

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to the marine diatom *Skeletonema costatum*

PMRA Submission Number: 2001-1027

EPA MRID Number {454050-16}

Data Requirement:

PMRA DATA CODE: marine algae:9.8.3 (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
{PMRA}

Date: March 28/02

Secondary Reviewer(s): Thomas M. Steeger, Ph.D.

Date: June 20, 2002

{EPA} *Thomas M Steeger*

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 MARINE DIATOM (*Skeletonema costatum*). Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601, (410) 822-8600. BASF Study Number: 46664.



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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, the cultures of the marine diatom *Skeletonema costatum* were exposed to BAS 510 F at mean measured concentrations of 0.23, 0.46, 0.99, 1.9 and 3.5 mg a.i/L under static conditions in accordance with the OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II*. The 96-hr NOAEC (cell counts) was 3.5 mg ai/L. The 96-hr EC₅₀ based on cell density was >3.5 mg a.i/L. Little or no inhibition was observed; 16% inhibition was seen at 0.46 mg ai/L, and 15% at 1.9 mg ai/l, after 96 hours. However, at 72 hours, cell counts at these concentrations were actually higher than in the pooled control, and seemed to fluctuate during the course of the study. The highest treatment level caused an increase in algal cell density (10%). Analysis of the data by Dunnett's test indicated no significant differences among treatments and control. After 96 hours, no changes in cell shape or size were noted, however, there was evidence of aggregation of cells and adherence to the test chambers in all control and treatment groups.

This toxicity study is classified as acceptable and satisfies the guideline requirements for an acute algal toxicity study using the marine diatom *Skeletonema costatum*.

Results Synopsis

Test Organism: marine diatom *Skeletonema costatum*

Test Type: Static

96 hr EC₅₀ (cell counts): >3.5 mg ai/L

96 hr NOAEC (cell counts): 3.5 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*

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
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 [test material].

Parameter	Values	Comments
Water solubility at 20°C	4.69 mg/L	low solubility
Vapour pressure	7×10^{-9} mbar @ 20 °C	not volatile
UV absorption	UV molecular extinction: 1.53×10^3 at 290 nm	-
pKa	does not dissociate in water	-
Kow	2.96	Not likely to bioconcentrate

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2. Test organism:

Name: marine diatom *Skeletonema costatum*

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.

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b) Definitive Study

BAS 510 F: Experimental Parameters

Parameter	Details	Remarks ----- Criteria
<u>Acclimation</u> Period: Culturing media and conditions: (same as test or not) Health: (any toxicity observed)	at least two weeks prior to test initiation in test media	----- <i>EPA recommends two week acclimation period.</i> <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>
<u>Test system</u>	Static	-----
Incubation facility	Environmental chamber at 20± 2°C	-----
Duration of the test	96 hr	----- <i>EPA requires: 96 - 120 hours</i> <i>OECD requires: 72 hours</i>
<u>Test vessel</u> Material: (glass/polystyrene) Size: Fill volume:	250-mL Erlenmeyer flasks with a final volume of 100 mL were used	----- <i>OECD recommends 250 ml conical flasks when the volume of the test solution is 100 ml or use a culturing apparatus.</i>

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Parameter	Details	Remarks ----- Criteria
<p><u>Details of growth medium</u></p> <p>Name:</p> <p>pH at test initiation:</p> <p>pH at test termination:</p> <p>Chelator used:</p> <p>Carbon source:</p>	<p>ASTM saltwater algal medium (ASTM Standard Guide 1218-90E)</p> <p>7.9</p> <p>8.4 - 8.9</p> <p>Na₂EDTA (15 mg/L)</p> <p>-</p>	<p>-----</p> <p><i>EPA recommends 20X-AAP medium and no chelators.</i></p> <p><i>OECD recommends the medium pH after equilibration with air be ~8 with less than .001 mmol/l of chelator, if used.</i></p>
<p>If non-standard nutrient medium was used, detailed composition provided (Yes/No)</p>	<p>Yes</p>	<p>-----</p>
<p><u>Dilution water</u></p> <p>Source:</p> <p>Type:</p> <p>pH: 8</p> <p>Salinity: 30 ppt</p> <p>Water pretreatment (if any):</p> <p>Intervals of water quality measurement: once</p>	<p>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 artificial saltwater at a salinity of approximately 30 parts per thousand (‰). The pH was adjusted to 8.0 ± 0.1 using 0.1 N NaOH, and the medium was sterilized by filtration (0.22 μm) prior to use.</p>	<p>-----</p> <p><u>pH:</u> <i>EPA : Skeletonema costatum= ~8.0</i> <i>Others = ~7.5 from beginning to end of the test.</i> <i>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.</i></p> <p><u>salinity:</u> <i>EPA: 30-35 ppt. EPA is against the use of dechlorinated water.</i></p>
<p>Indicate how the test material is added to the medium (added directly or used stock solution)</p>	<p>stock solution was added to test medium</p>	<p>-----</p>
<p>Aeration or agitation</p>	<p>agitated at 100 rpm</p>	<p>-----</p> <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>

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Parameter	Details	Remarks ----- Criteria
Initial cells density	77,000 cells/mL	<p>this cell counts exceeds the recommended level more than 10x</p> <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>
<u>Number of replicates</u> Control: Solvent control: Treated ones:	3 3 3	<p>-----</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>
<u>Test concentrations</u> Nominal: Measured:	0.25, 0.50, 1.0, 2.0 and 4.0 mg/L 0.23, 0.46, 0.99, 1.9 and 3.5 mg/L	<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>

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Parameter	Details	Remarks
		Criteria
Solvent (type, percentage, if used)	dimethyl formamide	
Method and interval of analytical verification: Limit of Detection: Limit of Quantitation:	0 and 96 hr using HPLC with UV detection - 0.12 mg ai/L	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	20.2 - 21.0°C 16-hr light white light, 4310±650 lux (3,710 - 4,140 lux)	<u>Temperature:</u> EPA: <i>Skeletonema</i> : 20°C, Others: 24-25°C; OECD recommended the temperature in the range of 21 to 25°C maintained at ± 2°C <u>Photoperiod:</u> EPA: <i>S. costatum</i> 14 hr light/ 10 hr dark, Others: Continuous; OECD: continuous uniform illumination <u>Light intensity:</u> EPA: <i>Anabaena</i> : 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%); OECD: approximately 8000 Lux measured with a spherical collector
<u>Reference chemical, if used</u> Name: Concentrations:	-	
Other parameters, if any	-	

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2. Observations:

Table 1: Observation

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> <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 12x that at 24 hr.	Cell growth was low, (not 16x higher at test termination vs day 1) ----- <i>EPA requires control cell count at termination to be $\geq 2X$ initial count or by a factor of at least 16 during the test.</i> <i>OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.</i>

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Water quality was acceptable (Yes/No)	yes	-----
Were raw data included?	Yes	-----

II. RESULTS AND DISCUSSION:

A. INHIBITORY EFFECTS:

Little or no inhibition was observed; 16% inhibition was seen at 0.46 mg ai/L, and 15% at 1.9 mg ai/L, after 96 hours. However, at 72 hours, cell counts at these concentrations were actually higher than in the pooled control, and seemed to fluctuate during the course of the study. The highest treatment level resulted in an increase in algal cell density (10%). Analysis of the data by Dunnett's test indicated no significant differences among treatments and control. After 96 hours, no changes in cell shape or size were noted, however, there was evidence of aggregation of cells and adherence to the test chambers in all control and treatment groups.

Table 2: Effect of BAS 510 F on growth of marine diatom *Skeletonema costatum*

Treatment: mean measured concentration (mg a.i./L)	cell count 24 hours (cell/mL)	cell count 48 hours (cell/mL)	cell count 72hours (cell/mL)	96 hours	
				cell count (cell/mL)	% inhibition
Negative Control	206,333	626,667	1,830,000	2,373,333	--
Solvent Control	207,667	560,000	2,145,000	2,593,333	--
Pooled Control	207,000	593,333	1,987,500	2,483,333	--
0.23	196,000	546,667	2,225,000	2,840,000	-14
0.46	191,000	510,000	2,405,000	2,086,667	16
0.99	207,667	555,000	2,205,000	2,786,667	-12
1.9	206,000	500,000	2,180,000	2,100,000	15
3.5	175,333	270,000*	2,235,000	2,740,000	-10

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Table 3: Statistical endpoint values.

Statistical Endpoint	96 hr Cell count/mL
NOEC	3.5 mg a.i./L
EC ₅₀ (95% C.I.)	>3.5 mg a.i./L
IC ₅₀ (mg a.i./L) (95% C.I.)	-
EC ₁₀ (95% C.I.)	>3.5 mg a.i./L
EC ₉₀ (95% C.I.)	>3.5 mg a.i./L
<u>Reference chemical, if used</u>	
NOEC EC ₅₀ (mg/L)	not used

B. REPORTED STATISTICS:

Calculations of cell densities and percent inhibition values, as well as statistical analyses, were conducted using "The SAS System for Windows", Version 8 or TOXSTAT, Version 3.5.

C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Verification was not necessary as no dose response exists and the highest test concentration produced increased cell densities compared to the pooled control.

D. STUDY DEFICIENCIES: The number of cells per mL at study initiation was 77000, which exceeds the recommended level of 10,000 cells/mL.

E. REVIEWER'S COMMENTS: No further comments.

F. CONCLUSIONS: The study is acceptable. The 96-hr EC₅₀ for cell density was > 3.5 mg ai/L. The 96-hr NOAEC was 3.5 mg ai/L.

III. REFERENCES:

Approved 04/01/01 C.K

Statistical Analysis System (SAS) Output follows:

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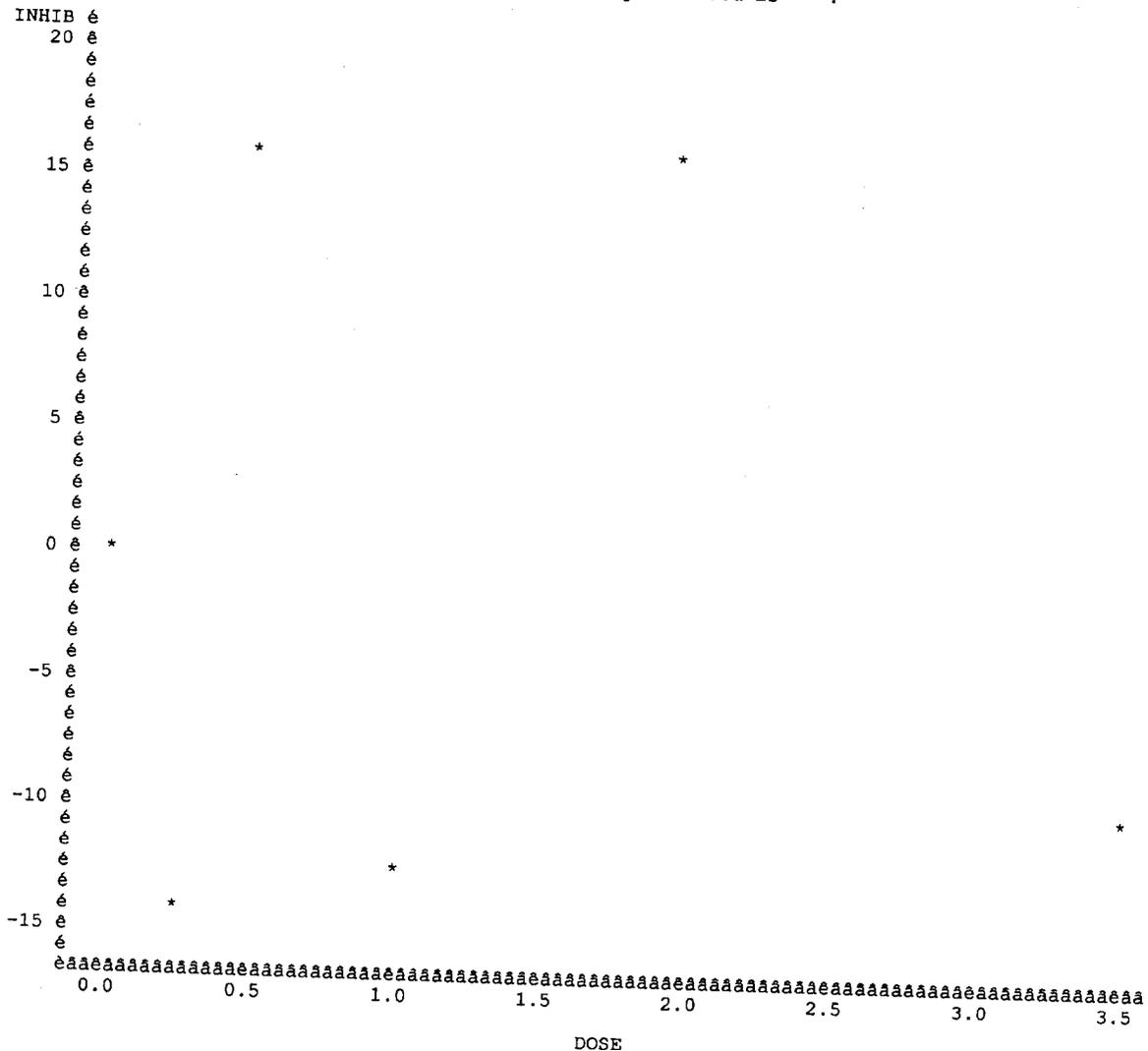
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Obs	DOSE	Average Cell Densities by Treatment		MEAN	SE	11
		TYPE	_FREQ_			
1	0.00	0	6	2483333.33	175530.94	
2	0.23	0	3	2840000.00	292802.55	
3	0.46	0	3	2086666.67	86666.67	
4	0.99	0	3	2786666.67	107289.85	
5	1.90	0	3	2100000.00	72111.03	
6	3.50	0	3	2740000.00	163707.06	

Obs	DOSE	Average Percent Inhibition of <i>Skeletonema</i> Cell Number by BAS 510F		INHIB	SE	12
		TYPE	_FREQ_			
1	0.00	0	6	-0.0000	7.0684	
2	0.23	0	3	-14.3624	11.7907	
3	0.46	0	3	15.9731	3.4899	
4	0.99	0	3	-12.2148	4.3204	
5	1.90	0	3	15.4362	2.9038	
6	3.50	0	3	-10.3356	6.5922	

Plot of Percent Inhibition of *Skeletonema* Cell Number over Increasing Concentrations of BAS 5 13

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



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Analysis of Variance for Percent Inhibition of Skeletonema Cell Number over Dose of BAS 510 14

The ANOVA Procedure

Class Level Information

Class	Levels	Values
DOSE	6	0 0.23 0.46 0.99 1.9 3.5

Number of observations 21

Dependent Variable: PRCNT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	2854.188802	570.837760	3.03	0.0438
Error	15	2829.386823	188.625788		
Corrected Total	20	5683.575625			

R-Square	Coeff Var	Root MSE	PRCNT Mean
0.502182	-1746.882	13.73411	-0.786207

Source	DF	Anova SS	Mean Square	F Value	Pr > F
DOSE	5	2854.188802	570.837760	3.03	0.0438

Dunnnett's t Tests for PRCNT

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

Alpha	0.05
Error Degrees of Freedom	15
Error Mean Square	188.6258
Critical Value of Dunnnett's t	2.86886

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

DOSE Comparison	Difference Between Means	Simultaneous 95% Confidence Limits
0.46 - 0	15.973	-11.888 43.834
1.9 - 0	15.436	-12.425 43.297
3.5 - 0	-10.336	-38.196 17.525
0.99 - 0	-12.215	-40.076 15.646
0.23 - 0	-14.362	-42.223 13.498

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