Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to the freshwater alga *Anabaena flos-aquae*.

PMRA Submission Number: 2001-1027 **EPA MRID Number{454050-15}**

Data Requirement: PMRA DATA CODE: fresh water algae:9.8.2-3 (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'-chlororobiphenyl-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 **Date:** March 27/02
{PMRA}

Secondary Reviewer(s): Thomas M. Steeger, Ph.D **Date:** June 22, 2002
{EPA} Thomas M Steeger

Company Code: BAZ
Active Code: CHH-BAZ-4
Use Site Category: In Canada, this fungicide is proposed for use in 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, Ph.D., Catherine M. Holmes, February, 2001. BAS 510 F: A 96-HOUR TOXICITY TEST WITH THE FRESHWATER ALGA (*Anabaena flos-aquae*) Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601, (410) 822-8600. BASF Study Number: 46667.

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, the cultures of the freshwater alga *Anabaena flos-aquae* were exposed to BAS 510 F at reported mean-measured concentrations of 0.25, 0.53, 0.99, 2.0 and 4.2 mg a.i/L under static conditions in accordance with the OPPTS Number 850.5400: *Algal Toxicity, Tiers I and II*. However, the study failed to accurately measure dissolved test material. Under the conditions tested, the 96- hr NOEC was 4 mg/L (nominal). The 96-hr EC_{50} based on cell density was >4.0 mg/L (nominal). The % growth inhibition (biomass) in the treated algal culture as compared to the control ranged from -9 to 25%. The highest treatment level caused an increase in algal cell density.

This toxicity study is classified as supplemental since exposure concentrations were not properly characterized. However, since it appears that BAS 510F does not adversely affect *Anabaena flos-aquae* at the nominal concentrations tested and because higher concentrations would not likely impact the algae due to the solubility limit of the chemical, EFED is not requesting that the study be repeated.

Results Synopsis

Test Organism: *Anabaena flos-aquae*
Test Type: Static

96 hr EC_{50}: >4.2 mg a.i/L
NOEC: 4.2 mg ai/L

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 powder
Lot No./Batch No. :	N75
Purity:	96.9%
Stability of Compound Under Test Conditions:	Compound was stable, with 97-105% recovery at test termination
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	$7x10^{-9}$ mbar @ 20 °C	not volatile
UV absorption	UV molecular extinction: $1.53x10^3$ at 290 nm	-
pKa	does not dissociate in water	-
Kow	2.96	Not likely to bioconcentrate

2. Test organism:

Name: freshwater alga *Anabaena flos-aquae*

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 capricorntum, 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 mg ai/L.

b) Definitive Study

BAS 510 F: Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation 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	*acceptable* *EPA recommends two week acclimation period.* *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.*

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Parameter	Details	Remarks Criteria
Test system	Static	
Incubation facility	environmental chamber	
Duration of the test	96-hr	acceptable *EPA requires: 96 - 120 hours* *OECD requires: 72 hours*
Test vessel Material: (glass/polystyrene) Size: Fill volume:	250 mL Erlenmeyer flasks with a final volume of 100 mL were used	acceptable OECD recommends 250 ml conical flasks when the volume of the test solution is 100 ml or use a culturing apparatus.
Details of growth medium Name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	Freshwater Algal Medium ASTM medium (ASTM Standard Guide 1218-90E) 7.3 7.4 Na_2EDTA (0.3 mg/L) $NaHCO_3$, 15 mg/L	acceptable *EPA recommends 20X-AAP medium and no chelators.* *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)	Freshwater Algal Medium	

Parameter	Details	Remarks Criteria
Dilution water Source: Type: pH: 7.5 Total Organic Carbon: NA Particulate matter: NA Metals: not detected Pesticides: not detected Chlorine: NA Water pretreatment (if any): 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)	acceptable *pH:* *EPA : Skeletonema costatum= ~8.0* *Others = ~7.5 from beginning to end of the test.* *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.* *salinity:* *EPA: 30-35 ppt. EPA is against the use of dechlorinated water.*
Indicate how the test material is added to the medium (added directly or used stock solution)	stock solution was added to test medium	acceptable
Aeration or agitation	agitated contiuously at 100 rpm	acceptable *EPA recommends agitation only for Selenastrum sp. at 100 cycles per min and Skeletonema sp. at ~60 cycles per min. Aeration is not recommended.*
Initial cells density	10,000 cells/mL	acceptable *EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *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.*
Number of replicates Control: Solvent control: Treated ones:	 3 3 3	acceptable *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.* *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.*

Parameter	Details	Remarks Criteria
Test concentrations Nominal: Measured:	0.25, 0.50, 1.0, 2.0 and 4.0 mg/L 0.25, 0.53, 0.99, 2.0 and 4.2 mg a.i./L	acceptable *EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *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.*
Solvent (type, percentage, if used)	dimethyl formamide, 0.1 mL/L	acceptable
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	
Test conditions Temperature: Photoperiod: Light intensity and quality:	24±2°C Continuous white light, 2354 ± 91.54 (mean ± std. dev.)	acceptable *Temperature:* *EPA: Skeletonema: 20°C,* *Others: 24-25°C;* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C* *Photoperiod:* *EPA: S. costatum 14 hr light/ 10 hr dark,* *Others: Continuous;* *OECD: continuous uniform illumination* *Light intensity:* *EPA: Anabaena: 2.0 Klux (±15%),* *Others: 4 - 5 Klux (±15%);* *OECD: approximately 8000 Lux measured with a spherical collector*
Reference chemical, if used Name: Concentrations:	-	
Other parameters, if any	-	

2. Observations:

Table 1: Observation

Parameters	Details	Remarks *Criteria*
Parameters measured including the growth inhibition/other toxicity symptoms	cell density/mL, morphological effects	acceptable *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.*
Measurement technique for cell density and other end points	hemacytometer and microscope	acceptable *EPA recommends the measurement technique of cell counts or chlorophyll a* *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).*
Observation intervals	24, 48, 72, 96 hr	acceptable *EPA and OECD: every 24 hours.*
Other observations, if any	-	
Indicate whether there was exponential growth in the control	96 hr control cell counts were 17.5x that at 24 hr.	acceptable *EPA requires control cell count at termination to be ≥ 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Water quality was acceptable (Yes/No)	yes	acceptable
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. INHIBITORY EFFECTS:

Algal cell density inhibition ranged from 25% at the lowest treatment level to -9.6% (increase) at the highest concentration.

Table 2: Effect of BAS 510 F on algal growth (freshwater alga *Anabaena flos-aquae*)

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	53,667	135,333	211,667	836,667	--
Solvent Control	43,000	116,667	234,333	855,000	--
Pooled Control	48,333	126,000	223,000	845,833	--
0.25	61,333	113,667	281,333	631,667	25
0.53	37,333	70,000	195,333	945,000	-12
0.99	31,333	94,000	191,000	755,000	11
2.0	36,000	107,333	182,000	855,000	-1.1
4.2	47,667	83,000	234,000	926,667	-9.6

Table 3: Statistical endpoint values.

Statistical Endpoint	96 hr Cell count/mL
NOEC	4.2 mg a.i./L
EC_{50} (95% C.I.)	>4.2 mg a.i./L
IC_{50} (mg a.i./L) (95% C.I.)	-
EC_{10} (95% C.I.)	>4.2 mg a.i./L
EC_{90} (95% C.I.)	>4.2 mg a.i./L
Reference chemical, if used NOEC EC_{50} (mg/L)	not used

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 (3) and "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: Although a white precipitate was observed in the highest test concentration (4 mg/L) and the solution had to be sonicated, there is no discussion as to whether the test solution remained clear. Since the highest test concentration is close to the solubility limit (4.6 mg/L) of the test compound, the presence of a precipitate at 4 mg/L implies that under the conditions used, BAS 510F is sparingly soluble. EFED requires that when solubility is low, water samples must be centrifuged and/or filtered prior to analysis. This study failed to centrifuge and/or filter water samples prior to analysis. Therefore, it is uncertain whether the measured concentrations reported in this study accurately reflect the actual amount dissolved.

E. REVIEWER'S COMMENTS: Mean-measured concentrations may not accurately reflect dissolved chemical concentrations.

F. CONCLUSIONS: The study is supplemental since it failed to accurately measure dissolved test material. The 96-hr EC_{50} for cell density was > 4.0 mg /L (nominal). The NOEC was 4.2 mg/L (nominal). Since it appears that BAS 510F does not adversely affect *Anabaena flos-aquae* at the nominal concentrations tested and because higher concentrations would not likely impact the algae due to the solubility limit of the chemical, EFED is not requesting that the study be repeated.

III. REFERENCES:
Approved 04/01/01 C.K.