DP Barcode :. D183758
PC Code No : 080807
EEB Out : JAN 12 1993

JAN 12 1993

To: Walter Waldrop
Product Manager 71
Special Review and Reregistration Division (H7508W)

From: Anthony Maciorowski, Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 080807
Chemical Name : Simazine
Type Product : Herbicide
Product Name :
Company Name : Ciba-Geigy Corporation
Purpose : Data review for reregistration.

Action Code : 627 Date Due : 1/20/93
Reviewer : Tracy L. Perry

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)	425037-02	Y	122-1(A)		
71-2(B)			72-3(B)	425037-03	Y	122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)			123-1(B)		
71-5(A)			72-3(F)			123-2	425037-(04,05,06,07)	Y
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)

DP BARCODE: D183758 REREG CASE #

CASE: 819251 DATA PACKAGE RECORD DATE: 10/23/92
SUBMISSION: S427875 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 627 GENERIC DATA SUBMISSION
CHEMICALS: 080807 Simazine (ANSI) 100.00 %

ID#: 080807
COMPANY:
PRODUCT MANAGER: 71 WALTER WALDROP 703-308-8062 ROOM: CS1 3B3
PM TEAM REVIEWER: VENUS EAGLE 703-308-8045 ROOM: CS1 33B5
RECEIVED DATE: 10/08/92 DUE OUT DATE: 01/06/93

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 183758 EXPEDITE: N DATE SENT: 10/20/92 DATE RET.: / /
CHEMICAL: 080807 Simazine (ANSI)
DP TYPE: 001 Submission Related Data Package
ADMIN DUE DATE: 01/20/93 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	/ /	/ /
BRAN: EEB	/ /	/ /
SECT:	/ /	/ /
REVR :	/ /	/ /
CONTR:	/ /	/ /

* * * DATA REVIEW INSTRUCTIONS * * *

PLEASE REVIEW MRID'S 42503702 THRU 42503707
TO SEE IF THEY FULFILL GL'S 72-1, 72-3 AND 123-2.
THANKS

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JAN 12 1993

MEMORANDUM

SUBJECT: Simazine: review of data submitted in support of reregistration.

FROM: Anthony Maciorowski, Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Walter Waldrop, PM 71
Reregistration Branch
Special Review and Reregistration Division (H7508W)

Background

As part of the reregistration process for the List A herbicide, simazine, the registrant has submitted the following studies:

Murphy, D. and J.P. Swiegert. 1992. Simazine: A 96-Hour Flow-Through Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). Laboratory Project No. 108A-143. Conducted by Wildlife International, Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 425037-02.

Murphy, D. and J.P. Swigert. 1992. Simazine: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*). Project No. 108A-142. Conducted by Wildlife International, Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 425037-03.

Thompson, S.G. and J.P. Swigert. 1992. Simazine: A 14-Day Toxicity Test with Duckweed (*Lemna gibba* G3). Laboratory Project No. 108A-137. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 425037-04.

Thompson, S.G. and J.P. Swigert. 1992. Simazine: A 5-Day Toxicity Test with the Marine Diatom (*Skeletonema costatum*). Laboratory Project No. 108A-140. Conducted by Wildlife

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

MEMORANDUM

SUBJECT: Simazine: review of data submitted in support of reregistration.

FROM: Anthony Maciorowski, Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Walter Waldrop, PM 71
Reregistration Branch
Special Review and Reregistration Division (H7508W)

Background

As part of the reregistration process for the List A herbicide, simazine, the registrant has submitted the following studies:

Murphy, D. and J.P. Swiegert. 1992. Simazine: A 96-Hour Flow-Through Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*). Laboratory Project No. 108A-143. Conducted by Wildlife International, Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 425037-02.

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CONCURRENCES								
SYMBOL	H7507C	H7507C	H7507C					
SURNAME	S. Perry	Craven	[illegible]					
DATE	1/6/93	1/12/93	1-12-93					

OFFICIAL FILE CO 4

EPA Form 1320-1A (1/90) *Printed on Recycled Paper*

International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 425037-05.

Thompson, S.G. and J.P. Swigert. 1992. Simazine: A 5-Day Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*). Laboratory Project No. 108A-141. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 425037-06.

Thompson, S.G. and J.P. Swigert. 1992. Simazine: A 5-Day Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*). Laboratory Project No. 108A-138. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. MRID No. 425037-07.

Results

GDLN #	Test species	% AI	Results	MRID #	Fulfill Data Req.
72-3(a)	Sheepshead minnow	96.9	96-hour LC_{50} >4.3 ppm	425037-02	Yes
72-3(b)	Eastern oyster	96.9	96-hour EC_{50} >3.7 ppm	425037-03	Yes
123-2	Lemna gibba	96.9	14-day EC_{50} = 140 ppb	425037-04	Yes
123-2	Skeletonema costatum	96.9	5-day EC_{50} = 600 ppb	425037-05	Yes
123-2	Selenastrum capricornutum	96.9	5-day EC_{50} = 100 ppb	425037-06	Yes
123-2	Navicula pelliculosa	96.9	5-day EC_{50} = 90 ppb	425037-07	Yes

According to current Simazine labels, the maximum use rate for this herbicide is 18 lbs a.i./A for nonselective weed control on noncropland. Using this rate, aquatic EEC's (estimated environmental concentration) were determined by calculating the estimated runoff from 10 acres flowing into a 1 acre pond 6 feet deep (see Attachment A).

At an application rate of 18 lbs a.i./A, the EEC for runoff into a 6 foot pond is 110 ppb. As this value exceeds the EC_{50} values for two of the four aquatic plant species (Navicula, Selenastrum), Tier III aquatic plant testing is now required. After a few calculations, EEB has determined that use rates greater or equal to

5

15 lbs. a.i./A produce EEC's that exceed the lowest EC_{50} of 90 ppb for *Navicula* (see Attachment A).

Therefore, Tier III aquatic plant testing is required for use rates $\geq$ 15 lbs a.i./A. Prior to beginning a Tier III study, the registrant must submit a protocol to the Agency for review. However, at this time, all Tier III plant requirements are postponed pending development of a guidance document.

All applicable data requirements for simazine and their statuses can be found in the attached table. If you have any questions, please contact Tracy Perry at 305-6451 or Henry Craven at 305-5320.

ATTACHMENT A

EEC CALCULATION SHEET

For an unincoporated ground application of simazine at a rate of 18.0 lbs a.i./A:

Runoff

18.0	lbs	x	0.01 (1% runoff)	x	10 (A) (10 A drainage)	=	1.8	lbs (tot.runoff)

EEC of 1 lb a.i. direct application to 1 acre 6-foot deep pond = 61 ppb.

Therefore, EEC = 61 ppb x 1.8 lbs = 110 ppb (6 ft. pond)

For an unincoporated ground application of simazine at a rate of 15.0 a.i./A:

Runoff

15.0	lbs	x	0.01 (1% runoff)	x	10 (A) (10 A drainage)	=	1.5	lbs (tot.runoff)

Therefore, EEC = 61 ppb x 1.5 lbs = 91.5 ppb (6 ft. pond)

Date: 01/05/93
Case No: 819251
Chemical No: 080807

PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition [1]	Use Pattern [2]	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,B,C,D,E,J	YES	72798	NO
71-1(b) Acute Avian Oral, Quail/Duck	(TEP)	-	-	-	-
71-2(a) Acute Avian Diet, Quail	(TGAI)	A,B,C,D,E,J	YES	00022923	NO
71-2(b) Acute Avian Diet, Duck	(TGAI)	A,B,C,D,E,J	YES	00022923, 00139393	NO
71-3 Wild Mammal Toxicity	(TGAI)	-	-	-	-
71-4(a) Avian Reproduction Quail	(TGAI)	A,B,C,D,E,J	YES	163134	NO
71-4(b) Avian Reproduction Duck	(TGAI)	A,B,C,D,E,J	NO	43678	YES [3]
71-5(a) Simulated Terrestrial Field Study	(TEP)	-	-	-	-
71-5(b) Actual Terrestrial Field Study	(TEP)	-	-	-	-
72-1(a) Acute Fish Toxicity Bluegill	(TGAI)	A,B,C,D,E,J	YES	0043674	NO
72-1(b) Acute Fish Toxicity Bluegill	(TEP)	E,J	YES	40098001, 0002543	NO
72-1(c) Acute Fish Toxicity Rainbow Trout	(TGAI)	A,B,C,D,E,J	YES	43666	NO
72-1(d) Acute Fish Toxicity Rainbow Trout	(TEP)	E,J	YES	40245701	NO
72-2(a) Acute Aquatic Invertebrate Toxicity	(TGAI)	A,B,C,D,E,J	YES	0003503	NO
72-2(b) Acute Aquatic Invertebrate Toxicity	(TEP)	E,J	YES	40098001, 43676	NO
72-3(a) Acute Estu/Mari Tox Fish	(TGAI)	A,B,C,D,E,J	YES	42503702	NO
72-3(b) Acute Estu/Mari Tox Mollusk	(TGAI)	A,B,C,D,E,J	YES	42503703	NO
72-3(c) Acute Estu.Mari Tox Shrimp	(TGAI)	A,B,C,D,E,J	YES	23331	NO

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* In Bibliographic Citation column indicates study may be upgradeable

Date: 01/05/93
Case No: 819251
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PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition[1]	Use Pattern[2]	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/Marl Tox Fish	(TEP)	-	-	-	-
72-3(e) Acute Estu/Marl Tox Mollusk	(TEP)	-	-	-	-
72-3(f) Acute Estu/Marl Tox Shrimp	(TEP)	-	-	-	-
72-4(a) Early Life-Stage Fish	(TGAI)	A,B,C,D,E,J	YES	43676, 43668	NO
72-4(b) Live-Cycle Aquatic Invertebrate	(TGAI)	A,B,C,D,E,J	YES	43676	NO
72-5 Life-Cycle Fish	(TGAI)	-	-	-	-
72-6 Aquatic Org. Accumulation	(TGAI)	E,J	YES	43668, 43670	NO
72-7(a) Simulated Aquatic Field Study	(TEP)	E,J	NO	-	NO[4]
72-7(b) Actual Aquatic Field Study	(TEP)	-	-	-	-
122-1(a) Seed Germ./Seedling Emerg.	(TGAI)	-	-	-	-
122-1(b) Vegetative Vigor	(TGAI)	-	-	-	-
122-2 Aquatic Plant Growth	(TGAI)	-	-	-	-
123-1(a) Seed Germ./Seedling Emerg.	(TGAI)	A,B,C,D,E,J	NO	-	YES[5]
123-1(b) Vegetative Vigor	(TGAI)	A,B,C,D,E,J	NO	-	YES[5]
123-2 Aquatic Plant Growth	(TGAI)	A,B,C,D,E,J	YES	425037-04,05,06,07	YES[6]
124-1 Terrestrial Field Study	(TEP)	-	-	-	-
124-2 Aquatic Field Study	(TEP)	A,B,C,D,E,J	NO	-	YES[7]
141-1 Honey Bee Acute Contact	(TGAI)	A,B,C,D,E,J	YES	00036935	NO
141-2 Honey Bee Residue on Foliage	(TEP)	-	-	-	-
141-5 Field Test for Pollinators	(TEP)	-	-	-	-

6

* In Bibliographic Citation column indicates study may be upgradeable

1.Composition: TGAI=Technical grade of the active ingredient; PAIRA=Pure active ingredient, radiolabeled; TEP=Typical end-use product

2.Use Patterns: A=Terrestrial Food Crop; B=Terrestrial Feed Crop; C=Terrestrial Non-Food Crop; D=Aquatic Food Crop; E=Aquatic Non-Food Outdoor; F=Aquatic Non-Food Industrial; G=Aquatic Non-Food Residential; H=Greenhouse Food Crop; I=Greenhouse Non-Food Crop; J=Forestry; K=Outdoor Residential; L=Indoor Food; M=Indoor Non-Food; N=Indoor Medical; O=Indoor Residential; Z=Use Group for Site 00000

3. As stated in the Simazine Second Round Review (3/8/89) and as confirmed by the registrant in their April 7, 1992 meeting with EEB, the avian reproduction study with the mallard must be repeated. The maximum concentration tested (20 ppm) in the initial study was well below residue levels reasonably expected on waterfowl food items.

4. Based on new Agency policy, this data requirement is waived. However, EEB is still concerned with the risk to aquatic organisms associated with certain aquatic use patterns (i.e. fish ponds and hatcheries) and use rates greater than 10 lbs a.i./A (noncropland). These concerns need to be addressed by the registrant. One possible risk mitigation option was suggested by the registrant in its April 7, 1992 meeting with EEB. In this meeting, the registrant proposed cancelling all aquatic uses and use rates above 10 lbs a.i./A.

5. Required for all terrestrial non-food and aquatic uses.

6. The four aquatic studies conducted with *Selenastrum capricornutum*, *Lemna gibba*, *Navicula pelliculosa*, and *Skeletonema costatum* are all core. However, the aquatic plant study using *Anabaena flos-aquae* is still outstanding.

7. Tier III aquatic plant testing is required for use rates $\geq$ 15 lbs. a.i./A. Prior to beginning a Tier III study, the registrant must submit a protocol to the Agency for review. However, at this time, all Tier III requirements are postponed pending development of a guidance document.

DATA EVALUATION RECORD

1. **CHEMICAL:** Simazine.
Shaughnessey No. 080807.

2. **TEST MATERIAL:** Simazine technical; Batch Code D3303B10; ID No. FL-850614 ARS-16871; 96.9% active ingredient (a.i.); a white powder.

3. **STUDY TYPE:** 72-3. Acute Toxicity Test for Estuarine and Marine Fish. Species Tested: Sheepshead minnow (Cyprinodon variegatus).

4. **CITATION:** Murphy, D. and J.P. Swiegert. 1992. Simazine: A 96-Hour Flow-Through Acute Toxicity Test with the Sheepshead Minnow (Cyprinodon variegatus). Laboratory Project No. 108A-143. Conducted by Wildlife International, Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 425037-02.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 12/30/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven
Date: 1/7/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for an acute toxicity test using the sheepshead minnow (Cyprinodon variegatus). The 96-hour LC_{50} was >4.3 mg a.i./l (mean measured concentration). The NOEC was 4.3 mg a.i./l.

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

9. **BACKGROUND:**

11

MRID No. 425037-02

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Shaughnessey No. 080807.

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5. **REVIEWED BY:**

Charles G. Nace Jr., M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Charles G Nace Jr.
Date: 12/09/92

6. **APPROVED BY:**

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

Signature: Rosemary Graham Mora,
Date: 12/2/92

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

Signature: Henry T. Craven
Date: 1/7/92

Tracy L. Perry 12/30/92

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an acute toxicity test using the sheepshead minnow (*Cyprinodon variegatus*). The concentration levels tested were <100 mg/l but not high enough to produce a precise LC_{50}. The 96-hour LC_{50} was >4.3 mg a.i./l (mean measured concentration). The NOEC was 4.3 mg a.i./l.

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

9. **BACKGROUND:**

6.0 12

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile sheepshead minnows (Cyprinodon variegatus) of the same year class were obtained from in-house cultures. During holding, the fish were fed flaked food, salmon mash and/or salmon starter, live brine shrimp nauplii, and frozen brine shrimp nauplii (Artemia sp.). A 16-hour light photoperiod was maintained. The temperature was 21.2-22.6°C and the salinity was 25-26 parts per thousand (ppt). There were no observed signs of disease during the 14-day holding period.

The fish were acclimated to test conditions for 50 hours prior to test initiation. The fish were not fed during the acclimation period. At test termination, the average length and weight of the control sheepshead minnows were 22 mm (17-26 mm) and 0.36 g (0.25-0.56 g), respectively. Biomass loading during the test was 0.24 g/l at any given time or 0.02 g/l/day.

B. **Test System:** Test chambers were Teflon®-lined 25-l polyethylene aquaria filled with 15 l of test solution (15.2 cm deep). The test solution was delivered to the chambers using a continuous-flow proportional diluter system. A peristaltic pump delivered the stock test solutions and solvent to the mixing chambers. After mixing, the flow was split into replicate chambers. Twelve volume additions of test solution were added over a 24 hour period to each replicate.

The aquaria were placed in a water bath (22 ±1°C) under fluorescent lighting regulated to produce a photoperiod of 16-hours of light and 8-hours of darkness. Thirty-minute transition periods to simulate dawn and dusk were also provided. Light intensity at the surface of the water was 344 lux.

Dilution water was collected from the Indian River Inlet, DE. The water was filtered through a sand filter to remove particles >25 µm, stored in a tank, diluted with fresh well water, aerated with spray nozzles, filtered, and then sent to the diluter.

A stock solution (5.17 mg a.i./l) was prepared by dissolving the test material in dimethyl formamide (DMF) and sonicating. The stock was further diluted with solvent to prepare four additional stocks at

concentrations of 3.10, 1.90, 1.10, and 0.70 mg a.i./ml. The test solutions were prepared by mixing the stock solutions with dilution water in the diluter.

C. **Dosage:** Ninety-six-hour flow-through test. Based on preliminary data, five nominal concentrations of 0.8, 1.3, 2.2, 3.6, and 6.0 mg a.i./l, a solvent control, and a dilution water control were used. The solvent concentration in the treatment and solvent control solutions was 1.2 ml/l.

D. **Design:** Two replicates were used for each treatment and control. Fish were impartially distributed by twos until each replicate contained 10 fish (i.e., 20 fish/treatment). Fish survival and signs of sublethal and behavioral effects were recorded at 4, 24, 48, 72, and 96 hours. Fish were not fed during testing and the test solutions were not aerated.

Temperature was recorded continuously in one dilution water control vessel and at the beginning and end of the test in all vessels. The dissolved oxygen concentration (DO) and pH were measured in alternate replicates in each treatment and control group daily. Salinity was recorded at the beginning of the test in the dilution water control.

Samples of the test solutions were collected at the beginning and end of the study in all chambers to verify exposure concentrations. The concentration of active ingredient of the test material was measured using gas chromatography.

E. **Statistics:** The median lethal concentration (LC_{50}) was determined by visual inspection of the mortality data.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.86, 1.5, 2.6, 4.3, and 4.3 mg a.i./l (Table 1, attached). The relatively low concentrations measured at 6.0 mg a.i./l nominal test concentration were a reflection of the 3.5 mg a.i./l water solubility limit of simazine. A white precipitate was observed in the mixing chambers of the 2.6, 4.3, and 4.3 mg a.i./l (mean measured concentration) treatment groups during the test, suggesting that the water solubility of the test substance was exceeded.

There was no mortality in the dilution water control, solvent control, or treatment groups during the test. All organisms appeared normal in appearance and behavior throughout the test (Table 3, attached).

Based on the results of continuous and daily temperature monitoring, the temperature ranged from 21.5 to 22.3°C. The DO ranged from 5.8 to 6.9 mg/l, the salinity was 25 ppt, and pH ranged from 8.2 to 8.3.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour LC_{50} value for sheepshead minnows exposed to, simazine was >4.3 mg a.i./l. The 96-hour no mortality concentration and no observed effect concentration determined by visually examining the mortality data was 4.3 mg a.i./l.

The study was conducted under EPA GLP guidelines stated in 40 CFR, Part 160. A Quality Assurance statement was included in the report.

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

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

Test using euryhaline test species such as the sheepshead minnow should be performed at a salinity of 10-17 ppt. The salinity of the water in this test was 25 ppt.

The maximum recommended solvent concentration is 0.5 ml/l. The solvent concentration in this test was 1.2 ml/l.

The primary stock solution was prepared by dissolving the test material in DMF at a "concentration of 5.17 mg a.i./l." Additional stocks at "concentrations of 3.10, 1.90, 1.10, and 0.70 mg a.i./ml" were prepared by diluting the primary stock with DMF. The secondary stock solutions are more concentrated than the primary stock solutions. This is a discrepancy in the report.

B. **Statistical Analysis:** Since there was no mortality during the test, no LC_{50} could be calculated.

C. **Discussion/Results:** The test material was reported as having a solubility of 3.5 mg a.i./l in water. Attempts were made to increase the solubility of the test material by using a solvent (DMF) at higher than recommended concentrations and sonicating the test solutions. The solvent concentration used did not appear to affect the test since there was no mortality in the solvent control. These methods did not appear

to substantially increase solubility as the highest mean measured concentration was 4.3 mg a.i./l. From the results of the solvent screening test, the test material was insoluble in the other recommended solvents. A precipitate was reported in the three highest concentrations which would indicate that the solubility was exceeded.

The guidelines state that the requirement to test up to 100 ppm can be waived for poorly soluble chemicals if "techniques to maximize chemical dissolution in the test media have been exhausted..." Based on the above, the reviewer feels that simazine was tested up to its maximum solubility as the guidelines require.

This study is scientifically sound and meets the guideline requirements for an acute toxicity test using the sheepshead minnow (Cyprinodon variegatus). The 96-hour LC_{50} was >4.3 mg a.i./l (mean measured concentration). The NOEC was 4.3 mg a.i./l.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 11/13/92.

SIMAZINE 080807 RIN 1646-93

Page_____ is not included in this copy.

Pages 17 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.

✓ 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.

DATA EVALUATION RECORD

1. **CHEMICAL:** Simazine.
Shaughnessey No. 080807.

2. **TEST MATERIAL:** Simazine technical; Batch Code D3303B10; I.D. No. FL-850614 ARS-16871; 96.9% active ingredient (a.i.); a white powder.

3. **STUDY TYPE:** 72-3. Mollusc 96-Hour Flow Through Shell Deposition Study. Species Tested: Eastern Oyster (*Crassostrea virginica*).

4. **CITATION:** Murphy, D. and J.P. Swigert. 1992. Simazine: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*). Project No. 108A-142. Conducted by Wildlife International, Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 425037-03.

5. **REVIEWED BY:**

Charles G. Nace Jr., M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Charles G Nace Jr.
Date: 12/02/92

6. **APPROVED BY:**

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

Signature: Rosemary Graham Mora
Date: 12/2/92

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

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

Tracy L. Perry 12/30/92

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for an acute toxicity test using the eastern oyster (*Crassostrea virginica*). The concentration levels tested were <100 mg/l but not high enough to produce a precise EC_{50} The 96-hour EC_{50} was >3.7 mg a.i./l (mean measured concentration). The NOEC was 3.7 mg a.i./l.

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

9. **BACKGROUND:**

20

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 Pasadena, MD and were held under test conditions for at least 10 days prior to test initiation. The oysters were supplied with unfiltered natural seawater supplemented with algae (*Thalassiosira sp.*). During holding, the temperature was 21.4-22.7°C, the salinity was 22-26 parts per thousand (ppt), the pH was 7.6-8.2, and the dissolved oxygen concentration was 5.2-7.4 mg/l. A random sample of 20 oysters had an average length of 40 mm (38-48 mm). Mortality during the holding period was 2%.

B. **Test System:** Test chambers were Teflon®-lined 57-l polyethylene aquaria filled with 12.6 l of test solution (7.4 cm deep). The test solution was delivered to the chambers using a continuous-flow proportional diluter system. A peristaltic pump delivered the stock solutions and solvent to mixing chambers. The flow to the test chambers was 1 l/oyster/hour.

The aquaria were randomly placed in a water bath (22 ±1°C) under fluorescent lighting regulated to produce a photoperiod of 16-hours of light and 8-hours of darkness. Thirty-minute transition periods to simulate dawn and dusk were also provided. Light intensity at the surface of the water was 301 lux.

Dilution water was unfiltered seawater collected from the Indian River Inlet, DE, diluted with well water to a salinity of 25 ppt, aerated with spray nozzles, and then sent to the diluter.

A stock solution (5.17 mg a.i./l) was prepared by dissolving the test material in dimethyl formamide (DMF) and sonicating. Aliquots of the stock were further diluted with solvent to prepare four additional stocks at concentrations of 3.10, 1.90, 1.10, and 0.70 mg a.i./ml. The test solutions were prepared by mixing the stock solutions with dilution water in the diluter.

C. **Dosage:** Ninety-six-hour, flow-through test. Based on preliminary data, five nominal concentrations of 0.8, 1.3, 2.2, 3.6, and 6.0 mg a.i./l, a solvent control, and a dilution water control were selected for the

test. The solvent concentration in the treatment and solvent control solutions was 1.2 ml/l.

D. **Design:** Immediately prior to test initiation, 1-8 mm of shell periphery were removed from each oyster using a motorized grinder. The oysters were indiscriminately divided in groups of 20 and distributed to each of one test vessel per treatment. To maximize new shell growth, algae (*Thalassiosira*) were added to the test solutions.

Observations of mortality and toxicity signs were made daily. At the end of the test, the length of the longest finger of new shell growth on each oyster was measured to the nearest 0.05 mm. Shell growth inhibition in each treatment group was expressed as a percentage of the mean growth in the dilution water control.

Temperature was recorded continuously in the dilution water control vessel. The pH, salinity, and DO were measured in each treatment and control groups at test initiation, 48, and 96 hours.

Samples of the test solutions were collected at the beginning and end of the study to verify exposure concentrations. The concentration of active ingredient in the water samples was measured using gas chromatography (GC).

E. **Statistics:** The median effective concentration (EC_{50}) was determined by visual examination of the growth data.

12. **REPORTED RESULTS:** Mean measured concentrations were 0.80, 1.3, 2.3, 3.5, and 3.7 mg a.i./l (Table 1, attached). These values represent 62-105% of nominal concentrations. A white precipitate was observed in the mixing chambers at 2.3, 3.5, and 3.7 mg a.i./l (mean measured concentrations) during the test, suggesting that the water solubility of the test substance was exceeded.

There were no mortalities or observations of sublethal responses during the test. Oyster shell growth in the dilution water control averaged 4.8 mm over the 96-hour test period, while oyster shell growth in the solvent control group averaged 5.1 mm (Table 3, attached). There were no apparent treatment-related effects on new shell growth at any of the concentrations tested. Growth inhibition ranged

from 1.6% (1.3 mg a.i./l) to 11% (2.3 mg a.i./l) (Table 4, attached).

The temperature ranged from 21.6°C to 22.4°C; the dissolved oxygen concentration ranged from 6.4 to 7.1 mg/l; the salinity ranged from 23 to 25 ppt; and pH ranged from 7.9 to 8.1.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 96-hour EC_{50} value for eastern oysters exposed to simazine was estimated to be >3.7 mg a.i./l, the highest concentration tested. Based upon visual inspection of the data, the no observed effect concentration was 3.7 mg a.i./l.

This study included an EPA Good Laboratory Practice compliance statement and a Quality Assurance statement which stated that the study followed guidelines in 40 CFR Part 160.

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

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

In this study, the flow rate of the test solution was 1 l/oyster/hour. According to a protocol recommended by the SEP, each oyster should receive a minimum of 5 L of flow-through test solution per hour. However, the above method is considered acceptable because a supplemental diet was added.

The solvent concentration was 1.2 ml/l, which is higher than the recommended concentration of 0.5 ml/l.

The primary stock solution was prepared by dissolving the test material in DMF at a "concentration of 5.17 mg a.i./l." Additional stocks at "concentrations of 3.10, 1.90, 1.10, and 0.70 mg a.i./ml" were prepared by diluting the primary stock with DMF. The secondary stock solutions are more concentrated than the primary stock solutions. This is a discrepancy in the report.

B. **Statistical Analysis:** Since no concentration effected ≥50% of the test organisms, the calculation of an EC_{50} was not possible. The growth data was analyzed using Bonferroni's t-test in TOXSTAT. No significant differences were found at any concentration tested (see attached printout).

C. **Discussion/Results:** The test material was reported as having a solubility of 3.5 mg a.i./l in water. Attempts were made to increase the solubility of the test material by using a solvent (DMF) at higher than recommended concentrations and sonicating the test solutions. The solvent concentration used did not appear to affect the test since there was no mortality in the solvent control. These methods did not appear to substantially increase solubility as the highest mean measured concentration was 3.7 mg a.i./l. From the results of the solvent screening test, the test material was insoluble in the other recommended solvents. A precipitate was reported in the three highest concentrations which would indicate that the solubility was exceeded.

The guidelines state that the requirement to test up to 100 ppm can be waived for poorly soluble chemicals if "techniques to maximize chemical dissolution in the test media have been exhausted..." Based on the above, the reviewer feels that simazine was tested up to its maximum solubility as the guidelines require.

This study is scientifically sound and meets the guideline requirements for an acute toxicity test using the eastern oyster (Crassostrea virginica). The 96-hour EC_{50} was >3.7 mg a.i./l (mean measured concentration). The NOEC was determined to be 3.7 mg a.i./l.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 11/16/92.

SIMAZINE 080807 RIN 1646-93

Page_____ is not included in this copy.

Pages 25 through 27 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.

✓ 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.

TITLE: SIMAZINE - EASTERN OYSTER SHELL GROWTH
FILE: SIMAZINE.TOX
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	NEG. CONTROL	1	5.2500	5.2500
1	NEG. CONTROL	2	4.5000	4.5000
1	NEG. CONTROL	3	2.9000	2.9000
1	NEG. CONTROL	4	5.9500	5.9500
1	NEG. CONTROL	5	4.1000	4.1000
1	NEG. CONTROL	6	4.2000	4.2000
1	NEG. CONTROL	7	6.0500	6.0500
1	NEG. CONTROL	8	6.1500	6.1500
1	NEG. CONTROL	9	5.3000	5.3000
1	NEG. CONTROL	10	6.9000	6.9000
1	NEG. CONTROL	11	3.1000	3.1000
1	NEG. CONTROL	12	4.1500	4.1500
1	NEG. CONTROL	13	4.4000	4.4000
1	NEG. CONTROL	14	3.8500	3.8500
1	NEG. CONTROL	15	3.8000	3.8000
1	NEG. CONTROL	16	4.2500	4.2500
1	NEG. CONTROL	17	4.1000	4.1000
1	NEG. CONTROL	18	4.3500	4.3500
1	NEG. CONTROL	19	8.2000	8.2000
1	NEG. CONTROL	20	5.0500	5.0500
2	SOL. CONTROL	1	4.6000	4.6000
2	SOL. CONTROL	2	1.9000	1.9000
2	SOL. CONTROL	3	6.3500	6.3500
2	SOL. CONTROL	4	6.3000	6.3000
2	SOL. CONTROL	5	8.9000	8.9000
2	SOL. CONTROL	6	3.5500	3.5500
2	SOL. CONTROL	7	6.0500	6.0500
2	SOL. CONTROL	8	6.7500	6.7500
2	SOL. CONTROL	9	3.8000	3.8000
2	SOL. CONTROL	10	4.1500	4.1500
2	SOL. CONTROL	11	3.7000	3.7000
2	SOL. CONTROL	12	2.4500	2.4500
2	SOL. CONTROL	13	6.7000	6.7000
2	SOL. CONTROL	14	6.0000	6.0000
2	SOL. CONTROL	15	7.6000	7.6000
2	SOL. CONTROL	16	6.7000	6.7000
2	SOL. CONTROL	17	4.6000	4.6000
2	SOL. CONTROL	18	3.7000	3.7000
2	SOL. CONTROL	19	5.6500	5.6500
2	SOL. CONTROL	20	3.1000	3.1000
3	0.74	1	3.3000	3.3000
3	0.74	2	4.5000	4.5000
3	0.74	3	4.8500	4.8500
3	0.74	4	5.5500	5.5500
3	0.74	5	4.4500	4.4500
3	0.74	6	4.9000	4.9000
3	0.74	7	6.1500	6.1500
3	0.74	8	3.9000	3.9000
3	0.74	9	6.8500	6.8500
3	0.74	10	4.1000	4.1000

3	0.74	11	2.9000	2.9000
3	0.74	12	3.0500	3.0500
3	0.74	13	1.9000	1.9000
3	0.74	14	5.1000	5.1000
3	0.74	15	5.7000	5.7000
3	0.74	16	3.1000	3.1000
3	0.74	17	3.5500	3.5500
3	0.74	18	5.0500	5.0500
3	0.74	19	7.0000	7.0000
3	0.74	20	3.0500	3.0500
4	1.3	1	3.3000	3.3000
4	1.3	2	6.5000	6.5000
4	1.3	3	3.9000	3.9000
4	1.3	4	5.9500	5.9500
4	1.3	5	5.1000	5.1000
4	1.3	6	9.1000	9.1000
4	1.3	7	4.2500	4.2500
4	1.3	8	4.1500	4.1500
4	1.3	9	5.8000	5.8000
4	1.3	10	6.1500	6.1500
4	1.3	11	4.5000	4.5000
4	1.3	12	3.6000	3.6000
4	1.3	13	4.5500	4.5500
4	1.3	14	3.4500	3.4500
4	1.3	15	8.1500	8.1500
4	1.3	16	4.9000	4.9000
4	1.3	17	4.8500	4.8500
4	1.3	18	3.8000	3.8000
4	1.3	19	4.0500	4.0500
4	1.3	20	5.2500	5.2500
5	2.1	1	4.8000	4.8000
5	2.1	2	5.0500	5.0500
5	2.1	3	5.0000	5.0000
5	2.1	4	3.0000	3.0000
5	2.1	5	4.1500	4.1500
5	2.1	6	5.4500	5.4500
5	2.1	7	4.1000	4.1000
5	2.1	8	3.7500	3.7500
5	2.1	9	4.5000	4.5000
5	2.1	10	3.3500	3.3500
5	2.1	11	4.2000	4.2000
5	2.1	12	4.1500	4.1500
5	2.1	13	5.0500	5.0500
5	2.1	14	3.3000	3.3000
5	2.1	15	6.4000	6.4000
5	2.1	16	4.1000	4.1000
5	2.1	17	4.8500	4.8500
5	2.1	18	5.2000	5.2000
5	2.1	19	5.3000	5.3000
5	2.1	20	2.9000	2.9000
6	3.2	1	4.0500	4.0500
6	3.2	2	5.7500	5.7500
6	3.2	3	2.2000	2.2000
6	3.2	4	6.9500	6.9500
6	3.2	5	5.2000	5.2000
6	3.2	6	3.2000	3.2000
6	3.2	7	4.0500	4.0500
6	3.2	8	3.7000	3.7000
6	3.2	9	4.6000	4.6000
6	3.2	10	5.2500	5.2500

6	3.2	11	4.5500	4.5500
6	3.2	12	4.8000	4.8000
6	3.2	13	5.9000	5.9000
6	3.2	14	3.1000	3.1000
6	3.2	15	3.7500	3.7500
6	3.2	16	3.9500	3.9500
6	3.2	17	3.9500	3.9500
6	3.2	18	5.3000	5.3000
6	3.2	19	4.4000	4.4000
6	3.2	20	6.9000	6.9000
7	3.4	1	3.8000	3.8000
7	3.4	2	6.8000	6.8000
7	3.4	3	5.1000	5.1000
7	3.4	4	4.9000	4.9000
7	3.4	5	2.4000	2.4000
7	3.4	6	6.9500	6.9500
7	3.4	7	3.4000	3.4000
7	3.4	8	5.8500	5.8500
7	3.4	9	4.2000	4.2000
7	3.4	10	2.3500	2.3500
7	3.4	11	3.4000	3.4000
7	3.4	12	5.7000	5.7000
7	3.4	13	5.6000	5.6000
7	3.4	14	6.1500	6.1500
7	3.4	15	6.6500	6.6500
7	3.4	16	4.1500	4.1500
7	3.4	17	5.2000	5.2000
7	3.4	18	3.1500	3.1500
7	3.4	19	3.8000	3.8000
7	3.4	20	3.2000	3.2000

SIMAZINE - EASTERN OYSTER SHELL GROWTH
File: SIMAZINE.TOX Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	NEG. CONTROL	4.827	4.827		
2	SOL. CONTROL	5.127	5.127	-0.678	
3	0.74	4.448	4.448	0.859	
4	1.3	5.065	5.065	-0.537	
5	2.1	4.430	4.430	0.898	
6	3.2	4.577	4.577	0.565	
7	3.4	4.637	4.637	0.429	

Bonferroni T table value = 2.43 (1 Tailed Value, P=0.05, df=120,6)

SIMAZINE - EASTERN OYSTER SHELL GROWTH
File: SIMAZINE.TOX Transform: NO TRANSFORMATION

BONFERRONI T-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	NEG. CONTROL	20			
2	SOL. CONTROL	20	1.075	22.3	-0.300
3	0.74	20	1.075	22.3	0.380
4	1.3	20	1.075	22.3	-0.238
5	2.1	20	1.075	22.3	0.397
6	3.2	20	1.075	22.3	0.250
7	3.4	20	1.075	22.3	0.190

DATA EVALUATION RECORD

1. **CHEMICAL:** Simazine.
Shaughnessey No. 080807.

2. **TEST MATERIAL:** Simazine technical; ID No. FL-850614 ARS-16871; Batch Code No. D3303B10; 96.9% active ingredient; a white powder.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: Duckweed (*Lemna gibba*).

4. **CITATION:** Thompson, S.G. and J.P. Swigert. 1992. Simazine: A 14-Day Toxicity Test with Duckweed (*Lemna gibba* G3). Laboratory Project No. 108A-137. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 425037-04.

5. **REVIEWED BY:**

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

Signature: C Mark Mossler
Date: 12/4/92

6. **APPROVED BY:**

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

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

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

Signature: Henry T. Craven
Date: 1/8/93

Tracy L. Perry 12/29/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 growth and reproduction study using non-target aquatic plants. Based on mean measured concentrations, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to simazine technical were 0.05, 0.11, and 0.14 mg ai/l, respectively.

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

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: *Lemna gibba* G3 used in the test came from laboratory stock cultures. Cultures that had been actively growing for at least two weeks were used as test inoculum.

B. Test System: Test vessels used were covered 250-ml glass beakers. The test medium was M-Hoagland's medium (without EDTA or sucrose) with the pH adjusted to 5.0 ±0.1 and autoclaved.

One-hundred milliliters of the appropriate test or control solution were placed into each beaker. The test vessels were kept at a temperature of 25 ±2°C in a chamber located in a constant temperature room. Fluorescent tubes provided continuous illumination of 5.4-6.5 klux.

C. Dosage: Fourteen-day static-renewal test. Based on the results of rangefinding tests, six nominal concentrations of 0.025, 0.05, 0.1, 0.2, 0.4, and 0.8 mg active ingredient (ai)/l, a solvent [0.16 ml dimethylformamide (DMF)/l of medium], and a medium control were selected for the definitive test. Test concentrations were corrected for percent active ingredient of the test material.

A primary and five secondary stock solutions of the test material were prepared in DMF. The test solutions were prepared by adding an appropriate volume (160 µl) of the stock solutions to 1000 ml of medium.

D. Test Design: An inoculum of *Lemna gibba* consisting of 15-18 fronds, representing at least five plants, was added to each beaker (3 beakers per treatment). The beakers were indiscriminantly positioned in the growth chamber. Test solutions were renewed on days 3, 6, 9, and 12 and frond counts were made on test days 0, 3, 6, 9, 12, and 14. Observations of colony formation, tissue chlorosis and necrosis, root destruction, and changes in color were also made at these times.

The pH of the initial, renewal, and terminal test and control solutions were determined and the temperature of the chamber was recorded continuously. Water temperature inside the chamber was also monitored twice a day.

Samples of the test solutions were collected from the freshly prepared medium on days 0, 6, and 12. Samples of old test solutions were taken on days 3, 9, and 14 from a composite of all three replicates. The samples were analyzed by gas chromatography for the test material.

E. Statistics: The growth rate was computed from frond number data. The 14-day EC_{50} and associated 95% confidence interval were calculated using the binomial method on percent inhibition of growth rate versus mean measured concentration data. Plant and frond number, as well as percentage of dead, necrotic, and chlorotic fronds (for a total of 6 measured parameters) were also statistically analyzed. The no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) were determined statistically and by evaluating visual effects.

12. REPORTED RESULTS: The mean measured concentrations were 0.027, 0.054, 0.11, 0.23, 0.43, and 0.88 mg ai/l (Table 1, attached). The test material was not detected in the solvent or negative control solutions.

Percent inhibition of growth rate increased with increasing toxicant concentration, but was not significantly different from the negative control at any treatment level (Table 3, attached).

Plants in the 0.027 and 0.054 mg ai/l treatment groups showed growth equivalent to the negative control. Plants at 0.11 mg ai/l were similar to control plants, but growth was reduced by 9.1%. By days 6-9 onwards, there was an increase in colony breakup, smallness of frond, and root destruction in test solutions of $\geq$0.23 mg ai/l.

The pH ranged from 5.0 to 5.2 in all initial test solutions and the controls at test initiation and from 5.2 to 6.6 at renewal or test termination. The temperature ranged from 23.2 to 24.8°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 14-day EC_{50} was calculated to be 0.32 mg ai/l with a 95% confidence interval of 0.23-0.43 mg ai/l based on growth rate inhibition. Based on this same inhibition, the NOEC and LOEC were determined to be 0.054 and 0.11 mg ai/l, respectively.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160. However, characterization of the test substance is the responsibility of the sponsor.

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 type of lighting was not specified. Warm-white illumination is recommended.

The light intensity during the test (5.4-6.5 klux) was higher than recommended (5 klux).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the EC_{50} and analysis of variance (coupled with Dunnett's test) to verify the NOEC and LOEC. A t-test indicated that the two control groups were not significantly different, therefore, control data were pooled (see attached printout). The reviewer obtained a more conservative EC_{50} based on percent reduction in frond number (Appendix III, attached). Using probit analysis, the 14-day EC_{50} and 95% confidence interval were 0.14 mg ai/l and 0.12-0.15 mg ai/l, respectively (see attached printout). The slope of the probit curve was 2.6. The reviewer concurs that the NOEC and LOEC were 0.54 and 0.11 mg ai/l, respectively.

C. **Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a Tier 2 growth and reproduction study using non-target aquatic plants. Based on mean measured concentrations, the 14-day NOEC, LOEC, and EC_{50} for *L. gibba* exposed to simazine technical were 0.05, 0.11, and 0.14 mg ai/l, respectively.

D. **Adequacy of the Study:**

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

SIMAZINE 080807 RIN 1646-93

Page____ is not included in this copy.

Pages 36 through 39 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.

__✓__ FIFRA registration data.

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MOSSLER SIMAZINE LEMNA GIBBA 11-17-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.88	100	98	98	0
.43	100	92	92	0
.23	100	74	74	0
.11	100	39	39	0
.054	100	9	9	0
.027	100	7	7	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 .1379726

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
5	1.476464E-02	.1362415	.11952	.1546919

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	1.657331E-02	1	.1193168

SLOPE = 2.61347

95 PERCENT CONFIDENCE LIMITS = 2.277019 AND 2.949921

LC50 = .1357261

95 PERCENT CONFIDENCE LIMITS = .1206562 AND .152495

LC10 = 4.433221E-02

95 PERCENT CONFIDENCE LIMITS = 3.591378E-02 AND 5.263533E-02

****

Lemna frond number

Summary Statistics and ANOVA

Transformation = None

Group (Concentration (mg ai/l))	n	Mean	s.d.	cv%
1 = control	6	773.0000	178.5934	23.1
2 0.027	3	715.6667	11.0151	1.5
3 0.054	3	700.6667	33.6502	4.8
4* 0.11	3	474.6667	16.2891	3.4
5* 0.23	3	201.0000	12.5300	6.2
6* 0.43	3	62.3333	4.5092	7.2
7* 0.58	3	19.0000	1.0000	5.3

NOEC = 0.054 mg ai/l *

LOEC = 0.11 mg ai/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* mean measured

Minumum detectable difference for
t-tests with Bonferroni adjustment = -150.070334
This difference corresponds to -19.41 percent of control

```
**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
**************************************************
```

Between groups sum of squares = 2216839.166667 with 6 degrees of freedom.

Error mean square = 9580.745098 with 17 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

```
**************************************************
*                                                *
* Warning - the test for equality of variances   *
* is significant (p less than 0.01). The         *
* results of this analysis should be inter-      *
* preted with caution.                           *
*                                                *
**************************************************
```

```
èëëëëëëëëëëëëëëëëëëëëëëëëëëëëëëë£
¤ STUDENT'S T-TEST (two-tailed) ¤
àëëëëëëëëëëëëëëëëëëëëëëëëëëëëëëë¥

     Enter the name of the DATAFILE you wish to analyze:  lem
   (Press RETURN if you wish to skip directly to T evaluation)

  What are the SAMPLE NUMBERS of the 2 variables you want to compare?
                1 'neg cont'                 2 'sol cont'
    Means =     698.3333                     847.6667
Variances =     59621.34                     3392.335

     Are these INDEPENDENT or PAIRED samples? (I or P)    i

      T =  1.030386                         df =  4
                          p =  .3610527
    The MEANS of these 2 samples are NOT significantly different.

The confidence limits on the DIFFERENCE between the means of these samples
can be calculated as:
                       149.3334 +/- T(4) *  144.9295


          Do you want another T-TEST using this datafile?
```

DATA EVALUATION RECORD

1. **CHEMICAL:** Simazine.
Shaughnessey No. 080807.

2. **TEST MATERIAL:** Simazine technical; ID No. FL-850614 ARS-16871; Batch Code No. D3303B10; 96.9% active ingredient; a white powder.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Skeletonema costatum.*

4. **CITATION:** Thompson, S.G. and J.P. Swigert. 1992. Simazine: A 5-Day Toxicity Test with the Marine Diatom (*Skeletonema costatum*). Laboratory Project No. 108A-140. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 425037-05.

5. **REVIEWED BY:**

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

Signature: [signature]
Date: 11/4/92

6. **APPROVED BY:**

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

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

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

Signature: Henry T. Craven 11/7/19
Date: Tracy L. Perry 12/30/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant growth and reproduction study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *S. costatum* exposed to simazine technical were 0.25, 0.52, and 0.60 mg ai/l, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Species:** *Skeletonema costatum* cultures used in the test came from laboratory stock cultures. Cultures in an exponential growth phase were used as test inoculum.

B. **Test System:** Test vessels used were 250-ml sterile Erlenmeyer flasks capped with gauze-wrapped cotton stoppers. The test medium was saltwater algal medium (Appendix I, attached) with the pH adjusted to 7.8-8.1 and filter sterilized (0.2 µm).

One-hundred milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept at 20 ±2°C in an environmental chamber under 16 hours of fluorescent illumination (4.0-5.4 klux) per day. The test vessels were continuously shaken at 100 rpm.

C. **Dosage:** Five-day static test. Based on the results of preliminary tests, five nominal concentrations of 0.125, 0.25, 0.5, 1.0, and 2.0 mg active ingredient (ai)/l were selected for the test. A solvent [0.4 ml dimethylformamide (DMF)/l of nutrient solution] and a medium control were also prepared. Test concentrations were corrected for percent active ingredient.

A primary and four secondary stock solutions of the test material were prepared in DMF. The test solutions were prepared by adding an appropriate volume (400 µl) of the stock solutions to 1000 ml of medium.

D. **Test Design:** An inoculum of *S. costatum* cells (1 ml) designed to provide 10,000 cells/ml was added to each flask (3 flasks per treatment). Cell density was determined daily using a hemocytometer. Each sample of test solution was counted one time and ten grids were enumerated to estimate a mean cell density.

The pH was measured at the beginning and end of the study. Temperature within the growth chamber was monitored during the test.

At the beginning and end of the test, samples were removed from exposure and control solutions and analyzed by gas chromatography for the test material.

E. **Statistics:** All calculations were made using mean measured concentrations. The growth rate and percent inhibition of growth rate were computed from the treatment cell density data in comparison to pooled control data. The 5-day EC_{50} value and associated 95% confidence interval were calculated using the binomial method on growth rate inhibition versus mean measured concentration data. The no-observed-effect concentration (NOEC) was estimated using the Kruskal-Wallis test and by analysis of cell number data.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.13, 0.25, 0.52, 1.0, and 2.0 mg ai/l (Table 1, attached). No test material was detected in the control solutions.

The growth rates at all concentrations were not significantly different ($p \leq 0.05$) from the pooled control. However reductions of 9.8, 46.4, and 97.5% were observed by day 5 at the three highest test concentrations (0.52, 1.0, and 2.1 mg ai/l) and appeared to be treatment related.

The pH was 8.0 in all test solutions and the controls at test initiation and ranged from 7.7 to 8.7 at test termination. The temperature ranged from 20 to 24°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 5-day EC_{50} based on growth rate was calculated to be 1.04 mg ai/l with a 95% confidence interval of 1.0-2.1 mg ai/l. The NOEC based on growth rate was 0.25 mg ai/l.

Good Laboratory Practice (GLP) and Quality Assurance statements were included in the report indicating compliance with 40 CFR Part 160. However, test substance characterization was the responsibility of the sponsor.

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 lighting was not specified. Cool-white lighting is recommended.

The light intensity during the test (4.0-5.4 klux) was higher than recommended (4.0 klux).

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the EC_{50} and ANOVA (coupled with

ys

Dunnett's test) to determine the NOEC and lowest-observed-effect concentration (LOEC). The reviewer obtained the same results for the NOEC. Using the moving average angle method, the EC_{50} and 95% confidence interval are 0.60 mg ai/l and 0.56-0.65 mg ai/l, respectively, based on mean measured concentrations and percentage inhibition calculated from cell density data (Appendix III, attached) in comparison to the pooled control (see attached printouts).

C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant growth and reproduction study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *S. costatum* exposed to simazine technical were 0.25, 0.52, and 0.60 mg ai/l, respectively.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 11-18-92.

SIMAZINE 080807 RIN 1646-93

Page_____ is not included in this copy.

Pages 47 through 49 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.

__✓__ 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.

Skeletonema cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
Concentration (mg ai/l*)				
1 = control	6	1881666.6667	120733.8671	6.4
2 0.13	3	2018333.3333	107741.9757	5.3
3 0.25	3	2023333.3333	147507.0620	7.3
4* 0.52	3	1123333.3333	30138.5689	2.7
5* 1.0	3	170000.0000	47286.3617	27.8
6* 2.1	3	13000.0000	8660.2540	66.6

NOEC = 0.25 mg ai/l *
LOEC = 0.52 mg ai/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* reads measured concentrations

Minumum detectable difference for
t-tests with Bonferroni adjustment = -148298.532509
This difference corresponds to -7.88 percent of control

```
**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
**************************************************
```

Between groups sum of squares =********** with 5 degrees of freedom.

Error mean square = ********** with 15 degrees of freedom.

Bartlett's test p-value for equality of variances = .041

MOSSLER SIMAZINE SKELETONEMA COSTATUM 11-18-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2.1	100	99	99	0
1	100	91	91	0
.51	100	40	40	0
.25	100	0	0	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 .5733212

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	9.74382E-03	.6044361	.5567368	.6540095

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.8298723	5.536053	3.941953E-03

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

SLOPE = 5.619139

95 PERCENT CONFIDENCE LIMITS = .5002537 AND 10.73802

LC50 = .5888791

95 PERCENT CONFIDENCE LIMITS = .2129012 AND 1.491834

LC10 = .3499556

95 PERCENT CONFIDENCE LIMITS = 1.320984E-03 AND .5296836

****

DATA EVALUATION RECORD

1. **CHEMICAL:** Simazine.
Shaughnessey No. 080807.

2. **TEST MATERIAL:** Simazine technical; ID No. FL-850614 ARS-16871; Batch Code No. D3303B10; 96.9% active ingredient; a white powder.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Selenastrum capricornutum*.

4. **CITATION:** Thompson, S.G. and J.P. Swigert. 1992. Simazine: A 5-Day Toxicity Test with the Freshwater Alga (*Selenastrum capricornutum*). Laboratory Project No. 108A-141. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 425037-06.

5. **REVIEWED BY:**

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

Signature: [signature]
Date: 12/4/92

6. **APPROVED BY:**

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

Signature: [signature]
Date: 12/4/92

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

Signature: [signature] 1/7/93
Date: Tracy L. Perry 12/30/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant growth and reproduction study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *S. capricornutum* exposed to simazine technical were 0.03, 0.07, and 0.10 mg ai/l, respectively.

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

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: *Selenastrum capricornutum* cultures used in the test came from laboratory stock cultures. Cultures in an exponential growth phase were used as test inoculum.

B. Test System: Test vessels used were 250-ml sterile Erlenmeyer flasks capped with gauze-wrapped cotton stoppers. The test medium was freshwater algal medium with vitamins (Appendix I, attached) with the pH adjusted to 7.5 ±0.1 and filter sterilized (0.2 µm).

One-hundred milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept at 24 ±2°C in an environmental chamber under continuous fluorescent illumination (3.9-4.3 klux). The test vessels were continuously shaken at 100 rpm.

C. Dosage: Five-day static test. Based on the results of preliminary tests, six nominal concentrations of 0.031, 0.063, 0.125, 0.25, 0.5, and 1.0 mg active ingredient (ai)/l were selected for the test. A solvent [0.2 ml dimethylformamide (DMF)/l of nutrient solution] and a medium control were also prepared. Test concentrations were corrected for percent active ingredient.

A primary and five secondary stock solutions of the test material were prepared in DMF. The test solutions were prepared by adding an appropriate volume (200 µl) of the stock solutions to 1000 ml of medium.

D. Test Design: An inoculum of *S. capricornutum* cells (1 ml) designed to provide 10,000 cells/ml was added to each flask (3 flasks per treatment). Cell density was determined daily using a hemocytometer. Each sample of test solution was counted one time and ten grids were enumerated to estimate a mean cell density.

The pH was measured at the beginning and end of the study. Temperature within the growth chamber was monitored continuously during the test and water temperature within the chamber was measured twice daily.

At the beginning and end of the test, samples were removed from exposure and control solutions and analyzed by gas chromatography for the test material.

E. **Statistics:** All calculations were made using mean measured concentrations. The growth rate and percent inhibition of growth rate were computed from the treatment cell density data in comparison to pooled control data. Since inhibition was determined based on initial cell density, greater than 100% inhibition was possible due to the death (and subsequent decay) of the original cellular inoculum. The 5-day EC_{50} value and associated 95% confidence interval were calculated using the moving average method on growth rate inhibition versus mean measured concentration data. The no-observed-effect concentration (NOEC) was estimated using Dunnett's test and by analysis of cell number data.

12. **REPORTED RESULTS:** The mean measured concentrations were 0.034, 0.068, 0.13, 0.29, 0.54, and 1.0 mg ai/l (Table 1, attached). No test material was detected in the control solutions.

The growth rates at the two lowest concentration levels were not significantly different ($p \leq 0.05$) from the pooled control (Table 3, attached). Reductions of 15.1, 50.7, 71.1, and 104% were observed by day 5 at the four highest test concentrations and growth rates at these levels were significantly less than the pooled control growth rate.

The pH was 7.3 in all test solutions and the controls at test initiation and ranged from 7.6 to 8.2 at test termination. The temperature ranged from 23.5 to 24.2°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The 5-day EC_{50} based on growth rate was calculated to be 0.26 mg ai/l with a 95% confidence interval of 0.25-0.27 mg ai/l. The NOEC based on growth rate was 0.068 mg ai/l.

Good Laboratory Practice (GLP) and Quality Assurance statements were included in the report indicating compliance with 40 CFR Part 160. However, test substance characterization was the responsibility of the sponsor.

54

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 lighting was not specified. Cool-white lighting is recommended.

B. Statistical Analysis: The reviewer used EPA's Toxanal program to determine the EC_{50} and ANOVA (coupled with Dunnett's test) to determine the NOEC and lowest-observed-effect concentration (LOEC). The reviewer obtained a more conservative estimate of the NOEC (0.034 mg ai/l). Using the moving average angle method, the EC_{50} and 95% confidence interval are 0.10 mg ai/l and 0.09-0.11 mg ai/l, respectively, based on mean measured concentrations and percentage inhibition calculated using cell density data (Appendix III, attached) in comparison to the pooled control (see attached printouts).

C. Discussion/Results: This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant growth and reproduction study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *S. capricornutum* exposed to simazine technical were 0.03, 0.07, and 0.10 mg ai/l, respectively.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, 11-18-92.

55

SIMAZINE 080807 RIN 1646-93

Page____ is not included in this copy.

Pages 56 through 59 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.

__✓__ 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.

Selenastrum cell density

Summary Statistics and ANOVA

Transformation = None

Group (Concentration (mg ai/l *))	n	Mean	s.d.	cv%
1 = control	6	5076666.6667	436608.1386	8.6
2 0.034	3	4693333.3333	528141.3952	11.3
3*0.068	3	3920000.0000	693108.9380	17.7
4* 0.13	3	1993333.3333	265769.3235	13.3
5* 0.29	3	222000.0000	64210.5910	28.9
6* 0.54	3	61000.0000	11135.5287	18.3
7* 1.0	3	8000.0000	2645.7513	33.1

NOEC = 0.034 mg ai/l *
LOEC = 0.068 mg ai/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* - mean measured concentrations

Minumum detectable difference for
t-tests with Bonferroni adjustment = -602076.754891
This difference corresponds to -11.86 percent of control

```
**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
**************************************************
```

Between groups sum of squares =********** with 6 degrees of freedom.

Error mean square = ********** with 17 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

```
**************************************************
*                                                *
* Warning - the test for equality of variances   *
* is significant (p less than 0.01). The         *
* results of this analysis should be inter-      *
* preted with caution.                           *
*                                                *
**************************************************
```

MOSSLER SIMAZINE SELENASTRUM CAPRICORNUTUM 11-18-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1	100	100	100	0
.54	100	99	99	0
.29	100	96	96	0
.13	100	61	61	0
.065	100	23	23	0
.034	100	8	8	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 .1084148

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.0142983	.1019912	9.046592E-02	.1140162

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	2.027469E-02	1	.4348184

SLOPE = 3.371993

95 PERCENT CONFIDENCE LIMITS = 2.891858 AND 3.852129

LC50 = .1030486

95 PERCENT CONFIDENCE LIMITS = 9.291847E-02 AND .1141779

LC10 = 4.329228E-02

95 PERCENT CONFIDENCE LIMITS = 3.602794E-02 AND 5.014099E-02

****

DATA EVALUATION RECORD

1. **CHEMICAL:** Simazine.
Shaughnessey No. 080807.

2. **TEST MATERIAL:** Simazine technical; ID No. FL-850614 ARS-16871; Batch Code No. D3303B10; 96.9% active ingredient; a white powder.

3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Navicula pelliculosa*.

4. **CITATION:** Thompson, S.G. and J.P. Swigert. 1992. Simazine: A 5-Day Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*). Laboratory Project No. 108A-138. Conducted by Wildlife International Ltd., Easton, MD. Submitted by Ciba-Geigy Corporation, Greensboro, NC. EPA MRID No. 425037-07.

5. **REVIEWED BY:**

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

Signature: Mark A. Mossler
Date: 12/4/92

6. **APPROVED BY:**

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

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

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

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

Tracy L. Perry 12/30/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant growth and reproduction study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *N. pelliculosa* exposed to simazine technical were 0.03, 0.07, and 0.09 mg ai/l, respectively.

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

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Species: *Navicula pelliculosa* cultures used in the test came from laboratory stock cultures. Cultures in an exponential growth phase were used as test inoculum.

B. Test System: Test vessels used were 250-ml sterile Erlenmeyer flasks capped with gauze-wrapped cotton stoppers. The test medium was freshwater algal medium with vitamins and silica (Appendix I, attached) with the pH adjusted to 7.5 ±0.1 and filter sterilized (0.2 µm).

One-hundred milliliters of the appropriate test or control solution were placed into each flask. The test vessels were kept at 20 ±2°C in an environmental chamber under 16 hours of illumination (4.0-4.3 klux) per day. The test vessels were continuously shaken at 100 rpm.

C. Dosage: Five-day static test. Based on the results of preliminary tests, six nominal concentrations of 0.031, 0.063, 0.125, 0.25, 0.5, and 1.0 mg active ingredient (ai)/l were selected for the test. A solvent [0.2 ml dimethylformamide (DMF)/l of nutrient solution] and a medium control were also prepared. Test concentrations were corrected for percent active ingredient.

A primary and five secondary stock solutions of the test material were prepared in DMF. The test solutions were prepared by adding an appropriate volume (200 µl) of the stock solutions to 1000 ml of medium.

D. Test Design: An inoculum of *N. pelliculosa* cells (2.1 ml) designed to provide 10,000 cells/ml was added to each flask (3 flasks per treatment). Cell density was determined daily using a hemocytometer. Each sample of test solution was counted one time and ten grids were enumerated to estimate a mean cell density.

The pH was measured at the beginning and end of the study. Temperature within the growth chamber was monitored continuously during the test.

At the beginning and end of the test, samples were removed from exposure and control solutions and analyzed by gas chromatography for the test material.

E. Statistics: All calculations were made using mean measured concentrations. The growth rate and percent inhibition of growth rate were computed from the treatment cell density data in comparison to solvent control data. The 5-day EC_{50} value and associated 95% confidence interval were calculated using the binomial method on growth rate inhibition versus mean measured concentration data. The no-observed-effect concentration (NOEC) was estimated using Dunnett's test and by analysis of cell number data.

12. REPORTED RESULTS: The mean measured concentrations were 0.033, 0.066, 0.13, 0.25, 0.44, and 0.84 mg ai/l (Table 1, attached). No test material was detected in the control solutions.

Growth rate in the solvent control was 3.4% less than the negative control and was determined to be significantly different from the negative control. The growth rates at the two lowest concentration levels were not significantly different ($p \leq 0.05$) from the solvent control (Table 3, attached). Reductions of 19.4, 41.4, 70.2, and 95.2% were observed by day 5 at the four highest test concentrations and growth rates at these levels were significantly less than the solvent control growth rate.

The pH was 7.2 in all test solutions and the controls at test initiation and ranged from 5.6 to 8.4 at test termination. The temperature ranged from 20.2 to 21.0°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: The 5-day EC_{50} based on growth rate was calculated to be 0.30 mg ai/l with a 95% confidence interval of 0.25-0.44 mg ai/l. The NOEC based on growth rate was 0.033 mg ai/l.

Good Laboratory Practice (GLP) and Quality Assurance statements were included in the report indicating compliance with 40 CFR Part 160. However, test substance characterization was the responsibility of the sponsor.

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 lighting was not specified. Cool-white lighting is recommended.

The test temperature (20.2-21.0°C) was less than recommended (24°C).

The photoperiod (16 hours light) was less than recommended (continuous).

B. **Statistical Analysis:** Using a t-test (attached), the reviewer determined that the medium and solvent control were not significantly different. The reviewer used EPA's Toxanal program to determine the EC_{50} and ANOVA (coupled with Dunnett's test) to determine the NOEC and lowest-observed-effect concentration (LOEC). The reviewer obtained the same result for the NOEC. Using the moving average angle method, the EC_{50} and 95% confidence interval are 0.09 mg ai/l and 0.08-0.10 mg ai/l, respectively, based on mean measured concentrations and percent inhibition computed from cell density data (Appendix III, attached) in comparison to the pooled control data (see attached printouts).

C. **Discussion/Results:** There appears to be a typographical error in the conclusion section. The authors stated that the EC_{50} was 0.030 mg ai/l. The reviewer believes that this should be 0.3 mg ai/l, as reported in the study summary.

This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant growth and reproduction study. Based on mean measured concentrations, the 5-day NOEC, LOEC, and EC_{50} for *N. pelliculosa* exposed to simazine technical were 0.03, 0.07, and 0.09 mg ai/l, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 11-19-92.

65

SIMAZINE 080807 RIN 1646-93

Page_____ is not included in this copy.

Pages 66 through 69 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.

__✓__ 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.

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¤ STUDENT'S T-TEST (two-tailed) ¤
àëëëëëëëëëëëëëëëëëëëëëëëëëëëëëëëëë¥

        Enter the name of the DATAFILE you wish to analyze:  nav
     (Press RETURN if you wish to skip directly to T evaluation)

  What are the SAMPLE NUMBERS of the 2 variables you want to compare?
              1 'neg cont'                    2 'solv cont'
   Means =       2460000                         2040000
Variances =      6.37E+10                        9.099997E+09

        Are these INDEPENDENT or PAIRED samples? (I or P)    i

          T =  2.696151                           df =  4
                              p =  .0543105
       The MEANS of these 2 samples are NOT significantly different.

The confidence limits on the DIFFERENCE between the means of these samples
can be calculated as:
                          420000 +/- T(4) *  155777.6


                Do you want another T-TEST using this datafile?
```

MOSSLER SIMAZINE NAVICULA PELLICULOSA 11-19-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.84	100	99	99	0
.44	100	98	98	0
.25	100	90	90	0
.13	100	67	67	0
.066	100	42	42	0
.033	100	5	5	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 8.180144E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	1.376584E-02	9.176393E-02	.0819766	.1017967

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	1.927508E-02	1	8.990634E-02

SLOPE = 2.940376

95 PERCENT CONFIDENCE LIMITS = 2.532149 AND 3.348602

LC50 = 9.083802E-02

95 PERCENT CONFIDENCE LIMITS = 8.109453E-02 AND .1012155

LC10 = 3.360084E-02

95 PERCENT CONFIDENCE LIMITS = 2.708016E-02 AND 3.988179E-02

****

Navicula pelliculosa

Summary Statistics and ANOVA

Transformation = None

Group (concentration (mg ai/l *))	n	Mean	s.d.	cv%
1 = control	6	2250000.0000	286426.2558	12.7
2 0.033	3	2133333.3333	496621.9219	23.3
3*0.066	3	1311666.6667	135308.2900	10.3
4*0.13	3	738333.3333	156071.5648	21.1
5*0.25	3	232333.3333	72403.9594	31.2
6*0.44	3	51000.0000	18734.9940	36.7
7*0.84	3	13333.3333	4041.4519	30.3

NOEC = 0.033 mg ai/l *

LOEC = 0.066 mg ai/l *

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

* - mean measured concentration

Minumum detectable difference for
t-tests with Bonferroni adjustment = -371856.127730
This difference corresponds to -16.53 percent of control

```
**************************************************
*                                                *
* Note - the above value for the minimum         *
* detectable difference is approximate as        *
* the sample sizes are not the same for all of   *
* the groups.                                    *
*                                                *
**************************************************
```

Between groups sum of squares =********** with 6 degrees of freedom.

Error mean square = ********** with 17 degrees of freedom.

Bartlett's test p-value for equality of variances = .001

```
**************************************************
*                                                *
* Warning - the test for equality of variances   *
* is significant (p less than 0.01). The         *
* results of this analysis should be inter-      *
* preted with caution.                           *
*                                                *
**************************************************
```