

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyaella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

Data Requirement: PMRA DATA CODE: 9.3.4-1
EPA DP Barcode: D278418
OECD Data Point:
EPA Guideline: nonguideline

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, Regi Mathew.
{PMRA}

Date: February 1/2002

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

Date: April 25, 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: Catherine Holmes, John Aufderheide, Tom Leak, February, 2001. Acute Toxicity of BAS 510 F in Whole Sediment to the Amphipod, *Hyaella azteca*. ABC Laboratories, Inc., 7200 E. ABC Lane Columbia, Missouri 65202. ABC Study No. 46319.



2013373

1

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

EXECUTIVE SUMMARY:

The 240-hr acute toxicity of BAS 510 F to *Hyalella azteca* was studied under static conditions. Test organisms were exposed to control, solvent control, and, test chemical at nominal concentration of 6.37, 12.5, 25.8, 49.5, and 97.0 mg active ingredient (a.i.)/kilogram of dry sediment. Mortality was observed at study initiation and completion. The 10-day EC_{50} was >97 mg a.i./kg dry sediment. The 10-day NOEC based on mortality was 97 mg a.i./kg dry sediment. The 10-day NOEC based on dry weights is 26 mg ai./kg dry weight.

Based on the results of this study, BAS 510 F would be classified as practically non-toxic to *Hyalella azteca* in accordance with the classification system of the U. S. EPA.

This study is scientifically sound. As a nonguideline study, it is classified as supplemental and provides useful information for characterizing acute risk to sediment dwelling freshwater invertebrates.

Results Synopsis

Test Organism Age (eg. 1st instar): 7 days

Test Type: Static

EC_{50} : >97 mg a.i./kg dry sediment

NOEL: 97 mg a.i./kg dry sediment

Endpoint(s) Effected: Mortality

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

U.S. EPA Office of Pesticides, Prevention and Toxic Substances (OPPTS) Draft Guideline 850.1735.

COMPLIANCE:

This study was conducted in compliance with the US EPA Good Laboratory Practice Regulations.

A. MATERIALS:

1. Test Material

BAS 510 F

Description:	white powder
Lot No./Batch No. :	N75
Purity:	96.9%
Stability of Compound Under Test Conditions:	expires August 29/2002.
Storage conditions of test chemicals:	room temperature
Radiolabelled tracer:	¹⁴ C Diphenyl-U-BAS 510 was used along with the test material as a tracer for the analysis of sediment and water samples. Radio purity was 98-99%, with specific activity of 6.27 Mbq/mg (376000 dpm/mg)

Physicochemical properties of BAS 510 F

Parameter	Values	Comments
Water solubility at 20°C	4.69 mg/L	very insoluble
Vapour pressure	7x10 ⁻⁹ mbar @ 20 °C	non-volatile
UV absorption	UV molecular extinction: 1.53x10 ³ at 290 nm	-
pKa	does not dissociate in water	not affected by pH
Log Kow	2.96	Not likely to bioconcentrate, however, it is a borderline case.

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

2. Test organism:

Species: *Hyalella azteca*

Age at test initiation: 7 days

Source: collected from in house culture

B. STUDY DESIGN:

1. Experimental Conditions

a) Range Finding Test:

A range-finding test was performed from October 24 to November 3, 2000. The targeted nominal concentrations were 0.00 (control), 0.01, 0.1, 1.00, 10.0, and 100 mg active ingredient (a.i.)/kg dry sediment. Duplicate test chambers were prepared for each treatment on October 23, 2000. The test organisms were added the next day and the test was terminated after 10-days of exposure. The survival of the test organisms was recorded from the range-finding test and used to set the definitive nominal concentrations.

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Details	Remarks
		Criteria
<u>Acclimation:</u> Period: Conditions: Feeding: Health:	organisms were added to the test chambers after a day of acclimation; conditions were similar to test conditions. Test organisms fed an invertebrate food suspension daily during course of study.	
Duration of the test	10 days	
<u>Test condition:</u> Static/flow through	Static	

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

Parameter	Details	Remarks ----- Criteria
Aeration, if any	Gentle aeration	
<u>Test vessel:</u> Material: Size: Fill volume:	Glass beakers 1L 190 g sediment and 600 mL water	
Source of dilution water and sediment	The test water was laboratory blended freshwater that was biologically aged and passed through a UV sterilizer and polypropylene cartridge filters and demineralized by reverse osmosis. The artificial sediment used for the test was prepared by mixing the following ratio of constituents: 70% fine industrial sand, 20% kaolinite clay, and 10% sphagnum peat. The peat moss was sieved to a finely ground consistency and did not contain any visible plant remains. Calcium carbonate, CaCO₃, was added to the artificial sediment to adjust the pH to 5.87.	
<u>Water parameters:</u> Hardness: pH: Dissolved oxygen: Temperature: Pesticides: Intervals of water quality measurement	142-154 mg/L CaCO ₃ 6.37 - 8.47 4.7mg/L to 8.2 mg/L 22.1 - 23.8°C not detected daily	
<u>Number of replicates:</u> Control (dilution water): Solvent control: Treatments:	 8 8 8	

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

Parameter	Details	Remarks
		Criteria
<u>Number of organisms per replicate:</u>		
Control	10	
Solvent control:	10	
Treatments:	10	
<u>Treatment concentrations:</u>	Nominal: 6.54, 13.1, 26.0, 50.9, and 99.7 mg active ingredient (a.i.)/kilogram of dry sediment. Fine industrial sang (150 g) treated with appropriate stock solutions and acetone carrier evaporated to dryness. Dried artificial sediment (1493 g) mixed with dosed medium (sand) and 632 ml of blended freshwater added. Afterwards, 190 g of spiked sediment added to each treatment replicate. Mean measured sediment concentrations were: 6.37, 12.5, 25.8, 49.5, and 97.0 mg 14C-equivalents (eq.) per kilogram of dry sediment.	
Solvent (type, percentage, if used)	acetone was evaporated in fume hood	
Lighting	day:night-rhythm 16:8 h, 411.4 to 475.5 lux	
<u>Recovery of chemical:</u>		
Frequency of determination:	beginning and end of study	
Level of Quantitation:	0.0103 mg ai/L	
Level of Detection:	not stated, generally 1/10 of LOQ	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks
		Criteria
Parameters measured including the sublethal effects	Survival at termination of test Dry weight of surviving organisms	

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

Observation intervals	test initiation and termination	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Survival of amphipods ranged from 79-99%. The survival in the vehicle control (94%) was equal to the survival rate at the highest test concentration. The second highest concentration resulted in significantly lower survival compared to the controls.

Table 3: Effect of BAS 510 F on mortality of *Hyalella azteca*.

Treatment (mg a.i./L) [mean measured]	No. of organisms	Observation period	
		Day 10 (test termination)	
		No Dead	% mortality
Control	80	4	5
Solvent control	80	5	6
6.37	80	7	9
12.5	80	1	1.25
25.8	80	7	9
49.5	80	17	19*
97.0	80	5	6
NOEC	97 mg ai/kg of dry sediment		
EC ₅₀	> 97 mg ai/kg of dry sediment		

* Statistically significant vs. controls

C. REPORTED STATISTICS:

The no-observed-effect concentrations (NOEC) based on survival at day 10 were analyzed using contingency table methods. Pairwise comparisons between the control and each of the test concentrations were performed using a frequency analysis based on a simple chi-square statistic estimated from the two-way contingency table. The growth data collected on day 10 were analyzed with a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test. Prior to the means comparison tests, a Shapiro-Wilk's test (8) and a Levene's test (9) were conducted to test for normality and homogeneity of variance, respectively. The survival data did not meet the criteria for normality or homogeneity of variance. Therefore a non-parametric

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

analysis of these data was performed on the ranks of these values. The EC_{50} could not be calculated due to lack of mortality.

Dry biomass of the control animals was determined by Dunnett's test ($\alpha = 0.05$) to be statistically reduced compared to the average dry biomass of the vehicle control animals. Therefore, statistical analyses of the treatment values were performed against the individual dry biomass of the vehicle control animals. Treated animals exposed to 6.37, 12.5, 25.8 mg ^{14}C -eq/kg dry sediment were determined by Dunnett's test ($\alpha = 0.05$) to be significantly less than the vehicle control dry weights. Therefore, based on dry biomass (weight), the NOEC is 97 mg a.i./kg

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

The data were not re-analyzed by the reviewer based on the lack of mortality in the treatment groups. Although the second highest concentration caused significant mortality (19%) this was judged not to be treatment related as there was no dose response noted and the highest treatment concentration resulted in identical survival rates compared to the controls.

E. STUDY DEFICIENCIES:

Draft guideline 850.1735 recommends that there should be no aeration unless the dissolved oxygen drops below 40% saturation. Renewal or flow-through of overlying water is recommended during the test. Composition of feed is not provided in report; feeding rate is not listed in report.

F. REVIEWER'S COMMENTS: Interstitial water concentrations on Day 0: 0.0814, 0.171, 0.366, 0.785, and 1.18 mg ^{14}C -eq/L. Interstitial water concentration on Day 10: 0.049, 0.101, 0.229, 0.543, and 0.952 mg ^{14}C -eq/L. Mean (10-day) interstitial water concentrations are: 0.065, 0.136, 0.298, 0.664, and 1.07 ^{14}C -eq/L. Bound mean-sediment concentrations were 6.37, 12.5, 25.8, 49.5, and 97.0 mg ^{14}C -eq/kg of dry sediment.

Figure 1 illustrates dry weight treatment means. The analysis conducted by the study's authors reports that since the control means were significantly different from the solvent control, the two groups were not pooled and comparisons were made relative to the vehicle control. What is unclear is how treatments ranging from 6.37 to 25.8 mg/kg sediment were relatively consistent with the control; however, they were statistically different than the vehicle control. If statistical comparisons are made relative to the control, only the two highest treatment groups, *i.e.*, 49.5 and 97.0 mg a.i./kg sediment are significantly different and exhibit a dose responses. The graph suggests that the vehicle control animal dry weights were unusually high; however, control animals and those exposed to concentrations less than 49.5 mg ai.L were unaffected.

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

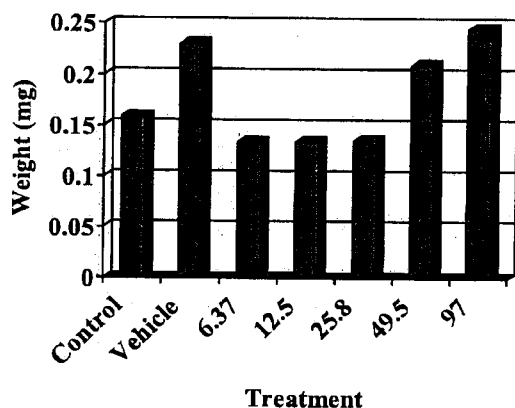


Figure 1 Mean dry weight of *Hyalella azteca* exposed to BAS 510 F in sediment.

since *Hyalella* dry weight in the three lowest test concentrations were significantly lower than the vehicle control.

G. CONCLUSIONS: This study is acceptable. Based on the EC_{50} of >97 mg ai/kg of dry sediment (> 1.07 mg a.i./L interstitial water), the test chemical is practically non-toxic to *Hyalella azteca*. When compared against the vehicle control and contingent upon a positive dose response curve, the NOEC would appear to be the highest dose tested, i.e., 97 mg ai/kg of dry sediment. However, when compared against the control, *Hyalella* dry weights were significantly different at the highest test concentrations and the NOEC is 12.5 mg a.i./L. Based on a significant effect related to dose and comparing against the solvent control, the study fails to establish an NOEC

III. REFERENCES:

Approved 04/01/01 C. K.

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

TITLE: Dry Weights *Hyalella azteca* to BAS 510F
 FILE: c:\hyalella
 TRANSFORM: NO TRANSFORM
 NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.1700	0.1700
1	Control	2	0.1300	0.1300
1	Control	3	0.1500	0.1500
1	Control	4	0.1900	0.1900
1	Control	5	0.2000	0.2000
1	Control	6	0.1220	0.1220
1	Control	7	0.1700	0.1700
1	Control	8	0.1380	0.1380
2	Vehicle	1	0.2000	0.2000
2	Vehicle	2	0.2750	0.2750
2	Vehicle	3	0.3110	0.3110
2	Vehicle	4	0.2560	0.2560
2	Vehicle	5	0.1500	0.1500
2	Vehicle	6	0.1200	0.1200
2	Vehicle	7	0.2560	0.2560
2	Vehicle	8	0.2700	0.2700
3	6.37	1	0.1330	0.1330
3	6.37	2	0.1110	0.1110
3	6.37	3	0.1220	0.1220
3	6.37	4	0.0900	0.0900
3	6.37	5	0.1400	0.1400
3	6.37	6	0.1400	0.1400
3	6.37	7	0.1880	0.1880
3	6.37	8	0.1500	0.1500
4	12.5	1	0.0890	0.0890
4	12.5	2	0.1400	0.1400
4	12.5	3	0.1200	0.1200
4	12.5	4	0.1400	0.1400
4	12.5	5	0.1400	0.1400
4	12.5	6	0.1100	0.1100
4	12.5	7	0.1600	0.1600
4	12.5	8	0.1700	0.1700
5	25.8	1	0.0890	0.0890
5	25.8	2	0.1330	0.1330
5	25.8	3	0.1400	0.1400
5	25.8	4	0.1670	0.1670
5	25.8	5	0.1670	0.1670
5	25.8	6	0.1220	0.1220
5	25.8	7	0.1000	0.1000
5	25.8	8	0.1600	0.1600
6	49.5	1	0.2000	0.2000
6	49.5	2	0.1710	0.1710
6	49.5	3	0.1110	0.1110
6	49.5	4	0.2570	0.2570
6	49.5	5	0.3000	0.3000
6	49.5	6	0.2500	0.2500
6	49.5	7	0.1500	0.1500
6	49.5	8	0.2330	0.2330

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

7	97.0	1	0.2000	0.2000
7	97.0	2	0.2560	0.2560
7	97.0	3	0.3200	0.3200
7	97.0	4	0.2330	0.2330
7	97.0	5	0.1800	0.1800
7	97.0	6	0.2560	0.2560
7	97.0	7	0.2110	0.2110
7	97.0	8	0.3000	0.3000

Dry Weights *Hyalella azteca* to BAS 510F
File: c:\hyalella Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	0.122	0.200	0.159
2	Vehicle	8	0.120	0.311	0.230
3	6.37	8	0.090	0.188	0.134
4	12.5	8	0.089	0.170	0.134
5	25.8	8	0.089	0.167	0.135
6	49.5	8	0.111	0.300	0.209
7	97.0	8	0.180	0.320	0.245

Dry Weights *Hyalella azteca* to BAS 510F
File: c:\hyalella Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.001	0.028	0.010
2	Vehicle	0.004	0.066	0.024
3	6.37	0.001	0.029	0.010
4	12.5	0.001	0.026	0.009
5	25.8	0.001	0.030	0.011
6	49.5	0.004	0.063	0.022
7	97.0	0.002	0.048	0.017

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

Dry Weights *Hyalella azteca* to BAS 510F
File: c:\hyalella Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.113	0.019	9.500
Within (Error)	49	0.097	0.002	
Total	55	0.211		

Critical F value = 2.34 (0.05,6,40)
Since F > Critical F REJECT Ho:All groups equal

Dry Weights *Hyalella azteca* to BAS 510F
File: c:\hyalella Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	0.159	0.159		
2	Vehicle	0.230	0.230	-3.175	
3	6.37	0.134	0.134	1.096	
4	12.5	0.134	0.134	1.124	
5	25.8	0.135	0.135	1.073	
6	49.5	0.209	0.209	-2.247	
7	97.0	0.245	0.245	-3.835	

Dunnett table value = 2.37 (1 Tailed Value, P=0.05, df=40,6)

Dry Weights *Hyalella azteca* to BAS 510F
File: c:\hyalella Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	8			
2	Vehicle	8	0.053	33.4	-0.071
3	6.37	8	0.053	33.4	0.025
4	12.5	8	0.053	33.4	0.025
5	25.8	8	0.053	33.4	0.024
6	49.5	8	0.053	33.4	-0.050
7	97.0	8	0.053	33.4	-0.086

Data Evaluation Report on the acute toxicity of BAS 510 F in whole sediment to the freshwater amphipod, *Hyalella azteca*

PMRA Submission Number 2001-1027

EPA MRID Number {454050-09}

Dry Weights *Hyalella azteca* to BAS 510F
File: c:\hyalella Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)			TABLE 1 OF 2		
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	8	0.159	0.159	0.158
2	Vehicle	8	0.230	0.230	0.158
3	6.37	8	0.134	0.134	0.158
4	12.5	8	0.134	0.134	0.158
5	25.8	8	0.135	0.135	0.158
6	49.5	8	0.209	0.209	0.209
7	97.0	8	0.245	0.245	0.245

Dry Weights *Hyalella azteca* to BAS 510F
File: c:\hyalella Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model)			TABLE 2 OF 2		
IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.158				
Vehicle	0.158	0.024		1.68	k= 1, v=49
6.37	0.158	0.024		1.76	k= 2, v=49
12.5	0.158	0.024		1.79	k= 3, v=49
25.8	0.158	0.024		1.80	k= 4, v=49
49.5	0.209	2.253	*	1.80	k= 5, v=49
97.0	0.245	3.844	*	1.81	k= 6, v=49

s = 0.045

Note: df used for table values are approximate when v > 20.