file 12-22-92

DP Barcode :D182756
PC Code No : 129099
EEB Out : 12/22/92

To: Dennis Edwards
Product Manager 19
Registration Division (H7505C)

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

Attached, please find the EEB review of...

Reg./File # : 003125-URU
Chemical Name : NTN 33893
Type Product : Insecticide
Product Name : Imidacloprid
Company Name : Miles Inc.
Purpose : Review studies.

Action Code : 612 Date Due : 1/16/93
Reviewer : Dana Lateulere

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)	422563-03, -04	S, Y	72-7(A)		
71-1(B)			72-2(B)			72-7(B)	422563-06, -10	NR*
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)	422563-05	Y	122-1(B)		
71-3			72-3(C)			122-2	422563-74	S
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	422563-75	N
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1	422730-03	Y
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)
N=Unacceptable (Study was rejected)/Nonconcur
NR* = Not reviewed at this time, will submit at a later date.

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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Subject: NTN 33893 (Imidacloprid), Data Evaluation Records.

To: Dennis Edwards, PM 19
Registration Division, H7505C

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

DEC 22 1992

EEB has reviewed the studies submitted by Miles Inc. for the pending registration of NTN 33893. The following is a summary of those studies:

1. England, D. and J.D. Bucksath. 1991. Acute Toxicity of NTN 33893 to Hyalella azteca. Report No. 101960. Prepared by ABC Laboratories, Inc., Columbia, MO. Submitted by Mobay Corporation, Stilwell, KS. EPA MRID No. 422563-03.

The study is scientifically sound but does not meet the guideline requirements for a static acute toxicity test using freshwater invertebrates. Hyalella azteca is not a recommended guideline species; the data will be used to supplement the NTN 33839 toxicity database. The purity of the test material was not reported. The 96-hour EC_{50} value was determined to be 55 µg/l (mean measured concentrations), respectively. Therefore, NTN 33893 is classified as very highly toxic to H. azteca. The 96-hour NOEC value was determined to be 0.35 µg/l mean measured concentration.

2. Wheat, J. and G.S. Ward. 1991. NTN 33893 Technical: Acute Effect on New Shell Growth of the Eastern Oyster, Crassostrea virginica. Report No. 101978. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422563-05.

The first study is not scientifically sound because the control oyster growth was less than the minimum requirement (2 mm). The second study is scientifically sound and meets the guideline requirements for a mollusc shell deposition study. Based on the results of the second study, the 96-hour EC_{50} was >145 mg a.i./l (mean measured concentration) which classifies NTN 33893 as practically non-toxic to eastern oysters. The NOEC could not be determined.

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3. Gagliano, G.G. 1991. Growth and Survival of the Midge (Chironomus tentans) Exposed to NTN 33893 Technical Under Static Renewal Conditions. Report No. 101985. Prepared by Mobay Corporation, Stilwell, KS. Submitted by Mobay Corporation, Kansas City, KS. EPA MRID No. 422563-04.

In this test, only the initial 48-hour period is "core". The remainder of the test is invalid because the dilution water control and solvent control appear to have been contaminated with the test material. The 48-hour LC_{50} value of 68.9 µg/l (mean measured concentration) classifies NTN 33893 as highly toxic to midge larvae. The 48-hour NOEC was 1.04 µg/l mean measured concentration.

4. Heimbach, F. 1989. Growth Inhibition of Green Algae (Scenedesmus subspicatus) Caused by NTN 33893 (Technical). Laboratory Report No. 100098. Conducted by Bayer AG, West Germany. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422563-74.

This study is scientifically sound but does not meet the guideline requirements for a Tier 1 non-target aquatic plant study. The test procedures deviated significantly from the recommended protocols. Exposure to NTN 33893 technical at a concentration of 10 mg ai/l (nominal) did not significantly reduce the growth of S. subspicatus over the 4-day test period.

5. Gagliano, G.G. and L.M. Bowers. 1991. Acute Toxicity of NTN 33893 Technical to the Green Alga (Selenastrum capricornutum). Report No. 101986. Conducted by Mobay Corporation, Stilwell, KS. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422563-75.

This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 non-target aquatic plant study. The control cultures did not grow logarithmically and light intensity was much greater than recommended.

6. Cole, J.H. 1990. The Acute Oral and Contact Toxicity to Honey Bees of Compound NTN 33893 Technical. Report No. 101321. Conducted by Huntingdon Research Centre Ltd., Cambridgeshire, UK. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422730-03.

This study is scientifically sound and fulfills the requirements for acute contact and oral studies with the honey bee. Acute contact and oral LD_{50} values of 0.078 and 0.0039 µg/bee, respectively, classify NTN 33893 technical as highly toxic to honey bees (Apis mellifera). The 48-hour contact and oral NOELs were 0.05 and 0.0015 µg/bee, respectively.

Note that MRID No.'s 422563-06 and 422563-10 have not been reviewed at this time. The review of these studies will take a

substantial amount of time. It was unnecessary to hold up the review and classification of the six studies noted above, as the two unreviewed studies are not needed for registration purposes. The two studies will be reviewed when time permits. For more information regarding this matter, please see memo to Dennis Edwards of 10/92, DP Barcode #D183139.

Questions regarding these reviews, contact Dana Lateulere at 308-2856.

DATA EVALUATION RECORD

1. **CHEMICAL:** NTN 33893.
Shaughnessey No. 129059.

2. **TEST MATERIAL:** NTN 33893 technical; 1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-*N*-nitro-1*H*-imidazol-2-amine; CAS No. 105827-78-9; Batch No. 9030211; 95.0% active ingredient; a tan powder.

3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: Midge (*Chironomus tentans*).

4. **CITATION:** Gagliano, G.G. 1991. Growth and Survival of the Midge (*Chironomus tentans*) Exposed to NTN 33893 Technical Under Static Renewal Conditions. Report No. 101985. Prepared by Mobay Corporation, Stilwell, KS. Submitted by Mobay Corporation, Kansas City, KS. EPA MRID No. 422563-04.

5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 9/28/92

Dana Spatolee 11/25/92 EFED/EB

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 9/28/92

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

Signature: Craven
Date: 12/4/92

7. **CONCLUSIONS:** In this test, only the initial 48-hour period is "core". The remainder of the test is invalid because the dilution water control and solvent control appear to have been contaminated with the test material. The 48-hour LC_{50} value of 68.9 µg/l (mean measured concentration) classifies NTN 33893 as highly toxic to midge larvae. The 48-hour NOEC was 1.04 µg/l mean measured concentration.

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

9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Second instar (12 days post-hatch) midge larvae (*Chironomus tentans*) were obtained from in-house cultures maintained in hard blended water. The cultures were fed a suspension of Tetramin® and cereal leaves five times per week. The temperature and photoperiod during culturing were 22 ±1°C and 16 hours of light.

B. Test System: Vessels used in the test were 1-l glass beakers containing 900 ml of test solution. Silica sand was used to provide a substrate depth of 0.5-1 mm. The beakers were randomly positioned in a water bath under a 16-hour light/8-hour dark photoperiod. Light intensity ranged from 40-60 ft-candles. Thirty-minute dawn and dusk simulations were used.

The primary stock solution (20 g a.i./l) was prepared by dissolving 2.1048 g of NTN 33893 in 100 ml of dimethylformamide (DMF) at 22°C. Three additional stocks were prepared by serial dilution. The test solutions were prepared by mixing an appropriate volume of appropriate stock with 1 l of dilution water.

The dilution water used was hard blended water (a mixture of treated city water and spring water) with a hardness of 118 mg/l, an alkalinity of 83 mg/l, and a pH of 8.1-8.2. The chlorine content of the water was monitored continuously to assure the residual chlorine remained <3 µg/l.

C. Dosage: Ten-day static-renewal test. Based on a preliminary test, seven nominal concentrations (0.33, 1.0, 3.0, 10, 33, 100, and 300 µg a.i./l), a solvent control (16.5 µl DMF/l), and a dilution water control were used.

D. Design: Ten midge larvae were randomly placed in each replicate chamber, two replicates per concentration. The loading was approximately 1 midge/90 ml. Test solutions were renewed every Monday, Wednesday, and Friday by siphoning the old test solutions out of the test chambers to a depth of approximately 1 cm. Fresh solutions were slowly added to avoid disturbing the test organisms. The fresh solutions were no more than 4 hours old at the time of renewal. The midges were

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fed the same food used in culturing at a rate of 0.5 ml/l of test solution.

All beakers were observed once every 24 hours for mortality and abnormal effects. At the end of the test, the midges were grouped by replicate, dried at 60°C for 24 hours, and weighed. The temperature, dissolved oxygen concentration (DO), conductivity, and pH were measured in alternating replicates of the control, solvent control, and the low, middle, and high concentrations on days 0, 3, 5, 7, and 10. The temperature of a centrally-located test beaker was also monitored continuously using a data logging device.

Samples of fresh test solutions were taken on days 0 and 5 to measure actual exposure concentrations. Old test solutions were analyzed on days 3 and 10. The concentration of NTN 33893 was determined using liquid chromatography.

E. Statistics: Dilution water control and solvent control growth data were compared using a t-test. All data were tested for normality (chi-square test) and homogeneity of variances (Bartlett's test). Survival data were analyzed using Fisher's Exact test. Test levels with significantly lowered survival were excluded from further analyses. Growth data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's test. The 24, 48, 72, 96, and 240-hour LC_{50} values and associated 95% confidence intervals were determined using a computer program developed by Stephan et al. (1978).

12. REPORTED RESULTS: No undissolved test substance was observed in the test chambers during the test. The mean measured concentrations were 0.67, 1.24, 3.39, 10.2, 34.5, 102, and 329 µg a.i./l (Table 2, attached). These values represented 99-203% of nominal concentrations. The control solutions were contaminated with the test material on three of five occasions. The average concentration in the dilution water control and solvent control was 0.20 and 0.15 µg/l, respectively. "No biological effects were observed in the controls and possible contamination of the samples may have occurred during sample extraction."

The mortality of midge larvae are given in Table 3 (attached). The 96-hour LC_{50} was 10.5 µg/l mean measured concentration (95% C.I. = 7.69-14.4 µg/l) using the probit method. The slope of the toxicity curve was 3.3. The 96-

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hour no-observed-effect concentration (NOEC), based on the lack of abnormal effects, was 1.24 µg/l.

After 10 days, survival at 3.39 µg/l was significantly lower than pooled control survival (Table 5, attached). Growth was significantly affected at 1.24 µg/l. The NOEC, based on survival and growth after 10 days was therefore 0.67 µg/l. The 10-day LC_{50} was 3.17 µg/l (95% C.I. = 1.24-10.2 µg/l).

On day 0 through 7, the DO ranged from 5.8 to 7.9 mg/l or 79 to 108% of saturation at 20°C. However, on day 10, DO was 2.0-4.0 mg/l "possibly due to an increased oxygen demand created by increased food in the test chambers" (Table 7, attached). The pH values ranged from 7.1 to 8.8. The temperature was 20.8-22.3°C.

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

Quality Assurance and Study Compliance 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 design differed from the SEP for freshwater invertebrate acute tests. Significant deviations are as follows:

This test was designed to gather survival and growth data, therefore, the midge larvae were fed during testing. The duration should have been 48 hours eliminating the addition of food to the vessels.

The test concentrations were approximately 30% of the next highest concentration. The SEP recommends that each nominal concentration be at least 60% of next highest.

The test solutions were as old as 4 hours at the time of renewal. The SEP states that the test solutions should be used within 30 minutes of preparation.

The DO at test termination ranged from 2.0 to 4.0 mg/l (22 to 43% of saturation at 20°C). Dissolved oxygen levels must remain above 40% of saturation during the test.

The author stated that conductivity was measured in alternating replicates of the control, solvent control, low, middle, and high concentration on days 0, 3, 5, 7, and 10. The results were not presented in the report.

B. **Statistical Analysis:** The reviewer used EPA's Toxanal program and mean measured concentrations to determine the 48, 96, and 240-hour LC_{50} values (see attached printouts 1-3). The results were similar to those of the author's.

Growth and survival at test termination were analyzed to verify the author's 10-day NOEC. Survival at concentrations ≥3.39 µg/l was significantly lower than survival in the solvent control (see attached printout 4). Average dry weight of surviving midges at concentrations ≥1.24 µg/l was significantly lower than the solvent control (see attached printout 4). These results are the same as those of the author's.

C. **Discussion/Results:** In this test, only the initial 48-hour period is "core". The remainder of the test is invalid because the dilution water control and solvent control appear to have been contaminated with the test material. The 48-hour LC_{50} value of 68.9 µg/l (mean measured concentration) classifies NTN 33893 as highly toxic to midge larvae. The 48-hour NOEC was 1.04 µg/l mean measured concentration.

D. **Adequacy of the Study:**

(1) **Classification:** Core for the initial 48-hour period only.

(2) **Rationale:** The remainder of the test is ~~in~~valid because the dilution water control and solvent control appear to have been contaminated with the test material.

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

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 09-16-92.

IMIDACLOPRID

Page_____ is not included in this copy.

Pages 10 through 13 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

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

_____ The document is not responsive to the request.

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

day 3 mean measured concentrations — 48-hour LC50 — Printout

RIFICI NTN 33893 CHIRONOMUS TENTANS 09-16-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
329.5	20	20	100	9.536742E-05
102.3	20	8	40	25.17223
34.3	20	7	35	13.1588
10.3	20	1	5	2.002716E-03
3.09	20	1	5	2.002716E-03
1.04	20	0	0	9.536742E-05
.54	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 10.3 AND 329.5 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 118.3196

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	6.572961E-02	68.94127	49.35569 - 98.45378	

98.45378

LMR 9/16/97

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1061265	1	7.839239E-02

SLOPE = 1.690331

95 PERCENT CONFIDENCE LIMITS = 1.139671 AND 2.240991

LC50 = 68.87281

95 PERCENT CONFIDENCE LIMITS = 44.81775 AND 111.5195

LC10 = 12.21

95 PERCENT CONFIDENCE LIMITS = 5.017858 AND 20.75928

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14

Printout 2

96-hour LC_{50}

RIFICI NTN 33893 CHIRONOMUS TENTANS 09-16-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
329	20	20	100	9.536742E-05
102	20	20	100	9.536742E-05
34.5	20	19	95	2.002716E-03
10.2	20	10	50	58.80985
3.39	20	1	5	2.002716E-03
1.24	20	0	0	9.536742E-05
.67	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 3.39 AND 34.5 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 10.2

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
6	5.135013E-02	11.43877	7.679475 - 17.03832

~~17.03832~~

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1373512	1	.9999185

SLOPE = 3.310458

95 PERCENT CONFIDENCE LIMITS = 2.083571 AND 4.537344

LC50 = 10.45897

95 PERCENT CONFIDENCE LIMITS = 7.686511 AND 14.42776

LC10 = 4.323704

95 PERCENT CONFIDENCE LIMITS = 2.343523 AND 6.104206

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Printout 3

10-day LC50

RIFICI NTN 33893 CHIRONOMUS TENTANS 09-16-92

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
329	20	20	100	9.536742E-05
102	20	20	100	9.536742E-05
34.5	20	20	100	9.536742E-05
10.2	20	20	100	9.536742E-05
3.39	20	11	55	41.19014
1.24	20	0	0	9.536742E-05
.67	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.24 AND 10.2 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 3.172205

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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MIDGE SURVIVAL AFTER 10 DAYS

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	20	0	
1	0.67 µg/l	20	0	
2	1.24	20	0	
3	3.39	20	11	*
4	10.2	20	20	*
5	34.5	20	20	*
6	102	20	20	*
7	329	20	20	*

422563-04, NTN 33893, midge dry weight

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

GRP1 (SOLVENT CRTL) MEAN =	2.2100	CALCULATED t VALUE =	1.5541
GRP2 (BLANK CRTL) MEAN =	2.0500	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	0.1600		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

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.

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	4	4.404	1.101	53.548
Within (Error)	5	0.103	0.021	
Total	9	4.507		

Critical F value = 5.19 (0.05,4,5)
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	2.210	2.210		
2	dilution contrl	2.050	2.050	1.116	
3	0.67 µg/l	2.060	2.060	1.046	
4	1.24	1.720	1.720	3.417	*
5	3.39	0.400	0.400	12.623	*

Dunnett table value = 2.85 (1 Tailed Value, P=0.05, df=5,4)

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	2			
2	dilution contrl	2	0.409	18.5	0.160
3	0.67 µg/l	2	0.409	18.5	0.150
4	1.24	2	0.409	18.5	0.490
5	3.39	2	0.409	18.5	1.810

PRINTOUT # 5

TITLE: 422563-04, NTN 33893, midge dry weight
FILE: a:42256304.dt1
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 5

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	2.2600	2.2600
1	solvent control	2	2.1600	2.1600
2	dilution contrl	1	1.9600	1.9600
2	dilution contrl	2	2.1400	2.1400
3	0.67 µg/l	1	2.0800	2.0800
3	0.67 µg/l	2	2.0400	2.0400
4	1.24	1	1.5200	1.5200
4	1.24	2	1.9200	1.9200
5	3.39	1	0.4200	0.4200
5	3.39	2	0.3800	0.3800

DATA EVALUATION RECORD

1. **CHEMICAL:** NTN 33893.
Shaughnessey No. 129059.

2. **TEST MATERIAL:** NTN 33893 technical; Batch No. 1717119/89: 96.2% active ingredient; and Batch No. 17129-90: 95.8% active ingredient; a yellow-colored powder.

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

4. **CITATION:** Wheat, J. and G.S. Ward. 1991. NTN 33893 Technical: Acute Effect on New Shell Growth of the Eastern Oyster, *Crassostrea virginica*. Report No. 101978. Prepared by Toxikon Environmental Sciences, Jupiter, FL. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422563-05.

5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 9/28/92 Interview
Dana J 11/25/92 EFED EET

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 9/28/92

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

Signature: Q. Westlake
Date: 12/14/92

7. **CONCLUSIONS:** The first study is not scientifically sound because the control oyster growth was less than the minimum requirement (2 mm). The second study is scientifically sound and meets the guideline requirements for a mollusc shell deposition study. Based on the results of the second study, the 96-hour EC_{50} was >145 mg a.i./l (mean measured concentration) which classifies NTN 33893 as practically non-toxic to eastern oysters. The NOEC could not be determined.

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. The oysters were held in the laboratory, in natural unfiltered seawater, for 2-6 days prior to testing. At the initiation of the holding period, 2-5 mm of shell margin was ground from each oyster with a grinding wheel to provide a smooth flattened edge. The salinity of the seawater ranged from 30 to 36 parts per thousand (ppt) and the temperature was 19.9-24.4°C.

The dilution water control oysters used in the first test had an average length (umbo to distal valve edge) of 21.5 (19.2-23.7) mm and an average wet weight of 0.31 (0.21-0.41) g. The control oysters used in the second test had an average length of 24.3 (19.5-28.0) mm and an average wet weight of 0.52 (0.35-0.86) g.

B. Test System: The test system for the two tests were different. "In the first test, the exposure system consisted of a glass head box fitted with glass tubing calibrated to provide unfiltered saltwater to each test chamber at a rate of approximately 400 ml/minute. This flow rate was sufficient to provide a minimum of approximately 1.2 l of dilution water per oyster per hour." The primary toxicant stock solution (384,800 mg a.i./l) was prepared in dimethylformamide (DMF). The solution was stirred overnight, allowed to settle for 1 day, then filtered. The filtrate concentration was 276,500 mg a.i./l. Four additional stock solutions were prepared by serial dilution. The stock solutions were continuously delivered to glass mixing boxes, where the test solutions were prepared. The test chambers were 29-l glass aquaria designed to maintain a solution height of 13 cm and a test volume of 19 l. The flow rate provided 30 volume additions/container/day.

The second test was performed using a glass head box fitted with glass tubing calibrated to provide a flow of dilution water of 365 ml/min. The flow of toxicant stock solution was approximately 135 ml/min giving a total flow rate of 500 ml/min (approximately 1.0 l/oyster/hour). The test containers were 11.3-l glass aquaria containing 5.4 l of solution at a depth of 6 cm. The flow rate provided 133 volume additions/container/day. The stock solution (500 mg

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a.i./l) for this test was prepared by mixing 104.4 g of NTN 33893 (Batch No. 17129-90) with 750 ml of seawater in a high speed blender. The mixture was diluted with 199.25 l of unfiltered seawater and stirred overnight.

All test chambers were randomly positioned in a water bath under a 16-hour light/8-hour dark photoperiod with 15-minute dawn and dusk simulations. Light intensity during the test was 304 to 508 lux.

Natural unfiltered seawater with a salinity of 30-35 ppt was used as test dilution water.

C. Dosage: Ninety-six-hour flow-through tests. Based on the results of a preliminary test, the first definitive test consisted of five nominal concentrations (2.6, 4.3, 7.2, 12.0, and 19.4 mg a.i./l), a dilution water control, and a solvent control (70 µl/l DMF). The second definitive test consisted of a single concentration (121.5 mg a.i./l) and a dilution water control.

D. Design: Just prior to test initiation, oysters which demonstrated shell growth during holding were carefully ground to remove all new shell growth. In the first test, the prepared oysters were impartially added, two at a time, to the test chambers for a total of 20 per concentration. In the second test, 30 oysters were used per concentration. One chamber was used per treatment in both tests. No supplemental food was added.

Observations of mortality and test solutions were made every 24 hours. At the end of the test, oyster growth was measured to the nearest 0.1 mm. The dissolved oxygen concentration (DO) 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 monitored hourly in the control chamber using a data logging device.

The test concentrations were measured using high pressure liquid chromatography fitted with an ultra-violet detector. During test 1, the solutions were measured at test initiation and termination. During test 2, the solutions were measured daily.

E. Statistics: Dilution water control and solvent control growth were compared using a t-test. Exposed oyster

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responses were compared to the pooled control using analysis of variance (ANOVA) and Dunnett's test. In the second test, the growth of exposed oysters were compared to that of the dilution water control using a t-test.

12. **REPORTED RESULTS:** The test systems functioned properly during the exposures. During the first test, the mean measured concentrations were 2.93, 5.14, 8.19, 14.2, and 23.3 mg a.i./l (Table 1, attached). These values ranged from 113 to 120% of nominal concentrations. Undissolved test material was observed in the two highest exposure levels throughout the exposure period. One observation of undissolved material was made in the 8.19 mg a.i./l concentration. In the single exposure test, the mean measured concentration was 145 mg a.i./l which was 119% of nominal concentration (Table 8, attached).

Mean new shell growth for the dilution water control and solvent control during the first test was 1.52 and 1.76 mm, respectively (Table 3, attached), and were not significantly different. Exposure to concentrations up to 23.3 mg a.i./l had no effect on new shell deposition, therefore the 96-hour EC_{50} for the first test was >23.3 mg a.i./l. The no-observed-effect concentration (NOEC) was 23.3 mg a.i./l.

In the single concentration test using 145 mg a.i./l, new shell growth was reduced by 22% compared to the dilution water control (Table 10, attached). This difference was statistically significant using the t-test. Mean new shell growth in the dilution water control was 2.89 mm. The 96-hour EC_{50} was >145 mg a.i./l and the NOEC could not be calculated. There was no mortality during either test.

Dissolved oxygen concentrations were at least 70% of saturation during both tests. The salinity during the first test was 32-35 ppt and 30 ppt during the second test. The pH values ranged from 7.6 to 8.1. The temperature during the first test was 20.1-22.5°C and 21.7-25.4°C during the second test.

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

A Good laboratory practice statement was included in the report, indicating that the study was conducted in accordance with Good Laboratory Practice Standards set forth in 40 CFR Part 160. The dates and types of quality assurance audits were also included.

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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:

An amendment to the SEP states that control oysters must deposit a minimum of 2 mm of new shell in 96 hours. At the end of the first test, the control and solvent control oysters deposited an average of 1.52 and 1.76 mm.

In this study, the flow rate of the test solution was about 1.0-1.2 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.

As the authors stated, the oysters were held in the laboratory for less than the required 10 days.

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 raw new shell deposition data from both tests were analyzed to determine the NOEC. The data from the first test did not meet the assumptions of normality and homogeneity of variances. The data were analyzed using the Kruskal-Wallis test. Average growth for several exposure groups were significantly higher than dilution water control and solvent control oysters (see attached printout 1). The NOEC for this test was 23.3 mg a.i./l. Growth inhibition >50% was not observed in this test, therefore EC_{50} calculations were not possible.

The data from the second test were analyzed using Student's t-test. Mean new shell growth in the exposure group was significantly lower than the control growth (see attached printout 1) therefore an NOEC could not be determined in this test. As above, an EC_{50} calculation was not possible.

C. Discussion/Results: Average new shell growth in control oysters (1.52 and 1.76 mm) at the conclusion of test 1 was lower than required (2.0 mm) in an amendment to the SEP. However, average growth in the control oysters during the second test was 2.89 mm. The test material could be considered practically non-toxic

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Pages 24 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.

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.

422563-05, NTN 33893 technical, new shell deposition
File: a:42256305.dt1 Transform: NO TRANSFORM

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

GRP1 (SOLVENT CRTL) MEAN =	1.7550	CALCULATED t VALUE =	1.4274
GRP2 (BLANK CRTL) MEAN =	1.5200	DEGREES OF FREEDOM =	38
DIFFERENCE IN MEANS =	0.2350		

TABLE t VALUE (0.05 (2),40) = 2.021 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2),40) = 2.704 NO significant difference at alpha=0.01

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	solvent control	1.755	1.755	1104.000
2	dilution contrl	1.520	1.520	802.000
3	2.93	1.715	1.715	1078.000
4	5.14	1.940	1.940	1435.000
5	8.19	2.540	2.540	2085.500
6	14.2	2.170	2.170	1727.500
7	23.3	2.110	2.110	1638.000

Calculated H Value = 36.089 Critical H Value Table = 12.590
Since Calc H > Crit H REJECT Ho:All groups are equal.

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 2 3 1 4 7 6 5
2	dilution contrl	1.520	1.520	\
3	2.93	1.715	1.715	. \
1	solvent control	1.755	1.755	. . \
4	5.14	1.940	1.940	. . . \
7	23.3	2.110	2.110	* . . . \
6	14.2	2.170	2.170	* . . . . \
5	8.19	2.540	2.540	* * * . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 12.804

Test 2 Statistical Evaluation - descriptive statistics

	N	MEAN	MEDIAN	TRMEAN	STDEV	SEMEAN
control	30	2.890	2.750	2.873	0.775	0.142
145 mg/l	30	2.237	2.200	2.258	0.959	0.175

	MIN	MAX	Q1	Q3
control	1.000	4.900	2.475	3.525
145 mg/l	0.000	4.000	1.575	3.000

TWOSAMPLE T FOR control VS 145 mg/l

	N	MEAN	STDEV	SE MEAN
control	30	2.890	0.775	0.14
145 mg/l	30	2.237	0.959	0.18

95 PCT CI FOR MU control - MU 145 mg/l: (0.20, 1.10)
TTEST MU control = MU 145 mg/l (VS NE): T= 2.90 P=0.0053 DF= 55

Mann-Whitney Confidence Interval and Test
control N = 30 Median = 2.7500
145 mg/l N = 30 Median = 2.2000
Point estimate for ETA1-ETA2 is 0.6000
95.2 pct c.i. for ETA1-ETA2 is (0.1999,1.1000)
W = 1090.0
Test of ETA1 = ETA2 vs. ETA1 n.e. ETA2 is significant at 0.0099
The test is significant at 0.0098 (adjusted for ties)

Test 2 RAW DATA

Printout # 2

ROW	control	145 mg/l
1	3.0	3.6
2	2.7	3.1
3	2.5	1.1
4	3.7	1.0
5	2.7	2.1
6	3.6	1.5
7	3.6	2.2
8	2.9	2.8
9	4.9	3.3
10	2.6	2.6
11	1.0	2.0
12	2.9	2.2
13	2.6	1.7
14	3.1	3.6
15	2.4	2.4
16	3.8	3.0
17	3.5	4.0
18	2.0	1.6
19	2.7	2.6
20	4.2	2.2
21	2.5	1.5
22	2.1	1.7
23	2.6	2.3
24	3.7	1.8
25	2.0	0.0
26	2.8	0.8
27	2.4	3.5
28	3.0	0.9
29	3.3	3.0
30	1.9	3.0

DATA EVALUATION RECORD

1. **CHEMICAL:** NTN 33893.
Shaughnessey No. 129059.

2. **TEST MATERIAL:** NTN 33893 technical (Imidacloprid); 1-[(6-chloro-3-pyrididyl)methyl]-4,S-dihydro-N-nitro-1H-imidazol-2-amine; Batch No. 890315 ELB 01; 99.8% active ingredient; a colorless crystal.

3. **STUDY TYPE:** Acute Contact LD_{50} and Oral LD_{50} Tests. Species Tested: Honey Bee (*Apis mellifera*). (# 141-1)

4. **CITATION:** Cole, J.H. 1990. The Acute Oral and Contact Toxicity to Honey Bees of Compound NTN 33893 Technical. Report No. 101321. Conducted by Huntingdon Research Centre Ltd., Cambridgeshire, UK. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422730-03.

5. **REVIEWED BY:**

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

Signature: [signature]
Date: 10/5/92

[signature]
11/25/92
EFED/EEB

6. **APPROVED BY:**

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

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Date: 10/5/92

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

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

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the requirements for acute contact and oral studies with the honey bee. Acute contact and oral LD_{50} values of 0.078 and 0.0039 µg/bee, respectively, classify NTN 33893 technical as highly toxic to honey bees (*Apis mellifera*). The 48-hour contact and oral NOELs were 0.05 and 0.0015 µg/bee, respectively.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Worker honey bees (*Apis mellifera*) were collected from the hives of Mr. R. Baker, Cambridgeshire, UK, within 3 to 4 hours prior to testing.

B. **Test System:** Bees were contained in cylindrical wire mesh cages (115 mm long and 40 mm in diameter). A glass feeding tube was inserted through the top of the cage and projected a 1.5 mm feeding hole. The bees were fed a 20% sugar/water solution. This food source was available *ad libitum* throughout the test (except during oral dosing). The cages were kept in a darkness and maintained at 25 ±1°C.

C. **Dosage:** Forty-eight hour acute contact and oral studies. Doses selected were based on preliminary rangefinding tests. For the contact study, the doses applied were 0.025, 0.05, 0.10, 0.20, and 0.40 µg/bee.

For the oral study, the doses that the bees ingested were 0.0015, 0.0031, 0.0063, 0.0125, and 0.025 µg/bee.

D. **Design:** The tests consisted of 5 treatment levels and a solvent control (contact) or sugar water control (oral). Two replicates of 10 bees each were used for each treatment and control.

For the contact study, bees were immobilized with carbon dioxide and dosed individually on the ventral side of the thorax with 1 µl of the appropriate test solution. Control bees were treated with 1 µl of dimethylformamide.

Oral exposure was accomplished by dissolving the test material in a 20% sugar/water solution. Feeding was done by supplying 0.2 ml of the test solutions in the feeding tube for the ten bees per cage to feed upon. Control bees were given a 20% sucrose solution. When all the test solution had been ingested (about 4 hours), the feeding tubes were replaced by tubes containing 20% sucrose solution.

Mortalities were recorded at 24 and 48 hours after treatment.

E. **Statistics:** The LD_{50} values and 95% confidence limits were calculated using probit analysis. Adjustments were made for control mortality with Abbott's correction.

12. **REPORTED RESULTS:** Percentage mortality for both tests is presented in the Table of Results (attached). The 48-hour LD_{50} for acute contact was determined to be 0.0081 µg/bee with a 95% confidence interval of 0.0055-0.0119 µg/bee. The 48-hour LD_{50} for oral ingestion was determined to be 0.0037 µg/bee with a 95% confidence interval of 0.0026-0.0053 µg/bee.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The author concluded that NTN 33893 is highly toxic to bees.

Quality Assurance and Good Laboratory Practice statements were included in the report indicating that the study was in compliance with the requirements of 40 CFR Part 160.

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

A. **Test Procedure:** The test procedures generally follow the protocols recommended by the SEP and Subdivision L guidelines, except for the following:

The age of the bees was not given and it is not known whether all test bees were at a uniform age.

No negative control group was included in the design for the contact study.

Observations of sublethal effects (if any) were not presented in the report.

B. **Statistical Analysis:** The reviewer calculated the LD_{50} values using probit analysis and obtained similar results for the oral study. For the contact study, the reviewer's LD value is 10 times greater than the author's. Therefore, the author probably made a typographical error in the results and summary sections since the data indicated only 20% mortality at the lowest dose (0.025 µg/bee). However, either the reviewer's value (0.078 µg/bee) or the author's value (0.0081 µg/bee) would classify the test substance as highly toxic to the bees.

Using EPA's Dunnett's test program, the reviewer determined that the no-observed-effect levels (NOEL) for

the oral and contact studies were 0.0015 and 0.05 µg/bee, respectively (see attached printouts).

C. **Discussion/Results:** This study is scientifically sound and fulfills the requirements for acute contact and oral studies with the honey bee. Acute contact and oral LD_{50} values of 0.078 and 0.0039 µg/bee, respectively, classify NTN 33893 technical as highly toxic to honey bees (*Apis mellifera*). The contact and oral NOELs were 0.05 and 0.0015 µg/bee, respectively.

D. **Adequacy of the Study:**

(1) **Classification:** Core.

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

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

15. **COMPLETION OF ONE-LINER:** Yes, 9-23-91.

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Page 34 is not included in this copy.

Pages _____ through _____ 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.

bee contact

Summary Statistics and ANOVA

Transformation = None

Group (dose (µg/bee))	n	Mean	s.d.	cv%
1 = control	2	10.0000	.0000	.0
2 0.025	2	8.0000	2.8284	35.4
3 0.05	2	7.0000	.0000	.0
4*0.1	2	4.5000	2.1213	47.1
5*0.2	2	2.0000	1.4142	70.7
6*0.4	2	.5000	.7071	141.4

NOEL = 0.05 µg/bee

*) 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 = -4.474623
This difference corresponds to -44.75 percent of control

Between groups sum of squares = 133.666667 with 5 degrees of freedom.

Error mean square = 2.500000 with 6 degrees of freedom.

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

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0058	0.0034	0.0086
EC 5.00	0.0124	0.0083	0.0168
EC10.00	0.0186	0.0133	0.0240
EC15.00	0.0245	0.0183	0.0306
EC50.00	0.0783	0.0675	0.0901
EC85.00	0.2498	0.2056	0.3205
EC90.00	0.3287	0.2628	0.4406
EC95.00	0.4937	0.3765	0.7090
EC99.00	1.0590	0.7337	1.7427

$y = 7.28 + 2.06(x)$

y = probit % inhibition

x = log(rate)

Bee oral ingestion

Summary Statistics and ANOVA

Transformation = None

Group (Dosage (µg/bee))	n	Mean	s.d.	cv%
1 = control	2	9.5000	.7071	7.4
2 0.0015	2	8.0000	1.4142	17.7
3* 0.0031	2	5.0000	1.4142	28.3
4* 0.0063	2	3.5000	2.1213	60.6
5* 0.0125	2	1.0000	1.4142	141.4
6* 0.025	2	.0000	.0000	.0

NOEL = 0.0015 µg/bee

*) 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 = -3.831838
This difference corresponds to -40.34 percent of control

Between groups sum of squares = 142.000000 with 5 degrees of freedom.

Error mean square = 1.833333 with 6 degrees of freedom.

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

NOTE: BECAUSE THERE WAS CONTROL MORTALITY, AND NONE
OF THE LOWER CONCENTRATIONS PRODUCED ZERO MORTALITY,
THE DATA HAS BEEN SUBJECTED TO ABBOTT'S CORRECTION.

MOSSLER NTN 33893 APIS MELLIFERA 9-23-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.025	19	19	100	1.907348E-04
.0125	19	17	89.4737	3.643036E-02
.0063	19	12	63.1579	17.96417
.0031	19	9	47.3684	50
.0015	19	3	15.7895	.2212524

THE BINOMIAL TEST SHOWS THAT .0015 AND .0125 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 3.485379E-03

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	.1617395	3.877385E-03	2.662001E-03	5.383747E-03

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1244218	1	.745034

SLOPE = 2.481616
95 PERCENT CONFIDENCE LIMITS = 1.606264 AND 3.356968

LC50 = 3.779504E-03 = 0.0038
95 PERCENT CONFIDENCE LIMITS = 2.678746E-03 AND 5.051707E-03

LC10 = 1.163246E-03
95 PERCENT CONFIDENCE LIMITS = 5.092623E-04 AND 1.800759E-03

****

DATA EVALUATION RECORD

1. **CHEMICAL:** NTN 33893.
Shaughnessey No. 129059.

2. **TEST MATERIAL:** NTN 33893 technical; 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1H-1,2,4-triazol-1-yl)-2-butanone; CAS No. 43121-43-3; Batch No. 9030211; 95% active ingredient; a tan powder.

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

4. **CITATION:** Gagliano, G.G. and L.M. Bowers. 1991. Acute Toxicity of NTN 33893 Technical to the Green Alga (*Selenastrum capricornutum*). Report No. 101986. Conducted by Mobay Corporation, Stilwell, KS. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422563-75.

5. **REVIEWED BY:**

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

Signature: [signature]
Date: 10/5/92

Dana Catteluccio 11/25/92 EFED EEB

6. **APPROVED BY:**

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

Signature: [signature]
Date: 10/5/92

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

Signature: [signature]
Date: 12/14/92

7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 non-target aquatic plant study. The control cultures did not grow logarithmically and light intensity was much greater than recommended.

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 Carolina Biological Supply, Burlington, NC. Stock cultures were maintained in algal nutrient medium under 18 hours of light/day.

B. Test System: Test vessels used were sterile glass 125-ml flasks fitted with steel caps. The test medium was the same as that used for culturing.

The test vessels were randomly placed on a shaker table (102 rpm) in an environmental chamber. Continuous cool-white illumination (800-900 footcandles) was provided and the temperature was monitored in a centrally located flask filled with medium.

A 240 g active ingredient (ai)/l stock was prepared by diluting 12.6374 g of the test material to 50 ml with dimethylformamide (DMF). Test solutions were created by addition of appropriate volumes of the stock to nutrient medium. The solvent control contained 0.5 ml of DMF/l of nutrient medium.

C. Dosage: Five-day growth and reproduction test. Based on the results of a preliminary test, five nominal concentrations of 15.6, 25.9, 43.2, 72, and 120 mg ai/l, and a solvent and medium control were selected for the definitive test.

D. Test Design: Fifty ml of the appropriate test or control solution were placed into each of three replicate flasks (3 per treatment level and the controls).

An inoculum of cells calculated to provide 10,000 cells/ml was introduced into each flask. Cell counts were performed using a microscope and hemocytometer on each test day. Each replicate was counted twice each day and eight fields were enumerated.

Samples were taken at test initiation and termination for analysis of the test material by high-performance liquid chromatography.

E. Statistics: All calculations were based on mean measured concentrations. Control data were pooled. The no-observed-effect concentration (NOEC) was

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estimated using analysis of variance (ANOVA) and Dunnett's test. The level of significance was $p \leq 0.05$.

12. **REPORTED RESULTS:** The mean measured concentrations ranged from 90 to 99% of nominal (Table 3, attached). The mean measured concentrations were 14.1, 24.1, 41.4, 69.5, and 119 mg ai/l. No undissolved test material was observed in the test solutions.

Cell counts and percent inhibition for each concentration after five days are given in Table 5 (attached). Both the 5-day EC_{50} and NOEC were determined to be >119 mg ai/l.

Temperature ranged from 23.8 to 25.6°C during the study. Although the pH and conductivity of the solutions were not measured due to laboratory error, these parameters are usually 6.6 and 428 µmhos, respectively.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the authors.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

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

A. **Test Procedure:** The test procedure and the report did not follow the SEP and Subdivision J guidelines, and the following deviations were noted:

The age of the inoculum was not reported.

The light intensity (8.6-9.7 klux) was higher than recommended (4 klux).

The amount of cellular inoculum (10,000 cells/ml) was greater than recommended (3000 cells/ml).

B. **Statistical Analysis:** Using the EPA's Dunnett's test program, the reviewer confirmed that all test concentrations did not significantly effect the growth of *S. capricornutum* (see attached printout).

C. **Discussion/Results:** Cellular growth of the pooled control only increased by five-fold. This may have been an indication that the light intensity was damaging to the cultures or that the culture used as

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inoculum was old or damaged. Average cell growth over a 5-day period is often 100-fold the original density.

This study is not scientifically sound and does not meet the guideline requirements for a Tier 2 non-target aquatic plant study.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** The control cultures did not grow logarithmically and and light intensity was much greater than recommended.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 9-23-92.

C/2

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

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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%
Concentration (mg ai/l*)				
1 = control	6	48333.3333	7814.5164	16.2
2 ~~14.1~~	3	53000.0000	2645.7513	5.0
3 24.1	3	53666.6667	3214.5503	6.0
4 41.4	3	51666.6667	1527.5252	3.0
5 69.5	3	54333.3333	3511.8846	6.5
6 119	3	53666.6667	4932.8829	9.2

NOEC = 119 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

* - based on mean measured conc.

Minumum detectable difference for
t-tests with Bonferroni adjustment = -7933.526637
This difference corresponds to -16.41 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 =116571428.571429 with 5 degrees of freedom.

Error mean square = 27866666.666667 with 15 degrees of freedom.

Bartlett's test p-value for equality of variances = .251

DATA EVALUATION RECORD

1. **CHEMICAL:** NTN 33893.
Shaughnessey No. 129059.

2. **TEST MATERIAL:** NTN 33893 technical; Batch No. 2/86; 92.8% active ingredient; a white powder.

3. **STUDY TYPE:** 122-2. Growth and Reproduction of Aquatic Plants - Tier 1. Species Tested: *Scenedesmus subspicatus*.

4. **CITATION:** Heimbach, F. 1989. Growth Inhibition of Green Algae (*Scenedesmus subspicatus*) Caused by NTN 33893 (Technical). Laboratory Report No. 100098, Conducted by Bayer AG, West Germany. Submitted by Mobay Corporation, Kansas City, MO. EPA MRID No. 422563-74.

5. **REVIEWED BY:**

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

Signature: [signature]
Date: 10/5/92

Dana Spatulla 11/25/92 EFED/EEB

6. **APPROVED BY:**

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

Signature: Louis M. Rifici
Date: 10/5/92

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

Signature: [signature]
Date: 12/14/92

7. **CONCLUSIONS:** This study is scientifically sound but does not meet the guideline requirements for a Tier 1 non-target aquatic plant study. The test procedures deviated significantly from the recommended protocols. Exposure to NTN 33893 technical at a concentration of 10 mg ai/l (nominal) did not significantly reduce the growth of *S. subspicatus* over the 4-day test period.

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, *Scenedesmus subspicatus*, came from laboratory stock cultures. Stock cultures were maintained in algal medium under 16-hours of illumination per day at 20°C. Transfers were made weekly to maintain active growth. The culture used as inoculum had been transferred to fresh medium three days before test initiation.

B. Test System: Test vessels used were 300-ml Erlenmeyer flasks. Each exposure flask was prepared by the addition of a stock solution prepared in deionized water and 10X algal medium.

The test vessels were kept in an incubator which provided 8000 lux illumination supplied by fluorescent lights. The temperature was 23 ±1°C and the vessels were agitated to suspend the algae.

C. Dosage: Four-day growth and reproduction test. Based on a preliminary test, one nominal concentration of 10 mg active ingredient (ai)/l and a medium control were selected for the definitive test.

D. Test Design: The exposure and control treatments were replicated three times. One-hundred milliliters of the appropriate test solution were placed into each flask. An inoculum of *Scenedesmus subspicatus* cells calculated to provide 10,000 cells/ml was aseptically introduced into each flask. A model was used to relate spectrophotometric absorbance with cell number and counts were performed on test days 1, 2, 3, and 4. Cells were also microscopically examined for any alterations in cell size or morphology. Growth rate and area under the growth curve were also determined.

Test temperature was recorded at test termination. The pH was measured daily.

E. Statistics: No statistical procedures were conducted on the data.

12. REPORTED RESULTS: No morphological abnormalities were observed for the exposed cells. The mean cell densities for the control and 10 mg ai/l treatment were 289 and 284 $x10^4$ cells/ml, respectively, after 96 hours (Table 3, attached). The growth rates for the control and 10 mg ai/l treatment were 5.90 and 5.88, respectively, after 96 hours. The areas

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under the growth curves for the control and 10 mg ai/l treatment were 6188 and 5809, respectively, after 96 hours. The EC_{50} (based on both area under the growth curve and biomass) was determined to be >10 mg ai/l and the no-observed-effect concentration (NOEC) was 10 mg ai/l.

The pH at initiation and termination ranged from 8.23 to 8.38 and from 8.07 to 8.15, respectively, in the test solutions and the controls. The temperature at test termination was 22.8°C.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: No conclusions other than those stated were made by the author.

Approximately 75 days prior to test initiation, a reference toxicant test under the same conditions used here was performed using potassium dichromate. The results were in agreement with a collaborative study.

Quality Assurance and Good Laboratory Practice statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

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

A. Test Procedure: The test procedure and the report did not meet the requirements of the SEP and Subdivision J guidelines. The following are deviations:

Light intensity during the test was 8 klux. The recommended light intensity is 4 klux.

It was not stated if the illumination was cool or warm. Guidelines recommend cool illumination.

The test was conducted for 4 days rather than the recommended 5 days.

The initial cell inoculum (10,000 cells/ml) was higher than recommended (3000 cells/ml).

The test temperature was not monitored during the study.

No justification was given as to why the author used *Scenedesmus subspicatus* rather than *Selenastrum capricornutum*.

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B. **Statistical Analysis:** Upon review of the cell density data, it is apparent that the test substance had little effect on cellular growth (2% inhibition).

C. **Discussion/Results:** This study is scientifically sound but does not meet the guideline requirements for a Tier 1 non-target aquatic plant study. Exposure to NTN 33893 technical at a concentration of 10 mg ai/l (nominal) did not significantly reduce the growth of *S. subspicatus* over the 4-day test period.

D. **Adequacy of the Study:**

(1) **Classification:** Supplemental.

(2) **Rationale:** The test procedures deviated significantly from the recommended protocols.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER:** Yes, 9-22-92.

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Pages _____ through _____ are not included.

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____ 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.

DATA EVALUATION RECORD

1. **CHEMICAL:** NTN 33893.
Shaughnessey No. 129059.

2. **TEST MATERIAL:** NTN 33893 technical; ABC Reference No. TS-4204; a light yellow powder.

3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Static Acute Toxicity Test. Species Tested: *Hyalella azteca*.

4. **CITATION:** England, D. and J.D. Bucksath. 1991. Acute Toxicity of NTN 33893 to *Hyalella azteca*. Report No. 101960. Prepared by ABC Laboratories, Inc., Columbia, MO. Submitted by Mobay Corporation, Stilwell, KS. EPA MRID No. 422563-03.

5. **REVIEWED BY:**

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

Signature: Louis M Rifici
Date: 9/28/92

Dana Spatulli 11/25/92 EFED/EEB

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 9/28/92

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

Signature: Anne Stavola
Date: 12/14/92

7. **CONCLUSIONS:** The study is scientifically sound but does not meet the guideline requirements for a static acute toxicity test using freshwater invertebrates. *Hyalella azteca* is not a recommended species in the SEP. The authors do not provide any justification for its use. In addition, the purity of the test material was not reported. The 48- and 96-hour EC_{50} values were 115.3 µg/l and 55 µg/l (mean measured concentrations), respectively. Therefore NTN 33893 is classified as highly toxic or very highly toxic to *H. azteca* depending on which LC_{50} is used. The 48- and 96-hour NOEC values were 0.97 µg/l and 0.35 µg/l mean measured concentrations. (96-hr LC_{50} used with this test species, CS)

8. **RECOMMENDATIONS:** The registrant should provide justification for using *H. azteca*, the registrant must also

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provide the lot/batch number and percentage active ingredient for the test material and the age of the test organisms used. Justification is not necessary for using H. azteca, the information will be used as supplemental data.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Juvenile *Hyalella azteca* (2-3 mm long) used in the test were obtained from in-house cultures. Adults were acclimated to the hard blended test water over a period of several days. The culture vessels were 1-gallon glass jars containing hard maple leaves as a primary food/substrate. A supplement of fish food, cereal leaves, and yeast was added 2-3 times weekly. The temperature was 20°C and the photoperiod was 16 hours of light.

B. **Test System:** Vessels used in the test were glass beakers containing 1000 ml of test solution. A 2" by 6" piece of nylon screen was placed in each test vessel as a substrate for the test animals. The beakers were placed in a water bath maintained at 20 ±2.0°C. Lighting was the same as that used in culturing.

Blended hard water (a well water and reverse-osmosis water mixture) with a hardness of 180 mg/l as $CaCO_3$, an alkalinity of 194 mg/l as $CaCO_3$, a pH of 8.3, and a conductivity of 430 µmhos/cm, was used as dilution water.

Two stock solutions (0.0001 mg/ml and 0.10 mg/ml) were prepared.

C. **Dosage:** Ninety-six-hour, static test. Based on preliminary testing, nine nominal concentrations (0.33, 1.0, 3.3, 10, 33, 100, 330, 1000, and 3000 µg/l) and a dilution water control were used.

D. **Design:** Ten *H. azteca* were impartially distributed to each test beaker. Two beakers were used per test level. The loading was approximately one organism per 100 ml of solution. All beakers were observed once daily to determine survival and abnormal effects.

The temperature, dissolved oxygen concentration (DO), and pH were measured in one replicate of the control, low, two middle, and high test concentrations daily.

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The temperature of the water bath was continuously monitored using a data logger.

Measured concentrations of NTN 33893 in the test solutions were determined at test initiation and termination using high performance liquid chromatography.

E. **Statistics:** The LC_{50} values and associated confidence intervals were determined using a computer program developed by Stephan et al. (1977).

12. **REPORTED RESULTS:** The mean measured concentrations were 0.35, 0.97, 3.5, 10, 34, 100, 340, 1000, and 3100 µg/l and averaged 102% of nominal concentrations (Table 5, attached). "The test material appeared to be stable in the system based on information supplied by the study sponsor and the consistent measurements at 0 and 96 hours."

The 48-hour LC_{50} value could not be determined due to insufficient mortality (Table 6, attached). The 96-hour LC_{50} was 526 µg/l (95% C.I. = 194-1263 µg/l) using the moving average method. The 48 and 96-hour EC_{50} values were 129 (95% C.I. = 85-193 µg/l) and 55 µg/l (95% C.I. = 34-93 µg/l), respectively (Table 7, attached). The 96-hour no-observed-effect concentration (NOEC) was 0.35 µg/l, based on the lack of mortality and abnormal effects at this level (Table 3, attached).

During the test, the temperature remained constant at 20°C. Dissolved oxygen concentrations ranged from 5.2 to 8.2 mg/l (60 to 94% of saturation at 20°C). The pH was 8.0-8.4.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors did not present any conclusions.

Quality assurance and study compliance statements were included in the report, indicating that the study was conducted in accordance with USEPA 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 in accordance with the SEP, except for the following:

Hyalella azteca is not a recommended species. The authors present no justification for using this species. In addition, the age and developmental stage of the organisms were not reported. It is possible

that 2 or more instars were present in the test population.

The test material was not adequately described. No lot or batch number or percentage active ingredient was provided in the report.

The recommended test temperature for amphipods is 17°C. The temperature during this test was 20°C.

The procedures used to prepare the test solutions and the time between test solution preparation and test initiation were not reported.

Fifteen to 30-minute dawn and dusk simulation periods are recommended in the SEP. These simulations were not used during the test.

The test concentrations were approximately 30% of the next highest concentration. The SEP recommends that each nominal concentration be at least 60% of next highest.

The dimensions of the test vessels were not reported.

B. Statistical Analysis: The reviewer calculated the 48 and 96-hour EC_{50} values using EPA's Toxanal computer program. The results were similar to those of the authors' (see attached printouts 1 and 2).

C. Discussion/Results: The study is scientifically sound but does not meet the guideline requirements for a static acute toxicity test using freshwater invertebrates. *Hyalella azteca* is not a recommended species in the SEP. The authors do not provide any justification for its use. In addition, the purity of the test material was not reported. The 48- and 96-hour EC_{50} values were 115.3 µg/l and 55 µg/l (mean measured concentrations), respectively. Therefore NTN 33893 is classified as highly toxic or very highly toxic to *H. azteca* depending on which LC_{50} is used. The 48- and 96-hour NOEC values were 0.97 µg/l and 0.35 µg/l mean measured concentrations. (96-hr values used with this test species) as

D. Adequacy of the Study:

(1) Classification: Supplemental.

(2) Rationale: The test species used is not recommended in the SEP. The authors do not

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provide any justification for its use. In addition, the percent active ingredient of the test material was not reported.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 09-16-92.

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_____ 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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48-hour EC_{50}

Printout 1

RIFICI NTN 33893 HYALELLA AZTECA 09-16-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
3100	20	20	100	9.536742E-05
1000	20	20	100	9.536742E-05
340	20	13	65	13.1588
100	20	10	50	58.80985
34	20	2	10	2.012253E-02
10	20	1	5	2.002716E-03
3.5	20	0	0	9.536742E-05
.97	20	0	0	9.536742E-05
.35	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 34 AND 1000 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 99.99999

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	.0513501	115.2767	74.90048	179.5673

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.0796061	1	.6815431

SLOPE = 1.821192

95 PERCENT CONFIDENCE LIMITS = 1.307351 AND 2.335033

LC50 = 127.7236

95 PERCENT CONFIDENCE LIMITS = 84.80026 AND 192.385

LC10 = 25.64045

95 PERCENT CONFIDENCE LIMITS = 11.76405 AND 42.33426

****

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96-hour EC_{50} Printout 2

RIFICI NTN 33893 HYALELLA AZTECA 09-16-92

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
3100	20	20	100	9.536742E-05
1000	20	20	100	9.536742E-05
340	20	19	95	2.002716E-03
100	20	8	40	25.17223
34	20	6	30	5.765915
10	20	1	5	2.002716E-03
3.5	20	1	5	2.002716E-03
.97	20	1	5	2.002716E-03
.35	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 10 AND 340 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 121.054

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
8	5.135013E-02	56.56807	34.35538	98.85121

98.85121

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2847988	2.965891	4.14002E-03

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

SLOPE = 1.479745
95 PERCENT CONFIDENCE LIMITS = .690056 AND 2.269433

LC50 = 67.48288
95 PERCENT CONFIDENCE LIMITS = 23.19101 AND 198.031

LC10 = 9.352712
95 PERCENT CONFIDENCE LIMITS = .6949445 AND 26.30639

****

C2