



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

JAN 11 1995

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OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Data review for Isoxaflutole (D219142, Chemical #123000, Case 286745, ID# 286745)

FROM: Renée Costello, Biologist *Renée Costello* 12/18/95
Environmental Risk Characterization Branch
Environmental Fate and Effects Division (7507C)

THRU: Elizabeth M. Leovey, Chief
Environmental Risk Characterization Branch
Environmental Fate and Effects Division (7507C) *[Signature]*

TO: Joanne Miller, PM 23
Registration Division (7505C)

The Environmental Risk Characterization Branch (ERCB) has completed the review of the data submitted in support of registration of Isoxaflutole, chemical number 123000. The following is a brief summary of the data reviewed:

Citation: RPA 201772 Technical - Acute Toxicity to the Freshwater Blue-green Alga, *Anabaena flos-aquae*. EPA MRID No. 435732-45

Conclusions: This study is scientifically sound and meets the guideline requirements for an algae acute toxicity test. Based on mean measured concentrations, the 120 hour EC_{50} of isoxaflutole technical is 0.18 mg ai/L. The NOEC is 0.0086 mg ai/L

Peer review: Mike Davy, Agronomist, ERCB *Michael Davy* 1/10/96

DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 122-2 OR 123-2 (TIER II)

1. CHEMICAL: Isoxaflutole PC Code No.: 123000

2. TEST MATERIAL: Isoxaflutole technical Purity: 96.8%

3. CITATION

Authors: James R. Hoberg

Title: RPA 201772 Technical - Acute Toxicity to
the Freshwater Blue-green Alga, *Anabaena*
flos-aquae

Study Completion Date: March 21, 1994

Laboratory: Springborn

Sponsor: Rhone-Poulenc

Laboratory Report ID: 94-3-5207

DP Barcode: D219142

MRID No.: 435732-45

4. REVIEWED BY: Renée Costello, Biologist, ERCB, EFED

Signature: *Renée Costello*

Date: 12/18/95

5. REVIEWED BY: Mike Davy, Agronomist, ERCB, EFED

Signature: *Mike Davy*

Date: 1-11-96

6. STUDY PARAMETERS

Definitive Test Duration: 120 hours

Type of Concentrations: Nominal and initial measured

7. CONCLUSIONS:

Results Synopsis

EC₅₀: 0.18 mg ai/L

95% C.I.: 0.14 - 0.24 mg ai/L

Probit Slope: 1.19

NOEC: 0.0086 mg ai/L

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS

1. Dose range progression was 30% in order to achieve an EC₅₀
and an NOEC.

This deviation did not effect the results of the study.

10. **SUBMISSION PURPOSE:** Product registration.

11. **MATERIALS AND METHODS**

A. Test Organisms

Guideline Criteria	Reported Information
Species <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Anabaena flos-aquae</i>
Initial Number of Cells 3,000 - 10,000 cells/mL	10,000 cells/mL
Nutrients Standard formula, e.g. 20XAAP	Standard AAP medium

B. Test System

Guideline Criteria	Reported Information
Solvent	Acetone
Temperature Skeletonema: 20°C Others: 24-25°C	25 °C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	1100 - 3300 Lux
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
pH Skeletonema: approx. 8.0 Others: approx. 7.5	7.4 - 8.5

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	30% in order to achieve an NOEC and EC ₅₀
Doses at least 5	0.0024, 0.0081, 0.027, 0.090, 0.30, and 1.0

Guideline Criteria	Reported Information
Controls negative and/or solvent	solvent and negative
Replicates per dose 3 or more	3
Duration of test 120 hours	120 hours
Daily observations were made?	Yes every 24 hours
Method of Observations	Cellular counts
Maximum Labeled Rate	0.18 lb ai/acre

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes
Control cell count at 120 hr $\geq 2X$ initial count?	$>2x$ from 24 hours
Initial chemical concentrations measured?	Yes
Raw data included?	Yes

Dose Response

Dose (mg ai/L)	Cell Density ($\times 10^4$ cells/mL)	% Inhibition	120-Hour pH
Control	89	N/A	8.5
Solvent Control	94	N/A	8.4
0.0020	92	-0.64	8.0
0.0086	85	6.8	7.9
0.028	73	20	7.8
0.087	62	32	7.9
0.23	49	46	7.9
0.99	12	87	7.7

DP Barcode: D219142

MRID No.: 435732-45

Statistical Results

Statistical Method: William's test

EC₅₀: 0.17 mg ai/L 95% C.I.: 0.031 - 1.1 mg ai/L

NOEC: 0.0086 mg ai/L

13. Verification of Statistical Results

Statistical Method: Toxanal

EC₅₀: 0.18 mg ai/L 95% C.I.: 0.14 - 0.24 mg ai/L

Probit Slope: 1.19 NOEC: 0.0086 mg ai/L

costello isoxaflutole anabaena

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.99	100	87	87	0
.23	100	46	46	0
.087	100	32	32	0
.028	100	20	20	0
.0086	100	7	7	0
.002	100	1	1	0

THE BINOMIAL TEST SHOWS THAT .23 AND .99 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 .2615028

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	4.185487E-02		.2055365 .1575311
.2775972			

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
3	2.302101E-02	1
.1337676		

SLOPE = 1.186771
95 PERCENT CONFIDENCE LIMITS = 1.006706 AND 1.366836

LC50 = .1820007
95 PERCENT CONFIDENCE LIMITS = .1423098 AND .2390341

LC10 = 1.548611E-02
95 PERCENT CONFIDENCE LIMITS = 1.009744E-02 AND .0217165

Anabaena cell densities
File: a:isoxaflutole\ana.dat

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	3	84.000	95.000	89.167
2	solvent control	3	88.250	100.750	93.833
3	0.0020	3	85.000	98.000	92.083
4	0.0086	3	80.750	90.000	85.250
5	0.028	3	64.500	84.500	72.833
6	0.087	3	53.000	68.250	62.083
7	0.23	3	43.500	53.750	49.417
8	0.99	3	10.250	14.250	11.750

Anabaena cell densities
I e: a:isoxaflutole\ana.dat

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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	30.583	5.530	3.193
2	solvent control	40.396	6.356	3.670
3	0.0020	43.271	6.578	3.798
4	0.0086	21.438	4.630	2.673
5	0.028	108.333	10.408	6.009
6	0.087	64.521	8.032	4.638
7	0.23	28.146	5.305	3.063
8	0.99	4.750	2.179	1.258

Anabaena cell densities
File: a:isoxaflutole\ana.dat

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	89.167	89.167	91.694
2	solvent control	3	93.833	93.833	91.694
3	0.0020	3	92.083	92.083	91.694
4	0.0086	3	85.250	85.250	85.250
5	0.028	3	72.833	72.833	72.833
6	0.087	3	62.083	62.083	62.083
7	0.23	3	49.417	49.417	49.417
8	0.99	3	11.750	11.750	11.750

Anabaena cell densities
 File: a:isoxaflutole\ana.dat

Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
control	91.694				
solvent control	91.694	0.474		1.75	k= 1, v=16
0.0020	91.694	0.474		1.83	k= 2, v=16
0.0086	85.250	0.734		1.86	k= 3, v=16
0.028	72.833	3.062	*	1.87	k= 4, v=16
0.087	62.083	5.077	*	1.88	k= 5, v=16
0.23	49.417	7.452	*	1.89	k= 6, v=16
0.99	11.750	14.513	*	1.89	k= 7, v=16

s = 6.533

Note: df used for table values are approximate when v > 20.