

16 6-13-00  
MRID No.: 445051-22

**DATA EVALUATION RECORD  
ALGAE OR DIATOM EC<sub>50</sub> TEST  
GUIDELINE 123-2 (TIER II)**

1. **CHEMICAL:** Mesotrione

PC Code No.: 122990

2. **TEST MATERIAL:** ZA1296

Purity: 96.8%

3. **CITATION**

Authors: D.V. Smyth, S.J. Kent, and N. Shillabeer

Title: ZA1296: Toxicity to the Blue-Green Alga  
*Anabaena flos-aquae*

Study Completion Date: July 13, 1996

Laboratory: Brixham Environmental Laboratory, Brixham  
Devon, UK

Sponsor: ZENECA Ag Products, Wilmington, DE

Laboratory Report ID: BL5801/B

DP Barcode: D245475

MRID No.: 445051-22

4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,  
Golder Associates Inc.

**Signature:** 

**Date:** 8/25/98

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist,  
Golder Associates Inc.

**Signature:** P. Kosalwat

**Date:** 8/25/98

5. **APPROVED BY:**

**Signature:** 

**Date:** 6/13/00

6. **STUDY PARAMETERS**

**Definitive Test Duration:** 120 hours

**Type of Concentrations:** Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills  
the guideline requirements for an algal toxicity test.

**Results Synopsis** - Based on mean measured concentrations

EC<sub>50</sub>: 132 ppm

95% C.I.: Not determined

NOEC: 56 ppm

Probit Slope: N/A

**8. ADEQUACY OF THE STUDY****A. Classification:** Core**B. Rationale:** N/A**C. Repairability:** N/A**9. GUIDELINE DEVIATIONS**

1. Initial cell density (20,000 cells/mL) was greater than recommended (3,000 cells/mL).
2. The initial pH of the two highest concentration levels (4.1 and 3.7 for 100 and 180 mg/L nominal concentrations, respectively) was much lower than recommended (7.5).

**10. SUBMISSION PURPOSE:****11. MATERIALS AND METHODS****A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Anabaena flos-aquae</i>
<b><u>Initial Number of Cells</u></b> 3,000 - 10,000 cells/mL	20,000 cells/mL
<b><u>Nutrients</u></b> Standard formula, e.g. 20XAAP	Algal medium by Miller et al. (1978)

**B. Test System**

Guideline Criteria	Reported Information
<b><u>Solvent</u></b>	None
<b><u>Temperature</u></b> Skeletonema: 20°C Others: 24-25°C	23.8 - 23.9°C

Guideline Criteria	Reported Information
<b><u>Light Intensity</u></b> Anabaena: 2.0 KLux ( $\pm 15\%$ ) Others: 4.0-5.0 KLux ( $\pm 15\%$ )	3.23 KLux
<b><u>Photoperiod</u></b> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<b><u>pH</u></b> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 3.7 - 7.5 Final: 3.6 - 7.8

**C. Test Design**

Guideline Criteria	Reported Information
<b><u>Dose range</u></b> 2X or 3X progression	1.8X
<b><u>Doses</u></b> at least 5	3.2, 5.6, 10, 18, 32, 56, 100, and 180 mg/L
<b><u>Controls</u></b> negative and/or solvent	Negative control
<b><u>Replicates per dose</u></b> 3 or more	3 (6 in the control)
<b><u>Duration of test</u></b> 120 hours	120 hours
<b>Daily observations were made?</b>	Yes
<b><u>Method of Observations</u></b>	Algal cell absorbance
<b><u>Maximum Labeled Rate</u></b>	Not reported

**12. REPORTED RESULTS**

Guideline Criteria	Reported Information
<b>Initial and 120 h cell densities were measured?</b>	Yes
<b>Control cell count at 120 hr <math>\geq 2X</math> initial count?</b>	Yes

Guideline Criteria	Reported Information
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response

Concentration (mg/L)		Algal cell absorbance*	% reduction	120-Hour pH
Nominal	Mean Measured			
Control	<0.018	1.92	--	7.8
3.2	3.2	1.83	5	7.8
5.6	5.4	1.86	3	7.7
10	9.9	1.85	4	7.7
18	17	1.87	3	7.8
32	32	1.78	7	7.7
56	54	1.94	0	7.6
100	96	<0.016	99	4.1
180	175	<0.016	99	3.6

\* Inoculum (Day 0) calculated cell absorbance = 0.006 (20,000 cells/mL)

Other Significant Results: Area under the growth curve was the most sensitive endpoint measured in this study.

Statistical Results for Area Under the Growth Curve

Statistical Method: Probit for the EC<sub>50</sub> and Dunnett's test for mean separation. Results based on nominal concentrations.

EC<sub>50</sub>: 54 mg/L

95% C.I.: 29 - 160 mg/L

Probit Slope: Not reported

NOEC: 32 mg/L

Statistical Results for Growth Rate

Statistical Method: Visual inspection and Dunnett's test for mean separation. Results based on nominal concentrations.

EC<sub>50</sub>: >180 mg/L

95% C.I.: N/A

Probit Slope: N/A

NOEC: 56 mg/L

**13. VERIFICATION OF STATISTICAL RESULTS**

Statistical Method: Binomial probability and visual inspection. Results based on mean measured concentrations.

EC<sub>50</sub>: 132 ppm

95% C.I.: Not determined

Probit Slope: N/A

NOEC: 56 ppm

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. Based on mean measured concentrations, the 120-hour EC<sub>50</sub> and NOEC for *Anabaena flos-aquae* exposed to ZA1296 were 132 and 56 ppm, respectively. This study is classified as **Core**.

MAX FEKEN ZA1296 ANABAENA 08-04-98

\*\*\*\*\*

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
175	100	99	99	0
96	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 131.5911

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.

\*\*\*\*\*