DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} 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 Freshwater Diatom

Study Completion Date: August 8, 1998

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

Sponsor: GB Biosciences Corporation
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P.O. Box 15458
Wilmington, DE 19850-5458

Laboratory Report ID: BL6423/B

MRID No.: 44908105

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: *Navicula pelliculosa*

Definitive Test Duration: 120 hours

Study Method: Static

Type of Concentrations: Nominal

7. CONCLUSIONS:

In this diatom EC_{50} test, *Navicula pelliculosa*, were exposed to Chlorothalonil at nominal concentrations of 0.90, 1.8, 3.5, 7.0, 14, 28, and 56 µg/L; mean measured concentrations were 1.1, 2.3, 4.0, 7.6, 14, 32, and 58 µg/L. By 120 hours, cell density in the nominal 0.90, 1.8, 3.5, 7.0, 14, 28, and 56 µg/L treatment groups was 106%, 100%, 99%, 65%, 61%, 3%, and 1%, respectively, of the solvent control. By 120 hours, area under the curve (biomass) in the nominal 0.90, 1.8, 3.5, 7.0, 14, 28, and 56 µg/L treatment groups was 102%, 91%, 91%, 54%, 48%, 1%, and 0%, respectively, of the solvent control. By 120 hours, growth rates in the nominal 0.90, 1.8, 3.5, 7.0, 14, 28, and 56 µg/L treatment groups were 101%, 100%, 100%, 93%, 92%, 42%, and 18%, respectively, of the solvent control.

The reviewer was unable to statistically verify the study authors' NOEC, LOEC, and EC_{50} estimates pertaining to biomass and growth rate. The study authors only provided replicate data for cell density and used these values to calculate biomass (area under the curve) and growth rate, which they then statistically analyzed. Although the study authors provided raw data for cell density, they did not estimate a NOEC, LOEC or EC_{50} for this endpoint. The reviewer's NOEC for cell density (3.9 µg/L) was identical to the NOEC reported by the study authors for biomass and growth rate. **The reviewer-calculated 120-hour EC_{50} value was 14 µg/L for cell density. The study author-calculated 120-hour EC_{50} value was 8.8 µg/L and 27 µg/L for biomass (area under the curve) and growth rate, respectively.**

This study is classified as CORE. This study supports the requirements for a diatom EC_{50} test (Subdivision J, §123-2 (TIER II)).

Results Synopsis

Cell density:

EC_{50}: 14 µg/L	95% C.I.: 12 and 17 µg/L
NOEC: 3.9 µg/L	Probit Slope: 4.49

Biomass (Area Under the Curve): (Note, these are study author reported values; reviewer was unable to verify due to a lack of replicate data)

EC_{50}: 8.8 µg/L 95% C.I.: 7.8-9.8 µg/L
NOEC: 3.9 µg/L Probit Slope: Not provided

Growth rate: (Note, these are study author reported values; reviewer was unable to verify due to a lack of replicate data)

EC_{50}: 27 µg/L 95% C.I.: 24-31 µg/L
NOEC: 3.9 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:

1. The initial pH (8.6-8.7) was higher than the guideline requirement (approximately 7.5).

2. The maximum labeled rate was not reported.

10. SUBMISSION PURPOSE: Reregistration

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Navicula pelliculosa*
Initial number of cells: 3,000 - 10,000 cells/mL	3,000 cells/mL
Nutrients:	

Guideline Criteria	Reported Information
Standard formula	Yes

B. Test System

Guideline Criteria	Reported Information
Solvent:	0.10 mL/L dimethylformamide
Temperature: *Skeletonema*: 20°C Others: 24-25°C	23.8-24.0°C
Light Intensity: *Anabaena*: 2.0 Klux (±15%) Others: 4.0-5.0 Klux (±15%)	4.3 Klux
Photoperiod: *Skeletonema*: 14 h light, 10 h dark, or 16 h light, 8 h dark Others: Continuous	Continuous
pH *Skeletonema*: approx. 8.0 Others: approx. 7.5	Initial 8.6-8.7; Final 7.7-7.8

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	2x
Doses: at least 5	Nominal: 0.90, 1.8, 3.5, 7.0, 14, 28, and 56 µg/L
Controls: Negative and/or solvent	Negative and solvent
Replicates per dose: 3 or more	Yes
Duration of test:	

Guideline Criteria	Reported Information
120 hours	120 hours
Daily observations were made?	Yes
Method of observations:	The number of cells was determined 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 ≥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 10^4 cells/mL)	% Reduction	120-hour pH
Control	132	<7>	7.8
Solvent control	142	--	7.7
1.1	151	<6>	7.7
2.3	142	0	7.8
4.0	140	1	7.8
7.6	92	35	7.8
14	86	39	7.8
32	3.91	97	7.8
58	0.92	99	7.8

< > 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	1.217	1	7.8
Solvent Control	1.232	--	7.7
1.1	1.244	<1>	7.7
2.3	1.232	0	7.8
4.0	1.228	0	7.8
7.6	1.132	7*	7.8
14	1.063	8*	7.8
32	0.513	58*	7.8
58	0.223	82*	7.8

< > 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 (µg/L)	Area Under the Growth Curve	% Reduction	120-hour pH
Negative Control	132.0	13	7.8
Solvent Control	152.1	--	7.7
1.1	155.1	<2>	7.7
2.3	139.1	9	7.8
4.0	138.1	9	7.8
7.6	81.7	46*	7.8
14	72.7	52*	7.8
32	2.3	99*	7.8
58	0.6	100*	7.8

< > Represents an increase in growth rate relative to the solvent control.
* Significant difference from the solvent control.

Other Significant Results: None.

Statistical Results

Statistical Method: EC_{50} values were determined using the probit method. Biomass areas were examined by one-way analysis of variance and using Dunnett's test.

Biomass (Area Under the Curve):

EC_{50}: 8.8 µg/L	95% C.I.: 7.8-9.8 µg/L
NOEC: 3.9 µg/L	Probit Slope: Not provided

Growth rate:

EC_{50}: 27 µg/L	95% C.I.: 24-31 µg/L
NOEC: 3.9 µg/L	Probit Slope: Not provided

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Only algal cell density were analyzed statistically because replicate data were not provided for area under the 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 EC_{50} estimate was performed using the method of Bruce and Versteeg via Nuthatch software.

Cell density:

EC_{50}: 14 µg/L	95% C.I.: 12 and 17 µg/L
NOEC: 3.9 µg/L	Probit Slope: 4.49

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

EC_{50}:	95% C.I.:
NOEC:	Probit Slope:

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

EC_{50}:	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 density (for which replicate data were provided), however, the study authors failed to estimate NOEC, LOEC or EC_{50} values for this endpoint. The NOEC and LOEC

for biomass and growth rate reported by the study authors were identical to those estimated by the reviewer for cell density; however, the EC_{50} values differed across endpoints.

The initial pH (8.6-8.7) was higher than the guideline requirement (approximately 7.5); this deviation probably did not impact the results of the study. The pH declined during the 120-hour test to a final pH range of 7.7-7.8.

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	89234.916	12747.845	32.523
Within (Error)	22	8623.075	391.958	
Total	29	97857.991		

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	139.000	139.000		
2	1.1	151.000	151.000	-0.909	
3	2.2	142.333	142.333	-0.253	
4	**3.9**	**140.000**	**140.000**	**-0.076**	
5	7.5	92.133	92.133	3.551	*
6	14	86.233	86.233	3.998	*
7	31	3.913	3.913	10.235	*

8	59	0.918	0.918	10.462 *

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 CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	9			
2	1.1	3	35.122	25.3	-12.000
3	2.2	3	35.122	25.3	-3.333
4	3.9	3	35.122	25.3	-1.000
5	7.5	3	35.122	25.3	46.867
6	14	3	35.122	25.3	52.767
7	31	3	35.122	25.3	135.087
8	59	3	35.122	25.3	138.082