

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

1. **CHEMICAL**: Chlorothalonil PC Code No.: 081901

2. **TEST MATERIAL**: Chlorothalonil Purity: 98.1%

3. **CITATION**:

Author: Smyth, D.V., Magor, S.E., and N. Shillabeer

Title: Chlorothalonil: Toxicity to the Marine Alga *Skeletonema costatum*

Study Completion Date: August 1, 1998

Laboratory: Brixham Environmental Laboratory
ZENECA Limited
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Laboratory Report ID: BL6422/B

MRID No.: 44908103

DP Barcode: D270160

4. **REVIEWED BY**: Dana Worcester, Senior Staff Scientist, Dynamac Corporation

Signature:

Date: 4/17/01

APPROVED BY: Kathleen Ferguson, Ph.D., Senior Staff Scientist, Dynamac Corporation

Signature:

Date: 4/17/01

5. **APPROVED BY**: Tim Bargar, Biologist, OPP/EFED/ERB III

Signature:

Date:

6. STUDY PARAMETERS

Scientific Name of Test Organism: *Skeletonema costatum*

Definitive Test Duration: 120 hours

Study Method: Static

Type of Concentrations: Nominal

7. CONCLUSIONS:

In this algae EC₅₀ test, *Skeletonema costatum* were exposed to Chlorothalonil at nominal concentrations of 1.5, 3.0, 6.0, 12, 24, 48, and 96 µg/L; mean measured concentrations were 1.6, 2.9, 5.8, 11, 22, 47, and 92 µg/L. By 120 hours, mean cell counts in the nominal 1.5, 3.0, 6.0, 12, 24, 48, and 96 µg/L treatment groups were 104%, 94%, 99%, 86%, 1%, 1%, and 1%, respectively, of the solvent control. Area under the growth curve (biomass) in the 1.5, 3.0, 6.0, 12, 24, 48, and 96 µg/L treatment groups was 104%, 97%, 97%, 76%, 0%, 0%, and 0%, respectively, of the solvent control, by 120 hours. The mean growth rate in the 1.5, 3.0, 6.0, 12, 24, 48, and 96 µg/L treatment groups was 101%, 99%, 100%, 97%, 5%, 9%, and 8%, respectively, of the solvent control by 120 hours. Statistically significant differences in area under the growth curve (biomass) were observed in the nominal 12, 24, 48, and 96 µg/L treatment groups; for growth rate, significant differences were observed in the 24, 48, and 96 µg/L treatment groups.

The reviewer was unable to statistically verify the study authors' NOEC, LOEC, and EC₅₀ estimates pertaining to biomass and growth rate. The study authors only provided replicate data for cell density and used these values to derive biomass (area under the curve) and growth rate, which they then statistically analyzed. Note, although the study authors did provide raw data for cell density, they did not estimate a NOEC, LOEC or EC₅₀. The reviewer's NOEC, LOEC, and EC₅₀ estimates for cell density were identical to those estimates reported by the study authors for biomass and growth rate. **The study author calculated 120-hour EC₅₀ value was 13 µg/L and 20 µg/L for biomass (area under the curve) and growth rate, respectively. The 120-hour NOEC value was 6 µg/L and 12 µg/L for biomass (area under the curve) and growth rate, respectively.**

This study is classified as CORE. This study supports the requirements for an algae EC₅₀ test (Subdivision J, §123-2 (TIER II)).

Results Synopsis

Cell density:

EC₅₀: 14 µg/L

NOEC: 5.9 µg a.i./L

95% C.I.: 6.5 and 30 µg/L

Probit Slope: 4.90

Biomass (Area Under the Curve): Note, these are study author reported values. Replicate data not provided; values could not be determined by reviewer.

EC₅₀: 13 µg/L

NOEC: 5.9 µg/L

95% C.I.: 12-14 µg/L

Probit Slope: Not provided

Growth rate: Note, these are study author reported values. Replicate data not provided; values could not be determined by reviewer.

EC₅₀: 20 µg/L

NOEC: 12 µg/L

95% C.I.: 18-22 µg/L

Probit Slope: Not provided

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

The maximum labeled rate was not reported.

10. SUBMISSION PURPOSE: Reregistration

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<p>Species: <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i></p>	<p><i>Skeletonema costatum</i></p>
<p>Initial number of cells: 3,000 - 10,000 cells/mL</p>	<p>10,000 cells/mL</p>
<p>Nutrients: Standard formula</p>	<p>Yes</p>

B. Test System

Guideline Criteria	Reported Information
Solvent:	0.10 mL/L dimethylformamide
Temperature: <i>Skeletonema</i> : 20°C Others: 24-25°C	19.8-20.0°C
Light Intensity: <i>Anabaena</i> : 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	4.1 Klux
Photoperiod: <i>Skeletonema</i> : 14 h light, 10 h dark, or 16 h light, 8 h dark Others: Continuous	16 h light, 8 h dark
pH <i>Skeletonema</i> : approx. 8.0 Others: approx. 7.5	Initial 8.1; Final 8.1-8.8

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	2x
Doses: at least 5	Nominal: 1.5, 3.0, 6.0, 12, 24, 48, and 96 µg/L
Controls: Negative and/or solvent	Negative and solvent
Replicates per dose: 3 or more	Yes
Duration of test: 120 hours	120 hours
Daily observations were made?	Yes
Method of observations:	The number of cells was determined

Guideline Criteria	Reported Information
	by electronic particle counting
Maximum labeled rate:	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes
Control cell count at 120 hr $\geq 2x$ initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response:**Cell density**

Initial Measured Concentration (µg/L)	Avg. Cell Density (x 10⁴ cells/mL)	% Reduction	120-hour pH
Control	59.8	--	8.7
Solvent control	62.0	--	8.6
1.6	64.8	<4>	8.7
2.9	59.9	6	8.6
5.8	61.4	1	8.7
11	53.1	14	8.8
22	0.653	99	8.2
47	0.771	99	8.1
92	0.753	99	8.1

< > Represents an increase in cell density relative to the solvent control.

Growth Rate

Initial Measured Concentration (µg/L)	Growth rate	% Reduction	120-hour pH
Control	0.955	--	8.7
Solvent Control	0.962	--	8.6
1.6	0.971	<1>	8.7
2.9	0.955	1	8.6
5.8	0.960	0	8.7
11	0.931	3	8.8
22	0.051	95*	8.2
47	0.085	91*	8.1
92	0.080	92*	8.1

< > Represents an increase in growth rate relative to the solvent control.

* Significantly different from solvent control.

Area Under the Growth Curve

Initial Measured Concentration (µg/L)	Area Under the Growth Curve	% Reduction	120-hour pH
Negative Control	132.4	--	8.7
Solvent Control	131.0	--	8.6
1.6	135.9	<4>	8.7
2.9	126.8	3	8.6
5.8	127.0	3	8.7
11	99.6	24*	8.8
22	0.5	100*	8.2
47	0.1	100*	8.1
92	0.0	100*	8.1

* Significantly different from solvent control.

Other Significant Results: None.

Statistical Results

Statistical Method: EC₅₀ values were determined using the probit method. Biomass areas were examined by one-way analysis of variance and using Dunnett's test.

Cell density: Estimates not provided by the study authors.

EC₅₀: 95% C.I.:
NOEC: Probit Slope:

Biomass (Area Under the Curve):

EC₅₀: 13 µg/L 95% C.I.: 12-14 µg/L
NOEC: 5.9 µg/L Probit Slope: Not provided

Growth rate:

EC₅₀: 20 µg/L 95% C.I.: 18-22 µg/L
NOEC: 12 µg/L Probit Slope: Not provided

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Only algal cell density data were analyzed statistically because replicate data were not provided for area under the growth curve or growth rate. After confirming normality and homogeneity of variance, negative and solvent control data were pooled because a two-tailed *t*-test revealed no significant difference. Treatment effects were assessed via Bonferroni-adjusted *t*-tests. An EC₅₀ estimate was performed using the method of Bruce and Versteeg via Nuthatch software.

Results Synopsis:**Cell density:**

EC₅₀: 14 µg/L 95% C.I.: 6.5 and 30 µg/L
NOEC: 5.9 µg a.i./L Probit Slope: 4.90

Biomass (Area Under the Curve): Replicate data not provided; values could not be determined.

EC₅₀: 95% C.I.:
NOEC: Probit Slope:

Growth rate: Replicate data not provided; values could not be determined.

EC₅₀: 95% C.I.:
NOEC: Probit Slope:

14. REVIEWERS' COMMENTS:

The reviewer's conclusions could not be compared directly to those of the study authors

because replicate data were not provided for the two endpoints analyzed statistically by the study laboratory (biomass and growth rate). The reviewer analyzed cell density (for which replicate data were provided), however, the study author failed to estimate NOEC, LOEC or EC₅₀ values for this endpoint. The NOEC and LOEC for biomass reported by the study authors were identical to those estimated for cell density by the reviewer. However, the study author-determined NOEC and LOEC for growth rate were one concentration higher than those values estimated for cell density.

The maximum labeled rate was not reported.

This study was conducted in accordance with UK Principles of Good Laboratory Practice (UK GLP Regulations 1997) and included a Quality Assurance Statement.

15. RESULTS OF STATISTICAL VERIFICATION:

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	22682.321	3240.332	187.530
Within (Error)	22	380.128	17.279	
Total	29	23062.449		

Critical F value = 2.46 (0.05,7,22)

Since F > Critical F REJECT Ho:All groups 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	GRPS 1&2 POOLED	61.244	61.244		
2	1.6	64.800	64.800	-1.283	
3	2.9	59.933	59.933	0.473	
4	5.9	61.433	61.433	-0.068	
5	12	53.067	53.067	2.951	*
6	23	0.653	0.653	21.865	*
7	47	0.771	0.771	21.822	*
8	93	0.753	0.753	21.829	*

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Bonferroni T table value = 2.66 (1 Tailed Value, P=0.05, df=22,7)

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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 DIFFERENCE CONTROL FROM CONTROL
1	GRPS 1&2 POOLED	9			
2	1.6	3	7.374	12.0	-3.556
3	2.9	3	7.374	12.0	1.311
4	5.9	3	7.374	12.0	-0.189
5	12	3	7.374	12.0	8.178
6	23	3	7.374	12.0	60.592
7	47	3	7.374	12.0	60.474
8	93	3	7.374	12.0	60.492