Data Evaluation Report on the acute toxicity of BAS 510 F to fresh water invertebrates - *Daphnia* sp.

PMRA Submission Number 2001-1027 **EPA MRID Number {454050-01}**

Data Requirement: PMRA DATA CODE: 9.3.2
EPA DP Barcode: D278418
OECD Data Point: IIA 8.3.1 and IIA 8.3.1.1
EPA Guideline: 72-2

Test material: BAS 510 F **Purity (%):** 94.4%
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 {PMRA} **Date:** January 24/02

Secondary Reviewer(s): Thomas M. Steeger, Ph.D {EPA} Thomas M Steeger **Date:** March 31, 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 (0.78 lbs a.i./Acre/application).

EPA PC Code: 128008

CITATION: G. Peter Dohmen, February, 2001. Effect of BAS 510 F on the Immobility of *Daphnia magna* STRAUS In a 48 Hour Static, Acute Toxicity Test. Ecology and Environmental Analytics BASF Aktiengesellschaft D-67114 Limburgerhof, Germany. Study # 41898.

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EXECUTIVE SUMMARY:

The 48-hr-acute toxicity of BAS 510 F to *Daphnia magna* STRAUS was studied under static conditions. Daphnids were exposed to control, solvent control, and, test chemical at nominal concentration of 0.5, 0.8, 1.5, 2.5, 4.3, 7.5 mg a.i/L (mean measured concentrations 0.49, 0.84, 1.59, 2.45, 3.57, and 5.13 mg a.i./L) for 48 hr. Mortality/immobilization were observed daily. The 48- hour EC_{50} was 5.33 mg a.i/L. The 48-hr- NOEC, based on immobilization was 1.59 mg a.i/L.

Based on the results of this study, BAS 510 F would be classified as moderately toxic to *Daphnia magna* in accordance with the classification system of the U. S. EPA.

Test solutions were not properly centrifuged and/or filtered prior to test chemical analysis; therefore, this study is classified as supplemental and does not satisfy the guideline requirements for an acute toxicity study with freshwater invertebrates. However, EPA is not requiring that the test be repeated.

Results Synopsis

Test Organism Age (eg. 1st instar): less than 24hr
Test Type: Static

LC_{50}: 5.33 mg a..i./L} 95% C.I.: 4 to 7.1 mg a.i./L
NOEL: 1.59 mg a.i./L
Endpoint(s) Effected: Immobility

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

The test was conducted according to in house SOPs based on OECD-guideline 202; EC Directive 79/831, update 1990; EPA 72-2 and OPPTS 850.1010, draft April 1996.

COMPLIANCE:

This study was conducted in compliance with the Good Laboratory Practice Regulations; Appendix I to §19a, Section 1, Chemikaliengeset of July 25, 1994 (Official Bulletin/Federal Republic of Germany, I 1994, P. 1703)."

A. MATERIALS:

1. Test Material BAS 510 F

Description:	Solid
Lot No./Batch No. :	N37
Purity:	94.4%
Stability of Compound Under Test Conditions:	Not stated
Storage conditions of test chemicals:	Not satated

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:

Species: *Daphnia magna* STRAUS,
Age at test initiation: less than 24 hours old at test initiation.
Source: collected from in house culture,

B. STUDY DESIGN:

1. Experimental Conditions

b) Definitive Study

Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation: Period: Conditions: Feeding: Health:	 not specified same as test 3 times per week with algae good	 *(EPA requires 7 day minimum acclimation period* *No feeding during study)*
Duration of the test	48 hr	*(EPA requires 96 hours, except daphnids which are 48 hours)*
Test condition: Static/flow through Type of dilution system- for flow through method. Flow rate Renewal rate for static renewal	 Static NA NA NA	 *(EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period)*

Parameter	Details	Remarks *Criteria*
Aeration, if any	none	
Test vessel: Material: *(glass/stainless steel)* Size: Fill volume:	 Glass beakers 50 mL	 *(EPA requires: size 20 mL or 3.9 L fill 200 mL)*
Source of dilution water	Synthetic medium (prepared using ultrapure, deionized water), M4 according to Elendt) is used for both culturing and testing.	*(EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water)*
Water parameters: Hardness: pH: Dissolved oxygen: Temperature: Total organic carbon Particulate matter Metals Pesticides Chlorine Intervals of water quality measurement	 2.48 mmol/L (248 mg/L as $CaCO_3$) 8.08 - 8.20 8.4 to 8.5 mg/L 20.6 - 22.8°C not stated not stated not stated not stated not stated 0 and 48 hours	Hardness exceeds EPA recommended range of 30 - 48 mg/L as $CaCO_3$. pH exceeds EPA recommended range of 7.2 - 7.6 *(hardness:* *EPA requires 40 - 48 mg/L as $CaCO_3$* *pH: EPA requires 7.2 - 7.6* *Temperature:* *EPA requires 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C;* OECD requires range of 18-22°C (±1°C) *Dissolved oxygen:* *EPA requires Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%)*
Number of replicates: Control (dilution water): Solvent control: Treatments:	 4 4 4	

Parameter	Details	Remarks *Criteria*
Number of organisms per replicate: Control (dilution water): Solvent control: Treatments:	 5 5 5	*(EPA/OECD require 5 treatment levels plus control* *EPA requires a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17oC, ≤ 0.5 g/L at > 17oC; flow-through: ≤ 1 g/L/day).*
Treatment concentrations: Nominal:	0 (control and solvent control), 0.5, 0.8, 1.5, 2.5, 4.3, 7.5 mg/L (nominal)	*(EPA requires a geometric series with each concentration being at least 60% of the next higher one)*
Solvent (type, percentage, if used)	acetone 0.01% (0.1 ml/L)	*(EPA requires solvents not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests)*
Lighting	day:night-rhythm 16:8 h; < 1,500 lux	*(EPA requires 16 hours light, 8 hours dark; OECD : optional light-dark cycle or complete darkness)*
Recovery of chemical: Frequency of determination Level of Quantitation Level of Detection	 beginning and end of study 0.3 mg/L not stated, generally 1/10 of LOQ	Recovery at the end of the study was low (42.3% of nominal) at the highest concentration. This is likely due to the low water solubility of the chemical; precipitation would occur after solvent evaporation.
Positive control {if used, indicate the chemical and concentrations}	none	

2. Observations:

Table 2: Observations

Parameters	Details	Remarks *Criteria*
Parameters measured including the sublethal effects	immobility	
Observation intervals	24 and 48 hr	
Water quality was acceptable (Yes/No)	Yes	reconstituted water
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION

A. MORTALITY:

Mortality of over 10%, indicated by immobility of Daphnids, was only observed at the two highest test concentrations.

Table 3: Effect of BAS 510 F on mortality of *Daphnia magna*.

Treatment (mg a.i./L) [mean measured]	No. of organisms	Observation period			
		Day 1		Day2	
		No Dead	% mortality	No Dead	% mortality
Control (dilution water only), if used	20	0	0	0	0
Solvent control, if used	20	0	0	0	0
0.49	20	0	0	0	0
0.84	20	0	0	1	5
1.59	20	1	5	1	5
2.45	20	1	5	2	10
3.57	20	1	5	3	15
5.13	20	1	5	11	55
NOEC	2.45 mg/L				

Table 3A Positive Control Study using Potassium Permanganate*

EC_{50}	5.33 mg/L
Positive control 48 hr NOEC 48 hr EC_{50}	Potassium dichromate 0.4 mg/L 0.91 mg/L

* Positive control study conducted in April 2000

C. REPORTED STATISTICS:

The NOEC (EC0) was calculated using ANOVA with William's test; = 0.05. The EC50 was determined by log-log analysis using the TOXSTAT 3.5 software.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Statistical Method: Probit analysis.
The results of the Probit analysis indicate that the LC_{50} value calculated by the applicant is valid.

EC_{50}: 6.19	95% C.I.: 4.4 - 15.5
Probit Slope: 2.60	95% C.I.: 1.20- 4.00

E. STUDY DEFICIENCIES:

Acclimation of the test organisms was not described. The pH range of 8.1-8.2 was higher than that recommended by the EPA (pH 7.2-7.6). Positive control using potassium permanganate was not run concurrently with study. Reported results for permanganate treatment are for April 2000; current study was conducted in June 2000. These are minor deficiencies.

F. REVIEWER'S COMMENTS: The solubility of the test material (4.6 mg/L) is a limiting factor in achieving the nominal concentrations of the two highest treatments, *i.e.*, 4.3 and 7.5 mg/L, where only 62.8% and 42.3% of nominal was measured in each, respectively. Although acetone was used as the co-solvent, the report does not state whether the test solutions at the two highest concentrations exhibited any signs of cloudiness. The appendix does state however that there was "some precipitation under test conditions during the course of the study which could be associated either with loss of solvent, or simply precipitation of finely dispersed material". The EPA Pesticide Reregistration Rejection Rate Analysis (EPA 738-R-94-035) requires that samples be centrifuged and/or filtered prior to analysis when the solubility of the test substance is in question.

G. CONCLUSIONS: This study is supplemental since test solutions were not centrifuged and/or filtered prior to analysis for test substance. Additionally, it is not clear whether the pH and/or water hardness may have impacted the solubility and/or toxicity of the test substance. Under the conditions tested and based on the EC_{50} of 5.33 mg/L, the test chemical is classified as moderately toxic (falls in the range of 1 to 10 mg a.i./L) to Daphnids. The EC0 (NOEC) was 1.59 mg/L.

III. REFERENCES: EPA, 194. Pesticide Reregistration Rejection Rate Analysis Ecological Effects, EPA 738-R-94-035.

Approved 04/01/01 C. K.

TITLE: BAS 510F
FILE: c:\BAS510
TRANSFORM: NO TRANSFORM NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	5.0000	5.0000
1	Control	2	5.0000	5.0000
1	Control	3	5.0000	5.0000
1	Control	4	5.0000	5.0000
1	Control	5	5.0000	5.0000
1	Control	6	5.0000	5.0000
1	Control	7	5.0000	5.0000
1	Control	8	5.0000	5.0000
2	0.49	1	5.0000	5.0000
2	0.49	2	5.0000	5.0000
2	0.49	3	5.0000	5.0000
2	0.49	4	5.0000	5.0000
3	0.84	1	4.0000	4.0000
3	0.84	2	5.0000	5.0000
3	0.84	3	5.0000	5.0000
3	0.84	4	5.0000	5.0000
4	1.59	1	4.0000	4.0000
4	1.59	2	5.0000	5.0000
4	1.59	3	5.0000	5.0000
4	1.59	4	5.0000	5.0000
5	2.45	1	5.0000	5.0000
5	2.45	2	4.0000	4.0000
5	2.45	3	4.0000	4.0000
5	2.45	4	5.0000	5.0000
6	3.57	1	5.0000	5.0000
6	3.57	2	4.0000	4.0000
6	3.57	3	4.0000	4.0000
6	3.57	4	4.0000	4.0000
7	5.13	1	2.0000	2.0000
7	5.13	2	2.0000	2.0000
7	5.13	3	2.0000	2.0000
7	5.13	4	3.0000	3.0000

BAS 510F
File: c:\BAS510 Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	8	5.000	5.000	5.000
2	0.49	4	5.000	5.000	5.000
3	0.84	4	4.000	5.000	4.750
4	1.59	4	4.000	5.000	4.750
5	2.45	4	4.000	5.000	4.500
6	3.57	4	4.000	5.000	4.250
7	5.13	4	2.000	3.000	2.250

BAS 510F
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SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.000	0.000	0.000
2	0.49	0.000	0.000	0.000
3	0.84	0.250	0.500	0.250
4	1.59	0.250	0.500	0.250
5	2.45	0.333	0.577	0.289
6	3.57	0.250	0.500	0.250
7	5.13	0.250	0.500	0.250

BAS 510F
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	23.875	3.979	24.869
Within (Error)	25	4.000	0.160	
Total	31	27.875		

Critical F value = 2.49 (0.05,6,25)
Since F > Critical F REJECT Ho:All groups equal

BAS 510F
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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	5.000	5.000		
2	0.49	5.000	5.000	0.000	
3	0.84	4.750	4.750	1.021	
4	1.59	4.750	4.750	1.021	
5	2.45	4.500	4.500	2.041	
6	3.57	4.250	4.250	3.062	*
7	5.13	2.250	2.250	11.227	*

Bonferroni T table value = 2.57 (1 Tailed Value, P=0.05, df=25,6)

BAS 510F
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BONFERRONI T-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	0.49	4	0.629	12.6	0.000
3	0.84	4	0.629	12.6	0.250
4	1.59	4	0.629	12.6	0.250
5	2.45	4	0.629	12.6	0.500
6	3.57	4	0.629	12.6	0.750
7	5.13	4	0.629	12.6	2.750

BAS 510F
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ANOVA TABLE

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BAS 510F
File: c:\BAS510 Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	8	5.000	5.000	5.000
2	0.49	4	5.000	5.000	5.000
3	0.84	4	4.750	4.750	4.750
4	1.59	4	4.750	4.750	4.750
5	2.45	4	4.500	4.500	4.500
6	3.57	4	4.250	4.250	4.250
7	5.13	4	2.250	2.250	2.250

BAS 510F
File: c:\BAS510 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	5.000				
0.49	5.000	0.000		1.71	k= 1, v=25
0.84	4.750	1.021		1.79	k= 2, v=25
1.59	4.750	1.021		1.82	k= 3, v=25
2.45	4.500	2.041	*	1.83	k= 4, v=25
3.57	4.250	3.062	*	1.84	k= 5, v=25
5.13	2.250	11.227	*	1.84	k= 6, v=25

s = 0.400
Note: df used for table values are approximate when v > 20.

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RESULTS CALCULATED USING THE MOVING AVERAGE METHOD
 SPAN          G              LC50          95 PERCENT CONFIDENCE LIMITS
 1              .5479185       4.919695      4.301712       7.888528


RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS     G              H             GOODNESS OF FIT PROBABILITY
 5              .2900499       1              .2174933

SLOPE     =      2.601086
95 PERCENT CONFIDENCE LIMITS  = 1.200238      AND     4.001934

LC50 =          6.19329
95 PERCENT CONFIDENCE LIMITS = 4.410136 AND  15.46311

LC10 =          2.012166
95 PERCENT CONFIDENCE LIMITS = 1.072287 AND  2.678968
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Steeger BAS 510F Acute Daphnid EC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
5.13	20	11	55	41.19014
3.57	20	3	15	.1288414
2.45	20	2	10	2.012253E-02
1.59	20	1	5	2.002716E-03
.84	20	1	5	2.002716E-03
.49	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 3.57 AND +INFINITY CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.919696

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.5479185	4.919695	4.301712	7.888528

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.2900499	1	.2174933

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