



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

8-3-95

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Pyriproxyfen: freshwater algae toxicity review

FROM: *Anthony F. Maciorowski* 8-3-95
Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Rick Keigwin/Joseph Travano
Product Manager 10
Registration Division (7505C)

Sumitomo Chemical Company, Ltd. submitted the following study to support pyriproxyfen:

Blasberg, J.W., S.L. Hicks, and D.L. Cramer. 1991. Acute toxicity of pyriproxyfen to *Selenastrum capricornutum* Printz. ABC Laboratories, Inc., Columbia, MO, Report #39543. MRID No. 436974-01.

The study is scientifically sound but does not fulfill the guideline requirement for a freshwater algae toxicity study. The study is supplemental, because it was conducted for only 72 hours, initial cell counts were not made for all concentrations, and light intensity was too high. The study must be repeated and conducted to conform with Subdivision J guideline requirements. The 72-hour EC_{50} value of 0.056 ppm classifies the technical pyriproxyfen as very highly toxic to freshwater invertebrates.

If you have any questions, please contact Bill Erickson at 305-6212 or Harry Craven at 305-5320.

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

1. CHEMICAL: Pyriproxyfen (129032)
2. TEST MATERIAL: Pyriproxyfen; 97.2%
3. CITATION:

Authors: Blasberg, J.W., S.L. Hicks, and D.L. Cramer
Title: Acute toxicity of pyriproxyfen to *Selenastrum capricornutum* Printz
Date: 1991
Laboratory: ABC Laboratories, Inc., Columbia, MO
Sponsor: Sumitomo Chemical Company, Ltd., Osaka, Japan
Report ID: #39543
MRID No.: 436974-01

4. REVIEWED BY:

William Erickson
Biologist
EEB/EFED/EPA

Signature:

Date:

W. Erickson
8/02/95

5. APPROVED BY:

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature:

Date:

Harry Craven
8/2/95

6. STUDY PARAMETERS:

Definitive Test Duration: 72 hours
Type of Concentrations: Mean measured

7. CONCLUSIONS: The study is scientifically sound but does not fulfill the guideline requirement for a freshwater algae toxicity study.

Results Synopsis:

72-hr EC₅₀: 0.056 mg/l
95% C.I.: 0.051-0.062 mg/l
NOEL: 0.020 mg/l

8. ADEQUACY OF THE STUDY: Supplemental. The study must be repeated and conducted in accordance with Subdivision J guideline requirements.

9. GUIDELINE DEVIATIONS:

1. The study was terminated after 72 hours (3 days); the guideline requirement is for at least a 5-day (120-hour) test.
2. Initial cell counts were made only for the negative and solvent controls.
3. Light intensity (8600 Lux) was higher than recommended (4000-5000 Lux).

10. SUBMISSION PURPOSE: Data to support EUP.

11. MATERIALS AND METHODS:

A. Test Organisms

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

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	Acetone
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	24 ± 2°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 klux (±15%) Others: 4.0-5.0 klux (±15%)	8600 Lux
<u>Photoperiod</u> <i>Skeletonema</i> : 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

Guideline Criteria	Reported Information
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	7.4

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	5
<u>Controls</u> negative and/or solvent	negative and solvent
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 120 hours	72 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Hemocytometer and microscope
<u>Maximum Labeled Rate</u>	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	No - initial cell counts at 0 hour were made only for controls; final counts were made at 72 h
Control cell count at 120 hr $\geq$ 2X initial count?	Cell counts in controls at 72 h were $>2X$ initial counts; unknown for the test concentrations for which initial counts were not made
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

72-hour Dose Response:

Dose (mg ai/L)	Cell Density (x 10 ⁴ cells/ml)	% Inhibition	72-Hour pH
Control	100	-	8.4
Solvent Control	100	-	8.4
0.020	100	0	8.3
0.038	84	16	8.4
0.069	47	53	8.3
0.15	3.8	96.2	7.9
0.33	1.1	98.9	7.4

Statistical Results:

Method: Nonlinear regression for EC₅₀;
Dunnett's Test for NOEC
72-h EC₅₀: 0.064 mg/l
95% C.I.: 0.058-0.069 mg/l
NOEC: 0.020 mg/l

13. VERIFICATION OF STATISTICAL RESULTS: (results attached)

Method: Probit Analysis for EC₅₀;
Williams' Test for NOEC
72-h EC₅₀: 0.056 mg/l
95% C.I.: 0.051-0.062 mg/l
NOEC: 0.020 mg/l

14. REVIEWER'S COMMENTS: The study is scientifically sound but does not fulfill the guideline requirement for a freshwater algae toxicity study. The study was conducted for only 3 days and did not conform to guideline requirements.

W. ERICKSON PYRIPROXYFEN SELANASTRUM EC50

	EXPOSED	DEAD	DEAD	PROB. (PERCENT)
.33	100	99	99	0
.15	100	97	97	0
.069	100	57	57	0
.038	100	24	24	0
.02	100	9	9	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 6.107487E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	1.318625E-02	6.380055E-02	5.727553E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
4	2.238142E-02	1

SLOPE = 3.435409
95 PERCENT CONFIDENCE LIMITS = 2.921458 AND 3.949361

LC50 = 5.662879E-02
95 PERCENT CONFIDENCE LIMITS = 5.123012E-02 AND 6.263128E-02

LC10 = 2.417451E-02
95 PERCENT CONFIDENCE LIMITS = 2.014725E-02 AND 2.791355E-02

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* Directory:                                     *
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*          July 21, 95                          4:26pm                    *
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*      W W W E      R  R   I    C      K K      S O  O
*      W W W E      R  R   I    C   C K  K  S   S O  O
*      W  W EEEEE R  R   III   CCC  K  K  SSS   OOO
*
*****
*
*      W  W EEEEE RRRR   III   CCC  K  K  SSS   OOO
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SELANASTRUM CELL COUNTS - PYRIPROXYFEN

File: A:PYR.DAT

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	CONTROL	3	101.000	110.000	105.000
2	.020	3	94.500	112.000	102.833
3	.038	3	72.500	95.000	84.100
4	.069	3	40.300	50.300	46.533
5	.15	3	2.560	4.780	3.817
6	.33	3	0.778	1.440	1.146

SELANASTRUM CELL COUNTS - PYRIPROXYFEN

File: A:PYR.DAT

Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	CONTROL	21.000	4.583	2.646
2	.020	77.083	8.780	5.069
3	.038	126.930	11.266	6.505
4	.069	29.563	5.437	3.139
5	.15	1.297	1.139	0.657
6	.33	0.114	0.337	0.195

SELANASTRUM CELL COUNTS - PYRIPROXYFEN

File: A:PYR.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	105.000	105.000	105.000
2	.020	3	102.833	102.833	102.833
3	.038	3	84.100	84.100	84.100
4	.069	3	46.533	46.533	46.533
5	.15	3	3.817	3.817	3.817
6	.33	3	1.146	1.146	1.146

SELANASTRUM CELL COUNTS - PYRIPROXYFEN

File: A:PYR.DAT

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

7

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	105.000				
.020	102.833	0.406		1.78	k= 1, v=12
.038	84.100	3.919	*	1.87	k= 2, v=12
.069	46.533	10.963	*	1.90	k= 3, v=12
.15	3.817	18.972	*	1.92	k= 4, v=12
.33	1.146	19.473	*	1.93	k= 5, v=12

s = 6.532

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