**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 the Marine Alga *Skeletonema costatum*

Study Completion Date: August 1, 1998

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

Sponsor: GB Biosciences Corporation
1800 Concord Pike
P.O. Box 15458
Wilmington, DE 19850-5458

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 $EC_{50}$ 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 $EC_{50}$ 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 $EC_{50}$. The reviewer's NOEC, LOEC, and $EC_{50}$ 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 $EC_{50}$ 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 $EC_{50}$ test (Subdivision J, §123-2 (TIER II)).

**Results Synopsis**

**Cell density:**

| | |
|---|---|
| $EC_{50}$: 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): Note, these are study author reported values. Replicate data not provided; values could not be determined by reviewer.**

| | |
|---|---|
| $EC_{50}$: 13 µg/L | 95% C.I.: 12-14 µg/L |
| NOEC: 5.9 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.**

| | |
|---|---|
| $EC_{50}$: 20 µg/L | 95% C.I.: 18-22 µg/L |
| NOEC: 12 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 |
|---|---|
| **Species:**<br>*Skeletonema costatum*<br>*Anabaena flos-aquae*<br>*Selenastrum capricornutum*<br>*Navicula pelliculosa* | *Skeletonema costatum* |
| **Initial number of cells:**<br>3,000 - 10,000 cells/mL | 10,000 cells/mL |
| **Nutrients:**<br>Standard formula | Yes |

**B. Test System**

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

**C. Test Design**

| Guideline Criteria | Reported Information |
|---|---|
| **Dose range:**<br>2x or 3x progression | 2x |
| **Doses:**<br>at least 5 | Nominal: 1.5, 3.0, 6.0, 12, 24, 48, and 96 µg/L |
| **Controls:**<br>Negative and/or solvent | Negative and solvent |
| **Replicates per dose:**<br>3 or more | Yes |
| **Duration of test:**<br>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 ≥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 | 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: $EC_{50}$ 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.**

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

**Biomass (Area Under the Curve):**

| | |
|---|---|
| $EC_{50}$: 13 µg/L | 95% C.I.: 12-14 µg/L |
| NOEC: 5.9 µg/L | Probit Slope: Not provided |

**Growth rate:**

| | |
|---|---|
| $EC_{50}$: 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 $EC_{50}$ estimate was performed using the method of Bruce and Versteeg via Nuthatch software.

**Results Synopsis:**

**Cell density:**

| | |
|---|---|
| $EC_{50}$: 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.**

| | |
|---|---|
| $EC_{50}$: | 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 (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 $EC_{50}$ 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:

44908103d
File: 8103d Transform: NO TRANSFORMATION

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

File: 8103d Transform: NO TRANSFORMATION

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 | * |

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

File: 8103d Transform: NO TRANSFORMATION

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 | 1.6 | 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 |