21 6-13-00

MRID No. 449017-10

DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} TEST
GUIDELINE 123-2 (TIER II)

1. **CHEMICAL:** Mesotrione PC Code No.: 122990

2. **TEST MATERIAL:** 4-(methylsulfonyl)-2-nitrobenzoic acid (MNBA)
A metabolite of mesotrione - 97.1% purity

3. **CITATION:**

Authors:	D.V. Smyth, S.J. Kent, and N. Shillabeer
Title:	MNBA: Toxicity to the Green Alga *Selenastrum capricornutum*
Study Completion Date:	July 31, 1997
Laboratory:	Brixham Environmental Laboratory, Devon, England
Sponsor:	ZENECA Ag Products, Wilmington, DE
Laboratory Report ID:	BL6066/B
MRID No.:	449017-10
DP Barcode:	D259964

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist,
Golder Associates Inc.

Signature: [signature] **Date:** 12/21/99

APPROVED BY: Max Feken, M.S., Environmental Toxicologist,
Golder Associates Inc.

Signature: [signature] **Date:** 12/21/99

5. **APPROVED BY:** James Goodyear, USEPA

Signature: [signature] **Date:** 6/13/00

6. **STUDY PARAMETERS:**

Definitive Test Duration: 72 hours
Type of Concentrations: Nominal

7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for an algal toxicity test using *Selenastrum capricornutum*.

Results Synopsis

EC_{50}: 42 mg/L 95% C.I.: not determined
Probit Slope: N/A NOEC: 30 mg/L

8. **ADEQUACY OF THE STUDY:**

A. Classification: Supplemental.

B. Rationale: The test was only conducted for 72 hours.

C. Repairability: No.

9. **GUIDELINE DEVIATIONS:** The test length (72 hours) was less then recommended (96 hours).

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

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

B. Test System

Guideline Criteria	Reported Information
Solvent	None
Temperature Skeletonema: 20°C Others: 24-25°C	24 ±1°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	9.0 KLux
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous lighting

Guideline Criteria	Reported Information
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 3.3 - 7.3 Final: 3.3 - 10.0

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	1.8X
Doses at least 5	3.2, 5.6, 10, 18, 32, 56, 100, and 180 mg/L
Controls negative and/or solvent	Medium and blank controls
Replicates per dose 3 or more	Control - 6 replicates Treatments - 3 replicates
Duration of test 120 hours	72 hours
Daily observations were made?	Yes
Method of Observations	Electronic cell counts
Maximum Labeled Rate	N/A

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and terminal cell densities were measured?	Yes
Control cell count at termination ≥2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes, samples collected at test initiation and termination were analyzed by HPLC.
Raw data included?	Yes

Measured Concentrations

Toxicant Concentration (mg/L)				
Nominal	0 hour	72 hour	Mean	Percent of Nominal
Control	<LOD	<LOD	<LOD	N/A
3.2	3.3	3.2	3.3	103
5.6	5.8	5.9	5.9	105
10	10	11	11	110
18	20	20	20	111
32	30	35	33	103
56	58	56	57	102
100	98	110	100	100
180	180	180	180	100

Note: Limit of determination (LOD) = 0.059 mg/L

Dose Response

Initial Measured Concentration (mg/L)	72-hr. Average Cell Density (x 10^4 cells/mL)	Inhibition (%)	Final pH
Control	288	N/A	9.8-10.0
3.3	282	2	9.5-10.0
5.8	282	2	9.4-9.5
10	293	-2*	9.7
20	277	4	9.3-9.5
30	295	-2	6.6-6.8
58	0	100	4.3-4.4
98	0	100	3.6-3.6
180	0	100	3.3

*Negative value indicates growth stimulation.

Other Significant Results: No signs of toxicity were reported.

Statistical Results

Statistical Methods: Results were based on both area under the growth curve and growth rate, using nominal concentrations. Probit analysis was used for EC_{50} estimation and Dunnett's test was used for NOEC determination. The more sensitive response was that of area under the growth curve (results reported below).

EC_{50}: 38 mg/L	95% C.I.: 35 - 42 mg/L
Probit Slope: not reported	NOEC: 32 mg/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Binomial probability was used for EC_{50} estimation and Williams' test was used for NOEC determination. The analyses were based on cell density and initial measured concentrations.

EC_{50}: 42 mg/L	95% C.I.: could not be determined
Probit Slope: N/A	NOEC: 30 mg/L

14. REVIEWER'S COMMENTS: This study is scientifically sound but does not fulfill the guideline requirements for an algal toxicity test since it was conducted for only 72 hours. Based on mean measured nominal concentrations, the 72-hour EC_{50} was 42 mg/L. The NOEC was determined to be 30 mg/L. This study can be categorized as **Supplemental**.

MNBA - 72 h cell density S.c.
File: sel Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN ($x10^4$)	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	6	288.167	16.969	16.969
2	3.3 ppm ai	3	281.667	16.778	16.892
3	5.8 ppm ai	3	281.667	16.762	16.892
4	10 ppm ai	3	293.000	17.113	16.892
5	20 ppm ai	3	276.667	16.633	16.892
6	30 ppm ai	3	295.333	17.175	16.892
7	58 ppm ai	3	0.377	0.541	0.541
8	98 ppm ai	3	0.187	0.425	0.461
9	180 ppm ai	3	0.260	0.496	0.461

MNBA - 72 h cell density S.c.
File: sel Transform: SQUARE ROOT(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	16.969				
3.3 ppm ai	16.892	0.209		1.72	k= 1, v=21
5.8 ppm ai	16.892	0.209		1.80	k= 2, v=21
10 ppm ai	16.892	0.209		1.83	k= 3, v=21
20 ppm ai	16.892	0.209		1.84	k= 4, v=21
30 ppm ai	16.892	0.209		1.85	k= 5, v=21
58 ppm ai	0.541	44.567	*	1.85	k= 6, v=21
98 ppm ai	0.461	44.784	*	1.85	k= 7, v=21
180 ppm ai	0.461	44.784	*	1.86	k= 8, v=21

s = 0.521
Note: df used for table values are approximate when v > 20.

MOSSLER MESOTRIONE SELENASTRUM CAPRICORNUTUM 11-11-99

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
98	100	100	100	0
58	100	100	100	0
30	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 41.71331

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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