

US EPA ARCHIVE DOCUMENT

**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

Study Completion Date: July 18, 1998

Laboratory: Brixham Environmental Laboratory
ZENECA Limited
Brixham, Devon, TQ5 8BA, UK

Sponsor: GB Biosciences Corporation
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Laboratory Report ID: BL6413/B

MRID No.: 44908104

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: *Anabaena flos-aquae*

Definitive Test Duration: 120 hours

Study Method: Static

Type of Concentrations: Nominal

7. CONCLUSIONS:

In this algae EC₅₀ test, *Anabaena flos-aquae* were exposed to Chlorothalonil at nominal concentrations of 5.0, 10, 20, 40, 80, 160, and 320 µg/L; mean measured concentrations were 4.9, 9.7, 20, 41, 79, 160, and 320 µg/L. By 120 hours cell density in the nominal 5.0, 10, 20, 40, 80, 160, and 320 µg/L treatment groups was 105%, 114%, 99%, 88%, 53%, 5%, and 1%, respectively, of the solvent control. By 120 hours, area under the growth curve (biomass) in the nominal 5.0, 10, 20, 40, 80, 160, and 320 µg/L treatment groups was 107%, 100%, 96%, 81%, 38%, 9%, and 3%, respectively, of the solvent control. By 120 hours, growth rates in the nominal 5.0, 10, 20, 40, 80, 160, and 320 µg/L treatment groups were 101%, 102%, 100%, 98%, 89%, 51%, and 26%, respectively, of the solvent control.

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 absorbance and used these values to calculate biomass (area under the curve) and growth rate, which they then statistically analyzed. As a result, the reviewer's NOEC and EC₅₀ estimates for cell absorbance differed (were higher) from the study authors' estimates for biomass and growth rate. **The study author calculated the 120-hour EC₅₀ value to be 74 µg/L and 200 µg/L for biomass (area under the curve) and growth rate, respectively. The 120-hour NOEC value was 20 µg/L and 40 µg/L for biomass (area under the curve) and growth rate, respectively.**

This study is classified as INVALID. This study does not support the requirements for an algae EC₅₀ test (Subdivision J, §123-2 (TIER II)).

Results Synopsis (NOTE: these are study author reported values)

Biomass (Area Under the Curve):

EC₅₀: 74 µg/L

95% C.I.: 45 to 150 µg/L

NOEC: 20 µg/L

Probit Slope: Not provided

Growth rate:

EC₅₀: 200 µg/L

95% C.I.: 94 to <320 µg/L

NOEC: 40 µg/L

Probit Slope: Not provided

8. ADEQUACY OF THE STUDY:

A. Classification: Invalid

B. Rationale: The initial cell density (20,000 cells/mL) was significantly higher than the guideline requirement (3,000-10,000 cells/mL).

C. Repairability: The initial cell density should be within the range stated in the guidelines.

9. GUIDELINE DEVIATIONS:

1. The initial cell density (20,000 cells/mL) was significantly higher than the guideline requirement (3,000-10,000 cells/mL).
2. The light intensity was slightly higher (2,870 lux) than the guideline requirement (2,000 ± 15% lux).
3. The maximum labeled rate was not reported.

10. SUBMISSION PURPOSE: Reregistration

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: <i>Skeletonema costatum</i> <i>Anabaena flos-aquae</i> <i>Selenastrum capricornutum</i> <i>Navicula pelliculosa</i>	<i>Anabaena flos-aquae</i>
Initial number of cells: 3,000 - 10,000 cells/mL	20,000 cells/mL
Nutrients: Standard formula	Yes

B. Test System

Guideline Criteria	Reported Information
Solvent:	0.10 mL/L dimethylformamide
Temperature: <i>Skeletonema</i> : 20°C Others: 24-25°C	24.0-24.1°C
Light Intensity: <i>Anabaena</i> : 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	2.9 Klux
Photoperiod: <i>Skeletonema</i> : 14 h light, 10 h dark, or 16 h light, 8 h dark Others: Continuous	Continuous
pH <i>Skeletonema</i> : approx. 8.0 Others: approx. 7.5	Initial 7.3-7.4; Final 7.4-7.8

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	2x
Doses: at least 5	Nominal: 5.0, 10, 20, 40, 80, 160, and 320 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 relative cell density was determined using a spectrophotometer

Guideline Criteria	Reported Information
Maximum labeled rate:	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes (see reviewer's comments)
Control cell count at 120 hr \geq2x initial count?	Yes (see reviewer's comments)
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response:**Cell density**

Initial Measured Concentration (µg/L)	Algal Cell Absorbance [Avg. Cell Density (x 10⁴ cells/mL)]*	% Reduction	120-hour pH
Control	2.330 [776.7]	<1>	7.8
Solvent control	2.300 [766.7]	--	7.8
4.9	2.420 [806.7]	<5>	7.8
9.7	2.630 [866.7]	<14>	7.8
20	2.270 [756.7]	1	7.8
41	2.020 [673.3]	12	7.7
79	1.220 [406.7]	47	7.7
160	0.122 [40.7]	95	7.4
320	0.028 [9.3]	99	7.4

* The study author reported algal cell absorbance values, and reported Inoculum (Day 0) calculated cell absorbance = 0.006 (2.00 x 10⁴ cells ml⁻¹). The reviewer calculated the average cell density (x10⁴) using the reported cell absorbance.

< > 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	1.193	0	7.8
Solvent Control	1.190	--	7.8
4.9	1.200	<1>	7.8
9.7	1.216	<2>	7.8
20	1.187	0	7.8
41	1.164	2	7.7
79	1.063	11*	7.7
160	0.602	49*	7.4
320	0.306	74*	7.4

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

* Significant difference from the solvent control.

Area Under the Growth Curve

Initial Measured Concentration ($\mu\text{g/L}$)	Area Under the Growth Curve	% Reduction	120-hour pH
Negative Control	3.4	<1>	7.8
Solvent Control	3.4	---	7.8
4.9	3.6	<7>	7.8
9.7	3.4	0	7.8
20	3.3	4	7.8
41	2.7	19*	7.7
79	1.3	62*	7.7
160	0.3	91*	7.4
320	0.1	97*	7.4

< > Represents an increase in area under the curve (biomass) relative to the solvent control.

* Significant difference from the solvent control.

Other Significant Results: None.

Statistical Results

Statistical Method: EC_{50} values were determined by a linear regression estimation procedure. Biomass areas were examined by one-way analysis of variance and using Dunnett's test.

Biomass (Area Under the Curve):

EC_{50} : 74 $\mu\text{g/L}$

NOEC: 20 $\mu\text{g/L}$

95% C.I.: 45-150 $\mu\text{g/L}$

Probit Slope: Not provided

Growth rate:

EC_{50} : 200 $\mu\text{g/L}$

NOEC: 40 $\mu\text{g/L}$

95% C.I.: 94-<320 $\mu\text{g/L}$

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: Replicate data were provided and verified for cell absorbance.

EC₅₀: 73 µg a.i./L

95% C.I.: 66 and 81 µg a.i./L

NOEC: 40 µg a.i./L

Probit Slope: 1.82

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 (area under the growth curve and growth rate). The reviewer analyzed cell absorbance (for which replicate data were provided), however, the study laboratory did not. It can be said that the authors' EC₅₀ estimates of 65 to 74 µg a.i./L for area under the growth curve are similar to the reviewer's estimate of 73 µg a.i./L, based on cell absorbance. The NOEC and LOEC reported by the study authors were one concentration lower than those reported by the reviewer due to the analysis of different endpoints.

The guideline requirements state that the initial cell count number should be 3,000-10,000 cells/mL. However, in this study, the initial number of cells was 20,000 cells/mL. The higher cell density could have had an impact on the results of the study.

The light intensity was slightly higher (2,870 lux) than the guideline requirement (2,000 lux ± 15%); this deviation probably did not impact the results of the study.

The study authors did not provide a clear conversion for cell absorbance to cell density (cells/mL). The reviewer calculated cell densities for each treatment group, assuming that the author’s note of 0.006 (2.00×10^4 cells/mL) was the conversion rate.

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 includes a Quality Assurance Statement.

15. REVIEWER’S STATISTICAL RESULTS:

449081-04
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	25.180	3.597	116.032
Within (Error)	22	0.681	0.031	
Total	29	25.861		

Critical F value = 2.46 (0.05,7,22)
Since F > Critical F REJECT Ho:All groups equal

449081-04
File: 449081-04d Transform: NO TRANSFORM

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	2.310	2.310		
2	4.8	2.420	2.420	-0.937	
3	9.5	2.627	2.627	-2.698	
4	20	2.267	2.267	0.369	
5	40	2.020	2.020	2.471	
6	75	1.222	1.222	9.272	*
7	160	0.122	0.122	18.643	*
8	320	0.028	0.028	19.444	*

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

449081-04
File: 449081-04d Transform: NO TRANSFORM

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	GRPS 1&2 POOLED	9			
2	4.8	3	0.312	13.5	-0.110
3	9.5	3	0.312	13.5	-0.317
4	20	3	0.312	13.5	0.043
5	40	3	0.312	13.5	0.290
6	75	3	0.312	13.5	1.088
7	160	3	0.312	13.5	2.188
8	320	3	0.312	13.5	2.282