

US EPA ARCHIVE DOCUMENT

**Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to the freshwater diatom *Navicula pelliculosa***

**PMRA Submission Number: 2001-1027**

**EPA MRID Number{454050-14}**

**Data Requirement:**

PMRA DATA CODE: fresh water algae:9.8.2-2 (TGAI)  
EPA DP Barcode: D278418  
OECD Data Point: fresh water algae: IIA 8.4.1; marine algae:  
IIA 8.4.1 (TGAI) and IIIA 10.2.1.11 (EP)  
EPA Guideline: 123-2

**Test material: BAS 510 F**

**Purity (%): 96.9%**

Common name: Nicobifen

Chemical name

IUPAC: 2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide

CAS name: 3-Pyridinecarboxamide, 2-chloro-N\_(4'-chloro[1.1'-biphenyl]-2-yl)

CAS No.: 188425-85-6

Synonyms:

**Primary Reviewer:** Peter Takacs and Peter Delorme  
{PMRA}

**Date:** March 14/02

**Secondary Reviewer(s):** Thomas M. Steeger, Ph.D.  
{EPA} *Thomas M Steeger*

**Date:** June 20, 2002

**Company Code:** BAZ

**Active Code:** CHH-BAZ-4

**Use Site Category:** In Canada, this fungicide is proposed for use on USC 13, 14 and 30; agricultural feed, food and turf uses. BAS 510 F is to be used 2-6 times per growing season depending on the crop, at a maximum recommended application rate of 875 g a.i./ha/application.

**EPA PC Code:** 128008

**CITATION:** Susan J. Palmer, Timothy Z. Kendall, Henry O. Krueger, Catherine M. Holmes, February, 2001. BAS 510 F: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER DIATOM (*Navicula pelliculosa*). Wildlife International, Ltd. 8598 Commerce Drive, Easton, Maryland 21601, (410) 822-8600. BASF Study Number: 63988.



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**EXECUTIVE SUMMARY:**

In a 96 hour acute toxicity study, the cultures of the freshwater diatom *Navicula pelliculosa*. were exposed to BAS 510 F at mean measured concentrations of 0.069, 0.14, 0.28, 0.60, 1.2 and 2.4 mg a.i/L under static conditions in accordance with the OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II* (1). The 96-hr NOEC was 0.14 mg ai/L. The 96-hr EC<sub>50</sub> based on cell density was 1.8 mg a.i/L. The % growth inhibition (biomass) in the treated algal culture as compared to the control ranged from 13-56%.

This toxicity study is classified as acceptable and satisfies the guideline requirements for an acute algal toxicity study.

**Results Synopsis**

Test Organism: diatom *Navicula pelliculosa*

Test Type: Static

96 hr EC<sub>50</sub>: 1.8 mg a.i./L , 95% C.I.: 1.3-2.6 mg a.i./L

96 hr EC<sub>10</sub>: 0.12 mg a.i./L

NOEC: 0.14 mg ai/L

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**I. MATERIALS AND METHODS**

**GUIDELINE FOLLOWED:**

The protocol was based on procedures outlined in the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II* (1).

**COMPLIANCE:**

This study was conducted in accordance with the Good Laboratory Practice Regulations as published by the US. EPA in 40 CFR Parts 160 and 792, 17 August 1989; OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau, 10 August 1984.

**A. MATERIALS:**

**1. Test Material**

BAS 510 F

**Description:** Solid white powder  
**Lot No./Batch No. :** N75  
**Purity:** 96.9%  
**Stability of Compound Under Test Conditions:** Not stated  
**Storage conditions of test chemicals:** ambient conditions

**Physicochemical properties of BAS 510 F.**

| Parameter                | Values  | Comments                     |
|--------------------------|---|------------------------------|
| Water solubility at 20°C | 4.69 mg/L   | low solubility               |
| Vapour pressure          | $7 \times 10^{-5}$ mbar @ 20 °C                       | not volatile                 |
| UV absorption            | UV molecular extinction: $1.53 \times 10^3$ at 290 nm | -                            |
| pKa                      | does not dissociate in water                          | -                            |
| Kow                      | 2.96  | Not likely to bioconcentrate |

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**2. Test organism:**

**Name:** Freshwater Diatom (*Navicula pelliculosa*)

*EPA requires a nonvascular species: For tier I testing, only one species, Selenastrum capricornutum, to be tested; for tier II testing, Skeletonema costatum, Anabaena flos-aquae, Selenastrum capricornutum, and a freshwater diatom is tested*

*OECD suggests that the following species are suitable: Selenastrum capricornutum, Scenedesmus subspicatus, and Chlorella vulgaris. If other species are used, the strain should be reported*

**Strain:** not specified

**Source:** Original algal cultures were obtained from UTEX - The Culture Collection of Algae at the University of Texas at Austin, and had been maintained in culture medium at Wildlife International, Ltd., Easton, Maryland. Algal cells used in this test were obtained from Wildlife International, Ltd. cultures that had been actively growing in culture medium for at least two weeks prior to test initiation.

**Age of inoculum:** not specified

**Method of cultivation:** not specified

**B. STUDY DESIGN:**

**1. Experimental Conditions**

**a) Range-finding Study:**

A range finding study was conducted to determine treatment levels. Test concentrations were 0, 0.024, 0.081, 0.27, 0.90, 3.0, 10 mg ai/L.

**b) Definitive Study**

**BAS 510 F: Experimental Parameters**

| Parameter   | Details   | Remarks<br>-----<br>Criteria  |
|---|---|---|
| <u>Acclimation</u><br><br>Period:<br>Culturing media and conditions: (same as test or not)<br>Health: (any toxicity observed) | at least two weeks prior to test initiation in test media | -----<br><br><i>EPA recommends two week acclimation period.</i><br><br><i>OECD recommends an amount of algae suitable for the inoculation of test cultures incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.</i> |

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| Parameter  | Details  | Remarks  |
|--|--|--|
|  |  | Criteria   |
| <u>Test system</u>   | Static   |  |
| Incubation facility  | Environmental chamber at 24 ± 2°C  |  |
| Duration of the test   | 96 hr  | EPA requires: 96 - 120 hours<br>OECD requires: 72 hours  |
| <u>Test vessel</u><br>Material: (glass/polystyrene)<br>Size:<br>Fill volume:   | 250-mL Erlenmeyer flasks with a final volume of 100 mL were used   | OECD recommends 250 ml conical flasks when the volume of the test solution is 100 ml or use a culturing apparatus.   |
| <u>Details of growth medium</u><br>Name:<br>pH at test initiation:<br>pH at test termination:<br>Chelator used:<br>Carbon source:  | ASTM medium (ASTM Standard Guide 1218-90E) with silica and selenium constituents<br>7.2<br>7.5 - 7.6<br>Na <sub>2</sub> EDTA (0.3 mg/L)<br>NaHCO <sub>3</sub> , 15 mg/L  | EPA recommends 20X-AAP medium and no chelators.<br>OECD recommends the medium pH after equilibration with air be ~8 with less than .001 mmol/l of chelator, if used.   |
| If non-standard nutrient medium was used, detailed composition provided (Yes/No)   | yes  |  |
| <u>Dilution water</u><br>Source:<br>Type:<br>pH: 7.5<br>Total Organic Carbon: NA<br>Particulate matter: NA<br>Metals: not detected<br>Pesticides: not detected<br>Chlorine: NA<br>Water pretreatment (if any):<br>Intervals of water quality measurement: once | Stock nutrient solutions were prepared by adding reagent-grade chemicals to purified Wildlife International, Ltd. well water. The test medium then was prepared by adding appropriate volumes of the stock nutrient solutions to purified well water (NANOpure® water) | <u>pH:</u><br>EPA : <i>Skeletonema costatum</i> = ~8.0<br>Others = ~7.5 from beginning to end of the test.<br>OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.<br><br><u>salinity:</u><br>EPA: 30-35 ppt. EPA is against the use of dechlorinated water. |

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| Parameter   | Details                                 | Remarks  |
|---|---|--|
| <i>Criteria</i>   |   |  |
| Indicate how the test material is added to the medium (added directly or used stock solution) | stock solution was added to test medium |  |
| Aeration or agitation   | agitated at 100 rpm                     | <p><i>EPA recommends agitation only for Selenastrum sp. at 100 cycles per min and Skeletonema sp. at ~60 cycles per min. Aeration is not recommended.</i></p>  |
| Initial cells density   | 10,000 cells/mL                         | <p><i>EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.</i></p> <p><i>OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.</i></p>   |
| <p><u>Number of replicates</u></p> <p>Control:<br/>Solvent control:<br/>Treated ones:</p>     | <p>3<br/>3<br/>3</p>                    | <p><b>EPA state that for Navicula tests, 4 replicates should be used.</b></p> <p><i>EPA requires a negative and/or solvent control with 3 or more replicates per doses. For Navicula sp. tests should be conducted with four replicate.</i></p> <p><i>OECD prefer three replicates at each test concentration and ideally twice that number of controls. When co-solvents are used, include a solvent control in the test.</i></p> |

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| Parameter   | Details  | Remarks<br>-----<br>Criteria   |
|---|--|--|
| <p><u>Test concentrations</u></p> <p>Nominal:<br/>Measured:</p>   | <p>0.078, 0.16, 0.31, 0.63, 1.3 and 2.5 mg/L<br/>0.069, 0.14, 0.28, 0.60, 1.2 and 2.4 mg/L</p> | <p><b>The lowest test concentration resulted in 13% inhibition of cell density</b></p> <p>-----</p> <p><i>EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.</i></p> <p><i>OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.</i></p> |
| <p>Solvent (type, percentage, if used)</p>  | <p>dimethylformamide (0.1 mL)</p>  | <p>-----</p>   |
| <p>Method and interval of analytical verification:<br/>Limit of Detection:<br/>Limit of Quantitation:</p> | <p>beginning and end of study using HPLC with UV detection<br/>-<br/>0.05 mg ai/L</p>          | <p>-----</p>   |
| <p><u>Test conditions</u></p> <p>Temperature:<br/>Photoperiod:<br/>Light intensity and quality:</p>       | <p>24±2°C<br/>Continuous<br/>white light, 4300±10% lux</p>                                     | <p>-----</p> <p><u>Temperature:</u><br/><i>EPA: Skeletonema: 20°C,<br/>Others: 24-25°C;<br/>OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C</i></p> <p><u>Photoperiod:</u><br/><i>EPA: S. costatum 14 hr light/ 10 hr dark,<br/>Others: Continuous;<br/>OECD: continuous uniform illumination</i></p> <p><u>Light intensity:</u><br/><i>EPA: Anabaena: 2.0 Klux (±15%),<br/>Others: 4 - 5 Klux (±15%);<br/>OECD: approximately 8000 Lux measured with a spherical collector</i></p>                                |
| <p><u>Reference chemical, if used</u></p> <p>Name:<br/>Concentrations:</p>                                | <p>not used</p>  | <p>-----</p>   |
| <p>Other parameters, if any</p>   | <p>-</p>   | <p>-----</p>   |

**2. Observations:**

Table 1: Observation

| Parameters  | Details   | Remarks<br>Criteria  |
|---|---|--|
| Parameters measured including the growth inhibition/other toxicity symptoms | cell density/mL, morphological effects              | <i>EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.</i>   |
| Measurement technique for cell density and other end points                 | hemacytometer and microscope                        | <i>EPA recommends the measurement technique of cell counts or chlorophyll a</i><br><br><i>OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).</i> |
| Observation intervals   | 24, 48, 72, 96 hr                                   | <i>EPA and OECD: every 24 hours.</i>   |
| Other observations, if any  | -   |  |
| Indicate whether there was exponential growth in the control                | 96-hr control cell counts were 22.4x that at 24 hr. | <i>EPA requires control cell count at termination to be <math>\geq 2X</math> initial count or by a factor of at least 16 during the test.</i><br><br><i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>   |
| Water quality was acceptable (Yes/No)                                       | yes   |  |
| Were raw data included?   | Yes   |  |

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**II. RESULTS AND DISCUSSION:**

**A. INHIBITORY EFFECTS:**

Algal cell density inhibition ranged from 13% at the lowest treatment level to 56% at the highest concentration.

**Table 2: Effect of BAS 510 F on algal growth (freshwater diatom *Navicula pelliculosa*.)**

| Treatment:<br>mean measured<br>concentration<br>(mg a.i./L) | mean cell count<br>24 hours<br>(cell/mL) | mean cell count<br>48 hours<br>(cell/mL) | mean cell count<br>72hours<br>(cell/mL) | 96 hours                        |              |
|---|--|--|---|---------------------------------|--------------|
|   |  |  |   | mean cell<br>count<br>(cell/mL) | % inhibition |
| Negative<br>Control   | 42,333                                   | 391,667                                  | 671,667                                 | 1,090,000                       |              |
| Solvent Control   | 52,000                                   | 453,667                                  | 731,667                                 | 1,021,667                       | -            |
| Pooled Control  | 47,167                                   | 422,667                                  | 701,667                                 | 1,055,833                       | -            |
| 0.069   | 53,667                                   | 439,667                                  | 685,000                                 | 920,000                         | 13           |
| 0.14  | 58,000                                   | 401,333                                  | 740,000                                 | 948,333                         | 10           |
| 0.28  | 51,000                                   | 391,000                                  | 805,000                                 | 851,667*                        | 19*          |
| 0.60  | 53,000                                   | 403,667                                  | 595,000*                                | 756,667*                        | 28*          |
| 1.2   | 46,000                                   | 242,667*                                 | 578,333*                                | 588,333*                        | 44*          |
| 2.4   | 20,333*                                  | 136,000*                                 | 292,667*                                | 461,667*                        | 56*          |

\* Statistically significant difference ( $p < 0.05$ ) from the pooled control replicates using Dunnett's test.

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**Table 3: Statistical endpoint values for BAS 510 F on algal growth of the freshwater diatom (*Navicula pelliculosa*.)**

| Statistical Endpoint                    | 96 hr Cell count/mL         |
|---|-----------------------------|
| NOEC                                    | 0.14 mg a.i./L              |
| EC <sub>50</sub> (95% C.I.)             | 1.8 (1.3-2.6) mg a.i./L     |
| IC <sub>50</sub> (mg a.i./L) (95% C.I.) | -                           |
| EC <sub>10</sub> (95% C.I.)             | 0.12 (0.046-0.33) mg a.i./L |
| EC <sub>90</sub> (95% C.I.)             | >2.4 mg a.i./L              |
| <u>Reference chemical, if used</u>      |                             |
| NOEC                                    | not used                    |
| EC <sub>50</sub> (mg/L)                 |                             |

**B. REPORTED STATISTICS:** [List the parameters that were analyzed and the statistical tests that were performed. May attach a copy of the statistical methods from the study with a statement that the reviewer has no objections to the analyses used.]

Calculations of cell densities and percent inhibition values, as well as statistical analyses, were conducted using "The SAS System for Windows" (Release 6.12). Inhibition values were calculated for each treatment group as the percent reduction in cell density relative to the pooled control replicates. The following formula was used:

$$\text{PERCENT INHIBITION} = (\text{MEAN RESPONSE}_{\text{CONTROL}} - \text{MEAN RESPONSE}_{\text{TREATMENT}} / \text{MEAN RESPONSE}_{\text{CONTROL}}) \times 100$$

Cell densities were analyzed statistically by linear interpolation versus concentration to determine the EC<sub>10</sub>, EC<sub>50</sub> and EC<sub>90</sub> value. The cell density data were evaluated for normality and homogeneity of variance ( $p = 0.05$ ) using the Shapiro-Wilk's and Levene's tests, respectively. Since the assumptions of normality and homogeneity of variances were met, the data were analyzed using analysis of variance (ANOVA) and Dunnett's test to compare the treatment data with the control data ( $p = 0.05$ ). The negative and solvent control data were compared using Student's t-test. Since the test indicated that there were no statistically significant differences ( $p > 0.05$ ) between the control groups, the data from the control groups were pooled for statistical analyses. Results of the statistical analyses, as well as an evaluation of the concentration response pattern, were used to determine the no-observed-adverse-effect-concentration (NOAEC) (*i.e.*, the highest test concentration that had no adverse inhibitory effect on growth) at test termination.

**C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:**

Statistical Method:

For the determination of the NOEC, a One-Way ANOVA with Student-Newman-Keuls method was used. The EC<sub>50</sub> was calculated using the Probit program.

ANOVA:

Power of performed test with alpha = 0.200: 1.000

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| Source of Variation | DF | SS               | MS               | F      | P      |
|---------------------|----|------------------|------------------|--------|--------|
| Between Treatments  | 6  | 852847619047.623 | 142141269841.271 | 16.662 | <0.001 |

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method) :

Comparisons for factor:

| Comparison        | Diff of Means | p | q      | P<0.05 |
|-------------------|---------------|---|--------|--------|
| control vs. Col 7 | 628333.333    | 7 | 11.783 | Yes    |
| control vs. Col 6 | 501666.667    | 6 | 9.408  | Yes    |
| control vs. Col 5 | 333333.333    | 5 | 6.251  | Yes    |
| control vs. Col 4 | 238333.333    | 4 | 4.469  | Yes    |
| control vs. Col 2 | 170000.000    | 3 | 3.188  | No     |
| control vs. Col 3 | 141666.667    | 2 | 2.657  | No     |

The re-analysis of the data indicates that the calculated NOEC of 0.14 mg ai/L is correct.

EC<sub>50</sub>:

The calculated 96-hr EC<sub>50</sub> for cell density is 1.93 mg ai/L (95% CI: 1.37 - 3.16 mg a.i./L), which is in good agreement with the reported value. Probit slope = 0.95669; EC<sub>10</sub> = 0.091 mg a.i./L

**D. STUDY DEFICIENCIES:** The lowest test concentration produced 13% inhibition; OECD recommends no effect at this concentration. The EPA guideline specifies that 4 replicates should be used with *Navicula* sp, however, only 3 were used in this study. The standard deviations of the mean cell counts should be detailed in the study report. These deficiencies are not thought by the reviewer to significantly change the results of the study.

**E. REVIEWER'S COMMENTS:** No further comments.

**F. CONCLUSIONS:** The study is acceptable. The EC<sub>50</sub> for biomass was more sensitive than that of growth rate inhibition (1.32 vs. 3.75 mg ai/L)

96 hr EC<sub>50</sub> (cell density): 1.8 mg ai/L  
NOEC: 0.14 mg ai/L

**III. REFERENCES:**

Approved 04/01/01 C.K.

**SAS Analysis Output Follows:**

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Average Cell Densities by Treatment

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| Obs | DOSE  | _TYPE_ | _FREQ_ | MEAN       | SE       |
|-----|-------|--------|--------|------------|----------|
| 1   | 0.000 | 0      | 6      | 1055833.33 | 55833.33 |
| 2   | 0.069 | 0      | 3      | 920000.00  | 35472.99 |
| 3   | 0.140 | 0      | 3      | 948333.33  | 60713.35 |
| 4   | 0.280 | 0      | 3      | 851666.67  | 6666.67  |
| 5   | 0.600 | 0      | 3      | 756666.67  | 72188.03 |
| 6   | 1.200 | 0      | 3      | 588333.33  | 43811.46 |
| 7   | 2.400 | 0      | 3      | 461666.67  | 31797.97 |

Average Inhibition by Treatment

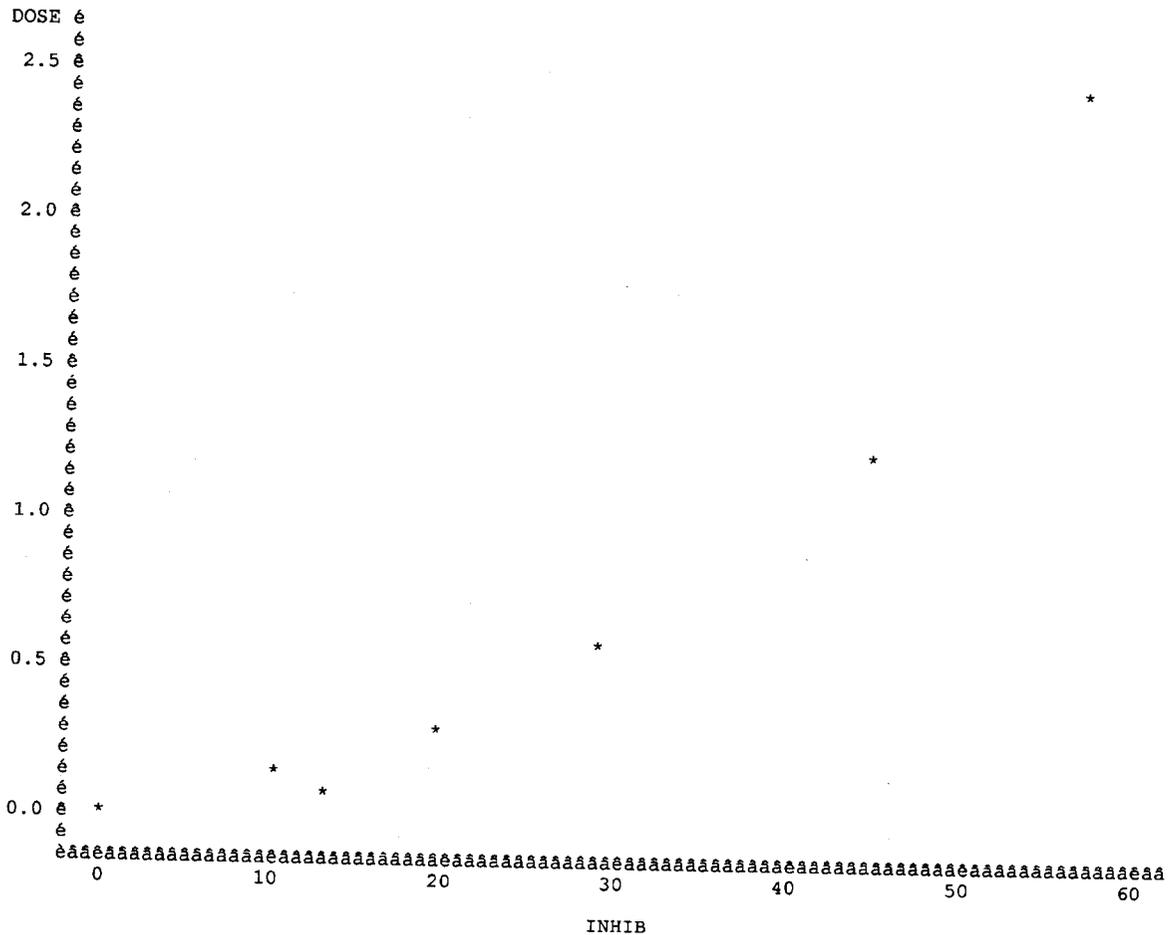
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| Obs | DOSE  | _TYPE_ | _FREQ_ | INHIB   | SE      |
|-----|-------|--------|--------|---------|---------|
| 1   | 0.000 | 0      | 6      | -0.0000 | 5.28808 |
| 2   | 0.069 | 0      | 3      | 12.8650 | 3.35972 |
| 3   | 0.140 | 0      | 3      | 10.1815 | 5.75028 |
| 4   | 0.280 | 0      | 3      | 19.3370 | 0.63141 |
| 5   | 0.600 | 0      | 3      | 28.3346 | 6.83707 |
| 6   | 1.200 | 0      | 3      | 44.2778 | 4.14947 |
| 7   | 2.400 | 0      | 3      | 56.2747 | 3.01165 |

Plot of Percent Inhibition of Navicula Cell Number over BAS 510F Concentration

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Plot of DOSE\*INHIB. Symbol used is '\*'.



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Analysis of Variance for Percent Inhibition of Cell Number by BAS 510 F

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The ANOVA Procedure

Class Level Information

| Class | Levels | Values                        |
|-------|--------|-------------------------------|
| DOSE  | 7      | 0 0.069 0.14 0.28 0.6 1.2 2.4 |

Number of observations 24

Dependent Variable: CELLS

| Source          | DF | Sum of Squares | Mean Square  | F Value | Pr > F |
|-----------------|----|----------------|--------------|---------|--------|
| Model           | 6  | 972069791667   | 162011631944 | 15.98   | <.0001 |
| Error           | 17 | 172304166667   | 10135539216  |         |        |
| Corrected Total | 23 | 1.144374E12    |              |         |        |

| R-Square | Coeff Var | Root MSE | CELLS Mean |
|----------|-----------|----------|------------|
| 0.849434 | 12.13261  | 100675.4 | 829791.7   |

| Source | DF | Anova SS     | Mean Square  | F Value | Pr > F |
|--------|----|--------------|--------------|---------|--------|
| DOSE   | 6  | 972069791667 | 162011631944 | 15.98   | <.0001 |

Dunnnett's t Tests for CELLS

NOTE: This test controls the Type I experimentwise error for comparisons of all treatments against a control.

|                                |          |
|--------------------------------|----------|
| Alpha                          | 0.05     |
| Error Degrees of Freedom       | 17       |
| Error Mean Square              | 1.014E10 |
| Critical Value of Dunnnett's t | 2.90374  |

Comparisons significant at the 0.05 level are indicated by \*\*\*.

| DOSE Comparison | Difference Between Means | Simultaneous 95% Confidence Limits |
|-----------------|--------------------------|------------------------------------|
| 0.14 - 0        | -107500                  | -314212 99212                      |
| 0.069 - 0       | -135833                  | -342545 70879                      |
| 0.28 - 0        | -204167                  | -410879 2545                       |
| - 0             | -299167                  | -505879 -92455                     |
| - 0             | -467500                  | -674212 -260788                    |
| - 0             | -594167                  | -800879 -387455                    |

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| Obs | DOSE  | N   | RESPONSE |
|-----|-------|-----|----------|
| 1   | 0.000 | 100 | 0        |
| 2   | 0.069 | 100 | 13       |
| 3   | 0.140 | 100 | 10       |
| 4   | 0.280 | 100 | 19       |
| 5   | 0.600 | 100 | 28       |
| 6   | 1.200 | 100 | 44       |
| 7   | 2.400 | 100 | 56       |

Probit Analysis of Percent Inhibition by BAS 510F

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Probit Procedure

Iteration History for Parameter Estimates

| Iter | Ridge | Loglikelihood | Intercept    | Log10(DOSE)  |
|------|-------|---------------|--------------|--------------|
| 0    | 0     | -415.88831    | 0            | 0            |
| 1    | 0     | -321.40836    | -0.250387046 | 0.7536705887 |
| 2    | 0     | -318.75217    | -0.272371846 | 0.9448186416 |
| 3    | 0     | -318.74371    | -0.273502708 | 0.9566452113 |
| 4    | 0     | -318.74371    | -0.273506849 | 0.9566883315 |

Model Information

|                        |              |
|------------------------|--------------|
| Data Set               | WORK.E       |
| Events Variable        | RESPONSE     |
| Trials Variable        | N            |
| Number of Observations | 6            |
| Number of Events       | 170          |
| Number of Trials       | 600          |
| Name of Distribution   | NORMAL       |
| Log Likelihood         | -318.7437133 |

Last Evaluation of the Negative of the Gradient

| Intercept    | Log10(DOSE)  |
|--------------|--------------|
| 6.4511987E-8 | -6.244935E-8 |

Last Evaluation of the Negative of the Hessian

|             | Intercept    | Log10(DOSE)  |
|-------------|--------------|--------------|
| Intercept   | 305.9289282  | -83.23039614 |
| Log10(DOSE) | -83.23039614 | 101.0710373  |

Algorithm converged.

Goodness-of-Fit Tests

| Statistic          | Value  | DF | Pr > ChiSq |
|--------------------|--------|----|------------|
| Pearson Chi-Square | 5.2477 | 4  | 0.2628     |
| L.R. Chi-Square    | 4.9863 | 4  | 0.2887     |

**Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to the freshwater diatom *Navicula pelliculosa***  
**PMRA Submission Number: 2001-1027** **EPA MRID Number{454050-14}**

Probit Procedure

Response-Covariate Profile

Response Levels 2  
 Number of Covariate Values 6

Since the chi-square is small ( $p > 0.1000$ ), fiducial limits will be calculated using a t value of 1.96.

Analysis of Parameter Estimates

| Variable    | DF | Estimate | Standard Error | Chi-Square | Pr > ChiSq | Label     |
|-------------|----|----------|----------------|------------|------------|-----------|
| Intercept   | 1  | -0.27351 | 0.06490        | 17.7582    | <.0001     | Intercept |
| Log10(DOSE) | 1  | 0.95669  | 0.11292        | 71.7810    | <.0001     |           |

Estimated Covariance Matrix

|             | Intercept | Log10(DOSE) |
|-------------|-----------|-------------|
| Intercept   | 0.004212  | 0.003469    |
| Log10(DOSE) | 0.003469  | 0.012751    |

Probit Model in Terms of Tolerance Distribution

| MU         | SIGMA     |
|------------|-----------|
| 0.28588919 | 1.0452725 |

Estimated Covariance Matrix for Tolerance Parameters

|       | MU       | SIGMA    |
|-------|----------|----------|
| MU    | 0.007908 | 0.008125 |
| SIGMA | 0.008125 | 0.015221 |

Probit Procedure

Probit Analysis on Log10(DOSE)

| Probability | Log10(DOSE) | 95% Fiducial Limits |         |
|-------------|-------------|---------------------|---------|
| 0.01        | -2.1458     | -2.7253             | -1.7782 |
| 0.02        | -1.8608     | -2.3572             | -1.5441 |
| 0.03        | -1.6801     | -2.1243             | -1.3951 |
| 0.04        | -1.5441     | -1.9494             | -1.2826 |
| 0.05        | -1.4334     | -1.8074             | -1.1908 |
| 0.06        | -1.3393     | -1.6869             | -1.1123 |
| 0.07        | -1.2567     | -1.5815             | -1.0433 |
| 0.08        | -1.1828     | -1.4874             | -0.9812 |
| 0.09        | -1.1156     | -1.4020             | -0.9245 |
| 0.10        | -1.0537     | -1.3237             | -0.8721 |
| 0.15        | -0.7975     | -1.0032             | -0.6511 |
| 0.20        | -0.5938     | -0.7558             | -0.4682 |
| 0.25        | -0.4191     | -0.5531             | -0.3018 |
| 0.30        | -0.2623     | -0.3821             | -0.1413 |
| 0.35        | -0.1169     | -0.2343             | 0.0181  |
| 0.40        | 0.0211      | -0.1025             | 0.1778  |
| 0.45        | 0.1545      | 0.0194              | 0.3379  |
| 0.50        | 0.2859      | 0.1355              | 0.4994  |
| 0.55        | 0.4172      | 0.2491              | 0.6633  |
| 0.60        | 0.5507      | 0.3628              | 0.8316  |
| 0.65        | 0.6887      | 0.4791              | 1.0069  |
| 0.70        | 0.8340      | 0.6006              | 1.1925  |
| 0.75        | 0.9909      | 0.7310              | 1.3937  |

**Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to the freshwater diatom *Navicula pelliculosa***

**PMRA Submission Number: 2001-1027**

**EPA MRID Number{454050-14}**

|      |        |        |        |
|------|--------|--------|--------|
| 0.80 | 1.1656 | 0.8755 | 1.6184 |
| 0.85 | 1.3692 | 1.0432 | 1.8809 |
| 0.90 | 1.6255 | 1.2536 | 2.2119 |
| 0.91 | 1.6873 | 1.3043 | 2.2920 |
| 0.92 | 1.7546 | 1.3594 | 2.3789 |
| 0.93 | 1.8285 | 1.4199 | 2.4746 |
| 0.94 | 1.9111 | 1.4875 | 2.5815 |
| 0.95 | 2.0052 | 1.5645 | 2.7035 |
| 0.96 | 2.1158 | 1.6549 | 2.8468 |
| 0.97 | 2.2518 | 1.7660 | 3.0231 |
| 0.98 | 2.4326 | 1.9136 | 3.2575 |
| 0.99 | 2.7176 | 2.1460 | 3.6272 |

Probit Analysis of Percent Inhibition by BAS 510F

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14:06 Thursday, June 20, 2002

Probit Procedure

Probit Analysis on DOSE

| Probability | DOSE      | 95% Fiducial Limits |           |
|-------------|-----------|---------------------|-----------|
| 0.01        | 0.00715   | 0.00188             | 0.01667   |
| 0.02        | 0.01378   | 0.00439             | 0.02857   |
| 0.03        | 0.02089   | 0.00751             | 0.04027   |
| 0.04        | 0.02857   | 0.01124             | 0.05217   |
| 0.05        | 0.03686   | 0.01558             | 0.06445   |
| 0.06        | 0.04579   | 0.02056             | 0.07721   |
| 0.07        | 0.05537   | 0.02621             | 0.09051   |
| 0.08        | 0.06565   | 0.03256             | 0.10442   |
| 0.09        | 0.07664   | 0.03963             | 0.11898   |
| 0.10        | 0.08837   | 0.04746             | 0.13426   |
| 0.15        | 0.15942   | 0.09926             | 0.22328   |
| 0.20        | 0.25478   | 0.17545             | 0.34024   |
| 0.25        | 0.38095   | 0.27983             | 0.49911   |
| 0.30        | 0.54670   | 0.41484             | 0.72231   |
| 0.35        | 0.76405   | 0.58300             | 1.04263   |
| 0.40        | 1.04972   | 0.78985             | 1.50574   |
| 0.45        | 1.42738   | 1.04565             | 2.17737   |
| 0.50        | 1.93148   | 1.36622             | 3.04375   |
| 0.55        | 2.61360   | 1.77476             | 4.60567   |
| 0.60        | 3.55391   | 2.30591             | 6.78603   |
| 0.65        | 4.88263   | 3.01364             | 10.15926  |
| 0.70        | 6.82387   | 3.98681             | 15.57839  |
| 0.75        | 9.79298   | 5.38249             | 24.75565  |
| 0.80        | 14.64242  | 7.50679             | 41.52943  |
| 0.85        | 23.40154  | 11.04599            | 76.01473  |
| 0.90        | 42.21432  | 17.93031            | 162.89970 |
| 0.91        | 48.67921  | 20.15188            | 195.86566 |
| 0.92        | 56.82924  | 22.87670            | 239.29845 |
| 0.93        | 67.37410  | 26.29785            | 298.27362 |
| 0.94        | 81.48005  | 30.72425            | 381.51079 |
| 0.95        | 101.20674 | 36.68523            | 505.19792 |
| 0.96        | 130.56693 | 45.17676            | 702.74133 |
| 0.97        | 178.57926 | 58.34607            | 1055      |
| 0.98        | 270.77991 | 81.95900            | 1809      |
| 0.99        | 521.86316 | 139.96682           | 4238      |