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

1. **CHEMICAL:** Cloransulam-methyl (DE-565) PC Code No.: 129116

2. **TEST MATERIAL:** DE-565 acid Purity: >96%
(A metabolite of DE-565)

3. **CITATION:**

Authors: D.P. Milazzo, L.M. Massaro, H.D. Kirk, J.M. Hugo, and M.D. Martin
Title: XDE-565 Acid: The Toxicity to the Green Alga, *Selenastrum capricornutum* Printz
Study Completion Date: May 16, 1995
Laboratory: The Dow Chemical Company, Midland, MI
Sponsor: DowElanco, Indianapolis, IN
Laboratory Report ID: DECO-ES-2906
DP Barcode: D252903
MRID No.: 447445-14

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

Signature: Mark A. Mossler **Date:** 4/5/99

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

Signature: P. Kosalwat **Date:** 4/5/99

5. **APPROVED BY:**

Signature: [signature] **Date:** 4/14/99

6. **STUDY PARAMETERS:**

Definitive Test Duration: 120 hours
Type of Concentrations: Initial measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC_{50} and NOEC for *S. capricornutum* exposed to DE-565 acid were 7.6 and 0.3 ppb, respectively.

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core.

B. **Rationale:** N/A.

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C. **Repairability:** N/A.

9. **GUIDELINE DEVIATIONS:** No guideline deviations of consequence were noted.

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	3,000 cells/mL
Nutrients Standard formula, e.g. 20XAAP	Algal assay medium

B. **Test System**

Guideline Criteria	Reported Information
Solvent	Acetone
Temperature Skeletonema: 20°C Others: 24-25°C	23.9 - 24.9°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.3-5.3 KLux
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Range of 7.2-7.8

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses Must be tested at the maximum label rate	3.1, 6.2, 12.6, 25.2, and 50.3 ppb
Controls Negative and/or solvent	Negative and solvent (100 µL/L) control groups
Replicates per dose 3 or more	Control groups - 6 replicates Treatment groups - 3 replicates
Duration of test 120 or 96 hours	120 hours
Daily observations were made?	Yes
Method of Observations	Cellular counts
Maximum Labeled Rate	Test material is a metabolite

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) Percent of nominal, Procedural recovery, Limit of quantitation (LOQ)	Samples analyzed by HPLC 89-100%, Procedural recovery not reported, LOQ = 2.2 ppb
Raw data included?	Yes

Analytical Results

Concentration (ppb)	Measured concentrations (ppb)		
Nominal	Hour of Study		
	0	72	120
Control	<LOQ	<LOQ	<LOQ
Solvent Control	<LOQ	<LOQ	<LOQ
3.1	3.0	3.1	3.0
6.2	5.6	5.8	5.6
12.6	11.6	11.4	11.4
25.2	22.6	22.7	24.1
50.3	44.6	46.5	46.0

Dose Response

Nominal Concentration (ppb)	Initial measured concentration (ppb)	Day 5 Avg. Cell Density (x 10^4 cells/mL)	% Inhibition*	pH
Control	<LOQ	83.2	N/A	7.8
Sol. Con.	<LOQ	75.4	N/A	7.7
3.1	3.0	58.1	23	7.4
6.2	5.6	30.3	60	7.3
12.6	11.6	38.4	49	7.3
25.2	22.6	23.2	69	7.2
50.3	44.6	13.4	82	7.2

*Comparison to the solvent control

Other Significant Results: No other results were reported.

Statistical Methods: linear regression and Dunnett's test

EC_{50}: 8.1 ppb
Probit Slope: N/A
95% C.I.: 2.0 - 33.0 ppb
NOEC: <3.0 ppb

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Methods: Moving average angle analysis was used to estimate the EC_{50} value. Williams' test detected a significant decrease in algal growth at all treatment levels. Consequently, the EC_5 from the probit model served as the NOEC.

EC_{50}: 7.6 ppb	95% C.I.: 6.0 - 9.5 ppb
Probit Slope: 1.2	NOEC/EC_5: 0.3 ppb

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. The 120-hour EC_{50} and NOEC for *S. capricornutum* exposed to DE-565 acid were 7.6 and 0.3 ppb, respectively. This study can be categorized as **Core**.

Selenastrum cell density
File: sel Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	6	753644.500	753644.500	753644.500
2	3.0 ppb	3	581111.000	581111.000	581111.000
3	5.6 ppb	3	303111.000	303111.000	343799.833
4	11.6 ppb	3	384488.667	384488.667	343799.833
5	22.6 ppb	3	232204.333	232204.333	232204.333
6	44.6 ppb	3	134169.000	134169.000	134169.000

Selenastrum cell density
File: sel Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	753644.500				
3.0 ppb	581111.000	4.127	*	1.75	k= 1, v=15
5.6 ppb	343799.833	9.804	*	1.84	k= 2, v=15
11.6 ppb	343799.833	9.804	*	1.87	k= 3, v=15
22.6 ppb	232204.333	12.474	*	1.88	k= 4, v=15
44.6 ppb	134169.000	14.819	*	1.89	k= 5, v=15

s = 59117.563
Note: df used for table values are approximate when v > 20.

NOEC < 3.0 ppb, ∴ NOEC = EC_5 = 0.3 ppb

MOSSLER DE565 ACID SELENASTRUM CAPRICORNUTUM 3-29-99

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
44.6	100	82	82	0
22.6	100	69	69	0
11.6	100	49	49	0
5.6	100	60	60.00001	0
3	100	23	23	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 9.953178

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	8.472753E-02	7.614035	6.014615	9.525196

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.9553584	6.018179	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

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

SLOPE = 1.161708

95 PERCENT CONFIDENCE LIMITS = 2.622628E-02 AND 2.297189

LC50 = 7.926308

95 PERCENT CONFIDENCE LIMITS = 3.939604E-06 AND 32.68049

LC10 = .639518

95 PERCENT CONFIDENCE LIMITS = 5.877472E-39 AND 2.999342

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Selenastrum cell density

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.0788	0.0000	0.9747
EC 5.00	0.3042	0.0000	1.9944
EC10.00	0.6250	0.0000	2.9583
EC15.00	1.0161	0.0000	3.9002
EC50.00	7.9263	0.0000	32.4829
EC85.00	61.8303	20.4790	% 100000002.0000E+12
EC90.00	100.5238	27.5933	% 100000002.0000E+12
EC95.00	206.5362	41.5910	% 100000002.0000E+12
EC99.00	797.1561	86.1488	% 100000002.0000E+12

TE - Upper limits greater than or equal to 1.E20 are really infinite