

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

1. **CHEMICAL:** Metolachlor (108801)
2. **TEST MATERIAL:** Metolachlor technical; 97.3%
3. **CITATION**

Author: Hoberg, J.R.
Title: Metolachlor technical - 5-day toxicity to the
freshwater diatom, *Navicula pelliculosa*, using
acetone as a carrier solvent
Lab. ID #: 94-12-5627
Sponsor: Ciba Crop Protection, Greensboro, NC
Test Lab: Springborn Laboratories, Inc., Wareham, MA
MRID #: 435413-02

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED/EPA

Signature: *W. Erickson*

Date: *3/01/95*

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: *Harry F. Craven*

Date: *3/01/95*

6. **STUDY PARAMETERS/RESULTS SYNOPSIS:**

Test Duration: 120 hours
Type of Concentrations: Mean measured
EC₅₀: 0.38 ppm ai
95% C.I.: 0.27-0.56 ppm ai
Slope: 0.89
NOEL: 3.7 ppb ai

7. **CONCLUSIONS:** The study is scientifically sound and satisfies the guideline requirement (123-2) for a Tier 2 aquatic plant growth study with a freshwater diatom. The EC₅₀ value of 0.38 ppm ai classifies metolachlor technical as highly toxic to *Navicula pelliculosa*.
8. **ADEQUACY OF THE STUDY:** Core.
9. **MAJOR GUIDELINE DEVIATIONS:** None.
10. **SUBMISSION PURPOSE:** Reregistration data.



2044736

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>Navicula pelliculosa</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	AAP

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	Acetone
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	24-25°C
<u>Light Intensity</u> Anabaena: 2.0 Lux (±15%) Others: 4.0-5.0 Lux (±15%)	3.5-4.8 Lux
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	7.5

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	3X
<u>Doses</u> at least 5	6
<u>Controls</u> negative and/or solvent	Negative and solvent
<u>Replicates per dose</u> 3 or more	3

Guideline Criteria	Reported Information
<u>Duration of test</u> 120 hours	120 hours
<u>Daily observations were made?</u>	Yes
<u>Method of Observations</u>	Cellular counts
<u>Maximum Labeled Rate</u>	Not reported

12. REPORTED RESULTS

Guideline Criteria	Reported Information
<u>Initial and 120 h cell densities were measured?</u>	Yes
<u>Control cell count at 120 hr ≥2X initial count?</u>	Yes
<u>Initial chemical concentrations measured?</u> (Optional)	Yes
<u>Raw data included?</u>	Yes

Dose Response:

Dose (mg ai/L)	Cell Density (x 10 ⁴ cells/ml)	% Inhibition	120-Hour pH
Control	116	N/A	9.4
Solvent Control	118	N/A	9.3
0.0007	117	0.6	9.3
0.0014	105	10.3	8.9
0.0025	91	22	8.4
0.0059	72	39	8.3
0.0140	60	49	8.0
0.0230	40	66	8.0

Other Significant Results: At test termination, bloated cells and cell fragments were observed in the 0.13, 0.43, and 1.3 mg ai/l treatment levels.

DP Barcode: D212142

MRID No.: 435413-01

Statistical Results:

Methods: Linear regression (EC_{50})
Williams' Test (NOEC)

EC_{50} : 0.4 ppm ai 95% C.I.: 0.17-0.97 ppm ai
NOEC: 0.0037 ppm ai

13. Verification of Statistical Results:

Methods: Probit Analysis (EC_{50})
Williams' Test (NOEC)

EC_{50} : 0.38 ppm ai 95% C.I.: 0.27-0.56 ppm ai
Slope: 0.89 NOEC: 0.0037 ppm ai

- 14. REVIEWER'S COMMENTS:** The study is scientifically sound and satisfies the guideline requirement (123-2) for a Tier 2 aquatic plant growth study with *Navicula pelliculosa*.

W. ERICKSON METOLACHLOR NAVICULA PELLICULOSA

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.3	100	66	66	0
.43	100	49	49	0
.13	100	39	39	0
.041	100	22	22	0
.013	100	10	10	0
.0037	100	1	1	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 .4584313

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	9.228689E-02		.3756784 .2538304

.6119041

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	2.941335E-02	1	.380309

SLOPE = .8915365
95 PERCENT CONFIDENCE LIMITS = .7386352 AND 1.044438

LC50 = .3825852
95 PERCENT CONFIDENCE LIMITS = .2764651 AND .5634822

LC10 = 1.439532E-02
95 PERCENT CONFIDENCE LIMITS = 8.138358E-03 AND 2.221422E-02

METOLACHLOR AQUATIC PLANT GROWTH - *Navicula pelliculosa*

SUMMARY STATISTICS

TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0	3	117.000	120.000	118.333
2	0.0037	3	111.000	121.000	116.667
3	0.013	3	104.000	106.000	105.000
4	0.041	3	88.000	95.000	91.667
5	0.13	3	66.000	78.000	71.667
6	0.43	3	59.000	61.000	60.333
7	1.3	3	34.000	50.000	40.667

SUMMARY STATISTICS

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0	2.333	1.528	0.882
2	0.0037	26.333	5.132	2.963
3	0.013	1.000	1.000	0.577
4	0.041	12.333	3.512	2.028
5	0.13	36.333	6.028	3.480
6	0.43	1.333	1.155	0.667
7	1.3	69.333	8.327	4.807

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0	3	118.333	118.333	118.333
2	0.0037	3	116.667	116.667	116.667
3	0.013	3	105.000	105.000	105.000
4	0.041	3	91.667	91.667	91.667
5	0.13	3	71.667	71.667	71.667
6	0.43	3	60.333	60.333	60.333
7	1.3	3	40.667	40.667	40.667

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
0	118.333				
0.0037	116.667	0.442		1.76	k= 1, v=14
0.013	105.000	3.539	*	1.85	k= 2, v=14
0.041	91.667	7.079	*	1.88	k= 3, v=14
0.13	71.667	12.388	*	1.89	k= 4, v=14
0.43	60.333	15.397	*	1.90	k= 5, v=14
1.3	40.667	20.618	*	1.91	k= 6, v=14

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3. **CITATION**

Author: Hoberg, J.R.
Title: Metolachlor technical - 5-day toxicity to the
freshwater green alga, *Selenastrum capricornutum*, using acetone as a carrier solvent
Lab. ID #: 94-12-5621
Sponsor: Ciba Crop Protection, Greensboro, NC
Test Lab: Springborn Laboratories, Inc., Wareham, MA
MRID #: 435413-01

4. **REVIEWED BY:**

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Biologist
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Signature:

Date:

W. Erickson
3/01/95

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature:

Date:

Harry T. Craven
3/01/95

6. **STUDY PARAMETERS/RESULTS SYNOPSIS:**

Test Duration: 120 hours
Type of Concentrations: Mean measured
EC₅₀: 10 ppb ai
95% C.I.: 6-20 ppb ai
Slope: 1.7
NOEL: 0.7 ppb ai

7. **CONCLUSIONS:** The study is scientifically sound and satisfies the guideline requirement (123-2) for a Tier 2 aquatic plant growth study with a freshwater green alga. The EC₅₀ value of 10 ppb ai classifies metolachlor technical as very highly toxic to *Selenastrum capricornutum*.
8. **ADEQUACY OF THE STUDY:** Core.
9. **MAJOR GUIDELINE DEVIATIONS:** None.
10. **SUBMISSION PURPOSE:** Reregistration data.

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	3000 cells/ml
<u>Nutrients</u> Standard formula, e.g. 20XAAP	AAP

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	Acetone
<u>Temperature</u> <i>Skeletonema</i> : 20°C Others: 24-25°C	24°C
<u>Light Intensity</u> <i>Anabaena</i> : 2.0 Lux (±15%) Others: 4.0-5.0 Lux (±15%)	3.8-4.8 Lux
<u>Photoperiod</u> <i>Skeletonema</i> : 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> <i>Skeletonema</i> : approx. 8.0 Others: approx. 7.5	7.5

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

Guideline Criteria	Reported Information
<u>Duration of test</u> 120 hours	120 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Cellular counts
<u>Maximum Labeled Rate</u>	Not reported

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?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response:

Dose (mg ai/L)	Cell Density ($\times 10^4$ cells/ml)	% Inhibition	120-Hour pH
Control	159	N/A	10.2
Solvent Control	158	N/A	10.2
0.0007	154	3.1	10.2
0.0014	144	9.4	10.0
0.0025	144	9.4	9.7
0.0059	104	35	9.6
0.0140	86	46	9.5
0.0230	28	82	8.5

Other Significant Results: At test termination, bloated cells and cell fragments were observed in the 0.0025, 0.0059, 0.014, and 0.023 mg ai/l treatment levels.

Statistical Results:

Methods: Linear regression (EC_{50})
Williams' Test (NOEC)

EC_{50} : 14 ppb ai 95% C.I.: 9.6-18 ppb ai
NOEC: 0.7 ppb ai

13. Verification of Statistical Results:

Methods: Probit (EC_{50})
Williams' Test (NOEC)

EC_{50} : 10 ppb ai 95% C.I.: 6-20 ppb ai
Slope: 1.7 NOEC: 0.7 ppb ai

- 14. REVIEWER'S COMMENTS:** The study is scientifically sound and satisfies the guideline requirement (123-2) for a Tier 2 aquatic plant growth study with *Selenastrum capricornutum*.

W. ERICKSON METOLACHLOR SELENASTRUM CAPRICORNUTUM

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.023	100	82	82	0
.014	100	46	46	0
.0059	100	35	35	0
.0025	100	9	9	0
.0014	100	9	9	0
.0007	100	3	3	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 1.473863E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	2.249374E-02		1.057443E-02
9.025015E-03		1.268971E-02	

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.1774439	3.740229	4.783149E-03

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

SLOPE = 1.737246
5 PERCENT CONFIDENCE LIMITS = 1.005447 AND 2.469045

LC50 = 1.063369E-02
5 PERCENT CONFIDENCE LIMITS = 6.823025E-03 AND .0202225

LC10 = 1.975339E-03
95 PERCENT CONFIDENCE LIMITS = 6.858153E-04 AND 3.358267E-03

METOLACHLOR AQUATIC PLANT GROWTH - *Selenastrum capricornutum*

SUMMARY STATISTICS

TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0	3	151.000	164.000	158.333
2	0.0007	3	148.000	161.000	154.333
3	0.0014	3	141.000	148.000	144.000
4	0.0025	3	143.000	146.000	144.000
5	0.0059	3	96.000	113.000	104.333
6	0.014	3	85.000	90.000	86.667
7	0.023	3	21.000	33.000	27.667

SUMMARY STATISTICS

TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0	44.333	6.658	3.844
2	0.0007	42.333	6.506	3.756
3	0.0014	13.000	3.606	2.082
4	0.0025	3.000	1.732	1.000
5	0.0059	72.333	8.505	4.910
6	0.014	8.333	2.887	1.667
7	0.023	37.333	6.110	3.528

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0	3	158.333	158.333	158.333
2	0.0007	3	154.333	154.333	154.333
3	0.0014	3	144.000	144.000	144.000
4	0.0025	3	144.000	144.000	144.000
5	0.0059	3	104.333	104.333	104.333
6	0.014	3	86.667	86.667	86.667
7	0.023	3	27.667	27.667	27.667

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
0	158.333				
0.0007	154.333	0.873		1.76	k= 1, v=14
0.0014	144.000	3.127	*	1.85	k= 2, v=14
0.0025	144.000	3.127	*	1.88	k= 3, v=14
0.0059	104.333	11.779	*	1.89	k= 4, v=14
0.014	86.667	15.633	*	1.90	k= 5, v=14
0.023	27.667	28.503	*	1.91	k= 6, v=14

S = 5.615