

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

Data Requirement:

PMRA DATA CODE: 9.5.2.4
EPA DP Barcode: D278418
OECD Data Point: IIA 8.2.1 and IIA 8.2.1.2
EPA Guideline: 72-3

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 Hemendra Mulye
{PMRA}

Date: May 13/02

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

Date: May 28, 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 (0.78 lbs a.i./Acre/application).

EPA PC Code: 128008

CITATION: Robert L. Boeri, Derek C. Wyskiel, Timothy J. Ward, Catherine M. Holmes. 2000. Flow-Through Acute Toxicity of BAS 510 F to the Sheepshead Minnow, *Cyprinodon variegatus*. T.R. Wilbury Laboratories, Inc. 40 Doaks Lane Marblehead, Massachusetts 01945. BASF Study Number 46668.



2013370

6

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, sheepshead minnow, (*Cyprinodon variegatus*) were exposed to BAS 510 F at nominal concentrations of 0 mg/L (control and solvent control), 0.52, 0.86, 1.4, 2.4, and 4.0 mg/L, under flow-through conditions. The 96-h LC_{50} was > 3.86 mg a.i./L (mean measured). Mortality and sublethal effects, including loss of equilibrium and lethargy were observed at the highest test concentration, thus the 96 hr NOEC was 2.33 mg ai/L. Based on the results of this study, BAS 510 F would be classified as moderately toxic to sheepshead minnow in accordance with the classification system of the U.S. EPA.

This toxicity study is classified as acceptable and satisfies the guideline requirement for acute estuarine/marine fish toxicity study.

Results Synopsis

Test Organism Size/Age(mean wet weight or length): 0.3 g

Test Type (Flowthrough, Static, Static Renewal): Flow-through

96 hr LC_{50} : > 3.86 mg a..i./L (solubility limit of test chemical)

96 hr NOEC (sublethal effects): 2.33 mg ai/L

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAD) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

OPPTS. 1996. U.S. EPA Prevention, Pesticides, and Toxic Substances. Ecological Effects Test Guidelines. OPPTS 850.1075. Fish Acute Toxicity Test, Freshwater and Marine. Public Draft. EPA 712-C-96-118. April 1996.

Protocol Deviations

The following deviations from the protocol occurred during the study:

1. Analytical samples were not collected in duplicate (due to the stability of the test substance and because initial sample volumes provided adequate volumes for re-analysis if required).
2. Acetone was used as the solvent rather than dimethylformamide.
3. Not all diluter flow splitter delivery volumes were within 10% between replicate test vessels (all splitter delivery volumes were within 13% between replicate test vessels).
4. The nominal value of the second lowest tested concentration was 0.86 mg/L rather than 0.88 mg/L.
5. To improve the analytical method, the analytical injection volume and run time were not as stated in the protocol.
6. Average length and weight were reported at test conclusion and not at test initiation. Average fish weight (0.3 gm) is less than recommended range of 0.5 to 5 gms.

These deviations did not adversely affect the outcome of the study and no other deviations from the protocol occurred.

COMPLIANCE:

With the exceptions noted, this study was conducted according to EPA Good Laboratory Practice Regulations (40 CFR 160).

A. MATERIALS:

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.

1. Test Material

BAS 510 F

Description:	Solid
Lot No./Batch No. :	N75
Purity:	96.9%
Stability of Compound	
Under Test Conditions:	stable

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

**Storage conditions of
test chemicals:**

stored at room temperature

Physicochemical properties of BAS 510 F (Nicobifen).

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	-
Log Kow	2.96	Some potential for bioaccumulation
photolysis	does not undergo photolysis	-
DT50 (water/sediment system)	342 day	Very persistent in the aquatic environment, partitions into sediment

2. Test organism:

Species: Sheepshead Minnow, *Cyprinodon variegatus*

Age at test initiation: juvenile

Weight at study initiation: 0.3 g (average weight at test conclusion)

(EPA requires: mean 0.5 - 5 g)

Length at study initiation: 2.73 cm (average length at test conclusion)

(EPA requires: Longest not > 2x shortest; OECD requires 2.0 ± 1.0 cm for bluegill and 5.0 ± 1.0 cm for rainbow trout)

Source: commercial supplier (Aquatic BioSystems, Fort Collins, Colorado)

B. STUDY DESIGN:

1. Experimental Conditions

b) Definitive Study

Table 1 . Experimental Parameters

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

Parameter	Details	Remarks ----- Criteria
<u>Acclimation:</u> Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	14 days similar to test conditions ad lib food during acclimation healthy	acceptable ----- (EPA requires minimum 14 days; no feeding during test; OECD requires minimum of 12 days)
Duration of the test	96 hr	acceptable ----- (EPA/OECD require 96 hour)
<u>Test condition:</u> Type of dilution system- for flow through method Flow rate	flow-through proportional diluter 6.6 volumes/24hr calibrated before study and checked twice daily	acceptable ----- (EPA requires: must provide reproducible supply of toxicant) (EPA requires: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)
Aeration, if any	None	acceptable ----- (EPA requires: no aeration; OECD permits aeration)
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Glass aquaria 20 L 15 L	acceptable ----- (EPA requires: size 19 L (5 gal) or 30 x 60 x 30 cm Fill volume: 15-30 L of solution)

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheephead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

Parameter	Details	Remarks ----- Criteria
Source of dilution water	Water used for acclimation of test organisms and for all toxicity testing was carbon filtered, natural seawater collected at T.R. Wilbury Laboratories in Marblehead, Massachusetts. Water was adjusted to a salinity of 15 to 17 parts per thousand with deionized water and stored in polyethylene tanks where it was aerated and recirculated through a 5 µm particle filter, activated carbon, and an ultraviolet sterilizer.	acceptable ----- (EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water); OECD permits dechlorinated tap water)
<u>Water parameters:</u> salinity pH Dissolved oxygen Total organic carbon Particulate matter Metals Pesticides Temperature Intervals of water quality measurement	15-17 ppt 7.7-8.0 7.3-8.4 mg/L 1.2 mg/L not reported OK ND 21.1 - 21.9 °C Recorded daily	acceptable ----- <u>(Hardness</u> EPA : 40 - 48 mg as CaCO ₃ /L OECD: 10 -250 mg as CaCO ₃ /L <u>pH</u> (EPA: 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8) OECD: 6.0 - 8.5 <u>Dissolved Oxygen</u> EPA: Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%) OECD: at least 80% saturation value. <u>Temperature:</u> EPA: estuarine/marine: 22 ± 1 °C OECD: 21 - 25°C for bluegill and 13 - 17°C for rainbow trout <u>Salinity</u> EPA: 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰) (EPA water quality: measured at beginning of test and every 48 hours)
<u>Number of replicates/groups:</u> Control (dilution water) solvent control Treatments:	2 2 2	acceptable ----- (EPA/OECD requires: Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series)

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAD) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

Parameter	Details	Remarks ----- Criteria
<u>Number of organisms per replicate /groups:</u>		acceptable
Control (dilution water):	10	
solvent control	10	
Treatments:	10	(EPA: $\geq 10/\text{concentration}$); OECD requires at least 7 fish/concentration)

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

Parameter	Details	Remarks
		Criteria
Biomass loading rate	0.22 g/L	acceptable <i>(EPA: static: ≤ 0.8 g/L at $\leq 17^{\circ}\text{C}$, ≤ 0.5 g/L at $> 17^{\circ}\text{C}$; flow-through: ≤ 1 g/L/day; OECD requires: maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through)</i>
<u>Test concentrations:</u> Nominal: Measured:	0 mg/L (control and solvent control), 0.52, 0.86, 1.4, 2.4, and 4.0 mg/L. 0, 0.490, 0.835, 1.39, 2.33, and 3.86 mg/L.	acceptable
Solvent (type, percentage, if used)	acetone (0.5 mL/L) an increased solvent concentration was used to facilitate the dissolution of the test material, which, at high concentrations tended to precipitate on the water surface.	acceptable <i>(EPA requires: not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD requires solvent not exceed 100 mg/L)</i>
Lighting	16 hrs light / 8 hrs dark	acceptable <i>(EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod)</i>
Feeding	none during study and 48 hrs prior to study	acceptable <i>(EPA/OECD requires: no feeding during the study)</i>
<u>Recovery of chemical:</u> Frequency of determination Level of Detection Level of Quantitation	 at start and at finish of test not stated 0.1 mg/L	acceptable
Positive control {if used, indicate the chemical and concentrations}	none	
Other parameters, if any	-	

2. Observations:

Table 2: Observations

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

Parameter	Details	Remarks Criteria
Parameters measured including the sublethal effects/toxicity symptoms	mortality and behavioural effects such as lethargy and loss of equilibrium	
Observation intervals	24, 48, 72 and 96 hours after start of exposure.	acceptable <i>(EPA/OECD requires: minimally every 24 hours)</i>
Water quality was acceptable (Yes/No)	yes	
Were raw data included?	Yes	
Other observations, if any	-	

II. RESULTS and DISCUSSION:

A. MORTALITY:

No control mortality occurred in either of the two controls. One fish died in the 1.39 mg ai/L exposure, after 48 hours, this was thought to be not treatment related as there was no mortality at the next higher treatment level. At the highest treatment group, one fish died at 24 hr and a second at 96 hr (10% mortality).

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

Table 2: Effect of BAS 510 F on mortality of sheepshead minnow.

Treatment (mg a.i./L) [mean measured]	No. of fish at start of study	Observation period					
		24 hr		48 hr		96 hr	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Solvent control	20	0	0	0	0	0	0
Control (dilution water only)	20	0	0	0	0	0	0
0.19	20	0	0	0	0	0	0
0.835	20	0	0	0	0	0	0
1.39	20	1	5	1	5	1	5
2.33	20	0	0	0	0	0	0
3.86	20	1	5	1	5	2	10
NOEC	2.33 mg ai/L						
LC ₅₀	>3.86 mg ai/L						

B. NON-LETHAL TOXICITY ENDPOINTS:

Sublethal effects were detected at the highest treatment level of 3.86 mg ai/L. The maximum number of fish affected at any time during the test was 3/20 (15 %). Affected fish exhibited one or both of the following sublethal effects: loss of equilibrium, lethargy.

C. REPORTED STATISTICS:

Results of the toxicity test could not be interpreted by standard statistical techniques (Stephan, 1983) at 24, 48, 72, or 96 hours because there was greater than 50% survival at all tested concentrations. The no observed effect concentration is the highest concentration of test substance not to cause toxicant-related mortality or sublethal effects.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Not required due to lack of mortality.

E. STUDY DEFICIENCIES: The concentration of solvent used (0.5 mL/L) exceeded the EPA recommended level of 0.1 mL/L, however, this adjustment was necessary in order to solubilize the test material. This level of acetone in test vessels did not result in any mortality or sublethal effects.

Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to sheepshead minnow, (*Cyprinodon variegatus*).

PMRA Submission Number 2001-1027

EPA MRID Number {454050-04}

F. REVIEWER'S COMMENTS:

Insoluble material observed as a small amount of white particulate matter settled on the bottom of the vessels (increasing with increasing test substance concentration) was noted in test vessels with means measured concentrations of 0.49 and 0.835 mg/L at 72 and 96 hours, in test vessels with a mean measured concentration of 1.39 mg/L at 48, 72, and 96 hours, and in test vessels with mean measured concentrations of 2.33 and 3.86 mg/L at 24, 48, 72, and 96 hours. Samples for analysis were prepared in triplicate and were analyzed both with and without filtration through a 0.22 μ m filter. Mean measured concentrations for the 0.5 mg/L, 3.0 mg/L, and 8.0 mg/L unfiltered samples were 0.498 mg/L, 2.98 mg/L, and 7.74 mg/L, respectively. Mean measured concentrations for the filtered samples were 0.496 mg/L, 2.92 mg/L and 6.94 mg/L, respectively. These results suggest that unfiltered samples gave a misleading impression of the amount in solution as concentrations increased and emphasizes the need for filtration prior to chemical analysis when a precipitate is observed. This study was well designed and followed the EPA guidelines as stated.

G. CONCLUSIONS: The study is acceptable. The 96-hr LC_{50} was estimated to be greater than the high concentration of 3.86 mg ai/L. The 96-hr NOEC based on both mortality and sublethal effects (lethargy, loss of equilibrium) was 2.33 mg ai/L.

III. REFERENCES:

Approved 04/01/01 C.K.