

US EPA ARCHIVE DOCUMENT

MRID No. 448065-01

**DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ 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 Freshwater Alga
(*Anabaena flos-aquae*)

Study Completion Date: April 8, 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-101A

DP Barcode: D255807

MRID No.: 448065-01

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 *Anabaena flos-aquae*. **Core** classification.

Results Synopsis

EC₅₀: 1.2 ppm ai

95% C.I.: 0.83 - 1.6 ppm ai

Probit Slope: N/A

NOEC: < 0.23 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 then recommended (120 hours).

10. SUBMISSION PURPOSE: To support captan use on crops where aquatic habitats and plantlife is expected to be subject to exposure.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u>	<i>Anabaena flos-aquae</i>
<u>Initial Number of Cells</u> 3,000 - 10,000 cells/mL	10,000 cells/mL
<u>Nutrients</u> Standard formula, e.g. 20XAAP	Freshwater algal medium

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	DMF (100 µL/L)
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	24.0-25.8°C
<u>Light Intensity</u> Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	1.9-2.4 KLux

Guideline Criteria	Reported Information
<p><u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous</p>	Continuous lighting
<p><u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5</p>	Initial: 7.4 - 7.5 Final: 7.5 - 8.2

C. Test Design

Guideline Criteria	Reported Information
<p><u>Dose range</u> 2X or 3X progression</p>	2X
<p><u>Doses</u> at least 5</p>	0.32, 0.64, 1.3, 2.6, and 5.1 mg ai/L
<p><u>Controls</u> negative and/or solvent</p>	Negative and solvent controls
<p><u>Replicates per dose</u> 3 or more</p>	3
<p><u>Duration of test</u> 120 hours</p>	96 hours
<p>Daily observations were made?</p>	Yes
<p><u>Method of Observations</u></p>	Cellular counts
<p><u>Maximum Labeled Rate</u></p>	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
<p>Initial and terminal cell densities were</p>	

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

Measured Concentrations

Nominal	Toxicant Concentration (mg ai/L)	
	0 hour	Percent of Nominal
Control	<LOQ	N/A
Solvent Control	<LOQ	N/A
0.32	0.23	73
0.64	0.51	80
1.3	0.88	68
2.6	2.16	83
5.1	4.89	96

Note: Mean method validation recovery = 98% and LOQ = 0.125 ppm ai

Dose Response

Initial Measured Concentration (mg ai/L)	96-hr. Average Cell Density ($\times 10^4$ cells/mL)	Inhibition* (%)	Final pH
Control	161.7	N/A	8.2
Solvent Control	148.0	N/A	8.0
0.23	137.7	11	7.9
0.51	100.0 ^a	35	7.9

Initial Measured Concentration (mg ai/L)	96-hr. Average Cell Density (x 10 ⁴ cells/mL)	Inhibition* (%)	Final pH
0.88	84.3 ^a	46	7.8
2.16	51.8 ^a	67	7.5
4.89	7.4 ^a	95	7.5

* Compared to the pooled control.

^aSignificantly 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 4.89 ppm ai treatment level. Cells from this solution were observed to recover to control levels after three days of reculturing in untreated algal medium, indicating algistatic effects.

Statistical Results for Cell Density

Statistical Method: Linear interpolation was used for EC₅₀ estimation and Bonferroni's test was used for NOEC determination.

EC₅₀: 1.2 ppm ai
Probit Slope: N/A

95% C.I.: 0.91 - 1.7 ppm ai
NOEC: 0.23 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Nonlinear regression was used for EC₅₀ estimation and Williams' test was used for NOEC determination. Comparison was made to the solvent control.

EC₅₀: 1.2 ppm ai
Probit Slope: N/A

95% C.I.: 0.83 - 1.6 ppm ai
Observed NOEC: <0.23 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 EC₅₀ was 1.2 ppm ai. The observed NOEC was determined to be < 0.23 ppm ai based on 11% cell density reductions beginning at this dosage level which appeared dose related and were below both pooled and solvent control cell density levels. This study can be categorized as **Core**.

Anabaena cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	1480000.000	1480000.000	1480000.000
2	0.23 ppm ai	3	1376666.667	1376666.667	1376666.667
3	0.51 ppm ai	3	1000000.000	1000000.000	1000000.000
4	0.88 ppm ai	3	843333.333	843333.333	843333.333
5	2.16 ppm ai	3	518333.333	518333.333	518333.333
6	4.89 ppm ai	3	74333.333	74333.333	74333.333

Anabaena cell density

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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED CALC. MEAN	SIG WILLIAMS	TABLE P=.05	DEGREES OF WILLIAMS	FREEDOM
Sol. Con.	1480000.000				
0.23 ppm ai	1376666.667	1.203	1.78	k= 1, v=12	
0.51 ppm ai	1000000.000	5.586 *	1.87	k= 2, v=12	
0.88 ppm ai	843333.333	7.410 *	1.90	k= 3, v=12	
2.16 ppm ai	518333.333	11.192 *	1.92	k= 4, v=12	
4.89 ppm ai	74333.333	16.359 *	1.93	k= 5, v=12	

s = 105235.714

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

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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.00	.	1630000	1400000	1410000	.	.	.
2	0.23	-0.63827	1340000	1420000	1370000	.	.	.
3	0.51	-0.29243	1120000	1030000	850000	.	.	.
4	0.88	-0.05552	845000	825000	860000	.	.	.
5	2.16	0.33445	695000	365000	495000	.	.	.
6	4.89	0.68931	111000	48000	64000	.	.	.

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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	0.029000	0.490000	1480000	437459
1	0.058513	0.462688	1442283	432235
2	0.063502	0.457264	1436507	432660
3	0.064697	0.456087	1435087	432731
4	0.064977	0.455814	1434754	432748
5	0.065042	0.455750	1434675	432753
6	0.065058	0.455735	1434657	432753
7	0.065061	0.455732	1434652	432754
8	0.065062	0.455731	1434651	432754
9	0.065063	0.455731	1434651	432754
10	0.065063	0.455731	1434651	432754

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	15878000.000	5292666.667
Residual	15	432753.749	28850.250
Uncorrected Total	18	16310753.749	

(Corrected Total) 17 9119342.757

Parameter	Estimate	Asymptotic		Asymptotic 95 %		
		Std. Error	Confidence Interval			
			Lower	Upper		
LOG_EC50	0.065	0.067591	-0.0790	0.2091		
SIGMA	0.456	0.059148	0.3297	0.5818		
C0	1434651.173	99410.618726	1222763.2605	1646539.0856		

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.707185222	-0.787459251
SIGMA	-0.707185222	1	0.5840980055
C0	-0.787459251	0.5840980055	1

anabaena cell density 5
 MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)

OBS CONC LOG_EC50 SIGMA C0 RESID_SS EC50

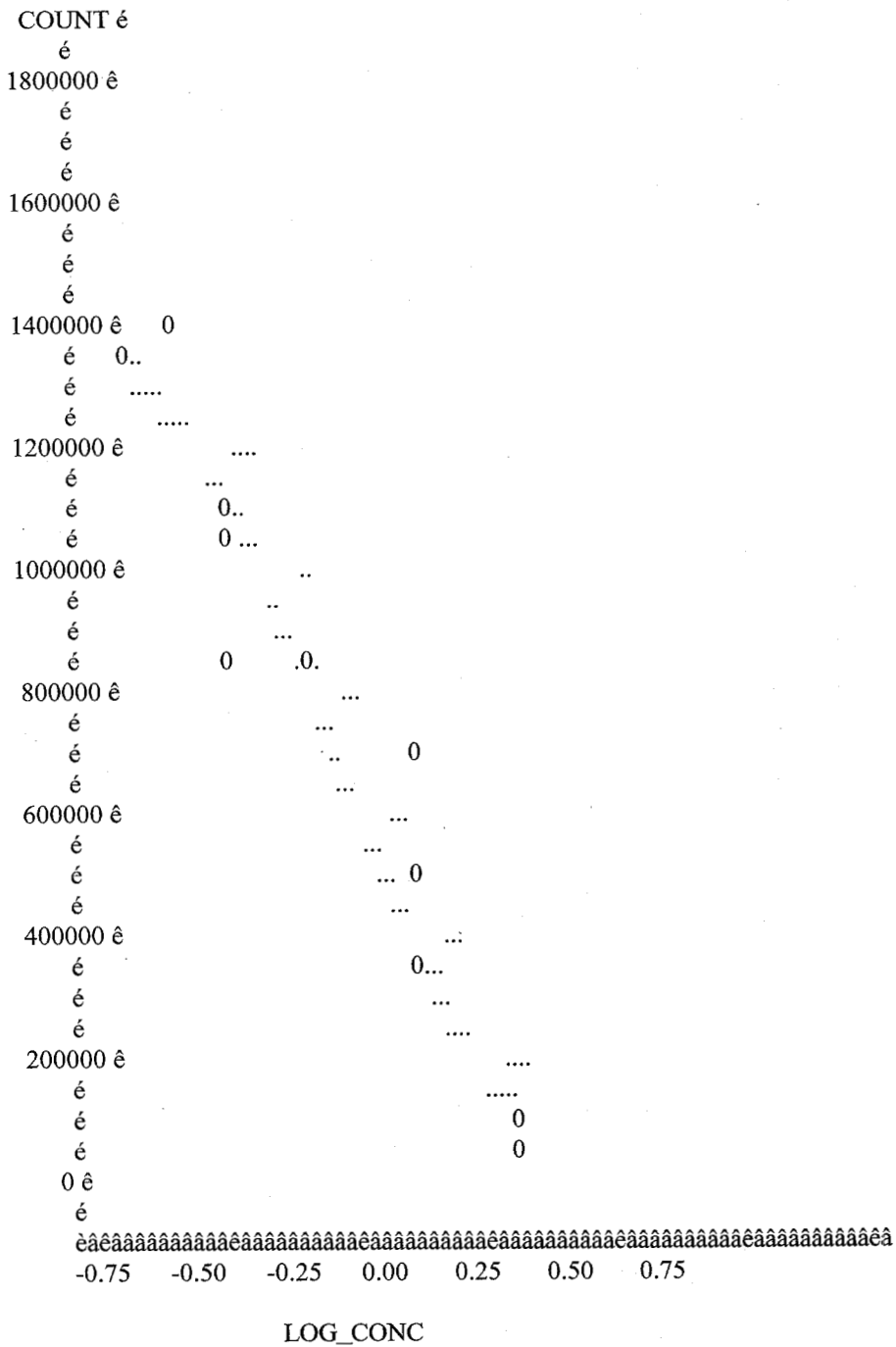
1 0 0.065063 0.45573 1434651.17 432753.75 1.16162
 anabaena cell density 6

MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)

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Plot of COUNT*LOG_CONC. Symbol used is 'O'.

Plot of PRED*LOG_CONC. Symbol used is '!'.
Note: The symbol used in the plot is actually a small circle 'O'.



NOTE: 1355 obs had missing values. 1284 obs hidden.

anabaena cell density 7
 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 0.23 0.51 0.88 2.16 4.89

Number of observations in data set = 36

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

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 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	4.20689E+12	8.41378E+11	75.97	0.0001
Error	12	1.32895E+11	1.10746E+10		
Corrected Total	17	4.33979E+12			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.969378	11.92998	105235.7	882111.1

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	5	4.20689E+12	8.41378E+11	75.97	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	5	4.20689E+12	8.41378E+11	75.97	0.0001

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

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General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	3	1480000.00	130000.000
0.23	3	1376666.67	40414.519
0.51	3	1000000.00	137477.271
0.88	3	843333.33	17559.423
2.16	3	518333.33	166232.769
4.89	3	74333.33	32746.501

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

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General Linear Models Procedure

Dunnnett'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= 1.107E10

Critical Value of Dunnnett's T= 2.502

Minimum Significant Difference= 214997

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Simultaneous Difference Between Means	Simultaneous Upper Confidence Limit	
0.23 - 0	-318330	-103333	111663	
0.51 - 0	-694997	-480000	-265003	***
0.88 - 0	-851663	-636667	-421670	***
2.16 - 0	-1176663	-961667	-746670	***
4.89 - 0	-1620663	-1405667	-1190670	***