Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to aquatic vascular plants *Lemna gibba.*

PMRA Submission Number 2001-1027 **EPA MRID Number{454050-13}**

Data Requirement: PMRA DATA CODE: 9.8.5 (TGAI)
EPA DP Barcode: D278418
OECD Data Point: IIA 8.6.1 (TGAI) and IIIA 10.8.2.1 (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 and Hemendra Mulye **Date:** April 4/02
{PMRA}

Secondary Reviewer(s): Thomas M. Steeger, Ph.D **Date:** June 18, 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, Ph.D., Catherine M. Holmes, February, 2001. BAS 510 F: A 7-DAY TOXICITY TEST WITH DUCKWEED (*Lemna gibba* G3). Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601 (410) 822-8600. BASF Study Number:64272

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EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, the freshwater floating aquatic vascular plant duck weed *Lemna gibba* were exposed to BAS 510 F at measured concentrations of 0.27, 0.50, 0.99, 2.0 and 3.9 mg a.i/L under static conditions in accordance with the U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.4400. The 7-day NOEC (based on frond necrosis) and IC_{50} (based on frond number) were 0.99 and >3.9 mg a.i/L, respectively. The percent frond growth inhibition in the treated culture as compared to the control ranged from 6.7-11% and was not statistically different from pooled controls.

The following abnormalities were noted: chlorosis and necrosis. This toxicity study is classified as acceptable and satisfies the guideline requirement for aquatic vascular plant toxicity study.

Results Synopsis

Test Organism: duck weed *Lemna gibba*
Test Type: Static

7day IC_{50}: > 3.9 mg a.i./L based on frond number
7 day NOEC: 0.99 mg a.i./L based on frond growth
Endpoint(s) Effected: frond growth and necrosis

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

U.S. Environmental Protection Agency Series 850 – Ecological Effects Test Guidelines (draft), OPPTS Number 850.4400: *Aquatic Plant Toxicity Test Using Lemna spp., Tiers I and II*.

COMPLIANCE:

US EPA 40 CFR Part 160, 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: 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	$7x10^{-9}$ 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: duckweed, *Lemna gibba* G3, *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: not stated
Source/Age of inoculum: The original duckweed cultures were obtained from the United States Department of Agriculture and have been maintained in culture medium at Wildlife International Ltd., Easton, Maryland. Duckweed plants used in the test were obtained from Wildlife International Ltd. cultures that had been actively growing in M-Hoagland's culture medium for at least two weeks prior to test initiation.

Method of cultivation: not stated

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study:
A study was conducted with six concentrations ranging from 0.024 to 10 mg ai/L. Inhibition in frond production was 6.9-39%. However, a dose response was not observed. Both the lowest and highest inhibition rates occurred near the middle of the concentration range.

b) Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Culturing media and conditions: (same as test or not) Health: (any toxicity observed)	Duckweed plants were obtained from Wildlife International Ltd. cultures that had been actively growing in M-Hoagland's culture medium for at least two weeks prior to test initiation.	
Test system Static	**static**	**unacceptable** *EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*

Parameter	Details	Remarks *Criteria*
Incubation facility	environmental chamber held at 25 ± 2°C	
Duration of the test	7 days	acceptable *EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel Material: (glass/polystyrene) Size: Fill volume:	250-mL glass beakers covered with disposable petri dishes, and contained 100 mL of test or control medium.	
Details of growth medium Name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	M-Hoagland's medium without EDTA 4.8 5.8 None tartaric acid	acceptable *EPA recommend the following culture media: Modified Hoagland's E+ or 20X-AAP. Chelators are not recommended.*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	-	
Dilution water Source/type: purified well water pH: 5.0 Total Organic Carbon: not stated Particulate matter: not stated Metals: not detected Pesticides: not detected Chlorine: not stated Water pretreatment (if any): Intervals of water quality measurement	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). The pH was adjusted to 5.0 ± 0.1 using 0.1 N NaOH and the medium was sterilized by autoclaving at approximately 121 C prior to use. Sampled at test initiation	acceptable *EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*

Parameter	Details	Remarks *Criteria*
Indicate how the test material is added to the medium (added directly or used stock solution)	stock solution was mixed into medium	
Aeration or agitation	not stated	
Sediment used **(for rooted aquatic vascular plants)** Origin: Textural classification (% sand, silt and clay): Organic carbon (%): Geographic location:	not used	
Number of replicates Control: Solvent control: Treatments:	3 3 3	
Number of plants/replicate	5	acceptable *EPA requires 5 plants.*
Number of fronds/plant	15	acceptable *EPA requires 3 fronds per plant.*
Test concentrations Nominal: Measured:	0.25, 0.50, 1.0, 2.0 and 4.0 mg ai/L 0.27, 0.50, 0.99, 2.0 and 3.9 mg ai/L	acceptable *EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	dimethylformamide at 0.1 ml/L	

Parameter	Details	Remarks *Criteria*
Method and interval of analytical verification: Limit of Quantitation: Limit of Detection:	study initiation and end 0.12 mg ai/L (based on the product of the lowest calibration standard (0.0600 mg a.i./L) and the dilution factor of the matrix blank samples (2.00).	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 24.7-25.4 °C continuous warm-white fluorescent lighting at 5000 lux.	acceptable *EPA temperature: 25°C* *EPA photoperiod: continuous* *EPA light: 5.0 Klux (±15%)*
Reference chemical (if used) Name: Concentrations:	not used	
Other parameters, if any	-	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks *Criteria*
Parameters measured	number of fronds, chlorosis, root damage, dead fronds, necrosis, and break-up of duckweed colonies.	
Measurement technique for frond number and other end points	fronds were counted at day 7	
Observation intervals	day 3, 5, 7	
Other observations, if any	-	

Indicate whether there was an exponential growth in the control	frond numbers at day 7 were 8.5x that at day 0 in both controls.	
Water quality was acceptable (Yes/No)	yes	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. INHIBITORY EFFECTS:

Percent inhibition of frond growth in the 0.27, 0.50, 0.99, 2.0 and 3.9 mg a.i./L treatment groups at test termination was 6.7, 9.5, 9.5, 11 and 11%, respectively. Inhibition of frond growth was not statistically significant (p >0.05) at any treatment level when compared to the pooled controls; therefore, the reduction [inhibition] in frond growth was not considered treatment-related. The mean percent chlorosis was <1% in any treatment group. However, the percentage of necrotic fronds was markedly higher in the 2.0 and 3.9 mg a.i./L treatment groups during the test than in the other treatment or control groups (38 and 59%, respectively, on Day 7). While the numbers of necrotic fronds in the 0.99 mg a.i./L treatment group on Day 5 and 7 appeared slightly higher than at lower concentrations, the necrosis was evident in only a small percentage of the population (mean percent necrosis of 4.7 and 2.4% on Days 5 and 7, respectively) and was not considered to be treatment-related.

[Briefly describe the phytotoxic inhibition including the effect on frond numbers, dry weight, growth rate, dose response relationship. Compare with reference standard, if used]

Describe other effects - Any change in frond development or appearance (increase or decrease in size, necrosis, chlorosis, sedimentation of test solutions, sinking of fronds, other abnormalities. There was or was not a major change in pH during the study. If there was no observed toxicity, state "There were no compound related phytotoxic effects."]

Table 3: Effect of BAS 510 F on frond number and necrosis of *Lemna gibba.*

Treatment (measured mg a.i./L)	Initial frond number	7days		
		frond number	frond % inhibition	necrotic fronds
Negative control	15	127	-	0
Solvent control (if used)	15	128	-	0
0.27	15	119	6.7	0
0.50	15	115	9.5	0
0.99	15	115	9.5	2.4
2.0	15	113	11	38
3.9	15	114	11	59

[If more than one endpoint parameter was measured, use a different table for other major endpoints.]

Table 4: Statistical endpoint values.

Statistical Endpoint	frond No.	growth rate	necrosis
NOEC (mg a.i./L)	-	-	0.99
LOEC (mg a.i./L)	-	-	2.0
IC_{50} (mg a.i./L)	> 3.9	-	-
other (IC_{25}/EC_{25})	-	-	-
Reference chemical NOEC IC_{50}/EC_{50}	-	-	-

B. REPORTED STATISTICS:

Statistical analyses were conducted using "TOXSTAT Version 3.5". A Student's t-test was used to determine any statistically significant differences ($p = 0.05$) in frond numbers between the negative and solvent control groups at test termination. The analysis showed no significant difference ($p > 0.05$) in frond numbers in the negative and solvent control. Therefore evaluation of the treatment groups was conducted relative to the pooled control replicates. The data were evaluated for normality and homogeneity of variances ($p = 0.05$) using the Shapiro-Wilks' and Levene's tests, respectively. Since

the data were normally distributed and the variances were homogeneous, statistically significant differences between the pooled control and the treatment groups were identified using analysis of variance (ANOVA) and Bonferroni's t-test. Results of the statistical analyses, as well as an evaluation of the concentration-response pattern and other observations of effects, were used in the determination of the no-observed-adverse-effect-concentration (NOAEC).

C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER: Not applicable as the IC_{50} exceeds the highest concentration used. The NOEC is acceptable to the reviewer based on evaluation of the raw data.

D. STUDY DEFICIENCIES: The test system used was a static one, whereas EPA 850.4400 recommends using a static renewal test to ensure nutrient availability and constant toxicant exposure. The analytical results indicate that the test material was stable during the 7 day exposure, with a recovery of 99-109% of nominal on day 7.

E. REVIEWER'S COMMENTS: Measured concentrations ranged from 93.8 to 105% of nominal on Day 0 and from 99.7 to 109% of nominal of Day 7.

F. CONCLUSIONS: This study is acceptable. Necrosis was more sensitive than frond growth as an endpoint.

IC_{50} > 3.9 mg ai/L (frond number) NOEC (necrosis): 0.99 mg ai/L

III. REFERENCES:

Approved 04/01/01 C.K.