15 C-13-00

MRID No.: 445051-21

DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} 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 Freshwater Diatom *Navicula pelliculosa*
Study Completion Date: June 29, 1996
Laboratory: Bixham Environmental Laboratory, Brixham, Devon, UK
Sponsor: ZENECA Ag Products, Wilmington, DE
Laboratory Report ID: BL5780/B
DP Barcode: D245475
MRID No.: 445051-21

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

Signature: [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: [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_{50}: 68 ppm 95% C.I.: Not determined

NOEC: 46 ppm Probit Slope: N/A

8. ADEQUACY OF THE STUDY

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS: None

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS

A. Test Organisms

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

B. Test System

Guideline Criteria	Reported Information
Solvent	None
Temperature Skeletonema: 20°C Others: 24-25°C	24.0 - 24.2°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	4.22 KLux
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

Guideline Criteria	Reported Information
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 6.3 - 8.3 Final: 7.1 - 8.1

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses at least 5	0.75, 1.5, 3.0, 6.0, 12, 24, 48, and 96 mg/L
Controls negative and/or solvent	Negative control
Replicates per dose 3 or more	3 (6 in the control)
Duration of test 120 hours	120 hours
Daily observations were made?	Yes
Method of Observations	Algal cell density
Maximum Labeled Rate	Not reported

12. REPORTED RESULTS

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

Dose Response

Concentration (mg/L)		Mean Cell Density (x 10^4 cells/mL)	% reduction	120-Hour pH
Nominal	Mean Measured			
Control	<0.015	149	--	7.8
0.75	0.70	166	-11*	7.8
1.5	1.4	181	-21	7.9
3.0	2.9	191	-28	8.0
6.0	5.7	202	-36	7.9
12	12	208	-39	8.0
24	23	210	-41	8.0
48	46	218	-46	8.1
96	92	5.24	96	7.1

*Negative value represents stimulation

Other Significant Results: Area under the growth curve was the most sensitive end-point measured in the study.

Statistical Results for Area Under the Growth Curve

Statistical Method: Probit method was used for calculating the EC_{50} and Dunnett's test for mean separation. Results based on nominal concentrations.

EC_{50}: 67 mg/L 95% C.I.: 61 - 74 mg/L

Probit Slope: Not reported NOEC: 48 mg/L

Statistical Results for Growth Rate

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

EC_{50}: >96 mg/L 95% C.I.: N/A

Probit Slope: N/A NOEC: 48 mg/L

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Binomial probability for EC_{50} and Williams' test for mean comparisons. Results based on mean measured concentrations.

EC_{50}: 68 ppm 95% C.I.: Not determined

Probit Slope: N/A NOEC: 46 mg/L

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_{50} and NOEC for *Navicula pelliculosa* exposed to ZA1296 were 68 and 46 ppm, respectively. This study is classified as **Core.**

MAX FEKEN ZA1296 NAVICULA 08-03-98

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
92	100	96	96	0
46	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 67.91513

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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ZA1296 - NAVICULA
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	CONTROL	6	149.333	149.333	185.926
2	0.70	3	166.000	166.000	185.926
3	1.4	3	181.000	181.000	185.926
4	2.9	3	190.667	190.667	185.926
5	5.7	3	201.667	201.667	185.926
6	12	3	207.667	207.667	185.926
7	23	3	210.000	210.000	185.926
8	46	3	217.667	217.667	185.926
9	92	3	5.237	5.237	5.237

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
CONTROL	185.926				
0.70 *	185.926	4.546	*	1.72	k= 1, v=21
1.4 *	185.926	4.546	*	1.80	k= 2, v=21
2.9 *	185.926	4.546	*	1.83	k= 3, v=21
5.7 *	185.926	4.546	*	1.84	k= 4, v=21
12 *	185.926	4.546	*	1.85	k= 5, v=21
23 *	185.926	4.546	*	1.85	k= 6, v=21
46 *	185.926	4.546	*	1.85	k= 7, v=21
92	5.237	17.900	*	1.86	k= 8, v=21

s = 11.385
Note: df used for table values are approximate when v > 20.

* - significantly higher than the control

NOEC = 46 ppm