

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 than 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

| Toxicant Concentration (mg ai/L) | | |
|----------------------------------|--------|--------------------|
| Nominal | 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 |

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

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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
 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 | -----RESPONSE----- | | |
|---------------|--------------------|------------|------------|
| | 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

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= 1.107E10

Critical Value of Dunnett'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 | |
|-----------------|-------------------------------------|------------|---------------------------------------|-------------------------------------|--|
| | Limit | Confidence | Between Means | 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 | *** | |