

MRID No. 448065-02

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

1. **CHEMICAL:** Captan PC Code No.: 081301  
 2. **TEST MATERIAL:** Captan technical Purity: 99.8%
3. **CITATION:** Authors: K.R. Drottar and H.O. Krueger  
Title: Captan: A 96-Hour Toxicity Test with the Marine Diatom  
 (*Skeletonema costatum*)  
Study Completion Date: April 9, 1999  
Laboratory: Wildlife International Ltd., Easton, MD  
Sponsor: Captan Stewardship Task Force - Tomen Agro, Inc., San  
 Francisco, CA, and Makhteshim-Agan of North America,  
 Inc., New York, NY  
Laboratory Report ID: 493A-102  
DP Barcode: D255807  
MRID No.: 448065-02

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

**Signature:**

**Date:**

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

**Signature:**

**Date:**

5. **APPROVED BY:** Brian Montague, Fisheries Biologist  
 Environmental Fate and Effects Division, 7507C

**Signature:**

**Date:** Oct. 29, 1999

6. **STUDY PARAMETERS:**

**Definitive Test Duration:** 96 hours  
**Type of Concentrations:** Initial measured

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

**Results Synopsis**  
 EC<sub>50</sub>: 0.18 ppm ai  
 Probit Slope: N/A

95% C.I.: 0.15 - 0.21 ppm ai  
 NOEC: 0.062 ppm ai



**8. ADEQUACY OF THE STUDY:**

- A. **Classification:** Core.
- B. **Rationale:** N/A.
- C. **Repairability:** N/A.

**9. GUIDELINE DEVIATIONS:**

1. The test length (96 hours) was less than recommended (120 hours).
2. Initial cell number was 7-8 times greater than recommended.

**10. SUBMISSION PURPOSE:** Supported to support captan use where aquatic habitat exposure is predicted.

**11. MATERIALS AND METHODS:**

**A. Test Organisms**

Guideline Criteria	Reported Information
<b><u>Species</u></b>	<i>Skeletonema costatum</i>
<b><u>Initial Number of Cells</u></b> 3,000 - 10,000 cells/mL	77,000 cells/mL
<b><u>Nutrients</u></b> Standard formula, e.g. 20XAAP	Saltwater algal medium with a salinity of 30 ppt

**B. Test System**

Guideline Criteria	Reported Information
<b><u>Solvent</u></b>	DMF (100 µL/L)
<b><u>Temperature</u></b> Skeletonema: 20°C Others: 24-25°C	19.9-21.2°C
<b><u>Light Intensity</u></b> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.9-4.7 KLux
<b><u>Photoperiod</u></b> Skeletonema: 14 h light, 10 h dark or	14 hours light, 10 hours dark

Guideline Criteria	Reported Information
16 h light, 8 h dark Others: Continuous	
<b>pH</b> Skeletonema: approx. 8.0 Others: approx. 7.5	Initial: 8.1 Final: 8.2 - 8.9

### C. Test Design

Guideline Criteria	Reported Information
<b>Dose range</b> 2X or 3X progression	2X
<b>Doses</b> at least 5	0.031, 0.063, 0.13, 0.25, 0.50, 1.0 mg ai/L
<b>Controls</b> negative and/or solvent	Negative and solvent controls
<b>Replicates per dose</b> 3 or more	3
<b>Duration of test</b> 120 hours	96 hours
<b>Daily observations were made?</b>	Yes
<b>Method of Observations</b>	Cellular counts
<b>Maximum Labeled Rate</b>	Not reported

### 12. REPORTED RESULTS:

Guideline Criteria	Reported Information
<b>Initial and terminal cell densities were measured?</b>	Yes
<b>Control cell count at termination <math>\geq 2X</math> initial count?</b>	Yes
<b>Initial chemical concentrations measured?</b> (Optional)	Yes, samples collected at test initiation were analyzed by GC.
<b>Raw data included?</b>	Yes

Measured Concentrations

Toxicant Concentration (mg ai/L)		
Nominal	0 hour	Percent of Nominal
Control	<LOQ	N/A
Solvent Control	<LOQ	N/A
0.031	0.0323	104
0.063	0.0619	98
0.13	0.127	98
0.25	0.247	99
0.50	0.533	107
1.0	1.14	114

Note: Mean method validation recovery = 116% and LOQ = 0.0125 ppm ai

Dose Response

Initial Measured Concentration (mg ai/L)	96-hr. Average Cell Density ( $\times 10^4$ cells/mL)	Inhibition* (%)	Final pH
Control	303.5	N/A	8.9
Solvent Control	298.6	N/A	8.8
0.032	273.7	9	8.7
0.062	257.3	15	8.8
0.127	189.7 <sup>a</sup>	37	8.7
0.247	94.4 <sup>a</sup>	69	8.4
0.533	14.4 <sup>a</sup>	95	8.2
1.14	3.0 <sup>a</sup>	99	8.2

\* Compared to the pooled control.

<sup>a</sup> Significantly reduced when compared to the pooled control ( $p \leq 0.05$ ).

Other Significant Results: The only sign of test material toxicity was enlarged cells at the 0.533 and 1.14 ppm ai treatment levels. Cells from these two solutions were observed to recover to control levels after six days of reculturing in untreated algal medium, indicating algistatic effects.

Statistical Results for Cell Density

Statistical Method: Linear interpolation was used for EC<sub>50</sub> estimation and Bonferroni's test was used for NOEC determination.

EC<sub>50</sub>: 0.18 ppm ai  
Probit Slope: N/A

95% C.I.: 0.11 - 0.21 ppm ai  
Statistical NOEC: 0.062 ppm ai  
Observed NOEC: 0.032 ppm ai

**13. VERIFICATION OF STATISTICAL RESULTS:**

Statistical Method: Nonlinear regression was used for EC<sub>50</sub> estimation and Williams' test was used for NOEC determination. Comparison was made to the solvent control.

EC<sub>50</sub>: 0.18 ppm ai  
Probit Slope: N/A

95% C.I.: 0.15 - 0.21 ppm ai  
NOEC: 0.062 ppm ai

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. Based on initial measured concentrations, the 96-hour EC<sub>50</sub> was 0.18 ppm ai. The NOEC was determined to be 0.062 ppm ai. This study can be categorized as **Core**.

Additional Comment EPA Reviewer: It should be noted that effects to cell density may actually beg

Skeletonema cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3 2986022.333	6.474	6.474
2	0.032 ppm ai	3 2737378.667	6.437	6.437
3	0.062 ppm ai	3 2573393.333	6.410	6.410
4	0.127 ppm ai	3 1897327.333	6.264	6.264
5	0.247 ppm ai	3 944215.333	5.973	5.973
6	0.533 ppm ai	3 144377.333	5.153	5.153
7	1.14 ppm ai	3 30046.000	4.477	4.477

Skeletonema cell density

File: skl Transform: LOG BASE 10(Y)

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE DEGREES OF WILLIAMS FREEDOM
Sol. Con.	6.474			
0.032 ppm ai	6.437	0.677	1.76	k= 1,v=14
0.062 ppm ai	6.410	1.159	1.85	k= 2,v=14
0.127 ppm ai	6.264	3.817 *	1.88	k= 3,v=14
0.247 ppm ai	5.973	9.094 *	1.89	k= 4,v=14
0.533 ppm ai	5.153	23.992 *	1.90	k= 5,v=14
1.14 ppm ai	4.477	36.261 *	1.91	k= 6,v=14

s = 0.067

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

skeletonema cell density

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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.000	. 2727883	3020228	3209956	.	.	.	.

2	0.032	-1.49485	2897456	2571681	2742999	.	.	.
3	0.062	-1.20761	2591255	2668290	2460635	.	.	.
4	0.127	-0.89620	2605015	1466739	1620228	.	.	.
5	0.247	-0.60730	1052045	937936	842665	.	.	.
6	0.533	-0.27327	125879	125598	181655	.	.	.

skeletonema cell density 12

MODEL: COUNT = C0 \* PROBNORM ((LOG\_EC50 - LOG\_CONC) / SIGMA)

WEIGHTED REGRESSION

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Non-Linear Least Squares Iterative Phase

Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	C0	Weighted SS
0	-0.813000	0.397000	2986022	759699
1	-0.759056	0.311596	2846424	558221
2	-0.756733	0.306151	2844015	554772
3	-0.756130	0.305514	2842544	554420
4	-0.756044	0.305423	2842334	554372
5	-0.756032	0.305411	2842304	554365
6	-0.756030	0.305409	2842300	554364
7	-0.756030	0.305409	2842299	554364
8	-0.756030	0.305409	2842299	554364
9	-0.756030	0.305408	2842299	554364

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	33848143.000	11282714.333
Residual	15	554364.270	36957.618
Uncorrected Total	18	34402507.270	
(Corrected Total)	17	22541345.574	

Parameter	Estimate	Asymptotic		Asymptotic 95 %		
		Std. Error	Confidence Interval		Lower	Upper
			Lower	Upper		
LOG_EC50	-0.756	0.03550	-0.8317	-0.6804		
SIGMA	0.305	0.03163	0.2380	0.3728		

C0 2842298.837 126639.37045 2572374.4349 3112223.2398

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.680546092	-0.670303707
SIGMA	-0.680546092	1	0.4753110713
C0	-0.670303707	0.4753110713	1

skeltonema cell density 13  
 MODEL: COUNT = C0 \* PROBNORM ((LOG\_EC50 - LOG\_CONC) / SIGMA)

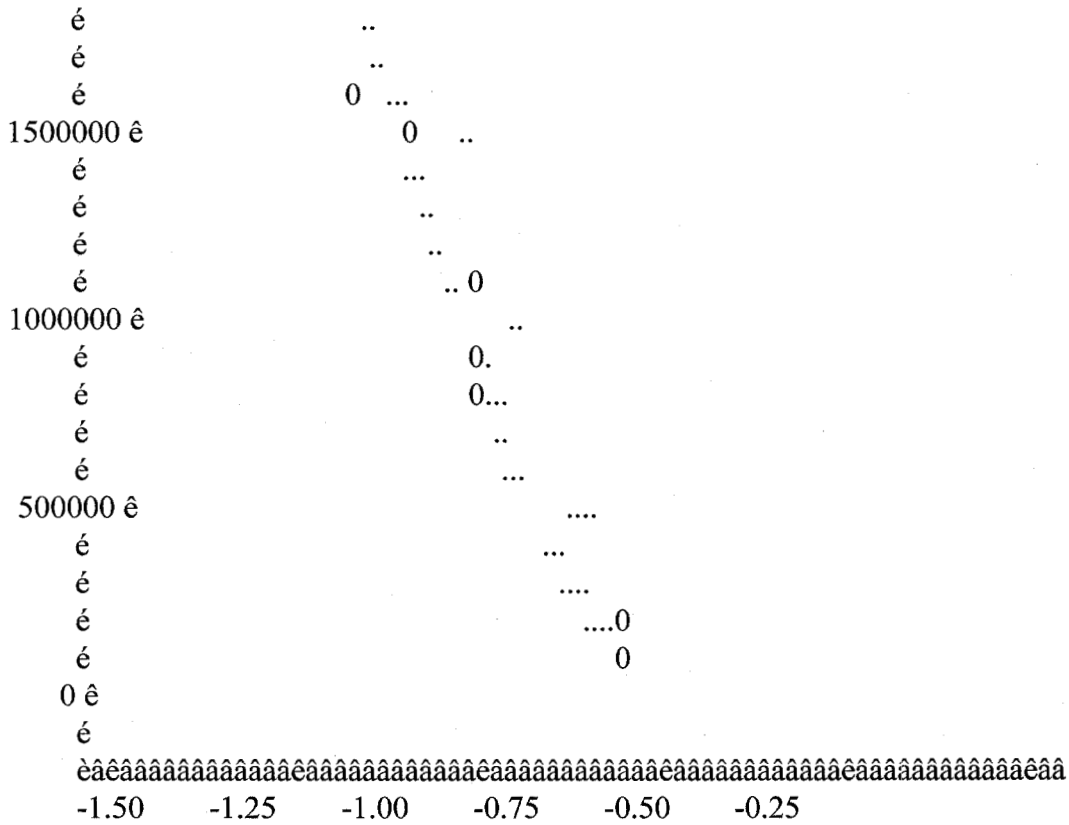
OBS	CONC	LOG_EC50	SIGMA	C0	RESID_SS	EC50
1	0	-0.75603	0.30541	2842298.84	554364.27	0.17538

skeltonema cell density 14  
 MODEL: COUNT = C0 \* PROBNORM ((LOG\_EC50 - LOG\_CONC) / SIGMA)  
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Plot of COUNT\*LOG\_CONC. Symbol used is '0'.  
 Plot of PRED\*LOG\_CONC. Symbol used is '!'.  
 (Note: The plots themselves are not visible in the provided image)

COUNT é  
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LOG\_CONC

NOTE: 1250 obs had missing values. 1169 obs hidden.

skeletonema cell density 15

COMPARISON OF MEANS FOR NOEL DETERMINATION

TEST IF TREATMENT IS LESS THAN CONTROL

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General Linear Models Procedure

Class Level Information

Class Levels Values

DOSE 6 0 0.032 0.062 0.127 0.247 0.533

Number of observations in data set = 36

NOTE: Due to missing values, only 18 observations can be used in this

analysis.

skeletonema cell density 16  
 COMPARISON OF MEANS FOR NOEL DETERMINATION  
 TEST IF TREATMENT IS LESS THAN CONTROL  
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General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	1.89827E+13	3.79653E+12	46.48	0.0001
Error	12	9.80179E+11	8.16816E+10		
Corrected Total	17	1.99628E+13			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.950900	15.19846	285799.9	1880452

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	1.89827E+13	3.79653E+12	46.48	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	1.89827E+13	3.79653E+12	46.48	0.0001

skeletonema cell density 17  
 COMPARISON OF MEANS FOR NOEL DETERMINATION  
 TEST IF TREATMENT IS LESS THAN CONTROL  
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General Linear Models Procedure

Level of	-----RESPONSE-----		
DOSE	N	Mean	SD

0	3	2986022.33	242849.985
0.032	3	2737378.67	162960.206
0.062	3	2573393.33	104973.469
0.127	3	1897327.33	617661.795
0.247	3	944215.33	104831.143
0.533	3	144377.33	32283.712

skeletonema cell density 18

COMPARISON OF MEANS FOR NOEL DETERMINATION  
TEST IF TREATMENT IS LESS THAN CONTROL

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General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 12 MSE= 8.168E10

Critical Value of Dunnett's T= 2.502

Minimum Significant Difference= 583889

Comparisons significant at the 0.05 level are indicated by '\*\*\*'.

DOSE Comparison	Simultaneous	Simultaneous	
	Lower Confidence Limit	Difference Between Means	
0.032 - 0	-832533	-248644	335246
0.062 - 0	-996518	-412629	171260
0.127 - 0	-1672584	-1088695	-504806 ***
0.247 - 0	-2625696	-2041807	-1457918 ***
0.533 - 0	-3425534	-2841645	-2257756 ***