

US EPA ARCHIVE DOCUMENT

MRID NO. 438698-09

**DATA EVALUATION RECORD  
ALGAE OR DIATOM EC<sub>50</sub> TEST  
GUIDELINE 122-2 OR 123-2 (TIER I OR II)**

1. **CHEMICAL:** Captan PC Code No.: 081301

2. **TEST MATERIAL:** Captan Purity: 90%

3. **CITATION:**

Authors: D.V. Smyth, S.A. Sankey, J.F. Tapp, and R.D. Stanley

Title: CAPTAN: Toxicity to the Green Alga *Selenastrum capricornutum*

Study Completion Date: November 16, 1990

Laboratory: Imperial Chemical Industries PLC, Group Environmental Laboratory, Brixham, U.K.

Sponsor: ICI Americas Inc., Fernhurst, U.K.

Laboratory Report ID: BL3954/B

DP Barcode: Not available.

MRID No.: 438698-09

4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Environmental Scientist, KBN Engineering and Applied Sciences, Inc.

**Signature:**

*[Handwritten Signature]* for RGM

**Date:**

*4/18/96*

**APPROVED BY:** Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

**Signature:**

*P. Kosalwat*

**Date:**

*4/18/96*

5. **APPROVED BY:**

**Signature:**

*Harry Craver*

**Date:**

*6/6/96*

6. **STUDY PARAMETERS**

**Scientific Name of Test Organism:** *Selenastrum capricornutum*

**Definitive Test Duration:** 96 hours

**Type of Concentrations:** Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 aquatic plant study. Based on the cell density of *Selenastrum capricornutum* exposed to Captan, the 96-hour NOEC, LOEC, and EC<sub>50</sub> for were 0.2, 0.38, and 1.77 ppm, respectively.

**Results Synopsis**

EC<sub>50</sub>: 1.77 ppm

NOEL: 0.2 ppm

95% C.I.: 1.55-2.03 ppm

Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

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

9. GUIDELINE DEVIATIONS:

1. The study was conducted for 96 hours; the guidelines require 120 hours.
2. The initial cell density (10,000 cells/ml) was more than three times higher than recommended (3,000 cells/ml).

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Skeletonema costatum Anabaena flos-aquae Selenastrum capricornutum Navicula pelliculosa	<i>Selenastrum capricornutum</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	Algal culture medium (Miller, et al., 1978)

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None
<u>Temperature</u> Skeletonema: 20°C Others: 24-25°C	23.8-23.9°C

Guideline Criteria	Reported Information
<p><b>Light Intensity</b>                      Anabaena: 2.0 Klux (±15%)                      (approx. 40 μE/m<sup>2</sup>/sec)                      Others: 4.0-5.0 Klux (±15%)                      (approx. 80-100 μE/m<sup>2</sup>/sec)</p>	7.7 Klux (104.9 μE/m <sup>2</sup> /sec)
<p><b>Photoperiod</b>                      Skeletonema:                      14 h light, 10 h dark or                      16 h light, 8 h dark                      Others: Continuous</p>	Continuous
<p><b>pH</b>                      Skeletonema: approx. 8.0                      Others: approx. 7.5</p>	7.3-7.4 at 0 hour 7.2-10.4 at 96 hours

**C. Test Design**

Guideline Criteria	Reported Information
<p><b>Dose range</b>                      2X or 3X progression</p>	2X
<p><b>Doses</b>                      at least 5</p>	0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 ppm
<p><b>Controls</b>                      negative and/or solvent</p>	Negative control
<p><b>Replicates per dose</b>                      3 or more</p>	Six replicates for the control and three replicates for each exposure concentration
<p><b>Duration of test</b>                      120 hours</p>	96 hours
<p><b>Daily observations were made?</b></p>	Yes
<p><b>Method of Observations</b></p>	Electronic particle counting
<p><b>Maximum Labeled Rate</b></p>	Not reported.

**12. REPORTED RESULTS**

Guideline Criteria	Reported Information
<p><b>Initial and 120 h cell densities were measured?</b></p>	Daily cell densities were reported.

Guideline Criteria	Reported Information
Control cell count at 120 hr $\geq 2X$ initial count?	Yes; 96-hour densities were $>2$ times the initial densities.
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response

Nominal Conc. (mg/L)	Initial Measured Conc. (mg/L)	96-hour Cell Density ( $\times 10^4$ cells/ml)	% Inhibition	96-Hour pH (Rep A/Rep B)
Control	<0.0079	328	N/A	10.3/10.3
0.2	0.2	312	5	10.4/10.4
0.4	0.38	289	12	10.3/10.4
0.8	0.83	284	13	10.3/10.2
1.6	1.5	201	39	10.0/9.9
3.2	2.9	103	69	8.0/8.7
6.4	5.8	31.7	90	7.4/7.5
12.8	11	10.7	97	7.3/7.2

Other Significant Results:

Statistical Results: Based on nominal test concentrations and growth rate.

Statistical Method: Linear Regression

EC<sub>50</sub>: 11 ppm

95% C.I.: 6.4-18 ppm

Probit Slope: N/A

NOEC: 0.8 ppm

Based on nominal test concentrations and mean area under the growth curve

Statistical Method: Linear Regression

EC<sub>50</sub>: 1.6 ppm

95% C.I.: 1.3-2.0 ppm

Probit Slope: N/A

NOEC: 0.2 ppm

13. **Verification of Statistical Results:** Based on initial measured concentrations and 96-hour cell density.

Statistical Method: Moving average method for EC<sub>50</sub> calculation and Williams' test for NOEC determination.

EC<sub>50</sub>: 1.77 ppm

95% C.I.: 1.55-2.03 ppm

Probit Slope: N/A

NOEC: 0.20 ppm

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for a Tier 2 aquatic plant study. Based on cell density, the 96-hour NOEC, LOEC, and EC<sub>50</sub> for *Selenastrum capricornutum* exposed to Captan were 0.2, 0.38, and 1.77 ppm, respectively. This study is classified as **Core**.

KOSALWAT CAPTAN SELENASTRUM CAPRICORNUTUM 04-12-96

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
11	100	97	97	0
5.8	100	90	90	0
2.9	100	69	69	0
1.5	100	39	39	0
.83	100	13	13	0
.38	100	12	12	0
.2	100	5	5	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 1.905244

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	1.448384E-02	1.768086	1.547388	2.029695

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	8.110029E-02	3.204047	6.786347E-03

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

SLOPE = 2.150826  
95 PERCENT CONFIDENCE LIMITS = 1.538311 AND 2.763341

LC50 = 1.761707  
95 PERCENT CONFIDENCE LIMITS = 1.296862 AND 2.404275

LC10 = .4523379  
95 PERCENT CONFIDENCE LIMITS = .2400941 AND .6734838

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Captan: Cell Density of Exposed S.capricornutum  
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Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.809	6.534	10.314	6.534	1.809
OBSERVED	0	10	8	9	0

Calculated Chi-Square goodness of fit test statistic = 6.9064  
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

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Shapiro - Wilk's test for normality

D = 7710.851

W = 0.971

Critical W (P = 0.05) (n = 27) = 0.923

Critical W (P = 0.01) (n = 27) = 0.894

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: Captan: Cell Density of Exposed S.capricornutum  
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 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	353.0000	353.0000
1	Control	2	364.0000	364.0000
1	Control	3	301.0000	301.0000
1	Control	4	302.0000	302.0000
1	Control	5	342.0000	342.0000
1	Control	6	305.0000	305.0000
2	0.2 ppm mmc	1	305.0000	305.0000
2	0.2 ppm mmc	2	322.0000	322.0000
2	0.2 ppm mmc	3	309.0000	309.0000
3	0.38 ppm mmc	1	308.0000	308.0000
3	0.38 ppm mmc	2	264.0000	264.0000
3	0.38 ppm mmc	3	295.0000	295.0000
4	0.83 ppm mmc	1	295.0000	295.0000
4	0.83 ppm mmc	2	280.0000	280.0000
4	0.83 ppm mmc	3	277.0000	277.0000
5	1.5 ppm mmc	1	163.0000	163.0000
5	1.5 ppm mmc	2	216.0000	216.0000
5	1.5 ppm mmc	3	223.0000	223.0000
6	2.9 ppm mmc	1	101.0000	101.0000
6	2.9 ppm mmc	2	111.0000	111.0000
6	2.9 ppm mmc	3	97.8000	97.8000
7	5.8 ppm mmc	1	33.0000	33.0000
7	5.8 ppm mmc	2	28.2000	28.2000
7	5.8 ppm mmc	3	33.8000	33.8000
8	11 ppm mmc	1	9.2600	9.2600
8	11 ppm mmc	2	7.9400	7.9400
8	11 ppm mmc	3	15.0000	15.0000

*mmc = mean measured concentrations at test initiation.*



Captan: Cell Density of Exposed S.capricornutum  
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	6	301.000	364.000	327.833
2	0.2 ppm mmc	3	305.000	322.000	312.000
3	0.38 ppm mmc	3	264.000	308.000	289.000
4	0.83 ppm mmc	3	277.000	295.000	284.000
5	1.5 ppm mmc	3	163.000	223.000	200.667
6	2.9 ppm mmc	3	97.800	111.000	103.267
7	5.8 ppm mmc	3	28.200	33.800	31.667
8	11 ppm mmc	3	7.940	15.000	10.733

Captan: Cell Density of Exposed S.capricornutum  
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	810.167	28.463	11.620	8.68
2	0.2 ppm mmc	79.000	8.888	5.132	2.85
3	0.38 ppm mmc	511.000	22.605	13.051	7.82
4	0.83 ppm mmc	93.000	9.644	5.568	3.40
5	1.5 ppm mmc	1076.333	32.808	18.941	16.35
6	2.9 ppm mmc	47.413	6.886	3.975	6.67
7	5.8 ppm mmc	9.173	3.029	1.749	9.56
8	11 ppm mmc	14.089	3.754	2.167	34.97

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	398635.460	56947.923	140.323
Within (Error)	19	7710.851	405.834	
Total	26	406346.311		

Critical F value = 2.54 (0.05,7,19)  
 Since F > Critical F REJECT Ho: All equal

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BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	327.833	327.833		
2	0.2 ppm mmc	312.000	312.000	1.112	
3	0.38 ppm mmc	289.000	289.000	2.726	*
4	0.83 ppm mmc	284.000	284.000	3.077	*
5	1.5 ppm mmc	200.667	200.667	8.927	*
6	2.9 ppm mmc	103.267	103.267	15.765	*
7	5.8 ppm mmc	31.667	31.667	20.791	*
8	11 ppm mmc	10.733	10.733	22.261	*

Bonferroni t table value = 2.70 (1 Tailed Value, P=0.05, df=19,7)

Captan: Cell Density of Exposed S.capricornutum  
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BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	6			
2	0.2 ppm mmc	3	38.417	11.7	15.833
3	0.38 ppm mmc	3	38.417	11.7	38.833
4	0.83 ppm mmc	3	38.417	11.7	43.833
5	1.5 ppm mmc	3	38.417	11.7	127.167
6	2.9 ppm mmc	3	38.417	11.7	224.567
7	5.8 ppm mmc	3	38.417	11.7	296.167
8	11 ppm mmc	3	38.417	11.7	317.100

Captan: Cell Density of Exposed S.capricornutum  
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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	327.833	327.833	327.833
2	0.2 ppm mmc	3	312.000	312.000	312.000
3	0.38 ppm mmc	3	289.000	289.000	289.000
4	0.83 ppm mmc	3	284.000	284.000	284.000
5	1.5 ppm mmc	3	200.667	200.667	200.667
6	2.9 ppm mmc	3	103.267	103.267	103.267
7	5.8 ppm mmc	3	31.667	31.667	31.667
8	11 ppm mmc	3	10.733	10.733	10.733

Captan: Cell Density of Exposed *S.capricornutum*  
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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	327.833				
0.2 ppm mmc	312.000	1.112		1.73	k= 1, v=19
0.38 ppm mmc	289.000	2.726	*	1.81	k= 2, v=19
0.83 ppm mmc	284.000	3.077	*	1.84	k= 3, v=19
1.5 ppm mmc	200.667	8.927	*	1.85	k= 4, v=19
2.9 ppm mmc	103.267	15.765	*	1.86	k= 5, v=19
5.8 ppm mmc	31.667	20.791	*	1.87	k= 6, v=19
11 ppm mmc	10.733	22.261	*	1.87	k= 7, v=19

s = 20.145

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