

US EPA ARCHIVE DOCUMENT

MRID NO. 438698-10

**DATA EVALUATION RECORD
ALGAE OR DIATOM EC₅₀ TEST
GUIDELINE 122-2 OR 123-2 (TIER I OR II)**

1. **CHEMICAL:** Captan PC Code No.: 081301
2. **TEST MATERIAL:** THPI Purity: 96%
(A metabolite of Captan)
3. **CITATION:**
Authors: S.J. Kent, S.A. Sankey, D.J. Morgan, and S.E. Magor
Title: THPI: Toxicity to the Green Alga *Selenastrum capricornutum*
Study Completion Date: September 2, 1994
Laboratory: Brixham Environmental Laboratory, ZENECA Limited, Brixham, U.K.
Sponsor: ZENECA Inc., Fernhurst, Haslemere, U.K.
Laboratory Report ID: BL5238/B
DP Barcode: Not available.
MRID No.: 438698-10
4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Environmental Scientist, KBN Engineering and Applied Sciences, Inc.
Signature:  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:  Date: 6/6/96
6. **STUDY PARAMETERS:**
Scientific Name of Test Organism: *Selenastrum capricornutum*
Definitive Test Duration: 72 hours
Type of Concentrations: Mean measured
7. **CONCLUSIONS:** This study is scientifically sound but does not fulfill the guideline requirements for a Tier 2 aquatic plant study. There was no justification why the study was conducted for only 72 hours (3 days). It appeared that the test was terminated while the algal growth was still in the middle of the exponential phase of the growth curves. The 72-hour EC₅₀ for *Selenastrum capricornutum* exposed to THPI was >180 ppm and the NOEC was 180 ppm.

Results Synopsis

EC₅₀: >180 ppm

95% C.I.: N/A

NOEL: 180 ppm

Probit Slope: N/A

8. ADEQUACY OF THE STUDY

A. Classification: Supplemental.

B. Rationale: The test was conducted for only 72 hours.

C. Repairability: No.

9. GUIDELINE DEVIATIONS:

1. The study was conducted for only 72 hours; the guidelines recommend 120 hours.
2. Initial cell concentration (10,000 cells/ml) was 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> <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<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	24 ±1°C

Guideline Criteria	Reported Information
<u>Light Intensity</u> Anabaena: 2.0 Klux ($\pm 15\%$) or $40 \mu\text{E}/\text{m}^2/\text{sec}$ Others: 4.0-5.0 Klux ($\pm 15\%$) or $80-100 \mu\text{E}/\text{m}^2/\text{sec}$	7.7 Klux ($94.4 \mu\text{E}/\text{m}^2/\text{sec}$)
<u>Photoperiod</u> Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous
<u>pH</u> Skeletonema: approx. 8.0 Others: approx. 7.5	6.71-7.47 at 0 hour 8.57-9.79 at 72 hours

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	1.8X
<u>Doses</u> at least 5	18, 32, 56, 100, and 180 ppm
<u>Controls</u> negative and/or solvent	Negative control.
<u>Replicates per dose</u> 3 or more	Six replicates for the control and three replicates for each exposure concentration.
<u>Duration of test</u> 120 hours	72 hours
Daily observations were made?	Yes
<u>Method of Observations</u>	Electronic particle counting
<u>Maximum Labeled Rate</u>	Not reported.

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Cell densities reported daily.
Control cell count at 120 hr $\geq 2X$ initial count?	Yes; 72 hour densities were >2 times the initial densities.
Initial chemical concentrations measured? (Optional)	Concentrations measured at 0 and 72 hours.
Raw data included?	Yes

Dose Response

Mean Measured Concentration (mg/L)	72-hour Cell Density ($\times 10^4$ cells/ml)	% Inhibition*	72-Hour pH (Rep A/Rep B)
Control	256	N/A	9.63/9.79
17	258	0	9.62/9.68
32	260	0	9.40/9.50
58	266	0	9.28/9.42
110	253	1.2	8.98/9.03
180	247	3.5	8.62/8.57

* Based on cell density.

Other Significant Results: None.Statistical Results: Based on the area under the growth curve and growth rate.

Statistical Method: N/A

EC₅₀: >180 ppm

95% C.I.: N/A

Probit Slope: N/A

NOEC: 180 ppm

THPI: 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	1.407	5.082	8.022	5.082	1.407
OBSERVED	1	6	7	7	0

Calculated Chi-Square goodness of fit test statistic = 2.5446
 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 = 2894.667

W = 0.975

Critical W (P = 0.05) (n = 21) = 0.908
 Critical W (P = 0.01) (n = 21) = 0.873

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

TITLE: THPI: Cell Density of Exposed S.capricornutum
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 TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	234.0000	234.0000
1	Control	2	267.0000	267.0000
1	Control	3	258.0000	258.0000
1	Control	4	267.0000	267.0000
1	Control	5	260.0000	260.0000
1	Control	6	248.0000	248.0000
2	18	1	244.0000	244.0000
2	18	2	270.0000	270.0000
2	18	3	260.0000	260.0000
3	32	1	242.0000	242.0000
3	32	2	278.0000	278.0000
3	32	3	259.0000	259.0000
4	56	1	266.0000	266.0000
4	56	2	268.0000	268.0000
4	56	3	264.0000	264.0000
5	100	1	242.0000	242.0000
5	100	2	260.0000	260.0000
5	100	3	256.0000	256.0000
6	180	1	243.0000	243.0000
6	180	2	270.0000	270.0000
6	180	3	228.0000	228.0000

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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	6	234.000	267.000	255.667
2	18	3	244.000	270.000	258.000
3	32	3	242.000	278.000	259.667
4	56	3	264.000	268.000	266.000
5	100	3	242.000	260.000	252.667
6	180	3	228.000	270.000	247.000

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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Control	161.867	12.723	5.194	4.98
2	18	172.000	13.115	7.572	5.08
3	32	324.333	18.009	10.398	6.94
4	56	4.000	2.000	1.155	0.75
5	100	89.333	9.452	5.457	3.74
6	180	453.000	21.284	12.288	8.62

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	626.286	125.257	0.649
Within (Error)	15	2894.667	192.978	
Total	20	3520.952		

Critical F value = 2.90 (0.05,5,15)
 Since F < Critical F FAIL TO 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	255.667	255.667		
2	18	258.000	258.000	-0.238	
3	32	259.667	259.667	-0.407	
4	56	266.000	266.000	-1.052	
5	100	252.667	252.667	0.305	
6	180	247.000	247.000	0.882	

Bonferroni t table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

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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	18	3	25.564	10.0	-2.333
3	32	3	25.564	10.0	-4.000
4	56	3	25.564	10.0	-10.333
5	100	3	25.564	10.0	3.000
6	180	3	25.564	10.0	8.667

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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	255.667	255.667	259.000
2	18	3	258.000	258.000	259.000
3	32	3	259.667	259.667	259.000
4	56	3	266.000	266.000	259.000
5	100	3	252.667	252.667	252.667
6	180	3	247.000	247.000	247.000

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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	259.000				
18	259.000	0.339		1.75	k= 1, v=15
32	259.000	0.339		1.84	k= 2, v=15
56	259.000	0.339		1.87	k= 3, v=15
100	252.667	0.305		1.88	k= 4, v=15
180	247.000	0.882		1.89	k= 5, v=15

s = 13.892
 Note: df used for table values are approximate when v > 20.