.0000	1.0000	21.8825	0.1700
CASE	133	3.0000	1.0000	19.9562	0.1590
CASE	134	3.0000	1.0000	20.8426	0.1500
CASE	135	3.0000	2.0000	28.5493	0.0930
CASE	136	3.0000	2.0000	22.4958	0.1750
CASE	137	3.0000	2.0000	23.6920	0.1410
CASE	138	3.0000	2.0000	22.5230	0.2650
CASE	139	3.0000	2.0000	23.7421	0.1860
CASE	140	3.0000	2.0000	24.5922	0.1600
CASE	141	3.0000	2.0000	22.3705	0.4120
CASE	142	3.0000	2.0000	23.3349	0.1660
CASE	143	3.0000	2.0000	23.8750	0.2380
CASE	144	3.0000	2.0000	22.4943	0.2250
CASE	145	3.0000	2.0000	20.6072	0.2170
CASE	146	3.0000	2.0000	21.1102	0.2060
CASE	147	3.0000	2.0000	17.5398	0.2560
CASE	148	3.0000	3.0000	19.4546	0.2180
CASE	149	3.0000	3.0000	24.1495	0.2180
CASE	150	3.0000	3.0000	25.0364	0.2280
CASE	151	3.0000	3.0000	22.2311	0.2460
CASE	152	3.0000	3.0000	24.9297	0.2290
CASE	153	3.0000	3.0000	23.1354	0.1950
CASE	154	3.0000	3.0000	23.7666	0.1300
CASE	155	3.0000	3.0000	24.0271	0.1870
CASE	156	3.0000	3.0000	24.8873	0.2850
CASE	157	3.0000	3.0000	21.6846	0.1750
CASE	158	3.0000	3.0000	22.1310	0.1680
CASE	159	3.0000	3.0000	23.5114	0.1460
CASE	160	3.0000	3.0000	21.2691	0.1450
CASE	161	3.0000	3.0000	20.3184	0.1060
CASE	162	3.0000	3.0000	19.9591	0.1810
CASE	163	3.0000	3.0000	21.9919	0.2210
CASE	164	3.0000	3.0000	21.5860	0.2210
CASE	165	3.0000	4.0000	25.0519	0.2670
CASE	166	3.0000	4.0000	26.0252	0.2940
CASE	167	3.0000	4.0000	25.9860	0.2210
CASE	168	3.0000	4.0000	24.7166	0.2310
CASE	169	3.0000	4.0000	23.0451	0.1750

CASE	170	3.0000	4.0000	23.8953	0.1400
CASE	171	3.0000	4.0000	22.0579	0.2850
CASE	172	3.0000	4.0000	21.2296	0.1860
CASE	173	3.0000	4.0000	21.7834	0.2110
CASE	174	3.0000	4.0000	22.3402	0.2010
CASE	175	3.0000	4.0000	22.1134	0.1540
CASE	176	3.0000	4.0000	21.1102	0.1960
CASE	177	3.0000	4.0000	22.6104	0.1370
CASE	178	3.0000	4.0000	20.0797	0.2880
CASE	179	3.0000	4.0000	20.3755	0.1710
CASE	180	4.0000	1.0000	26.6049	0.2650
CASE	181	4.0000	1.0000	25.6869	0.1890
CASE	182	4.0000	1.0000	25.2173	0.2300
CASE	183	4.0000	1.0000	24.0709	0.1700
CASE	184	4.0000	1.0000	23.7927	0.2810
CASE	185	4.0000	1.0000	21.9992	0.1280
CASE	186	4.0000	1.0000	20.7249	0.2580
CASE	187	4.0000	1.0000	21.0247	0.1900
CASE	188	4.0000	1.0000	22.3676	0.1090
CASE	189	4.0000	1.0000	21.7378	0.3070
CASE	190	4.0000	1.0000	19.6212	0.1410
CASE	191	4.0000	1.0000	18.1960	0.0940
CASE	192	4.0000	1.0000	19.0698	0.1630
CASE	193	4.0000	1.0000	18.4034	0.1220
CASE	194	4.0000	2.0000	23.3646	0.3120
CASE	195	4.0000	2.0000	24.6777	0.1810
CASE	196	4.0000	2.0000	25.6728	0.1990
CASE	197	4.0000	2.0000	22.5173	0.1530
CASE	198	4.0000	2.0000	24.4337	0.1980
CASE	199	4.0000	2.0000	24.5922	0.1450
CASE	200	4.0000	2.0000	21.6887	0.2220
CASE	201	4.0000	2.0000	23.9332	0.2580
CASE	202	4.0000	2.0000	23.6467	0.2320
CASE	203	4.0000	2.0000	21.2979	0.1610
CASE	204	4.0000	2.0000	20.8890	0.1920
CASE	205	4.0000	2.0000	21.3540	0.2880
CASE	206	4.0000	2.0000	23.1291	0.1250
CASE	207	4.0000	2.0000	23.0311	0.2260
CASE	208	4.0000	2.0000	18.9632	0.1790
CASE	209	4.0000	3.0000	28.5970	0.1660
CASE	210	4.0000	3.0000	25.7739	0.0780
CASE	211	4.0000	3.0000	24.7803	0.2640
CASE	212	4.0000	3.0000	25.3107	0.2310
CASE	213	4.0000	3.0000	21.7125	0.2310
CASE	214	4.0000	3.0000	18.2373	0.1420
CASE	215	4.0000	3.0000	23.8507	0.4280
CASE	216	4.0000	3.0000	21.9213	0.1600
CASE	217	4.0000	3.0000	20.8979	0.1970
CASE	218	4.0000	3.0000	20.9539	0.1530
CASE	219	4.0000	3.0000	19.4239	0.2980
CASE	220	4.0000	3.0000	19.8145	0.1260
CASE	221	4.0000	4.0000	20.7622	0.4530
CASE	222	4.0000	4.0000	29.7451	0.1910
CASE	223	4.0000	4.0000	23.3958	0.2960
CASE	224	4.0000	4.0000	26.4160	0.2870
CASE	225	4.0000	4.0000	25.7168	0.1570
CASE	226	4.0000	4.0000	24.7385	0.1700
CASE	227	4.0000	4.0000	26.4746	0.1370
CASE	228	4.0000	4.0000	21.4780	0.3070
CASE	229	4.0000	4.0000	25.2093	0.2820
CASE	230	4.0000	4.0000	21.8722	0.2700
CASE	231	4.0000	4.0000	19.6266	0.1180
CASE	232	4.0000	4.0000	21.2543	0.0720
CASE	233	4.0000	4.0000	19.6097	0.2240
CASE	234	4.0000	4.0000	16.7644	0.1660
CASE	235	5.0000	1.0000	26.9571	0.2530
CASE	236	5.0000	1.0000	27.2268	0.1930
CASE	237	5.0000	1.0000	23.5086	0.2850
CASE	238	5.0000	1.0000	25.4253	0.3070

CASE	239	5.0000	1.0000	23.1720	0.3400
CASE	240	5.0000	1.0000	26.0428	0.2020
CASE	241	5.0000	1.0000	23.5659	0.2770
CASE	242	5.0000	1.0000	27.8865	0.2400
CASE	243	5.0000	1.0000	25.3289	0.3630
CASE	244	5.0000	1.0000	24.7979	0.3130
CASE	245	5.0000	1.0000	21.2269	0.2300
CASE	246	5.0000	1.0000	22.8770	0.2060
CASE	247	5.0000	1.0000	23.5083	0.0880
CASE	248	5.0000	1.0000	19.5639	0.1470
CASE	249	5.0000	1.0000	17.0812	0.2370
CASE	250	5.0000	2.0000	23.1580	0.2440
CASE	251	5.0000	2.0000	25.2173	0.1930
CASE	252	5.0000	2.0000	22.7996	0.2620
CASE	253	5.0000	2.0000	25.7246	0.3140
CASE	254	5.0000	2.0000	25.0799	0.1950
CASE	255	5.0000	2.0000	22.0040	0.2560
CASE	256	5.0000	2.0000	23.6549	0.2530
CASE	257	5.0000	2.0000	24.5379	0.2320
CASE	258	5.0000	2.0000	24.0949	0.2210
CASE	259	5.0000	2.0000	22.9872	0.2960
CASE	260	5.0000	2.0000	21.4370	0.1780
CASE	261	5.0000	2.0000	22.0040	0.1860
CASE	262	5.0000	2.0000	21.5857	0.1950
CASE	263	5.0000	3.0000	25.4202	0.1360
CASE	264	5.0000	3.0000	26.6124	0.1180
CASE	265	5.0000	3.0000	22.7542	0.1890
CASE	266	5.0000	3.0000	22.4379	0.1430
CASE	267	5.0000	3.0000	24.2736	0.3340
CASE	268	5.0000	3.0000	21.2979	0.1190
CASE	269	5.0000	3.0000	23.0251	0.2940
CASE	270	5.0000	3.0000	20.2264	0.1950
CASE	271	5.0000	3.0000	22.2580	0.1520
CASE	272	5.0000	3.0000	18.8440	0.2240
CASE	273	5.0000	3.0000	20.9412	0.1720
CASE	274	5.0000	3.0000	20.8979	0.2090
CASE	275	5.0000	3.0000	19.3414	0.1970
CASE	276	5.0000	3.0000	19.8320	0.1480
CASE	277	5.0000	4.0000	25.1789	0.2330
CASE	278	5.0000	4.0000	22.1845	0.2210
CASE	279	5.0000	4.0000	23.5604	0.2250
CASE	280	5.0000	4.0000	23.8029	0.2310
CASE	281	5.0000	4.0000	22.3402	0.1830
CASE	282	5.0000	4.0000	18.7167	0.2820
CASE	283	5.0000	4.0000	23.5882	0.1150
CASE	284	5.0000	4.0000	24.9970	0.2260
CASE	285	5.0000	4.0000	22.7016	0.1560
CASE	286	5.0000	4.0000	24.1946	0.1900
CASE	287	5.0000	4.0000	23.0616	0.2280
CASE	288	5.0000	4.0000	20.2391	0.2730
CASE	289	6.0000	1.0000	25.4431	0.2370
CASE	290	6.0000	1.0000	24.4168	0.3080
CASE	291	6.0000	1.0000	24.6866	0.1880
CASE	292	6.0000	1.0000	24.9637	0.1450
CASE	293	6.0000	1.0000	22.7368	0.2390
CASE	294	6.0000	1.0000	21.5411	0.2550
CASE	295	6.0000	1.0000	21.6130	0.2340
CASE	296	6.0000	1.0000	24.1495	0.2050
CASE	297	6.0000	1.0000	23.5110	0.1450
CASE	298	6.0000	1.0000	23.4904	0.1600
CASE	299	6.0000	1.0000	21.1942	0.2980
CASE	300	6.0000	1.0000	21.4791	0.1870
CASE	301	6.0000	1.0000	20.4151	0.1800
CASE	302	6.0000	1.0000	20.0209	0.2160
CASE	303	6.0000	1.0000	22.4152	0.2840
CASE	304	6.0000	1.0000	23.5110	0.1690
CASE	305	6.0000	2.0000	26.0902	0.2620
CASE	306	6.0000	2.0000	22.1631	0.3030
CASE	307	6.0000	2.0000	22.3054	0.1580

CASE	308	6.0000	2.0000	24.9090	0.1700
CASE	309	6.0000	2.0000	21.3883	0.1920
CASE	310	6.0000	2.0000	23.2082	0.2380
CASE	311	6.0000	2.0000	22.5230	0.2670
CASE	312	6.0000	2.0000	24.0116	0.2410
CASE	313	6.0000	2.0000	20.6652	0.2010
CASE	314	6.0000	2.0000	25.7029	0.2290
CASE	315	6.0000	2.0000	22.9549	0.1290
CASE	316	6.0000	2.0000	19.3376	0.2190
CASE	317	6.0000	2.0000	18.4446	0.1820
CASE	318	6.0000	2.0000	24.4006	0.0970
CASE	319	6.0000	3.0000	27.2111	0.1700
CASE	320	6.0000	3.0000	22.8770	0.3010
CASE	321	6.0000	3.0000	22.0919	0.3040
CASE	322	6.0000	3.0000	24.1946	0.2450
CASE	323	6.0000	3.0000	23.2721	0.2380
CASE	324	6.0000	3.0000	23.3819	0.1620
CASE	325	6.0000	3.0000	23.0027	0.2240
CASE	326	6.0000	3.0000	22.2580	0.1910
CASE	327	6.0000	3.0000	21.7619	0.1690
CASE	328	6.0000	3.0000	26.7505	0.2150
CASE	329	6.0000	3.0000	21.1151	0.1850
CASE	330	6.0000	3.0000	20.2965	0.2070
CASE	331	6.0000	3.0000	19.3577	0.1730
CASE	332	6.0000	3.0000	20.7712	0.0880
CASE	333	6.0000	3.0000	17.6142	0.1170
CASE	334	6.0000	3.0000	17.6413	0.0940
CASE	335	6.0000	4.0000	20.8705	0.2500
CASE	336	6.0000	4.0000	21.1025	0.2390
CASE	337	6.0000	4.0000	24.3387	0.2560
CASE	338	6.0000	4.0000	23.7652	0.1950
CASE	339	6.0000	4.0000	24.9090	0.2500
CASE	340	6.0000	4.0000	22.8282	0.1770
CASE	341	6.0000	4.0000	21.6678	0.1330
CASE	342	6.0000	4.0000	23.2791	0.1460
CASE	343	6.0000	4.0000	24.5488	0.2550
CASE	344	6.0000	4.0000	22.2434	0.1540
CASE	345	6.0000	4.0000	23.2582	0.2150
CASE	346	6.0000	4.0000	23.2624	0.2410
CASE	347	6.0000	4.0000	21.7834	0.2380
CASE	348	6.0000	4.0000	19.9627	0.1760
CASE	349	7.0000	1.0000	20.3339	0.1850
CASE	350	7.0000	1.0000	21.3521	0.1570
CASE	351	7.0000	1.0000	25.5599	0.1410
CASE	352	7.0000	1.0000	24.3227	0.2920
CASE	353	7.0000	1.0000	20.7155	0.1670
CASE	354	7.0000	1.0000	22.3817	0.1430
CASE	355	7.0000	1.0000	25.5760	0.1760
CASE	356	7.0000	1.0000	22.8152	0.1290
CASE	357	7.0000	1.0000	24.7558	0.2860
CASE	358	7.0000	1.0000	22.2347	0.1800
CASE	359	7.0000	1.0000	21.7586	0.2000
CASE	360	7.0000	1.0000	21.3566	0.1630
CASE	361	7.0000	1.0000	21.0830	0.3090
CASE	362	7.0000	1.0000	20.0559	0.3170
CASE	363	7.0000	1.0000	20.2642	0.1810
CASE	364	7.0000	1.0000	18.4989	0.2400
CASE	365	7.0000	2.0000	24.8993	0.2060
CASE	366	7.0000	2.0000	21.8622	0.1800
CASE	367	7.0000	2.0000	25.5457	0.1790
CASE	368	7.0000	2.0000	24.5711	0.2650
CASE	369	7.0000	2.0000	21.1346	0.3000
CASE	370	7.0000	2.0000	24.0207	0.2570
CASE	371	7.0000	2.0000	24.4125	0.2240
CASE	372	7.0000	2.0000	22.7574	0.2420
CASE	373	7.0000	2.0000	23.7149	0.1840
CASE	374	7.0000	2.0000	22.0388	0.1490
CASE	375	7.0000	2.0000	19.5255	0.1670
CASE	376	7.0000	2.0000	21.7767	0.2570

CASE	377	7.0000	3.0000	23.0311	0.1900
CASE	378	7.0000	3.0000	21.6070	0.2100
CASE	379	7.0000	3.0000	19.0736	0.2130
CASE	380	7.0000	3.0000	20.5884	0.2210
CASE	381	7.0000	3.0000	22.3217	0.1890
CASE	382	7.0000	3.0000	22.2696	0.1670
CASE	383	7.0000	3.0000	22.6343	0.2170
CASE	384	7.0000	3.0000	22.7126	0.1320
CASE	385	7.0000	3.0000	23.3594	0.2090
CASE	386	7.0000	3.0000	23.1991	0.1210
CASE	387	7.0000	3.0000	20.0897	0.1880
CASE	388	7.0000	3.0000	19.8516	0.1500
CASE	389	7.0000	3.0000	18.4989	0.2180
CASE	390	7.0000	3.0000	21.8800	0.1850
CASE	391	7.0000	3.0000	20.9966	0.1290
CASE	392	7.0000	3.0000	20.4874	0.1730
CASE	393	7.0000	3.0000	18.7249	0.1470
CASE	394	7.0000	4.0000	21.9864	0.2470
CASE	395	7.0000	4.0000	22.8763	0.2260
CASE	396	7.0000	4.0000	22.6218	0.1850
CASE	397	7.0000	4.0000	24.5780	0.2140
CASE	398	7.0000	4.0000	24.8759	0.1390
CASE	399	7.0000	4.0000	22.8883	0.1810
CASE	400	7.0000	4.0000	19.3748	0.2690
CASE	401	7.0000	4.0000	20.2084	0.1510
CASE	402	7.0000	4.0000	23.0311	0.2020
CASE	403	7.0000	4.0000	24.0553	0.2700
CASE	404	7.0000	4.0000	19.4521	0.1360
CASE	405	7.0000	4.0000	21.1140	0.1330
CASE	406	7.0000	4.0000	20.9662	0.1980
CASE	407	7.0000	4.0000	19.6998	0.1720
CASE	408	7.0000	4.0000	19.4405	0.0970
CASE	409	7.0000	4.0000	16.8566	0.1300

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THE FOLLOWING RESULTS ARE FOR:
TRT = 1.0000

TOTAL OBSERVATIONS: 60

	REP	WEIGHT	LENGTH
N OF CASES	60	60	60
MINIMUM	1.0000	0.0790	16.5198
MAXIMUM	4.0000	0.3510	27.3945
MEAN	2.6000	0.2047	22.4444
STANDARD DEV	1.1379	0.0601	2.2885

THE FOLLOWING RESULTS ARE FOR:
TRT = 2.0000

TOTAL OBSERVATIONS: 62

	REP	WEIGHT	LENGTH
N OF CASES	62	62	62
MINIMUM	1.0000	0.0790	17.6669
MAXIMUM	4.0000	0.4190	27.6489
MEAN	2.4194	0.2055	22.6071
STANDARD DEV	1.1242	0.0738	2.6258

THE FOLLOWING RESULTS ARE FOR:
TRT = 3.0000

TOTAL OBSERVATIONS: 57

	REP	WEIGHT	LENGTH
N OF CASES	57	57	57
MINIMUM	1.0000	0.0930	17.5398
MAXIMUM	4.0000	0.4120	28.5493
MEAN	2.6140	0.2048	22.7738
STANDARD DEV	1.0980	0.0598	2.1054

THE FOLLOWING RESULTS ARE FOR:
TRT = 4.0000

TOTAL OBSERVATIONS: 55

	REP	WEIGHT	LENGTH
N OF CASES	55	55	55
MINIMUM	1.0000	0.0720	16.7644
MAXIMUM	4.0000	0.4530	29.7451
MEAN	2.4727	0.2059	22.6554
STANDARD DEV	1.1362	0.0784	2.7595

THE FOLLOWING RESULTS ARE FOR:
TRT = 5.0000

TOTAL OBSERVATIONS: 54

	REP	WEIGHT	LENGTH
N OF CASES	54	54	54
MINIMUM	1.0000	0.0880	17.0812
MAXIMUM	4.0000	0.3630	27.8865
MEAN	2.4259	0.2204	23.0589
STANDARD DEV	1.1261	0.0614	2.2946

THE FOLLOWING RESULTS ARE FOR:
TRT = 6.0000

TOTAL OBSERVATIONS: 60

	REP	WEIGHT	LENGTH
N OF CASES	60	60	60
MINIMUM	1.0000	0.0880	17.6142
MAXIMUM	4.0000	0.3080	27.2111
MEAN	2.4667	0.2058	22.5852
STANDARD DEV	1.1270	0.0540	2.0676

THE FOLLOWING RESULTS ARE FOR:
TRT = 7.0000

TOTAL OBSERVATIONS: 61

	REP	WEIGHT	LENGTH
N OF CASES	61	61	61
MINIMUM	1.0000	0.0970	16.8566
MAXIMUM	4.0000	0.3170	25.5760
MEAN	2.5410	0.1948	21.8799
STANDARD DEV	1.1485	0.0510	2.0050

SUMMARY STATISTICS FOR REP

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 0.1352 DF= 6 PROBABILITY = 1.0000

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	2.2355	6	0.3726	0.2925	0.9404
WITHIN GROUPS	511.9992	402	1.2736		

SUMMARY STATISTICS FOR WEIGHT

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 17.1269 DF= 6 PROBABILITY = 0.0088

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	0.0189	6	0.0032	0.7886	0.5793
WITHIN GROUPS	1.6061	402	0.0040		

SUMMARY STATISTICS FOR LENGTH

BARTLETT TEST FOR HOMOGENEITY OF GROUP VARIANCES

CHI-SQUARE = 10.4273 DF= 6 PROBABILITY = 0.1078

ANALYSIS OF VARIANCE

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	F	PROBABILITY
BETWEEN GROUPS	45.7362	6	7.6227	1.4177	0.2063
WITHIN GROUPS	2161.4601	402	5.3768		

KOLMOGOROV-SMIRNOV ONE SAMPLE TEST USING STANDARD NORMAL DISTRIBUTION

VARIABLE	N-OF-CASES	MAXDIF	PROBABILITY (2-TAIL)
REP	409.0000	0.8413	0.0000
WEIGHT	409.0000	0.5287	0.0000
LENGTH	409.0000	1.0000	0.0000

DATA EVALUATION RECORD

120301

1. **CHEMICAL:** Thidiazuron. Shaughnessey No.: ~~None listed~~.

2. **TEST MATERIAL:** Thidiazuron, SN 49537; N-phenyl-N'-1,2,3-thiadiazol-5-ylurea; 99.3% active ingredient; Batch No. 7/9.82; a beige colored powder with little lumps.

3. **STUDY TYPE:** Marine Fish Acute Toxicity Test. Species Tested: *Cyprinodon variegatus*.

4. **CITATION:** Schupner, J.K. and B.J. Stachura. 1991. The Static Acute Toxicity of Thidiazuron to the Sheepshead Minnow, *Cyprinodon variegatus*. Performed by NOR-AM Chemical Company, NOR-AM Research Center, Pikeville, North Carolina. Study No. 504AW. Submitted by NOR-AM Chemical Company. EPA MRID No. 418461-01.

5. **REVIEWED BY:**

Alvaro A. Yamhure,
Aquatic Biologist
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature:

Date: 1/6/93

6. **APPROVED BY:**

Dan Rieder,
Head Section 3
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature:

Date: 1-6 93

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a 96-hour static acute toxicity test using marine fish. Given the low solubility of the toxicant (Thidiazuron) in sea water and the low toxicity to the test species - LC_{50}>36 mg/l- (*Cyprinodon variegatus*) **this study is rated as core;** however, in the future we expect the registrant to follow our recommendations on alternative ways of increasing the solubility of test compounds [see Page 4;Section 14(C) of this review]. The NOEL is therefore established as <36 mg/l, the highest measured test concentration used.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Young of the year sheepshead minnows were obtained from Aquatic BioSystems, Inc., Fort Collins, Colorado. The fish were reared in synthetic seawater (17.3-20.5°C) in 200-l fiberglass fish tanks under 16 hours of light (150 footcandles) and were fed salmon started *ad libitum*. The fish were acclimated to test dilution water (synthetic seawater) and test temperature (22 ±1°C) for at least 48 hours prior to test initiation. The fish were not fed during the acclimation period. No mortality was observed during the acclimation period. At the time of test initiation, the fish were 148 days old.

At test termination, the mean weight of the fish was 0.386 ±0.119 g, and the mean standard length was 2.3 ±0.23 cm. The organism loading rate was 0.26 g/l.

B. **Test System:** The test was conducted in 19-l glass fish tanks (24.5 cm high X 39.4 cm long X 20.2 cm wide) with a test solution volume of 15 l and depth of 19 cm. Test vessels were randomly positioned in a water bath and covered with glass sheets to prevent evaporation.

The photoperiod was 16 hours of light (117 foot candles) and 8 hours of darkness, with gradual intensity changes at dawn and dusk.

The diluent was synthetic seawater (Instant Ocean salts and deionized well water) adjusted to a salinity of 17 parts per thousand (ppt) with deionized water.

C. **Dosage:** Ninety-six-hour acute static test. Prior to test initiation, a range finding study was performed using the nominal test concentrations of 0.31, 1.5, 3.1, 15.0, 31.0 mg/l; no mortality was observed.

It was determined before the definitive test that 36 mg/l was the maximum solubility of the test material in seawater at the test temperature. Therefore, based on the results of the range finding test, only one test concentration at the water solubility limit (36 mg/l) was used. A solvent control (0.5 ml solvent/l) and untreated control were also used in this study.

A stock solution (72 mg/ml) in the solvent, dimethylformamide (DMF), was prepared and added to 15 l of diluent in each of triplicate vessels to obtain the nominal concentration of 36 mg/l.

D. **Design:** Ten fish were randomly distributed, by twos, to each test tank. Three tanks (replicates) were used for the treatment and each control. Fish were not fed during the test.

Observations of mortality and abnormal effects were made every 24 hours (±1 hour) after test initiation. Dissolved oxygen concentration, pH, temperature, and salinity were determined in each replicate at test initiation, 48 hours, and at test termination. The temperature in the water bath was monitored continuously.

The concentration of thidiazuron in filtered samples from each test vessel was determined at test initiation and test termination using high pressure liquid chromatography.

E. **Statistics:** Statistical analysis of the data was not necessary.

12. **REPORTED RESULTS:** The mean measured concentrations of the triplicate vessels were 36, 36, and 34 mg/l. (Table 3, attached). This mean measured concentration of 36 mg/l is the solubility of the test material in seawater at 22°C.

The 96-hour LC_{50} was >36 mg/l. During the study, no mortality or sublethal effects were noted at the test concentration or the controls (Table 1, attached).

During the study, the dissolved oxygen concentrations were maintained at 4.7-8.3 mg/l, the salinity was 17-19 ppt, and the pH was 7.6-8.2. The temperature in the water bath was maintained at 21.8-23.0°C (mean 22.5 ±0.3°C), and the temperature in the test vessels was 21.2-22.3°C.

13. **STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Since there was no mortality or abnormal behavior and appearance evident during the course of this study, Thidiazuron may be considered non-toxic under the conditions of this test."

A GLP compliance statement was included in the report indicating that this study was conducted in accordance with

GLP as defined in 40 CFR Part 160. This statement was signed by the Study Director and sponsor representatives.

A Quality Assurance Statement was included and signed by a GLP Quality Assurance Inspector of the preforming laboratory.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated from the SEP or Subdivision E as follows:

The pH (8.2) of the control at test initiation was higher than the pH (7.7-8.0) recommended in the guidelines for a euryhaline fish species.

B. Statistical Analysis: No statistical analysis was necessary.

C. Discussion/Results: The above mentioned deviation probably did not affect the results of the study. However, an accurate LC_{50} was not established by this study. Although the test was conducted with the concentration at the maximum seawater solubility of the test material, it was not clearly demonstrated that techniques to maximize chemical dissolution in the test media had been exhausted. These techniques may include the use of different solvent, minor changes in environmental conditions (e.g., temperature and pH) or using a flow-through system to conduct the test.

Because of the apparent solubility problems with the test material and because the registrant had not been alerted to EEB's policy regarding procedures to be attempted when this type of solubility problems are encountered, this study is rated as scientifically sound and as fulfilling guideline requirements **(core)** for an acute static toxicity test for marine fish. An accurate LC_{50} based on mean measured concentrations could not be determined. The approximate value of the 96-hour LC_{50} was found to be >36 mg/l, which was the reported maximum solubility of the test material in the solvent system used. Based on this LC_{50}, thidiazuron is classified, at most, as moderately toxic to *Cyprinodon variegatus*. The NOEC was 36 mg/l mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, September 24, 1991.

THIDIAZURON

Page_____ is not included in this copy.

Pages 45 through 46 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.

120301

Shaughnessey # ~~NONE GIVEN~~ Chemical Name Thidiazuron Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} - pp (95% C.L.) Control Mortality (%) - Solvent Control Mortality (%) - Slope - # Animals/Level - Temperature - 48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) Comments:	_____	_____
96-Hour LC_{50} Species: Cyprinodon variegatus Lab: NOR-AM MRID # 418461-01	99.3%	LC_{50} - 36 ppm* (95% C.L. NA) Control Mortality (%) - 0 Solvent Control Mortality (%) - 0 Slope - NA # Animals/Level - 30 Temperature - 21.8-23°C 96-Hour Dose Level ppm* /(% Mortality) 36 (0), ( ), ( ), ( ), ( ) Comments: *Based on nominal concentration of test substance solubility. An accurate LC_{50} was not established by this study.	Cary 9/24/91	Supplemental

DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron. 120301
Shaughnessey No. ~~102301~~.

2. **TEST MATERIAL:** Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) technical; Batch No. 7/9.82; 99.3% active ingredient; a beige powder.

3. **STUDY TYPE:** Freshwater Fish Acute Static Toxicity Test. Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).

4. **CITATION:** Schupner, J.K. and B.J. Stachura. 1991. The Acute Toxicity of Thidiazuron Technical to the Bluegill Sunfish, *Lepomis macrochirus*, in a Static System. Laboratory Project ID No. 511AW. Prepared and submitted by NOR-AM Chemical Company, Pikeville, NC. EPA MRID No. 420692-01.

5. **REVIEWED BY:**

Alvaro A. Yamhure,
Aquatic Biologist
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature:
Date: 12/16/92

6. **APPROVED BY:**

Dan Rieder,
Head Section 3
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature: Dan Rieder
Date: 1-6 93

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a 96-hour static acute toxicity test using ~~marine~~ fish. Given the low solubility of the toxicant (Thidiazuron) in sea water and the low toxicity to the test species - LC_{50}>32 mg/(*Lepomis macrochirus*) **this study is rated as core;** however, in the future we expect the registrant to follow our recommendations on alternative ways of increasing the solubility of test compounds [see Page 4;Section 14(C) of this review]. The NOEC to the bluegill sunfish is therefore established at 32 mg/l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Bluegill sunfish (*Lepomis macrochirus*) were obtained from Aquatic Research Organisms in Hampton, NH. The fish (approximately 4 months old) were maintained in well water in a flow-through system and were fed salmon starter *ad libitum*.

Fish were acclimated to the test temperature and dilution water for 73 hours prior to test initiation and feeding was discontinued. No mortality occurred during acclimation. Mean weight and length of control fish taken at test termination were 2.019 ±0.898 g and 40 ±5.4 mm, respectively. Biomass loading rate was 0.301 g/l.

B. **Test System:** Test chambers were 67-liter glass aquaria (75 x 30 x 30 cm), each containing 67 l of test solution. The depth of the solution was 29.7 cm. The aquaria were covered with glass sheets to prevent evaporation and entry of foreign material.

The laboratory environment was maintained on a 16-hour daylight photoperiod (153 foot candles) with a dawn/dusk simulation period. The test aquaria were maintained at 22 ±1°C in a water bath.

Soft blended water (a mixture of well water and deionized well water) was used as dilution water (Appendix II, attached). A 77 mg ai/ml stock solution was prepared in dimethylformamide (DMF). A 33 ml aliquot of the stock was added to 67 liters of dilution water to create a concentration of 38 mg ai/l. The solutions were stirred for 24 hours after preparation.

C. **Dosage:** Based on a range-finding test, one nominal concentration (38 mg ai/l) of thidiazuron technical, a dilution water control and a solvent control (0.5 ml DMF/l) were used.

D. **Design:** Ten bluegill were randomly distributed to each aquarium (three replicate aquaria per concentration or control). Test chambers were randomly positioned in the water bath. Fish were not fed and test solutions were not aerated during the test. Observations of

mortality and sublethal responses were made every 24 hours.

The temperature, dissolved oxygen (D.O.), pH, and specific conductance were measured in all test chambers at 0, 48, and 96 hours. Temperature was also monitored continuously in the water bath.

Thidiazuron concentrations were measured by high performance liquid chromatography from samples taken at test initiation and termination. The samples were filtered (0.45 μm) before the analysis.

E. Statistics: No statistical analyses were performed.

12. REPORTED RESULTS: The mean measured concentration of thidiazuron was 32 mg/l. This value represents 84% of the nominal concentration (Table 3, attached). A precipitate was noted in all test aquaria.

The responses of bluegill are given in Table 1 (attached). The 96-hour LC_{50} was determined to be greater than 32 mg/l. This is the maximum solubility of the test material in soft, blended water at 22.3°C. One mortality occurred but it was not considered to be treatment related. No abnormal behavior was noted during the course of the study.

All dissolved oxygen readings were $\geq$62% of saturation during the first 48 hours and $\geq$48% of saturation throughout the remainder of the study. The pH ranged from 5.9 to 6.9 and the temperature from 21.8 to 23.2°C. Conductivity was between 100 and 120 μmhos/cm.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The authors concluded that the test material may be considered non-toxic under the conditions of the test.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the guidelines with the following exceptions:

Fish were acclimated for a shorter period (73 hours) than recommended (2 weeks).

The fish were added to the test solutions 24 hours after preparation. A time period of no more than 30 minutes is recommended by the SEP.

The temperature varied by more than 1°C over the study period.

B. **Statistical Analysis:** Upon review of the mortality data, the reviewer agrees that the LC_{50} was >32 mg ai/l. The no-observed-effect concentration (NOEC) was 32 mg ai/l.

C. **Conclusions:** The food analysis indicated some detectable mercury, heptachlor epoxide, and delta-BHC. However, these amounts were low and probably did not influence the results. The test type conducted by the authors (i.e., 30 fish, one concentration) applies to a concentration of 100 mg/l or greater.
The only concentration tested (32 mg ai/l) is near the limit of water solubility for thidiazuron under the conditions of this test. However, the authors did not demonstrate whether: 1) techniques to maximize chemical dissolution in the test media had been exhausted (e.g. different solvents, minor changes in environmental conditions, etc.), and 2) testing with a more soluble formulation was available.

The 96-hour LC_{50} of >32 mg ai/l (mean measured concentration) classifies thidiazuron technical as slightly toxic to bluegill sunfish. The NOEC was 32 mg ai/l. Because of the apparent solubility problems with the test material, because the registrant had not been alerted to EEB's policy regarding procedures to be attempted when this type of solubility problems are encountered and because of the slight toxicity of the toxicant to the test species (slight toxicity,by EEB standards for aquatic organisms is >10 -100 ppm),this study is rated as scientifically sound and as fulfilling guideline requirements **(core)** for an acute static toxicity test for fresh water fish. An accurate LC_{50} based on mean measured concentrations could not be determined. The approximate value of the 96-hour LC_{50} was found to be >32 mg/l, which was the reported maximum solubility of the test material in the solvent system used. Based on this LC_{50}, thidiazuron is

classified, at most, as slightly toxic to *Lepomis macrochirus*. The NOEC was 32 mg/l mean measured concentration.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 12-19-91 (and corrected by EEB).

THIDIAZURON

Page_____ is not included in this copy.

Pages 53 through 55 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.

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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.

Shaughnessey # ~~120301~~ 120301 Chemical Name Thidiazuron Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} = pp (95% C.L.) Control Mortality (%) = Solvent Control Mortality (%) = Slope = # Animals/Level = Temperature = 48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) Comments:	_____	_____

96-Hour LC_{50}	99.3%	LC_{50} = >32 mg ai/l* pp (95% C.L. N/A) Control Mortality (%) = 0
		 Solvent Control Mortality (%) = 0
		 Slope = N/A # Animals/Level = 30
		 Temperature = 22°C
		 96-Hour Dose Level mg ai/l* pp /(% Mortality)
		 32 (3), ( ), ( ), ( ), ( )
		 Comments: NOEC = 32 mg ai/l*
		 * Based on mean measured conc. of ai.
			M. Widstrom 12/19/91	Supplemental

Species: Lepomis macrochirus

Lab: NOR-AM

MRID # 420692-01

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DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron. 120301
Shaughnessey No. ~~102301.~~

2. **TEST MATERIAL:** Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-5-ylurea) technical; Batch No. 7/9.82; 99.3% active ingredient; a beige powder.

3. **STUDY TYPE:** Freshwater Fish Acute Static Toxicity Test. Species Tested: Rainbow Trout (*Oncorhynchus mykiss*).

4. **CITATION:** Schupner, J.K. and B.J. Stachura. 1991. The Acute Toxicity of Thidiazuron Technical to the Rainbow Trout, *Oncorhynchus mykiss*, in a Static System. Laboratory Project ID No. 512AW. Prepared and submitted by NOR-AM Chemical Company, Pikeville, NC. EPA MRID No. 420692-02.

5. **REVIEWED BY:**

Alvaro A. Yamhure,
Aquatic Biologist
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature:
Date: 12/16/92

6. **APPROVED BY:**

Dan Rieder,
Head Section 3
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature:
Date: 1-6-93

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a 96-hour static acute toxicity test using ~~rainbow~~ fish. Given the low solubility of the toxicant (Thidiazuron) in water and the low toxicity to the test species (*Oncorynchus mykiss*) - LC_{50} >19 mg/l- **this study is rated as core;** however, in the future we expect the registrant to follow our recommendations on alternative ways of increasing the solubility of test compounds [see Page 4;Section 14(C) of this review]. The NOEC is therefore established as 19 mg/l and thidiazuron is found to be slightly toxic to the rainbow trout.

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

57

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Rainbow trout (*Oncorhynchus mykiss*) were obtained from Aquatic Research Organisms in Hampton, NH. The fish (32 days old) were maintained in well water in a flow-through system and were fed salmon starter *ad libitum*.

Fish were acclimated to the test temperature and dilution water for 6 days prior to test initiation and feeding was discontinued 3 days prior to testing. No mortality occurred during acclimation. Mean weight and length of control fish taken at test termination were 0.414 ±0.088 g and 32 ±1.9 mm, respectively. Biomass loading rate was 0.276 g/l.

B. **Test System:** Test chambers were 19.5-liter glass aquaria (39.4 x 24.5 x 20.2 cm), each containing 15 l of test solution. The depth of the solution was 19 cm. The aquaria were covered with glass sheets to prevent evaporation and entry of foreign material.

The laboratory environment was maintained on a 16-hour daylight photoperiod (153 foot candles) with a dawn/dusk simulation period. The test aquaria were maintained at 12 ±1°C in a water bath.

Soft blended water (a mixture of well water and deionized well water) was used as dilution water (Appendix II, attached). A 77 mg ai/ml stock solution was prepared in dimethylformamide (DMF). A 7.4 ml aliquot of the stock was added to 15 liters of dilution water to create a concentration of 38 mg ai/l. The solutions were stirred for 24 hours after preparation.

C. **Dosage:** Based on a range-finding test, one nominal concentration (38 mg ai/l) of thidiazuron technical, a dilution water control and a solvent control (0.49 ml DMF/l) were used.

D. **Design:** Ten trout were randomly distributed to each aquarium (three replicate aquaria per concentration or control). Test chambers were randomly positioned in the water bath. Fish were not fed and test solutions were not aerated during the test. Observations of

mortality and sublethal responses were made every 24 hours.

The temperature, dissolved oxygen (D.O.), pH, and specific conductance were measured in all test chambers at 0, 48, and 96 hours. Temperature was also monitored continuously in the water bath.

Thidiazuron concentrations were measured by high performance liquid chromatography from samples taken at test initiation and termination. The samples were filtered (0.45 μm) before analysis.

E. Statistics: No statistical analyses were performed.

12. REPORTED RESULTS: The mean measured concentration of thidiazuron was 19 mg/l. This value represents 50% of the nominal concentration (Table 3, attached). A precipitate was noted in all test aquaria.

The responses of trout are given in Table 1 (attached). The 96-hour LC_{50} was determined to be greater than 19 mg/l. This is the maximum solubility of the test material in soft, blended water at 12°C. One mortality occurred but it was not considered to be treatment related. No abnormal behavior was noted during the course of the study.

All dissolved oxygen readings were $\geq$82% of saturation during the study. The pH ranged from 6.5 to 6.9 and the temperature from 11.5 to 12.8°C. Conductivity was 80 μmhos/cm throughout the test.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The authors concluded that the test material may be considered non-toxic under the conditions of the test.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the guidelines with the following exceptions:

Fish were acclimated for a shorter period (6 days) than recommended (2 weeks).

The fish were added to the test solutions 24 hours after preparation. A time period of no more than 30 minutes is recommended by the SEP.

The temperature varied by more than 1°C over the study period.

B. **Statistical Analysis:** Upon review of the mortality data, the reviewer agrees that the LC_{50} was >19 mg ai/l. The no-observed-effect concentration (NOEC) was 19 mg ai/l.

C. **Discussion/Results:** The food analysis indicated some detectable mercury, heptachlor epoxide, and delta-BHC. However, these amounts were low and probably did not influence the results.

This study is scientifically sound and meets the guideline requirements **(core)** for a static freshwater fish toxicity study. The test type conducted by the authors (i.e., 30 fish, one concentration) applies to a concentration of 100 mg/l or greater. The only concentration tested (19 mg ai/l) is near the limit of water solubility for thidiazuron under the conditions of this test. However, the authors did not demonstrate whether: 1) techniques to maximize chemical dissolution in the test media had been exhausted (e.g. different solvents, minor changes in environmental conditions, etc.), and 2) testing with a more soluble formlation was available. The 96-hour LC_{50} of >19 mg ai/l (mean measured concentration) classifies thidiazuron technical as slightly toxic to rainbow trout. The NOEC was 19 mg ai/l.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 12-19-91 (Amended by EEB).

THIDIAZURON

Page_____ is not included in this copy.

Pages 61 through 63 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.

Shaughnessey # 120301 ~~102304~~ Chemical Name Thidiazuron Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} = pp (95% C.L.) Control Mortality (%) = Solvent Control Mortality (%) = Slope = # Animals/Level = Temperature = 48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) Comments:	_____	_____

96-Hour LC_{50}	99.3%	LC_{50} = >19 ~~pp~~ mg ai/l* (95% C.L. N/A) Control Mortality (%) = 0
		 Solvent Control Mortality (%) = 0
		 Slope = N/A # Animals/Level = 30
		 Temperature = 12 °C
		 96-Hour Dose Level ~~pp~~ mg ai/l* /(% Mortality)
		 19 (3), ( ), ( ), ( ), ( )
		 Comments: NOEC = 19 mg ai/l*
		 * Based on mean measured conc. of ai
			M. Mossler 12/14/91	Supplemental

Species: Oncorhynchus mykiss

Lab: NOR-AM

MRID # 420692-02

6 of

DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron. Shaughnessey No.: 120301 ~~None listed.~~

2. **TEST MATERIAL:** Thidiazuron, SN 49537; N-phenyl-N'-1,2,3-thiadiazol-5-ylurea; 99.3% active ingredient; Batch No. 7/9.82; a beige colored powder with little lumps.

3. **STUDY TYPE:** Marine Shrimp Acute Toxicity Test. Species Tested: *Mysidopsis bahia*.

4. **CITATION:** Schupner, J.K. 1991. The Static Acute Toxicity of Thidiazuron to the Mysid Shrimp, *Mysidopsis bahia*. Performed by NOR-AM Chemical Company, NOR-AM Research Center, Pikeville, North Carolina. Study No. 503AW. Submitted by NOR-AM Chemical Company. EPA MRID No. 418461-02.

5. **REVIEWED BY:**

Alvaro A. Yamhure,
Aquatic Biologist
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature:
Date: 12/16/92

6. **APPROVED BY:**

Dan Rieder,
Head Section 3
Ecological Effects Branch
Environmental Fate and
Effects Division - H7507C

Signature: Daniel Rieder
Date: 1-6-93

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements **(core)** for an acute static toxicity test using marine shrimp. Two important environmental testing values (pH and salinity)were above the maximum recommended and test mysids (96 hours old) used were older than the advisable age (24 hours old). We at EEB recognize that the age of the shrimp issue has not been sufficiently emphasized to the registrants and will not hold registrants responsible until appropiate official collective notice is issued.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile mysid shrimp were obtained from Aquatic BioSystems, Inc., Fort Collins, Colorado. The mysids were reared in synthetic seawater in 19-l glass fish tanks under 16 hours of light (139 footcandles) and were fed *Artemia* nauplii. The mysids were acclimated to test dilution water (synthetic seawater) and test temperature (22 ±1°C) for at least 48 hours prior to test initiation. The mysids were fed *Artemia* nauplii *ad libitum* during the acclimation period. No mortality was observed during the acclimation period. At the time of test initiation the mysids were 4 days old (96 hours).

B. **Test System:** The test was conducted in 1-l glass beakers (15.5 cm high X 10.2 cm diameter) with a test solution volume of 800 ml and depth of 10 cm. Test vessels were randomly positioned in a water bath and covered with glass sheets to prevent evaporation or contamination.

The photoperiod was 16 hours of light (139 foot candles) and 8 hours of darkness, with gradual intensity changes at dawn and dusk.

The diluent was synthetic seawater (Instant Ocean salts and deionized well water) adjusted to a salinity of 20 parts per thousand (ppt) with deionized water.

C. **Dosage:** Ninety-six-hour acute static test. Based on the results of two range finder studies, five nominal test concentrations (0.52, 0.86, 1.4, 2.4, and 4.0 mg/l) were selected for the study. A solvent control (0.5 ml solvent/l) and untreated control were also used in the study.

An 8 mg/ml stock solution was prepared in dimethylformamide (DMF). Appropriate amounts of stock solution were added to 2 l of diluent to prepare the nominal test concentrations.

D. **Design:** Ten mysid shrimp were randomly distributed, by twos, to each beaker. Two beakers (replicates) were used per concentration. The organism loading during the test was 10 mysids/800 ml. Mysids of each replicate were fed two drops of *Artemia* nauplii twice daily during the test.

Observations of mortality and abnormal effects were made every 24 hours (±1 hour). Dissolved oxygen concentration, pH, temperature, and salinity were determined in each replicate at test initiation, 48 hours, and at test termination. The temperature in the water bath was monitored continuously.

The concentration of thidiazuron present in each test vessel was determined at test initiation and test termination, using high pressure liquid chromatography.

E. **Statistics:** The mortality data was analyzed with Toxdat (Stephan, 1985). The 96-hour LC_{50} (with 95% confidence limits) was determined using the probit method.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.58, 0.94, 1.4, 2.2, and 3.9 mg/l, which represent 92-109% of nominal concentrations (Table 3, attached). Mean test concentrations were used to determine the LC_{50} values.

The 96-hour LC_{50} (95% confidence interval) was 3.2 mg/l (2.8-3.7 mg/l). During the study, mortality and sublethal effects were noted at the highest test concentration (3.9 mg/l) (Table 1, partial, attached). No sublethal effects were noted in the controls or in the remaining concentrations (0.58-2.2 mg/l). A single mortality was noted at the 0.94 and 2.2 mg/l and in the solvent control.

During the study, the dissolved oxygen concentrations were maintained at 5.6-7.7 mg/l. The test solutions had a salinity of 20-21 ppt and a pH of 7.9-8.2. The temperature in the water bath was maintained at 22.0-22.9°C (mean 22.3 ±0.23°C). The temperature in the test vessels was 21.9-22.7°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions, other than those stated above, were presented in the report.

A GLP compliance statement was included in the report indicating that this study was conducted in accordance with GLP as defined in 40 CFR Part 160. This statement was signed by the Study Director and sponsor representatives.

A Quality Assurance Statement was included and signed by a GLP Quality Assurance Inspector of the performing laboratory.

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

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the guidelines, but deviated from best practices as follows:

Four-day-old mysids were used as test animals. Since its Designation E: 729 - 88 standard procedure, ASTM has indicated that 24-hour old mysids are to be used in this type of test. The test type conducted by the authors (i.e., 30 fish, one concentration) applies to a concentration of 100 mg/l or greater. The only concentration tested (32 mg ai/l) is near the limit of water solubility for thidiazuron under the conditions of this test. However, the authors did not demonstrate whether: 1) techniques to maximize chemical dissolution in the test media had been exhausted (e.g. different solvents, minor changes in environmental conditions, etc.), and 2) testing with a more soluble formulation was available. The 96-hour LC_{50} of >32 mg ai/l (mean measured concentration) classifies thidiazuron technical as slightly toxic to bluegill sunfish. The NOEC was 32 mg ai/l.

The pH (8.2) and salinity (20 ppt) of the control at test initiation were higher than the pH (7.7-8.0) and salinity (10-17 ppt) recommended for a euryhaline shrimp species. The combination of older shrimp with higher than recommended pH and salinity are not likely to produce and adequate evaluation of toxicity/risk to the test species or the other species of the ecosystem it is suppose to represent. We at EEB recognize that the age of the shrimp issue has not been sufficiently emphasized to the registrants.

The volume capacity of the test vessels (1 l) and the solution volume (800 ml) were less than the volume capacity (3.9 l) and solution volume (2-3 l) recommended in the guidelines.

B. Statistical Analysis: The reviewer used EPA's Toxanal to determine the LC_{50} value and 95% confidence interval based on mean measured concentrations. The reviewer's values were the same as those of the author (printout, attached).

C. Discussion/Results: Both, pH and salinity were above required values. Four-day old mysids were used in this

study, instead of 24-hour old mysids. Older mysids may be less sensitive than younger, 24-hour old mysids.

This study is scientifically sound and fulfills the guideline requirements **(core)** for an acute static toxicity test using marine shrimp. The 96-hour LC_{50} of 3.2 mg/l mean measured concentration indicates that thidiazuron may be moderately toxic to *Mysidopsis bahia*. The NOEC was 1.4 mg/l.

D. **Adequacy of the Study:**

(1) Classification: Core
(2) Rationale: N/A
(3) Repairability: N/A

15. **COMPLETION OF ONE-LINER:** Yes, October 15, 1991 (amended by EEB).

THIDIAZURON

Page_____ is not included in this copy.

Pages 70 through 71 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.

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Rosemary Graham Mora Thidiazuron Mysidopsis bahia 9-24-91

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3.9	20	15	75	2.069473
2.2	20	1	5	2.002716E-03
1.4	20	0	0	9.536742E-05
.94	20	1	5	2.002716E-03
.58	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.2 AND 3.9 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.239656

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.159231	3.239656	2.889576	3.773436

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	3.548348	6.904162	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 5.129264
95 PERCENT CONFIDENCE LIMITS =-4.532761 AND 14.79129

LC50 = 3.281521
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 1.855566
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

****

Shaughnessey # 120301 ~~None listed~~ Chemical Name Thidiazuron Chemical Class _____ Page ____ of ____

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
48-Hour EC_{50}	_____	EC_{50} - pp (95% C.L.) Control Mortality (%) - Solvent Control Mortality (%) -		
Species:		Slope - # Animals/Level - Temperature -		
Lab:		48-Hour Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( )		
MRID #		Comments:		
96-Hour LC_{50}	99.3%	LC_{50} - 3.2 ppm* (95% C.L. moving average 2.9 - 3.8) Control Mortality (%) - 0 Solvent Control Mortality (%) - 5		
Species: Mysidopsis bahia		Slope - NA # Animals/Level - 20 Temperature - 22-22.9C	RAM 10/15/91	Supplemental
Lab: NOR-AM		96-Hour Dose Level ppm*/(% Mortality) 0.58 (0), 0.94 (5), 1.4 (0), 2.2 (5), 3.9 (75)		
MRID # 418461-02		Comments: * Based on measured concentrations. Test organisms (96 hours) were older than allowed (≤ 24 hours) pH and temperature higher than recommended	12/9/92	

DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron.
Shaughnessey No. 120301.

2. **TEST MATERIAL:** Thidiazuron technical; N-phenyl-N'-1,2,3-thidiazol-5-yl urea; CAS No. 51707-55-2; Batch No. 7/9.82; 99.3% active ingredient; a yellowish powder.

3. **STUDY TYPE:** Mollusc 96-Hour Shell Deposition Study.
Species Tested: Eastern Oyster (*Crassostrea virginica*).

4. **CITATION:** Ward, G.S. 1991. Thidiazuron Technical: Acute Effect on New Shell Growth of, the Eastern Oyster, *Crassostrea virginica*. Laboratory Project ID. J9101006b. Nor-Am Study No. 505 AW. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Nor-Am Chemical Company, Pikeville, NC. EPA MRID No. 421320-01.

5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 4/1/92 12/16/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 4/1/92

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

Signature: Henry T. Craven
Date: 1/7/93

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a shell deposition test using the eastern oyster. The 96-hour EC_{50}, based on the percent difference in shell deposition compared to the control, was 5.4 mg/l mean measured concentration. The 96-hour LC_{50} was 4.3 mg/l mean measured concentration. Therefore, thidiazuron technical is classified as moderately toxic to eastern oysters. The NOEC was reported as 1.06 mg/l (mean measured concentration).

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Eastern oysters (*Crassostrea virginica*) were obtained from a commercial supplier in Dennis, MA. Upon arrival, approximately 2 to 5 mm of new shell growth was removed from each oyster using a fine grit grinding wheel. The oysters were then placed in flowing, unfiltered natural seawater for two days prior to testing. The salinity of the seawater was 28 parts per thousand (ppt) and the temperature was 28.3-28.7°C.

The test oysters had an average length (umbo to distal valve edge) of 24.7 (20.4-29.7) mm and appeared to be in good condition prior to test initiation. Just prior to initiation, 140 oysters which had demonstrated growth during holding were selected and all new shell growth removed.

B. **Test System:** The test system was a proportional diluter calibrated to provide test solution or dilution water (control) to each test chamber at a rate of approximately 220 ml/minute, sufficient to provide a minimum of approximately 0.06 l per oyster per hour. The test chambers were 11.3-l glass tanks (42 x 21.5 x 12.5 cm high) designed to maintain a solution height of 6 cm and a test volume of 5.4 l. The chambers were positioned in a water bath under a 16-hour light/8-hour dark photoperiod. Light intensity during the test was 292 to 358 lux. No aeration was used during the test.

Natural unfiltered seawater with a salinity of 28-32 ppt was used as dilution water. The water was aerated prior to use.

A thidiazuron stock solution (15 mg a.i./l) was prepared by adding 2.8506 g of thidiazuron technical to 190 l of unfiltered seawater. The solution was stirred for 24 hours prior to use in the test system. The stock was pumped directly to the diluter's chemical mixing chamber and served as the highest test solution.

C. **Dosage:** Ninety-six-hour flow-through test. Based on the results of a preliminary test, five nominal concentrations (1.1, 1.9, 3.2, 5.4, 9.0, and 15 mg a.i./l) and a dilution water control were used.

D. **Design:** The test system was allowed to operate for 1 day prior to test initiation. Prepared oysters were impartially added to the test chambers for a total of 20 per chamber. One chamber was used per treatment.

Observations of mortality were made every 24 hours. At the end of the test, oyster growth was measured. The dissolved oxygen and pH of the test solutions were measured in each chamber at the beginning of the test and at each 24-hour observation. The salinity of the dilution water control was measured daily. The temperature was also monitored hourly in the control chamber using a data logging device.

Thidiazuron concentrations were measured by HPLC from samples taken at test initiation and termination. The test concentrations were verified prior to test initiation.

E. **Statistics:** The 96-hour median effective concentration (EC_{50}), 96-hour median lethal concentration (LC_{50}), and associated 95% confidence intervals (C.I.) were calculated using the probit method. Statistical differences between the treatment and control groups were determined using analysis of variance (ANOVA) and Dunnett's test.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.722, 1.06, 2.32, 3.39, 6.39, and 10.4 mg/l, ranging from 56 to 72% of nominal (Table 1, attached). Measured concentrations were generally consistent between sampling days. No undissolved test material was observed in the test solutions.

New shell growth data were presented in Table 2 (attached). Mean new shell growth in the control was 2.4 mm. The percent difference in new shell growth compared to the control ranged from +12% at 1.06 mg/l to -81% at 10.4 mg/l (Table 3, attached). The 96-hour EC_{50}, based on mean measured concentrations, was 5.18 mg/l (95% C.I. = 2.10-1568 mg/l). Survival of oysters ranged from 100% at 0.722 and 1.06 mg/l to 5% at 10.4 mg/l. The 96-hour LC_{50}, based on measured concentrations, was 4.28 mg/l (95% C.I. = 3.6-5.12 mg/l). The no-observed-effect concentration (NOEC) was 1.06 mg/l.

Dissolved oxygen at test initiation ranged from 4.9 to 5.9 mg/l or 63 to 77% of saturation and remained above 53% of saturation for the remainder of the test. The pH values ranged from 7.2 to 7.9. The temperature, as recorded by a

data logger, was 26.3-29.1°C. The salinity was 28 to 32 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author presented no conclusions.

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.

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

A. **Test Procedure:** The test procedures were generally in accordance with the SEP, APHA (1981), and Anonymous (1976), except for the following:

In this study, the flow rate of the test solution was about 0.06 l/oyster/hour. According to the protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976), each oyster should receive a minimum of 5 L of flow-through test solution per hour.

The author did not state whether the test containers were randomly positioned in the water bath.

The method used to measure the oysters was not described in the report.

The oysters should be arranged in the test aquaria with the cupped-valve down and the anterior hinged ends oriented in one direction. The authors did not describe the positioning of the oysters.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the 96-hour EC_{50} value and obtained similar results (see attached printout 1). The 96-hour LC_{50} was calculated and agreed with the author's (see attached printout 2). The slope of the probit line was 4.8. The NOEC was determined using the new shell deposition data and Steel's Many-One rank test (Toxstat Version 3.3). Mean shell growth at concentrations ≥6.39 mg/l were significantly different from the control (see attached printout 4). The author's NOEC was more conservative than the reviewer's.

C. **Discussion/Results:** Shell deposition by the control oysters was somewhat variable. The relative standard deviation for control growth was 54.2% and may have

weakened the results. However, the author's NOEC value is very conservative. The mortality of oysters in this test is worth noting because it is uncommon, the shell deposition of oysters which subsequently died was used in the analysis of the results, and the lethal effects were more sensitive than the sublethal endpoint, shell growth.

This study is scientifically sound and meets the guideline requirements for a shell deposition test using the eastern oyster. The 96-hour EC_{50}, based on the shell deposition results, was 5.4 mg/l mean measured concentration. The 96-hour LC_{50} was 4.3 mg/l mean measured concentration. Therefore, thidiazuron technical is classified as moderately toxic to eastern oysters. The NOEC was reported as 1.06 mg/l (mean measured concentration).

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 03-10-92.

THIDIAZURON

Page_____ is not included in this copy.

Pages 79 through 81 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) _____.

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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.

Pk #4 1 Shell deposition - inhibition

RIFICI THIDIAZURON CRASSOSTREA VIRGINICA 3-4-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
10.4	100	81	81	0
6.39	100	54	54	0
3.39	100	29	29	0
2.32	100	21	21	0
1.06	100	0	0	0
.722	100	21	21	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 5.788214

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	4.719424E-02	5.384021	4.768189	6.156776

6.157

LMC 3/4/92

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.860028	14.26092	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 1.759543

95 PERCENT CONFIDENCE LIMITS = .1277833 AND 3.391302

LC50 = 5.204737

95 PERCENT CONFIDENCE LIMITS = 2.102321 AND 2514.959

LC10 = .9876889

95 PERCENT CONFIDENCE LIMITS = 1.098699E-07 AND 2.339744

****

printout 2 : oyster mortality

RIFICI THIDIAZURON CRASSOSTREA VIRGINICA 3-4-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
10.4	20	19	95	0
6.39	20	16	80	0
3.39	20	8	40	0
2.32	20	1	5	0
1.06	20	0	0	0
.722	20	0	0	0

THE BINOMIAL TEST SHOWS THAT 3.39 AND 6.39 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.945683

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	6.572957E-02	4.32715	3.565411	5.3906

5.3906

LMK 3/4/92

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1073587	1	.824177

SLOPE = 4.803724

95 PERCENT CONFIDENCE LIMITS = 3.229753 AND 6.377694

LC50 = 4.281563

95 PERCENT CONFIDENCE LIMITS = 3.600149 AND 5.115299

LC10 = 2.329293

95 PERCENT CONFIDENCE LIMITS = 1.642086 AND 2.867115

****

TITLE: 421320-01, THIDIAZURON, NEW SHELL GROWTH
FILE: A:42132001.DAT
TRANSFORM: 1/(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	CONTROL	1	2.8000	0.5976
1	CONTROL	2	1.9000	0.7255
1	CONTROL	3	3.6000	0.5270
1	CONTROL	4	1.4000	0.8452
1	CONTROL	5	2.4000	0.6455
1	CONTROL	6	5.0000	0.4472
1	CONTROL	7	2.0000	0.7071
1	CONTROL	8	5.5000	0.4264
1	CONTROL	9	4.4000	0.4767
1	CONTROL	10	1.1000	0.9535
1	CONTROL	11	2.4000	0.6455
1	CONTROL	12	3.7000	0.5199
1	CONTROL	13	2.4000	0.6455
1	CONTROL	14	0.8000	1.1180
1	CONTROL	15	1.7000	0.7670
1	CONTROL	16	1.2000	0.9129
1	CONTROL	17	1.3000	0.8771
1	CONTROL	18	1.7000	0.7670
1	CONTROL	19	1.6000	0.7906
1	CONTROL	20	2.0000	0.7071
2	0.722 mg/l	1	3.7000	0.5199
2	0.722 mg/l	2	1.8000	0.7454
2	0.722 mg/l	3	1.3000	0.8771
2	0.722 mg/l	4	1.0000	1.0000
2	0.722 mg/l	5	1.0000	1.0000
2	0.722 mg/l	6	1.8000	0.7454
2	0.722 mg/l	7	1.5000	0.8165
2	0.722 mg/l	8	2.2000	0.6742
2	0.722 mg/l	9	0.8000	1.1180
2	0.722 mg/l	10	2.1000	0.6901
2	0.722 mg/l	11	2.6000	0.6202
2	0.722 mg/l	12	3.7000	0.5199
2	0.722 mg/l	13	1.6000	0.7906
2	0.722 mg/l	14	1.9000	0.7255
2	0.722 mg/l	15	0.7000	1.1952
2	0.722 mg/l	16	2.5000	0.6325
2	0.722 mg/l	17	0.7000	1.1952
2	0.722 mg/l	18	1.8000	0.7454
2	0.722 mg/l	19	1.4000	0.8452
2	0.722 mg/l	20	3.5000	0.5345
3	1.06	1	3.0000	0.5774
3	1.06	2	2.0000	0.7071
3	1.06	3	39.0000	0.1601
3	1.06	4	3.2000	0.5590
3	1.06	5	1.8000	0.7454
3	1.06	6	2.8000	0.5976
3	1.06	7	1.9000	0.7255
3	1.06	8	2.5000	0.6325
3	1.06	9	3.0000	0.5774
3	1.06	10	1.0000	1.0000
3	1.06	11	2.2000	0.6742
3	1.06	12	4.3000	0.4822
3	1.06	13	3.4000	0.5423
3	1.06	14	4.7000	0.4613
3	1.06	15	2.3000	0.6594
3	1.06	16	3.1000	0.5680
3	1.06	17	1.3000	0.8771
3	1.06	18	2.3000	0.6594
3	1.06	19	2.6000	0.6202
3	1.06	20	3.3000	0.5505
4	2.32	1	1.1000	0.9535
4	2.32	2	3.5000	0.5345
4	2.32	3	3.0000	0.5774
4	2.32	4	2.2000	0.6742
4	2.32	5	1.5000	0.8165
4	2.32	6	0.6000	1.2910
4	2.32	7	1.5000	0.8165
4	2.32	8	2.3000	0.6594
4	2.32	9	1.2000	0.9129
4	2.32	10	3.4000	0.5423

4	2.32	11	1.7000	0.7670
4	2.32	12	0.8000	1.1180
4	2.32	13	2.0000	0.7071
4	2.32	14	1.3000	0.8771
4	2.32	15	0.8000	1.1180
4	2.32	16	2.6000	0.6202
4	2.32	17	2.1000	0.6901
4	2.32	18	36.0000	0.1667
4	2.32	19	1.4000	0.8452
4	2.32	20	1.4000	0.8452
5	3.39	1	1.8000	0.7454
5	3.39	2	1.8000	0.7454
5	3.39	3	1.7000	0.7670
5	3.39	4	1.8000	0.7454
5	3.39	5	1.9000	0.7255
5	3.39	6	3.2000	0.5590
5	3.39	7	1.6000	0.7906
5	3.39	8	2.3000	0.6594
5	3.39	9	2.6000	0.6202
5	3.39	10	2.7000	0.6086
5	3.39	11	2.1000	0.6901
5	3.39	12	2.3000	0.6594
5	3.39	13	0.7000	1.1952
5	3.39	14	1.9000	0.7255
5	3.39	15	1.2000	0.9129
5	3.39	16	1.5000	0.8165
5	3.39	17	1.2000	0.9129
5	3.39	18	1.1000	0.9535
5	3.39	19	1.4000	0.8452
6	6.39	1	1.4000	0.8452
6	6.39	2	1.4000	0.8452
6	6.39	3	2.1000	0.6901
6	6.39	4	1.0000	1.0000
6	6.39	5	1.4000	0.8452
6	6.39	6	1.5000	0.8165
6	6.39	7	2.0000	0.7071
6	6.39	8	2.0000	0.7071
6	6.39	9	2.4000	0.6455
6	6.39	10	1.4000	0.8452
6	6.39	11	1.5000	0.8165
6	6.39	12	1.3000	0.8771
6	6.39	13	1.3000	0.8771
6	6.39	14	1.4000	0.8452
7	10.4	1	1.3000	0.8771
7	10.4	2	1.0000	1.0000
7	10.4	3	0.6000	1.2910
7	10.4	4	2.3000	0.6594
7	10.4	5	1.4000	0.8452
7	10.4	6	2.4000	0.6455

Chi-square test for normality: actual and expected frequencies
Data FAIL normality test.

Bartletts test for homogeneity of variance
Data FAIL homogeneity test at 0.01 level. Try another transformation.

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	CONTROL	2.445				
2	0.722 mg/l	1.880	361.50	325.00	20.00	
3	1.06	4.485	461.50	325.00	20.00	
4	2.32	3.520	366.50	325.00	20.00	
5	3.39	1.832	335.50	308.50	19.50	
6	6.39	1.579	189.00	239.00	17.00	*
7	10.4	1.500	53.50	174.50	13.00	*

Critical values use k = 6, are 1 tailed, and alpha = 0.05

Shaughnessey # 120301 Chemical Name Thiadiazuron Chemical Class _____ Page 1 of 1

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

96 48-Hour EC_{50}	99.3	EC_{50} = 5.4 * ppm (95% C.L. Moving Average 4.8 - 6.2) Control Mortality (%) = 0
		Solvent Control Mortality (%) = N/A
		Species: Crassostrea virginica Slope = N/A # Animals/Level = 20
		Temperature = 26-29°C
		Lab: Tetratech Environmental Services
		96 48-Hour Dose Level ppm* /(% Effect)
		0.722 (21) 1.06 (0), 2.32 (21), 3.39 (29), 6.39 (54), 10.4 (81)
		MRID # 421320-01
		Comments: * mean measured concentration
			LMR 3/10/92	Core

96-Hour LC_{50}	99.3	LC_{50} = 4.28 * ppm (95% C.L. Prob. + 3.6 - 5.1) Control Mortality (%) = 0
		Solvent Control Mortality (%) = N/A
		Species: Crassostrea virginica Slope = 4.8 # Animals/Level = 20
		Temperature = 26-29°C
		Lab: Tetratech Environmental Sciences
		96-Hour Dose Level ppm* /(% Mortality)
		0.722 (0) 1.06 (0), 2.32 (5), 3.39 (40), 6.39 (80), 10.4 (95)
		MRID # 421320-01
		Comments: * mean measured concentration
			LMR 3/10/92	Core

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DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron.
Shaughnessey No. 120301.

2. **TEST MATERIAL:** Thidiazuron technical; CAS No. 51707-55-2; Batch No. 7/9.82; 99.3% active ingredient; an off-white granule.

3. **STUDY TYPE:** Freshwater Invertebrate Flow-Through Life-Cycle Toxicity Test. Species Tested: *Daphnia magna*.

4. **CITATION:** Blakemore, G.C. and M. Muckerman. 1991. Chronic Toxicity of Thidiazuron to *Daphnia magna* Under Flow-Through Test Conditions. Final Report No. 39114. Nor-Am Study No. 507-AW. Prepared by ABC Laboratories, Inc., Columbia, MO. Submitted by Nor-Am Chemical Company, Pikeville, NC. EPA MRID No. 421320-02.

5. **REVIEWED BY:**

Alvaro A. Yamhure, Aquatic Biologist
Ecological Effects Branch
Environmental Fate and Effects Division
H7505C

Date: 12/16/92

Signature:

6. **APPROVED BY:**

Dan Rieder, Head, Section 3
Ecological Effects Branch
Environmental Fate and Effects Division
H7507C

Date:

Signature: 1.6.93

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a chronic, flow-through toxicity test using the freshwater invertebrate, *Daphnia magna*. Statistically significant effects on daphnid length were observed at all tested concentrations; therefore, an MATC value for this statistic could not be established. The 21-day EC_{50} based on immobilization was 0.68 mg/l mean measured concentration. This study is rated **supplemental.**

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** *Daphnia magna* (<24 hours old) were obtained from in-house cultures. The primary culture was obtained from the Columbia National Fisheries Research Laboratory in Columbia, MO. The cultures were housed in a temperature-controlled area (20 ±2°C) on a 16-hour daylight photoperiod with 30-minute dawn/dusk simulations. The light intensity was maintained at 40-80 ft-candles using cool white fluorescent tubes.

Adult daphnids were fed a suspension of algae (*Selenastrum capricornutum* and *Ankistrodesmus falcatus*) supplemented with a trout chow and yeast suspension.

B. **Test System:** A 0.5-l proportional diluter intermittently delivered an average of 5.3 ml/chamber/minute (or 7.6 volume replacements per day) to each of four replicate test vessels per concentration. Flow splitting boxes were used to mix and divide each test solution. To minimize turbulence, the solutions were introduced into the test vessels using 14-gauge hypodermic needles. Thidiazuron was allowed to run through the system for 4 days before use. The functioning of the diluter was verified analytically prior to test initiation. Diluter operation was observed twice daily during the study.

The test vessels were 1-liter glass beakers with notched drains covered by 50-mesh stainless steel screen. The test chambers were positioned in a temperature-controlled water bath set to maintain 20 ±2°C. The photoperiod was 16-hour light/8-hour dark with 30-minute transitions between light and dark. The light intensity of the cool-white fluorescent tubes was 55-60 ft-candles.

The dilution water was a mixture of soft-blended water and well water with a final hardness of 162-188 mg/l as $CaCO_3$. The characteristics of the well water used are given in Table I (attached).

The test substance was dissolved in dimethylformamide (DMF). The resulting stock solution was delivered to the diluter using a syringe dispenser.

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C. **Dosage:** Twenty-one-day, flow-through, life-cycle chronic toxicity test. Based on a preliminary test, five nominal concentrations (0.09, 0.18, 0.38, 0.75, and 1.5 mg a.i./l), a dilution water control, and a solvent control (0.05 ml DMF/l) were selected for the test.

D. **Design:** Four chambers were used for each concentration with ten randomly-placed daphnids per chamber. All chambers were observed daily for mortality, abnormal effects, and the release of the first brood. Young were counted every Monday, Wednesday, and Friday by removing the adult with a smooth glass pipet and pouring the test solution through a 50-mesh stainless steel screen. The collected young were placed in sample jars, counted, and discarded. The solution was collected and replaced along with the adult daphnids back into the chamber. The test chambers were cleaned on each counting day. At test termination, the daphnids were measured.

The daphnids were uniformly fed an algal suspension (*Selenastrum capricornutum* and *Ankistrodesmus falcatus*) providing at least 4 x 10^8 cells/l to each replicate. Each replicate was also supplemented twice daily with 1 ml of a trout chow/yeast suspension (2.5 mg solids/ml) giving a final suspended solids concentration of 5 mg/l.

The dissolved oxygen concentration (DO), temperature, and pH were measured in alternating, duplicate replicates of the dilution water control, solvent control, low, middle, and high concentrations on days 0, 4, 7, 14, and 21. The temperature of the water bath was recorded continuously with a thermal data logger. The temperature, hardness, DO, pH, alkalinity, and conductivity of the dilution water were determined daily.

Thidiazuron concentrations were measured by HPLC from samples taken on days 0, 4, 7, 11, 14, and 21.

E. **Statistics:** Daphnid survival, growth (length), and reproduction (time to first brood and young/adult reproduction days) were analyzed statistically. Survival was analyzed using frequency analysis to compare the concentrations to the control and a one-tailed Fisher's Exact test and the Chi-Square statistic to determine which test concentrations exhibited

responses significantly less than the control. Continuous data (daphnid reproduction and growth) were tested for normal distribution (Shapiro-Wilks) and homogeneity of variance. Reproduction in each replicate within a concentration was compared using t-tests. One-way analysis of variance (ANOVA) and Dunnett's test were used to determine significant differences from the controls. Length data were analyzed using ANOVA and Dunnett's test. The level of significance in all tests was $P \leq 0.05$. The dilution water control and solvent control data were pooled prior to each analysis. The 21-day EC_{50} was calculated using a computer program developed by Stephan (1978).

12. **REPORTED RESULTS:** The mean measured concentrations were 0.10, 0.15, 0.34, 0.69, and 1.4 mg/l (Table II, attached). These values represented 110, 83, 89, 92, and 93% of nominal. Measured concentrations were fairly consistent between sampling days. The test material was detected in the solvent control solution at test initiation. The authors explained that the contamination was considered to have happened during sample preparation because no other contamination was observed during the test. A diluter malfunction on day 10 resulted in 56 volume turnovers on that day.

Survival of daphnids at 0.34, 0.69, and 1.4 mg/l was significantly affected compared to the pooled controls (Table IV, attached). The 21-day EC_{50}, based on immobilization, was 0.68 mg/l. The two highest concentrations were excluded from further analyses.

The length in days to first brood release and the number of young per adult reproductive day were unaffected at the analyzed concentrations ≤ 0.34 mg/l (Table V, attached). The values for these two parameters at the two highest test concentrations were not included in the statistical analysis. All young produced during the study appeared normal. Light discoloration of the adult daphnids was commonly observed at concentrations ≥ 0.34 mg/l (Table VI, attached).

Adult daphnid lengths at 0.10, 0.15, and 0.34 mg/l were significantly lower than those of the pooled controls (Table 4, attached). The lengths of the two highest test concentrations were excluded from statistical analysis.

The authors discounted the findings of the length data and determined the maximum acceptable toxicant concentration

(MATC) limits using the reproduction and survival data. The MATC limits were estimated to be >0.15 and <0.34 mg/l (mean measured concentrations) resulting in a geometric mean MATC of 0.23 mg/l.

The results of water quality monitoring were presented in Table 7 (attached). The pH of the test solutions ranged from 8.22 to 8.49 Dissolved oxygen saturation ranged from 83 to 95% at 20°C. The temperature of the test solutions at the daily recordings was 19-21°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author presented no conclusions other than those previously mentioned.

Quality Assurance and GLP Compliance Statements were included in the report indicating adherence to USEPA GLP Regulations (40 CFR 160).

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

A. **Test Procedure:** Since an SEP for conducting daphnid flow-through chronic tests are not available at the present time, ASTM guidelines were used in the data validation process. The test procedures were generally in accordance with protocols recommended by ASTM (1985), but deviated as follows:

The authors used an average of 7.6 volume replacements per day. ASTM recommends 4 to 6 volume replacements per day.

Dry weight is preferable to length measurements. The authors used length as the growth endpoint in this test.

B. **Statistical Analysis:** The 21-day EC_{50} was determined using EPA's Toxanal program. The results were the same as the authors' (see attached printout 1). The 95% confidence interval was 0.5-1.0 mg/l. Survival after 21 days was analyzed using the non-parametric Steel's Many-One rank test (Toxstat Version 3.3) due to zero variance in the solvent control data. Survival at the two highest concentrations (0.69 and 1.4 mg/l) was significantly lower than survival in the solvent control (see attached printouts 2 and 3). This result was less conservative than the authors'. Survival in the dilution water control and solvent control were not significantly different.

The reviewer used one-way ANOVA and Dunnett's test (Toxstat Version 3.3) to analyze the average number of young produced per adult reproductive day after 21 days. Reproduction was not affected by the concentrations of Thidiazuron (see attached printouts 4 and 5).

Adult daphnid length was analyzed using two-way ANOVA (Crunch Version 3) and Bonferroni's test. One-hundred percent mortality occurred in replicate A of the 1.4 mg/l test level. To avoid an empty cell in the analysis, the reviewer used the mean of all lengths at this level as a single observation for replicate A (see attached printout 6). In agreement with the authors, the lengths of exposed daphnids were significantly lower than control daphnids (see attached printout 7). There was no statistical difference between dilution water control and solvent control lengths.

C. **Discussion/Results:** The authors discount the results of the analysis of daphnid length because the data do not follow the same dose-response of the other parameters (i.e. survival). However, the reduction in length in exposed daphnids was highly significant and consistent with each exposure. When replicate means are examined (see attached printout 6), a dose-response becomes apparent. The trend towards decreasing size with increasing concentration is masked by a few values (i.e. 4.02 mm at exposure level 2, replicate 3; 4.21 mm at exposure level 3, replicate 3; and 3.95 mm at exposure level 4, replicate 4) when only the exposure level means are examined.

This study is scientifically sound but does not meet the guideline requirements for a chronic, flow-through toxicity test using the freshwater invertebrate, *Daphnia magna*. Exposure effects were observed at all tested concentrations. The 21-day EC_{50} was 0.68 mg/l mean measured concentration. The MATC was less than 0.10 mg/l mean measured concentration, the lowest concentration tested.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** Exposure effects were observed at all tested concentrations. An MATC could not be determined.

(3) Repairability: No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 03-03-92.(Amended by EEB)

REFERENCES: ASTM. 1985. Proposed Standard Practice for Conducting Daphnia magna Chronic Toxicity Tests in a Flow-Through System. Draft No. 4.

THIDIAZURON

Page_____ is not included in this copy.

Pages 94 through 99 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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____ Description of quality control procedures.

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RIFICI THIDIAZURON DAPHNIA MAGNA 3-3-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.4	40	25	62.5	0
.69	40	27	67.5	0
.34	40	9	22.5	0
.15	40	3	7.500001	0
.1	40	3	7.500001	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 .5269616

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1262785	.6781466	.5041287	1.010816

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.5416265	2.619309	4.904115E-02

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

SLOPE = 1.855258
95 PERCENT CONFIDENCE LIMITS = .4898759 AND 3.220641

LC50 = .6917053
95 PERCENT CONFIDENCE LIMITS = .3503267 AND 3.83212

LC10 = .1430146
95 PERCENT CONFIDENCE LIMITS = 4.691082E-03 AND .2949949

TITLE: 421320-02, THIDIAZURON, 21-DAY SURVIVAL
FILE: 42132002.DT1
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	1.0000	1.4120
1	SOLVENT CONTROL	2	1.0000	1.4120
1	SOLVENT CONTROL	3	1.0000	1.4120
1	SOLVENT CONTROL	4	1.0000	1.4120
2	DILUT. CONTROL	1	1.0000	1.4120
2	DILUT. CONTROL	2	0.9000	1.2490
2	DILUT. CONTROL	3	0.9000	1.2490
2	DILUT. CONTROL	4	1.0000	1.4120
3	0.09 mg/l	1	1.0000	1.4120
3	0.09 mg/l (0.10)	2	1.0000	1.4120
3	0.09 mg/l	3	1.0000	1.4120
3	0.09 mg/l	4	0.7000	0.9912
4	0.18	1	0.9000	1.2490
4	0.18 (0.15)	2	1.0000	1.4120
4	0.18	3	0.8000	1.1071
4	0.18	4	1.0000	1.4120
5	0.38	1	1.0000	1.4120
5	0.38 (0.34)	2	0.6000	0.8861
5	0.38	3	0.9000	1.2490
5	0.38	4	0.6000	0.8861
6	0.75	1	0.6000	0.8861
6	0.75 (0.69)	2	0.1000	0.3218
6	0.75	3	0.5000	0.7854
6	0.75	4	0.1000	0.3218
7	1.5	1	0.0000	0.1588
7	1.5 (1.4)	2	0.1000	0.3218
7	1.5	3	0.5000	0.7854
7	1.5	4	0.9000	1.2490

nominal concentrations

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance
These two tests can not be performed because at least one group has zero variance.
Data FAIL to meet homogeneity of variance assumption.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.4120	CALCULATED t VALUE =	1.7321
GRP2 (BLANK CRTL) MEAN =	1.3305	DEGREES OF FREEDOM =	6
DIFFERENCE IN MEANS =	0.0815		

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

421320-02, THIDIAZURON, 21-DAY SURVIVAL
File: 42132002.DT1 Transform: ARC SINE(SQUARE ROOT(Y))

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	SOLVENT CONTROL	1.412				
2	DILUT. CONTROL	1.331	14.00	10.00	4.00	
3	0.09 mg/l (0.10)	1.307	16.00	10.00	4.00	
4	0.18 (0.19)	1.295	14.00	10.00	4.00	
5	0.38 (0.41)	1.108	12.00	10.00	4.00	
6	0.75 (0.87)	0.579	10.00	10.00	4.00	*
7	1.5 (1.4)	0.629	10.00	10.00	4.00	*

Critical values use k = 6, are 1 tailed, and alpha = 0.05

TITLE: 421320-02, THIDIAZURON, YOUNG/ADULT REPRODUCTIVE DAY
FILE: A:42132002.DT2
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	SOLVENT CONTROL	1	5.0000	5.0000
1	SOLVENT CONTROL	2	6.1000	6.1000
1	SOLVENT CONTROL	3	5.7000	5.7000
1	SOLVENT CONTROL	4	5.3000	5.3000
2	DILUT. CONTROL	1	5.6000	5.6000
2	DILUT. CONTROL	2	5.2000	5.2000
2	DILUT. CONTROL	3	4.9000	4.9000
2	DILUT. CONTROL	4	6.1000	6.1000
3	0.10 mg/l	1	5.7000	5.7000
3	0.10 mg/l	2	4.9000	4.9000
3	0.10 mg/l	3	5.4000	5.4000
3	0.10 mg/l	4	7.3000	7.3000
4	0.15	1	5.2000	5.2000
4	0.15	2	5.2000	5.2000
4	0.15	3	7.0000	7.0000
4	0.15	4	6.5000	6.5000
5	0.34	1	4.3000	4.3000
5	0.34	2	4.0000	4.0000
5	0.34	3	7.6000	7.6000
5	0.34	4	5.0000	5.0000
6	0.69	1	5.8000	5.8000
6	0.69	2	4.2000	4.2000
6	0.69	3	5.1000	5.1000
6	0.69	4	4.8000	4.8000
7	1.4	1	4.9000	4.9000
7	1.4	2	5.9000	5.9000
7	1.4	3	5.0000	5.0000
7	1.4	4	4.4000	4.4000

TWOSAMPLE T FOR CONTROL VS SOLVENT

	N	MEAN	STDEV	SE MEAN
CONTROL	4	5.450	0.520	0.26
SOLVENT	4	5.525	0.479	0.24

95 PCT CI FOR MU CONTROL - MU SOLVENT: (-0.98, 0.83)

TTEST MU CONTROL = MU SOLVENT (VS NE): T= -0.21 P=0.84 DF= 5

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

421320-02, THIDIAZURON, YOUNG/ADULT REPRODUCTIVE DAY
File: A:42132002.DT2 Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	3.424	0.571	0.673
Within (Error)	21	17.797	0.847	
Total	27	21.221		

Critical F value = 2.57 (0.05,6,21)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	SOLVENT CONTROL	5.525	5.525		
2	DILUT. CONTROL	5.450	5.450	0.115	
3	0.10 mg/l	5.825	5.825	-0.461	
4	0.15	5.975	5.975	-0.691	
5	0.34	5.225	5.225	0.461	
6	0.69	4.975	4.975	0.845	
7	1.4	5.050	5.050	0.730	

Dunnett table value = 2.46 (1 Tailed Value, P=0.05, df=20,6)

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	SOLVENT CONTROL	4			
2	DILUT. CONTROL	4	1.601	29.0	0.075
3	0.10 mg/l	4	1.601	29.0	-0.300
4	0.15	4	1.601	29.0	-0.450
5	0.34	4	1.601	29.0	0.300
6	0.69	4	1.601	29.0	0.550
7	1.4	4	1.601	29.0	0.475

DAPHNID LENGTH

Analysis of Variance File: thidiazu Date: 03-03-1992

n's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors: C	R	N	Mean	Median	S.D.
*	*	212	3.9548		0.2631
1	*	40	4.1838		0.1516
2	*	38	4.1711		0.1532
3	*	37	3.8473		0.1787
4	*	37	3.8608		0.2025
5	*	31	3.8661		0.3040
6	*	13	3.7500		0.1414
7	*	16	3.6731		0.1852
*	1	56	3.9263		0.2284
*	2	47	3.9404		0.2618
*	3	56	4.0098		0.2612
*	4	53	3.9396		0.2972
1	1	10	4.1150		0.1132
1	2	10	4.2600		0.1612
1	3	10	4.2100		0.1468
1	4	10	4.1500		0.1599
2	1	10	4.1850		0.1313
2	2	9	4.0667		0.1299
2	3	9	4.1389		0.1294
2	4	10	4.2800		0.1549
3	1	10	3.9000		0.1374
3	2	10	3.7750		0.2125
3	3	10	3.8200		0.1513
3	4	7	3.9143		0.2035
4	1	9	3.8000		0.1369
4	2	10	3.8250		0.2031
4	3	8	4.0188		0.2137
4	4	10	3.8250		0.2045
5	1	10	3.7550		0.2192
5	2	6	3.7500		0.1673
5	3	9	4.2056		0.2404
5	4	6	3.6583		0.2178
6	1	6	3.7417		0.1463
6	2	1	3.7000		0.0000
6	3	5	3.7300		0.1483
6	4	1	3.9500		0.0000
7	1	1	3.6700		0.0000
7	2	1	3.8000		0.0000
7	3	5	3.6700		0.1754
7	4	9	3.6611		0.2162

Fmax for testing homogeneity of between subjects variances: Not defined

Analysis of Variance Dependent variable: LENGTH

Source	df	SS (H)	MSS	F	P
Between Subjects	211	14.6035			
C (CONC)	6	6.6867	1.1145	36.456	0.0000
R (REP)	3	0.4081	0.1360	4.450	0.0048
CR	18	1.8838	0.1047	3.423	0.0000
Subj w Groups	184	5.6249	0.0306		

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	4.184	6	3.750
2	4.171	7	3.673
3	3.847		
4	3.861		
5	3.866		

Comparison	Bon-ferroni	Dunnett
1 > 2		
1 > 3	0.0000	0.0100
1 > 4	0.0000	0.0100
1 > 5	0.0000	0.0100
1 > 6	0.0000	0.0100
1 > 7	0.0000	0.0100
2 > 3	0.0000	N.A.
2 > 4	0.0000	N.A.
2 > 5	0.0000	N.A.
2 > 6	0.0000	N.A.
2 > 7	0.0000	N.A.
3 < 4		N.A.
3 < 5		N.A.
3 > 6		N.A.
3 > 7	0.0224	N.A.
4 < 5		N.A.
4 > 6		N.A.
4 > 7	0.0093	N.A.
5 > 6		N.A.
5 > 7	0.0094	N.A.
6 > 7		N.A.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

dephnid length

Data listing File: thidiazu Date: 03-03-1992

FILTER: None

Obs.	CONC	REP	LENGTH
1	1	1.00	4.05
2	1	1.00	4.30
3	1	1.00	4.20
4	1	1.00	4.15
5	1	1.00	4.00
6	1	1.00	4.05
7	1	1.00	4.25
8	1	1.00	4.15
9	1	1.00	4.05
10	1	1.00	3.95
11	1	2.00	4.00
12	1	2.00	4.20
13	1	2.00	4.05
14	1	2.00	4.45
15	1	2.00	4.30
16	1	2.00	4.40
17	1	2.00	4.25
18	1	2.00	4.50
19	1	2.00	4.20
20	1	2.00	4.25
21	1	3.00	4.20
22	1	3.00	4.10
23	1	3.00	4.30
24	1	3.00	4.40
25	1	3.00	4.20
26	1	3.00	4.05
27	1	3.00	4.20
28	1	3.00	4.30
29	1	3.00	4.40
30	1	3.00	3.95
31	1	4.00	4.50
32	1	4.00	4.05
33	1	4.00	4.00
34	1	4.00	4.15
35	1	4.00	4.05
36	1	4.00	4.30
37	1	4.00	4.15
38	1	4.00	4.00
39	1	4.00	4.05
40	1	4.00	4.25
41	2	1.00	4.20
42	2	1.00	4.05
43	2	1.00	4.30
44	2	1.00	4.25
45	2	1.00	4.05
46	2	1.00	4.15
47	2	1.00	4.35
48	2	1.00	4.25
49	2	1.00	4.30
50	2	1.00	3.95
51	2	2.00	3.85
52	2	2.00	4.20

53	2	2.00	4.00
54	2	2.00	4.25
55	2	2.00	4.15

Data listing File: thidiazu Date: 03-03-1992

FILTER: None

Obs.	CONC	REP	LENGTH
56	2	2.00	3.95
57	2	2.00	4.05
58	2	2.00	4.00
59	2	2.00	4.15
60	2	3.00	4.00
61	2	3.00	4.15
62	2	3.00	4.25
63	2	3.00	4.05
64	2	3.00	3.95
65	2	3.00	4.10
66	2	3.00	4.25
67	2	3.00	4.35
68	2	3.00	4.15
69	2	4.00	4.55
70	2	4.00	4.20
71	2	4.00	4.05
72	2	4.00	4.30
73	2	4.00	4.15
74	2	4.00	4.50
75	2	4.00	4.20
76	2	4.00	4.30
77	2	4.00	4.35
78	2	4.00	4.20
79	3	1.00	3.75
80	3	1.00	3.90
81	3	1.00	3.85
82	3	1.00	4.00
83	3	1.00	4.00
84	3	1.00	3.65
85	3	1.00	3.80
86	3	1.00	4.10
87	3	1.00	3.95
88	3	1.00	4.00
89	3	2.00	3.90
90	3	2.00	3.85
91	3	2.00	4.00
92	3	2.00	3.95
93	3	2.00	3.55
94	3	2.00	3.50
95	3	2.00	3.40
96	3	2.00	3.80
97	3	2.00	3.85
98	3	2.00	3.95
99	3	3.00	3.85
100	3	3.00	3.70
101	3	3.00	3.90
102	3	3.00	4.00
103	3	3.00	3.95
104	3	3.00	3.80
105	3	3.00	4.00
106	3	3.00	3.55
107	3	3.00	3.65

108	3	3.00	3.80
109	3	4.00	3.75
110	3	4.00	3.55

Data listing File: thidiazu Date: 03-03-1992

FILTER: None

Obs.	CONC	REP	LENGTH
111	3	4.00	4.00
112	3	4.00	4.15
113	3	4.00	4.00
114	3	4.00	4.05
115	3	4.00	3.90
116	4	1.00	3.65
117	4	1.00	3.80
118	4	1.00	3.75
119	4	1.00	3.80
120	4	1.00	3.55
121	4	1.00	3.90
122	4	1.00	3.85
123	4	1.00	4.00
124	4	1.00	3.90
125	4	2.00	3.90
126	4	2.00	4.00
127	4	2.00	3.60
128	4	2.00	3.95
129	4	2.00	3.85
130	4	2.00	3.50
131	4	2.00	4.00
132	4	2.00	4.10
133	4	2.00	3.75
134	4	2.00	3.60
135	4	3.00	4.20
136	4	3.00	4.25
137	4	3.00	4.10
138	4	3.00	4.00
139	4	3.00	3.75
140	4	3.00	3.65
141	4	3.00	4.05
142	4	3.00	4.15
143	4	4.00	3.75
144	4	4.00	3.95
145	4	4.00	4.00
146	4	4.00	3.85
147	4	4.00	4.10
148	4	4.00	3.80
149	4	4.00	3.65
150	4	4.00	3.40
151	4	4.00	4.00
152	4	4.00	3.75
153	5	1.00	3.40
154	5	1.00	3.95
155	5	1.00	4.05
156	5	1.00	4.00
157	5	1.00	3.80
158	5	1.00	3.55
159	5	1.00	3.70
160	5	1.00	3.75
161	5	1.00	3.85
162	5	1.00	3.50

163	5	2.00	3.50
164	5	2.00	3.95
165	5	2.00	3.80

Data listing File: thidiazu Date: 03-03-1992

FILTER: None

Obs.	CONC	REP	LENGTH
166	5	2.00	3.90
167	5	2.00	3.65
168	5	2.00	3.70
169	5	3.00	3.80
170	5	3.00	4.00
171	5	3.00	4.25
172	5	3.00	4.25
173	5	3.00	3.95
174	5	3.00	4.40
175	5	3.00	4.30
176	5	3.00	4.55
177	5	3.00	4.35
178	5	4.00	3.70
179	5	4.00	3.95
180	5	4.00	3.30
181	5	4.00	3.55
182	5	4.00	3.70
183	5	4.00	3.75
184	6	1.00	3.75
185	6	1.00	3.95
186	6	1.00	3.50
187	6	1.00	3.80
188	6	1.00	3.70
189	6	1.00	3.75
190	6	2.00	3.70
191	6	3.00	3.65
192	6	3.00	3.75
193	6	3.00	3.75
194	6	3.00	3.95
195	6	3.00	3.55
196	6	4.00	3.95
197	7	1.00	3.67
198	7	2.00	3.80
199	7	3.00	3.70
200	7	3.00	3.55
201	7	3.00	3.95
202	7	3.00	3.50
203	7	3.00	3.65
204	7	4.00	3.80
205	7	4.00	3.65
206	7	4.00	3.95
207	7	4.00	3.50
208	7	4.00	3.80
209	7	4.00	3.75
210	7	4.00	3.20
211	7	4.00	3.70
212	7	4.00	3.60

Shaughnessey # 120301 Chemical Name Thiobacarb Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
Chronic Fish		Concentrations Tested (pp___) = _____		
		MATC = >_____<_____ pp___.		
Species:		Effected Parameters = _____		
Lab:		Control Mortality (%) = Solvent Control Mortality (%) =		
MRID #		Comments:		
Chronic Invertebrate	99.3	Concentrations Tested (pp*M) = 0.10, 0.15, 0.31, 0.69, 1.4	L MK 3/3/12	Supplemental
Species: Daphnia magna		MATC = >_____<0.10 pp*M.		
Lab: ABC Laboratories		Effected Parameters = Daphnid length, Survival		
MRID # ~~421-32~~ 421320-02		Control Mortality (%) = 5.0 Solvent Control Mortality (%) = 0		
		Comments:		

*Mean measured concentrations

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DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron.
Shaughnessey No. ~~none listed~~ 120301.

2. **TEST MATERIAL:** Thidiazuron technical; N-phenyl-N'-1,2,3-thiadiazol-5-ylurea; CAS No. 51707-55-2; Batch code 7/9.82; 99.3% purity; a beige powder.

3. **STUDY TYPE:** Non-Target Plants: Vegetative Vigor Phytotoxicity Test - Tier 1. Species Tested: Soybean, Lettuce, Carrot, Tomato, Cucumber, Radish, Oat, Wheat, Corn, and Onion.

4. **CITATION:** Downey, S.S. 1990. Investigation into the Phytotoxic Effects of Thidiazuron on Vegetative Vigor (Tier 1). Laboratory Report ID. No. 502 AW. Conducted by NOR-AM Chemical Company, Environmental Sciences Department, Pikeville, NC. Submitted by NOR-AM Chemical Company, Wilmington, DE. EPA MRID No. 418191-01.

5. **REVIEWED BY:**

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

Signature: Mark Mossler
Date: 10/14/91 12/16/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 10/14/91 Michael Barry 11-23-92

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

Signature: Dana Rico 11/24/92
Date: Henry T. Craven 11/25/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 1 vegetative vigor non-target plant phytotoxicity test. Onion, wheat, carrot, cucumber, lettuce, soybean, and tomato were all significantly affected in some parameter observed. The NOEC for these species was therefore <0.2 lb ai/A. The NOEC for the remaining three species (corn, oat, and radish) was

0.2 lb ai/A. A summary of the species and parameters affected by greater than 25% is presented in Table 19 (attached).

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 six families (i.e., soybean, lettuce, carrot, tomato, cucumber, and radish). Monocotyledon plants were represented by four species from two families (i.e., corn, oat, wheat, and onion). Cultivars, lot number, and seed source were provided in the report.

B. **Test System:** Twenty pots were filled with 2.5 cm of perlite or pea gravel over which 5.0 cm (12.5 cm for large-seeded species) of a low organic matter soil (1.0%) was added. Excess seed of each crop were planted per pot so that each pot could be thinned to 5 vigorous plants. Oat, onion, wheat, carrot, lettuce, and tomato seed were covered with 0.6 cm of soil and corn, cucumber, radish, and soybean were covered with 2.5 cm of soil. Each pot was labeled accordingly and watering was accomplished by sub-irrigation until emergence.

Plants were allowed to grow for 8-31 days after planting. All plants had between 2 and 4 leaves upon application. The plants were sprayed with a hand-held sprayer equipped with a single nozzle held at 30 cm. The sprayer was calibrated for 0.2 lb ai/A prior to application by setting the pressure to 27 psi and all pots were placed within the spray swath.

After application, the pots were placed in a glasshouse with a 16-hour supplemented photoperiod, a temperature of 16-36°C, and a relative humidity of 15-100%. Irrigation was accomplished by top-watering (avoiding foliage) and the plants were fertilized with a slow-release fertilizer or liquid fertilizer as needed.

C. **Dosage:** The test solution was prepared by adding 18.13 g of thidiazuron to 900 ml of dimethylformamide (DMF). The DMF solution was brought to 1500 ml with

water. The resulting concentration corresponded to a treatment rate of 0.2 lb ai/A applied in 2 gal/A. Control plants were sprayed with 60% aqueous DMF.

D. **Design:** Each treatment/crop combination was replicated twenty times (i.e., 5 plants/pot, 20 pots/treatment or control). The plants were rated for spray scorch 2 days after application (DAA). At 7, 13, 21, 28, and 35 DAA, plants were assessed for abnormalities. Observations and measurements were terminated or taken respectively when abnormalities had either decreased to a very low level or showed no signs of improvement. Plant height and fresh weight were assessed at this time.

E. **Statistics:** A mean fresh weight per plant was determined for each pot. Growth inhibition was calculated using the following equation:

$$\% \text{ growth inhibition} = \frac{(\text{cont. growth} - \text{treat. growth})}{\text{cont. growth}} \times 100$$

The control and treatment damage, height, and fresh weight were compared to each other using a t-test.

12. **REPORTED RESULTS:**
Table 1 (attached) summarizes the results from the leaf scorch ratings taken at 2 DAA. Only lettuce was significantly affected by thidiazuron. However, this amount of damage was minimal (i.e., 15% leaf damage).

Chlorosis was observed in several species (cucumber, wheat, radish, soybean). The effect was however minimal in the latter three species (less than 4% at any timepoint). Chlorosis in cucumber reached 41% in comparison to the control at 13 DAA, but declined to 0% at 28 DAA (Table 3, attached).

A significant amount of necrosis was observed at the final observation period for treated wheat, cucumber, and lettuce plants (Table 5, attached). Wheat demonstrated only 3% necrosis in comparison to the controls 28 DAA. Cucumber was heavily damaged (83% necrosis) by 28 DAA. All lettuce plants were dead and necrotic (100%) by 7 DAA.

Three species exhibited a darkening of leaves upon treatment with thidiazuron. In carrot and soybean plants, the effect decreased to 7 and 3%, respectively, by the end of the observation periods. Cucumber demonstrated 28% leaf darkening by 28 DAA.

Four species demonstrated deformities. Carrot and soybean deformity declined to 9 and 1% by 35 and 28 DAA, respectively. Cucumber exhibited decreasing deformity over time in contrast to tomato, which showed increasing damage over time. The damage for cucumber and tomato was 48 and 90% by 28 and 35 DAA, respectively.

Suckering or stimulation of the axial meristems was apparent in treated soybeans, increasing to 78% by termination. A minor degree of increased reddening was exhibited by treated tomato plants, but never exceeded 3% and declined to zero by termination.

Cucumber and tomato demonstrated reductions in height (52 and 44% by 28 and 35 DAA, respectively - Table 15, attached). However, these species did not show a concomitant decrease in fresh weight. Onion, wheat, lettuce, and soybean demonstrated significant reductions in fresh weight by the end of the observation periods (Table 17, attached). Fresh weight of these four species was inhibited by 29, 23, 100, and 17%, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Four species of monocotyledons and six species of dicotyledons were treated with the maximum field application rate of thidiazuron. No abnormalities exceeding 25% at the final rating were observed in corn, oat, wheat, carrot, and radish. In the remainder of the species (onion, cucumber, lettuce, soybean, and tomato) effects greater than 25% were observed at the final rating and thus trigger Tier 2 testing."

The Quality Assurance Unit of NOR-AM Chemical Company stated that Good Laboratory Practice (GLP) Standards were employed. Statements of compliance to GLPs and QA were enclosed in the report.

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

A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines except for the following:

Plants should have been individually weighed so that variance within replicates could be determined.

B. **Statistical Analysis:** Dunnett's comparison test was conducted on onion fresh weight data because there was no variation in lettuce fresh weight (all tissue was dead and inhibition was therefore 100% for all

replications). The reviewer obtained the same results as the author.

C. **Discussion/Results:** Applications of thidiazuron significantly scorched lettuce 15%. Only wheat and soybean demonstrated significant amounts of plant chlorosis by the end of the observation period. The magnitude of the response was minimal (1 and 7% for wheat and soybean, respectively). Cucumber did demonstrate an additional 12% chlorosis over the controls, but this was non-significant. Wheat demonstrated significant signs of necrosis but the response was small (3%). In contrast, cucumber and lettuce sustained large amounts of necrosis (83 and 100%, respectively). Soybean, carrot, and cucumber demonstrated significant signs of leaf darkening at the end of the study (3, 7, and 28%, respectively). Four species exhibited leaf deformities at the end of the study. Soybean, carrot, cucumber, and tomato were affected by 1, 9, 48, and 90%, respectively. Suckering or tillering was observed in soybean plants. This condition affected 78% of the population. A 3% increase in anthocyanin content was witnessed for treated tomato plants. The NOEC for six of the test species (cucumber, lettuce, tomato, wheat, carrot, and soybean) was <0.2 lb ai/A and the NOEC for the remaining species was 0.2 lb ai/A based on these physiological parameters.

Tomato and cucumber were the only species to demonstrate significant decreases in plant height. These two species were reduced by 44 and 52%, respectively. Reductions in fresh weight were observed between the controls and plants treated with 0.2 lb ai/A thidiazuron for four species tested. Soybean, wheat, onion, and lettuce biomass was reduced by 17, 23, 29, and 100%, respectively. The NOEC for tomato, cucumber, lettuce, soybean, wheat, and onion was <0.2 lb ai/A and the NOEC for the remaining four species was 0.2 lb ai/A based on these vegetative parameters.

The author stated that height was not significantly reduced for lettuce. However, the reviewer fails to understand how height cannot be significantly different when all of the plants were dead by 7 DAA. However, the significance of the weight parameter for lettuce demonstrates that this species was severely injured by applications of thidiazuron. Additionally, only four lettuce plants were used in some of the treatment and control pots rather than five. Since all plants were

dead by 7 DAA, the reviewer feels than the number of plants used adequately reflects the toxicity of the test material.

This study is scientifically sound and meets the guideline requirements for a Tier 1 vegetative vigor non-target plant phytotoxicity test. Onion, wheat, carrot, cucumber, lettuce, soybean, and tomato were all significantly affected in some parameter observed. The NOEC for these species was therefore <0.2 lb ai/A. The NOEC for the remaining three species (corn, oat, and radish) was 0.2 lb ai/A.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

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

THIDIAZURON

Page_____ is not included in this copy.

Pages 121 through 128 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.

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____ 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.

onion fresh weight

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	20	2.9440	1.0438	35.5
2*	20	2.0850	.8477	40.7

NOEC = < 0.216 ac/A.

raw data from Appendix XXIII (attached)

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

Minumum detectable difference for Dunnett's test = -.508156
This difference corresponds to -17.26 percent of control

Between groups sum of squares = 7.378810 with 1 degrees of freedom.

Error mean square = .904110 with 38 degrees of freedom.

Bartlett's test p-value for equality of variances = .368

DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron. 120301
Shaughnessey No. ~~none listed~~.

2. **TEST MATERIAL:** Thidiazuron technical; N-phenyl-N'-(1,2,3-thidiazol-5'yl)-urea; CAS No. 51707-55-2; Batch No. 7/9.82; 99.3% active ingredient; a beige crystalline solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 1. Species Tested: *Selenastrum capricornutum*.

4. **CITATION:** Hughes, J.S. 1990. The Toxicity of Thidiazuron Technical to *Selenastrum capricornutum*. Laboratory Project No. B643-01-3. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by NOR-AM Chemical Company, Pikeville, NC. EPA MRID No. 417611-04.

5. **REVIEWED BY:**

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

Signature: Mark Mossler 12/16/92
Date: 10/14/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
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Signature: P. Kosalwat
Date: 10/14/91

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

Signature: Michael Davy 11-23-92
Date: Henry T. Craven 11/24/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 1 non-target aquatic plant study. Exposure of *S. capricornutum* to 0.15 mg ai/l thidiazuron (nominal concentration) for 5 days resulted in a 17% reduction in growth. The NOEC was 0.15 mg ai/l.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, *Selenastrum capricornutum*, came from laboratory stock cultures originally obtained from the University of Texas Culture Collection, Austin, TX. Stock cultures were maintained in synthetic algal assay procedure nutrient medium (AAP; Miller et al., 1978) under 4306 lux illumination, and a temperature of 24 ±2°C. The culture flasks were shaken continuously at 100 oscillations per minute. Transfers were made to maintain logarithmic growth. The culture used as inoculum had been transferred to fresh medium seven days before test initiation.

B. **Test System:** All glassware was cleaned according to EPA methods and autoclaved before use. Test vessels used were 250-ml Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 7.5 ±0.1 and filtered (0.22 µm).

The test vessels were kept in an incubator with environmental conditions like those employed in culturing.

C. **Dosage:** Five-day growth and reproduction test. For a Tier 1 test, one nominal concentration (0.15 mg ai/l), a control and a solvent control [N,N-dimethylformamide (DMF)] were used.

A primary stock solution of 5.0 mg ai/ml was prepared by adding 0.0504 g of thidiazuron technical to 10 ml of solvent. The material went into solution after inverting the flask several times. A secondary stock (0.5 mg ai/ml) was prepared from the primary stock. The test solution was prepared by diluting 0.075 ml of the secondary stock solution to 250 ml of sterile medium. A medium and solvent (0.3 ml DMF/l) control were also prepared.

D. **Test Design:** Fifty milliliters of the appropriate test solution were placed into each flask (3 replicate flasks per treatment and the controls).

An inoculum of *Selenastrum capricornutum* cells calculated to provide 3000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.226 ml per flask. The flasks were randomly repositioned each working day to minimize spatial

differences in the incubator. Cell counts were performed using an electronic particle counter on test days 3, 4, and 5. Three counts per replicate, using sample volumes of 0.1 to 2.0 ml, were made on each counting day.

The pH was measured at test initiation (initial test solution) and termination (replicates combined).

E. **Statistics:** Percent inhibition was computed by comparing the treatment cell densities with those of the solvent control using the following formula:

$$\%I = \frac{C - T}{C} \times 100$$

where C = mean growth in the solvent control and
T = mean growth in treated culture.

A t-test was performed to determine if the mean day 5 cell count in the treatment cultures was significantly different from that in the solvent control.

12. **REPORTED RESULTS:** Cell counts for the control, solvent control, and exposure concentration are given in Table 3 (attached).

Growth of *Selenastrum capricornutum* was inhibited by 17.5% when exposed to the test concentration. The cell growth in the medium control was not significantly different from growth in the solvent control.

The pH at initiation and termination ranged from 7.40 to 7.46 and from 8.36 to 8.44, respectively, in the test solution and the controls.

The 5-day EC_{50} was greater than the concentration equivalent to direct application at the maximum field rate.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Tier 2 testing is not required.

Quality Assurance and Good Laboratory Practice statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160, under the Federal Insecticide, Fungicide, and Rodenticide Act.

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

A. Test Procedure: The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

The dissolved oxygen and conductivity of the test solution were not measured.

Light intensity during the test was 4.306 klux. The recommended light intensity is 4 klux.

The temperature in the incubator was given as 24 ±2°C The report did not state whether the temperature was measured during the test period or, if measured, what the results were.

Cell growth measurements were not taken daily. Measurements were made on days 3, 4, and 5 only.

B. Statistical Analysis: The reviewer used Dunnett's test to analyze the 5-day data to determine the NOEC value. The results obtained by the reviewer are in agreement with the author's. None of the treatment or control densities were significantly different. The NOEC for *S. capricornutum* was 0.15 mg ai/l.

C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for a Tier 1 non-target aquatic plant study. Exposure of *S. capricornutum* to 0.15 mg ai/l thidiazuron (nominal concentration) for 5 days resulted in a 17% reduction in growth. The NOEC was 0.15 mg ai/l.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 9/24/91.

THIDIAZURON

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Pages _____ through _____ are not included.

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____ 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.

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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.

selenastrum cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	3	2363333.3333	180092.5688	7.6
2	3	2183333.3333	144683.5628	6.6
3	3	2126666.6667	155670.5924	7.3

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

1= solvent control
2= control
3= 0.15 mg ai/l
Raw Data from Table 3 (attached)
NOEC = 0.15 mg ai/l

Minumum detectable difference for Dunnett's test = -307284.363416
This difference corresponds to -13.00 percent of control

Between groups sum of squares =********** with 2 degrees of freedom.

Error mean square = ********** with 6 degrees of freedom.

Bartlett's test p-value for equality of variances = .957

Shaughnessey # 120301 Chemical Name Thidiazuron Chemical Class ________ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
14-Day EC_{50} Species: Lab: MRID #		EC_{50} = pp 95% C.L. ( ) Slope = # plants/vessel = Temperature = 14-Day Dose Level pp /(% Effect) ( ), ( ), ( ), ( ), ( ) Comments:		

5-Day EC_{50}
Species: Selenastrum capricornutum
Lab: Malcolm Pirnie
MRID # 417611-04
	99.3%	EC_{25} + EC_{50} = >0.15 mg a.i./l* 95% C.L. (N/A)
		Slope = N/A # Cells/ml = 3,000
		Temperature = 24±2°C
		5-Day Dose Level mg a.i./l* /(% Effect)
		0.15 (17), ( ), ( ), ( ), ( )
		Comments:
		* Based on nominal concentrations of active ingredient
		NOEC = 0.15 mg a.i./l*
			M. Hossler
			9/24/91
				Core

DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron. 120301
Shaughnessey No. ~~none listed~~.

2. **TEST MATERIAL:** Thidiazuron technical; N-phenyl-N'-1,2,3-thiadiazol-5-ylurea; CAS No. 51707-55-2; Batch code 7/9.82; 99.3% purity; a beige powder.

3. **STUDY TYPE:** Non-Target Plants: Seedling Emergence Phytotoxicity Test - Tier 1. Species Tested: Soybean, Lettuce, Carrot, Tomato, Cucumber, Radish, Oat, Wheat, Corn, and Onion.

4. **CITATION:** Downey, S.S. 1991. Investigation into the Phytotoxic Effects of Thidiazuron on Seedling Emergence (Tier 1). Laboratory Report ID. No. 501 AW. Conducted by NOR-AM Chemical Company, Environmental Sciences Department, Pikeville, NC. Submitted by NOR-AM Chemical Company, Wilmington, DE. EPA MRID No. 418213-01.

5. **REVIEWED BY:**

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

Signature: Mark A. Mossler
Date: 10/14/91 12/16/92

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 10/14/91

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

Signature: Michael Barry 11-23-92 / Henry T. Craven 11/25/92
Date: 11-24-92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 1 seedling emergence non-target plant phytotoxicity test. The NOEC of thidiazuron for all species except oat and tomato was 0.2 lb ai/A. The NOEC for oat and tomato was <0.2 lb ai/A. All effects were less than 25%.

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 six families (i.e., soybean, lettuce, carrot, tomato, cucumber, and radish). Monocotyledon plants were represented by four species from two families (i.e., corn, oat, wheat, and onion). Cultivars, lot number, and seed source were provided in the report.

B. **Test System:** Ten pots were filled with 2.5 cm of perlite over which 7.5 cm of a low organic matter soil (1.6%) was added. Twenty seeds of each crop were planted per pot and 0.6 cm of the soil was used to cover the seeds. Each pot was labeled accordingly.

The pots were sprayed with a hand-held sprayer equipped with a single nozzle at 30 cm above the pots. The sprayer was calibrated for 0.2 lb ai/A prior to application by setting the pressure to 27 psi and all pots were placed within the spray swath.

After application, the pots were placed in a growth chamber with a 15-hour photoperiod, a temperature of 20-21°C, and a relative humidity of 26-78%. Irrigation was accomplished using a matting which provided water to the pots by capillary action.

C. **Dosage:** The test solution was prepared by adding 12.09 g of thidiazuron to 600 ml of dimethylformamide (DMF). The DMF solution was brought to 1000 ml with water. The resulting concentration corresponded to a treatment rate of 0.2 lb ai/A applied in 2 gal/A. Control plants were sprayed with 60% aqueous DMF.

D. **Design:** Each treatment/crop combination was replicated ten times (i.e., 20 seeds/pot, 10 pots/treatment or control). At 5, 10, and 17 days after application (DAA), plants were assessed for number of emerged seedlings and any abnormalities. At 17 DAA, all plants were harvested at soil level and the fresh weight of plants per pot was recorded.

E. **Statistics:** Mean fresh weight per plant was obtained by dividing the total weight of plant tissue from each pot by the number of plants that emerged.

The control and treatment emergence and fresh weight were compared to each other using a t-test.

12. **REPORTED RESULTS:**
Table 1 (attached) summarizes the results from the emergence data. No significant differences in percent emergence existed for any of the test species except oat and carrot 17 DAA. The emergence of these two species was inhibited by 21 and -56% (growth stimulation), respectively.

Table 3 (attached) summarizes the results from the fresh weight data. No significant decreases in fresh weight existed for any of the test species except tomato 17 DAA. The weight of this species was inhibited by 17%. The fresh weight of cucumber increased by 19% when treated with thidiazuron in comparison to the solvent controls. The ranges of height of the treated and control plants were similar for the test species.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"The maximum field application rate of thidiazuron had little effect on the emergence rates and growth of the seedlings of 10 species of plant. At the final rating point (17 days post-treatment), the emergence of carrots was promoted by 56% and that of oats inhibited by 21%. Cucumber and tomato showed minor effects on fresh weights but these were less than 25%."

The Quality Assurance Unit of NOR-AM Chemical Company stated that Good Laboratory Practice (GLP) Standards were employed. Statements of compliance to GLPs and QA were enclosed in the report.

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

A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines except for the following:

Plants should have been individually weighed so that variance within replicates could be determined.

B. **Statistical Analysis:** Dunnett's comparison test was conducted on oat percent emergence data (the most sensitive species). The reviewer obtained the same results as the author.

C. **Discussion/Results:** Although the mean plant height was presented in Appendix IV, the author did not include the raw data for this parameter. However, upon review of the mean plant height, it seems that the test

material did not affect the growth of plants for the tested species.

In the summary, the author stated that the test material was applied in 20 gallons of water per acre. However, in the methods and results section, it was stated that the test material was applied in 2 gallons of water. The solution calculations agree with the latter. Two gallons is an extremely small amount of diluent to apply to an acre and is usually done by airplane. Although a low gallonage nozzle was used for application, no data as to the area of the spray plot or the volume of material applied were submitted.

In the results section, the author stated that a control containing 68% DMF was used. The reviewer feels that this is a typographical error and should read 60%.

No significant decreases in emergence were observed between the control and plants treated with 0.2 lb ai/A thidiazuron for the ten species tested except for oat 17 DAA. The emergence of this species was inhibited by 21%. The NOEC for all plants except oat was 0.2 lb ai/A. The NOEC for oat was <0.2 lb ai/A. Carrot emergence was 31% for the control plants 17 DAA. However, the treated carrot plants demonstrated a higher emergence than the controls, indicating that the test compound was not affecting the emergence of the plants. Carrot is a very slowly emerging plant and the test period for this species probably should have been extended until a higher emergence was witnessed.

No significant decreases in plant fresh weight were observed between the control and plants treated with 0.2 lb ai/A thidiazuron for the ten species tested except for tomato 17 DAA. Tomato fresh weight was inhibited by 17%. The fresh weight NOEC for all plants except tomato was 0.2 lb ai/A. The fresh weight NOEC for tomato was <0.2 lb ai/A.

This study is scientifically sound and meets the guideline requirements for a Tier 1 seedling emergence non-target plant phytotoxicity test. The NOEC for all species except oat and tomato was 0.2 lb ai/A. The NOEC for oat and tomato was <0.2 lb ai/A.

D. **Adequacy of the Study:**

(1) Classification: Core.

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

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

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

THIDIAZURON

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oat emergence

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	10	76.5000	13.1339	17.2
2*	10	60.5000	17.0701	28.2

NOEC = 0.216 ai/A

raw data from Appendix II (attached)

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

Minumum detectable difference for Dunnett's test = -11.782924
This difference corresponds to -15.40 percent of control

Between groups sum of squares = 1280.000000 with 1 degrees of freedom.

Error mean square = 231.944444 with 18 degrees of freedom.

Bartlett's test p-value for equality of variances = .438

DATA EVALUATION RECORD

1. **CHEMICAL:** Thidiazuron. 120301
Shaughnessey No. ~~none listed~~.

2. **TEST MATERIAL:** Thidiazuron technical: N-phenyl-N'-1,2,3-thiadiazol-5-ylurea; CAS No. 51707-55-2; Batch code 7/9.82; 99.3% purity; a beige powder.

3. **STUDY TYPE:** Non-Target Plants: Seed Germination Phytotoxicity Test - Tier 1. Species Tested: Soybean, Lettuce, Carrot, Tomato, Cucumber, Radish, Oat, Wheat, Corn, and Onion.

4. **CITATION:** Downey, S.S. 1991. Investigation into the Phytotoxic Effects of Thidiazuron on Seed Germination (Tier 1). Laboratory Report ID. No. 500 AW. Conducted by NOR-AM Chemical Company, Environmental Sciences Department, Pikeville, NC. Submitted by NOR-AM Chemical Company, Wilmington, DE. EPA MRID No. 417862-03.

5. **REVIEWED BY:**

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Date: 10/14/91

6. **APPROVED BY:**

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Date: 10/14/91

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

Signature: Michael Barrett 11-23-92 Daniel Rieder 11-24-92 Henry T. Craven 11/25/92
Date:

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 1 seed germination non-target plant phytotoxicity test. The NOEC for all test species was 0.2 lb ai/A.

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 six families (i.e., soybean, lettuce, carrot, tomato, cucumber, and radish). Monocotyledon plants were represented by four species from two families (i.e., corn, oat, wheat, and onion). Cultivars, lot number, and seed source were provided in the report.

B. **Test System:** A sterile filter-paper was placed in the bottom of a plastic petri plate (90 mm diameter, 12 mm depth). Two milliliters of the test or control solution were added to each plate of ten seeds for each of the ten species tested. Two circles of filter paper were placed over the seeds.

The treated and control plates were placed in separate light-tight boxes along with a container of water to maintain humidity. The boxes were placed in a growth chamber set at 19-23°C with 36-66% humidity. The plates were checked daily and watered with 1 or 2 ml of either control or treatment solution as needed.

C. **Dosage:** The test solution was prepared by adding 60 mg of the test material to 100 ml of acetone. One milliliter of this solution was brought to 1.0 l in sterilized, glass-distilled water. The resulting concentration was 0.6 ppm thidiazuron with a acetone concentration of 0.1%. This concentration corresponds to a treatment rate of 0.2 lb ai/A. The control solution contained 0.1 ppm of acetone in water.

D. **Design:** Each treatment/crop combination was replicated ten times (i.e., 10 seeds/plate, 10 plates/treatment or control). After incubation, the seeds were removed from the petri plates and the radicle lengths were observed. Seeds were considered germinated if the radicle was at least 5 mm long. Percent seed germination was calculated at 5, 9, and 14 days after treatment. Noticeable abnormalities between control and treatment seeds were noted.

E. **Statistics:** The control and treatment germination were compared to each other using a t-test.

12. **REPORTED RESULTS:**
Table 1 (attached) summarizes results for the germination test. No significant difference in percent germination existed for any of the test species upon full germination. The NOEC value for all species was 0.2 lb ai/A.

Various species demonstrated a few moldy or discolored seeds towards the end of the experiment. Several species had a little shorter radicle length than the controls and cucumber demonstrated slight inhibition of radicle formation.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"Treatment of 10 species of seeds with the equivalent of the maximum field application rate of thidiazuron had no effects on the final germination rates of any species."

The Quality Assurance Unit of NOR-AM Chemical Company stated that Good Laboratory Practice (GLP) Standards were employed. Statements of compliance to GLPs and QA were enclosed in the report.

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

A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines.

B. **Statistical Analysis:** Dunnett's comparison test was conducted on soybean percent germination (the species with the largest difference between treatment and control with the treatment mean being less than the control). The reviewer obtained the same results as the author (attached).

C. **Discussion/Results:** The author stated that the control solution contained 0.1 ppm acetone. The treatment solution contained 1 ml of acetone in 1 l of distilled water. This is a 0.1% acetone solution, not a 0.1 ppm solution. In actuality, this would be a 789 ppm acetone solution. Since the author stated that the controls were treated with water containing the same amount of acetone as the treatment plates, the reviewer feels that the validity of the experiment was not compromised.

Although the mean radicle length was presented in Appendix V, the author did not include the raw data for radicle length. However, upon review of the mean radicle length, it seems that the test material did not affect the growth of the radicle in the tested species,

except for oat (1-6.5 cm for the treatment plants vs. 8 cm for the control plants).

No significant decreases in germination were observed between the control and seeds treated with 0.2 lb ai/A thidiazuron for the ten species tested. The NOEC for all plants was 0.2 lb ai/A.

This study is scientifically sound and meets the guideline requirements for a Tier 1 seed germination non-target plant phytotoxicity test.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

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

THIDIAZURON

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____ Identity of product inert ingredients.

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____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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soybean germination

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
1 = control	10	100.0000	.0000	.0
2	10	98.0000	4.2164	4.3

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

NOEC = 0.2 lb ai/A

Raw data from Appendix V - (attached)

Minumum detectable difference for Dunnett's test = -2.306667
This difference corresponds to -2.31 percent of control

Between groups sum of squares = 20.000000 with 1 degrees of freedom.

Error mean square = 8.888889 with 18 degrees of freedom.

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