

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

PMRA Submission Number{2001-1027}

EPA MRID Number{454050-05}

Data Requirement:

PMRA DATA CODE: 9.3.3
EPA DP Barcode: D278418
OECD Data Point: IIA 8.3.2.1
EPA Guideline: 72-4

Test material: BAS 510 F

Purity (%): 96.3 %

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:
{PMRA}

Peter Takacs, Peter Delorme

Date: January 30/2002

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

Date: April 1, 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: Hisgen, February, 2001. BAS 510 F Determination of the chronic effect on the reproduction of the water flea *Daphnia magna* STRAUS. Experimental Toxicology and Ecology, BASF, Ludwigshafen, Germany. Study # 00/0618/51/2.



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EXECUTIVE SUMMARY:

The 21-day-chronic toxicity of BAS 510 F to *Daphnia magna* was studied under static renewal conditions. Daphnids were exposed to control, and test chemical at measured concentrations of 0.031, 0.08, 0.16, 0.31, 0.63, 1.31, 2.6 mg a.i./L. The 21-day LC₅₀/EC₅₀ could not be calculated due to lack of mortality. The 21-day NOEC based on production of young by parents was 1.31 mg a.i./L. The sublethal effects included were production of young, survival of young and abortion of eggs. Production of offsprings in the treated groups indicated that BAS 510 F had a significant effect on the reproduction at concentrations greater than 1.31 mg a.i./L. The most sensitive end point was production of young.

This study is classified as supplemental and does not fulfill the guideline requirements for a chronic toxicity study with freshwater invertebrates; the study fails to provide information on the chronic effects of BAS 510F on the growth (length and weight) of daphnids.. Additionally, since the solubility of the test material appeared to be an issue in this study, EPA is uncertain whether the mean measured concentrations reported in this study accurately reflect the dissolved concentration of BAS 510F. The laboratory must provide data on daphnid growth; otherwise this study must be repeated.

Results Synopsis

Test Organism Age (eg. 1st instar): not stated
Test Type: Static Renewal

NOEC: 1.31 mg a.i./L

LOEC: 2.63 mg a.i./L

Endpoint(s) Effected: production of young

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

OECD-Guideline for testing of chemicals, No. 202, 4. April 1984: *Daphnia* sp., Acute Immobilization Test and Reproduction Test. Part.11 -Reproduction Test.
EEC Guideline XI/691/86, Draft 4: "Prolonged toxicity study with *Daphnia magna*: effects on reproduction".

COMPLIANCE:

This study was conducted in accordance with the GLP provisions of the "Chemicals Act" (Chemikaliengesetz; Bundesgesetzblatt 1994, Teil I, 29.07.94; FR Germany) and with the "OECD Principles of Good Laboratory Practice" (Pans, 1981). This is confirmed by the signature of the study director.

A. MATERIALS:

1. Test Material

Description: powder

Lot No./Batch No. : N46

Purity: 96.3%

Stability of Compound

Under Test Conditions: Stable until 12/2001

Storage conditions of

test chemicals: stored under ambient conditions

Physicochemical properties of BAS 510 F.

Parameter	Values	Comments
Water solubility at 20°C	4.69 mg/L	very insoluble
Vapour pressure	7×10^{-9} mbar @ 20 °C	non-volatile
UV absorption	UV molecular extinction: 1.53×10^3 at 290 nm	-
pKa	does not dissociate in water	not affected by pH
Kow	2.96	moderately lipophilic

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2. Test organism:

Species: *Daphnia magna* STRAUS

Age of parental stock: 2 - 4 weeks with individuals of 2 - 24 h

Source: The clone of *Daphnia magna* STRAUS 1820 used was supplied by the Institut National de Recherche Chimique Appliquee, France, in 1978. From this date on this clone was cultured and bred continuously in the Laboratory of Ecology of BASF AG in Ludwigshafen.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: In an acute preliminary test with daphnids the value for the EC50 (48h) was 9,1 mg/l. (Project-No.: 00/0618/50/v2, from 18.08.00, non GLP)

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
<u>Parental acclimation:</u> Period: Conditions: same as test Feeding: Health: (any mortality observed)	Acclimation was not used, due to similar conditions during the test. During the test daphnids were fed live green algae (<i>Scenedesmus subspicatus</i> , <i>Desmodesmus subspicatus</i>), cultured in a synthetic medium) daily. Yes Every Monday, Wednesday and Friday, the solution was changed.	(EPA requires consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period)
<u>Test condition:</u> Static renewal: Renewal rate/procedure for static renewal		
Aeration, if any	not stated	
Duration of the test	21 days	

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Parameter	Details	Remarks
		Criteria
<u>Test vessel</u> Material: Size: Fill volume:	glass beaker 100ml 50 ml	
Source of dilution water	M4 medium was used to dilute the stock solution	----- <i>(EPA: Unpolluted well or spring water that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details))</i>

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Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u>		
Hardness	2.2-3.2 mmol/L	Hardness exceeds EPA recommended 140 - 160 mg/L as CaCO ₃ ; pH exceeds EPA recommended 7.6 - 8.0
pH	7.7 - 8.1	
Dissolved oxygen	8.0 - 9.5 mg/L	
Temperature	19.9 - 20.5 °C	
Total organic carbon	not stated	
Particulate matter	not stated	
Metals	not stated	
Pesticides	not stated	
Chlorine	not stated	
Interval of water quality measurement	every 2-3 days	
<u>Number of replicates:</u>		
Control (dilution water):	10	
Treatment:	10	
<u>Number of organisms:</u>		
For growth and reproduction:	10	
For survival test:	no separate test was done for survival of parents	
<u>Treatment concentrations:</u>		
Nominal:	0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, mg/L	
Measured:	0, 0.03, 0.08, 0.16, 0.31, 0.63, 1.31, 2.6 mg/L	
Solvent (type, percentage, if used)	not stated	
Lighting	16 hr light: 8 hr dark	
<u>Recovery of the chemical:</u>		
Frequency of determination	day 0, 2, 7, 9, 16, 19	
Level of Quantitation	0.005 mg/L	
Level of Detection	0.0005 mg/L	

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2. Observations:

Table 2: Observations

Parameters	Details	Remarks
		<i>Criteria</i>
Data end points measured (list)	Survival of first-generation daphnids Number of young produced per female aborted eggs	<i>(EPA requires: - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs)</i>
Observation intervals	daily	
Water quality was acceptable (Yes/No)	Yes	
Were raw data included?	Yes	

II. RESULTS AND DISCUSSION

A. MORTALITY:

No mortality of parent organisms was observed at any test concentration. Significantly fewer young were produced (54% of control) at the highest test concentration of 2.63 mg ai/L.

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Table 3: Effect of BAS 510 F on growth and survival of *Daphnia* sp.

Treatment (mg a.i./L) measured conc.	Parent mortality (dead or immobile)		Mean number of living young per parent at day 21	Mean number of dead young per parent at day 21	Mean number of aborted eggs per parent at day 21
	No Dead	%			
Control	0	0	166.2	0	1.6
0.031	0	0	167.4	0.2	0.9
0.08	0	0	163.6	0.7	1.8
0.16	0	0	168.2	0.5	2.1
0.31	0	0	164.3	0.6	1.1
0.63	0	0	166.5	0.2	2.8
1.31	0	0	162.5	0	2.6
2.63	0	0	91.6	0	2.2
NOEC	1.31mg/L				
LOEC	2.63 mg/L				

B. EFFECT ON REPRODUCTION AND CHRONIC/SUBLETHAL EFFECTS :

The most sensitive endpoint was production of young. Only the highest test concentration caused significant inhibition in young production, thus the LOEC was 2.63 mg/L and the NOEC was 1.31 mg/L. No apparent effects were seen on adult survival, death of young or aborted eggs.

C. REPORTED STATISTICS:

To determine the LOEC and the NOEC, Duncan's multiple range test was used.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER :

Statistical Method: Since the response data in the highest treatment group was not normally distributed a Kruskal-Wallis One Way Analysis of Variance on Ranks test was used with Dunnett's method for multiple comparison.

Control vs. highest treatment concentration (2.63 mg/L):

Comparison	Diff of Ranks	p	q'	P<0.05
Col 8 vs cont	100.000	2	3.780	Yes

The differences in the median values among the treatment groups are greater than would be

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expected by chance; there is a statistically significant difference ($P = <0.001$). All other concentrations were statistically not different from the control.

E. STUDY DEFICIENCIES:

The EPA requirement for data on growth (dry weight and length [optional] of the first generation daphnids) was not reported, however, this study followed OECD guideline 202, which does not require the inclusion of this endpoint; however, EPA guideline 72-4 does require data on growth. The OECD guideline 202 recommends the use of at least 40 individuals per concentration; this study used only 10 individuals per concentration, albeit in separate vessels.

F. REVIEWER'S COMMENTS:

Although fewer than recommended organisms were used per treatment level, there was no mortality of parent daphnids at any concentration during the 21 days of the study. The young production per parent was more than sufficient, therefore the results are deemed acceptable by the reviewer.

The analytical results indicate that exposure solution samples were diluted with acetonitrile/water so the final solutions have an acetonitrile content of approximately 20%; however, the report does not indicate what solvent was used to prepare the treatment solutions. The reader must assume that the stock solution (3.2 mg/L) was prepared using M4 medium since the highest exposure concentration was 3.2 mg/L. In previous studies (acute daphnid MRID 454050-01) with BAS 510F, the limited solubility (4.69 mg/L) required the use of a co-solvent (acetone) and even then the compound was observed to precipitate at higher exposure concentrations. In this study, it appears that solubility is again an issue with recoveries ranging from 67 - 95% of nominal. However, it also appears the stock solutions may have been made up in M4 medium. It is possible that the media contributed to further limiting the solubility (salting out) of BAS 510F and that ideally, water samples should have been filtered using 0.2 um filter prior to analysis to better assure accurate analysis.

EPA requires data on growth; this study failed to provide any data on either dry weight or length of daphnia offspring.

G. CONCLUSIONS: The study is scientifically sound and provides useful information on the chronic effects of BAS 510F on freshwater invertebrate reproduction and survival; however, the study has failed to provide any information on the effects of BAS 510 F on daphnia growth. Additionally, since the solubility of the test material appeared to be an issue in this study, EPA is uncertain whether the mean measured concentrations reported in this study accurately reflect the dissolved concentration of BAS 510F. The laboratory must provide data on daphnid growth; otherwise this study must be repeated.

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III. REFERENCES:

Approved 04/01/01 C.K.

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TITLE: Chronic Daphnid Life Cycle Using BAS 510F

FILE: c:\DAP_C

TRANSFORM: NO TRANSFORM

NUMBER OF GROUPS: 8

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	0	1	1.0000	1.0000
1	0	2	1.0000	1.0000
1	0	3	2.0000	2.0000
1	0	4	3.0000	3.0000
1	0	5	2.0000	2.0000
1	0	6	1.0000	1.0000
1	0	7	1.0000	1.0000
1	0	8	1.0000	1.0000
1	0	9	2.0000	2.0000
1	0	10	2.0000	2.0000
2	0.05	1	0.0000	0.0000
2	0.05	2	0.0000	0.0000
2	0.05	3	2.0000	2.0000
2	0.05	4	0.0000	0.0000
2	0.05	5	3.0000	3.0000
2	0.05	6	0.0000	0.0000
2	0.05	7	1.0000	1.0000
2	0.05	8	3.0000	3.0000
2	0.05	9	0.0000	0.0000
2	0.05	10	0.0000	0.0000
3	0.1	1	1.0000	1.0000
3	0.1	2	1.0000	1.0000
3	0.1	3	2.0000	2.0000
3	0.1	4	3.0000	3.0000
3	0.1	5	3.0000	3.0000
3	0.1	6	0.0000	0.0000
3	0.1	7	1.0000	1.0000
3	0.1	8	1.0000	1.0000
3	0.1	9	5.0000	5.0000
3	0.1	10	1.0000	1.0000
4	0.2	1	1.0000	1.0000
4	0.2	2	2.0000	2.0000
4	0.2	3	1.0000	1.0000

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4	0.2	4	2.0000	2.0000
4	0.2	5	3.0000	3.0000
4	0.2	6	4.0000	4.0000
4	0.2	7	3.0000	3.0000
4	0.2	8	2.0000	2.0000
4	0.2	9	2.0000	2.0000
4	0.2	10	1.0000	1.0000
5	0.4	1	2.0000	2.0000
5	0.4	2	0.0000	0.0000
5	0.4	3	0.0000	0.0000
5	0.4	4	0.0000	0.0000
5	0.4	5	0.0000	0.0000
5	0.4	6	1.0000	1.0000
5	0.4	7	1.0000	1.0000
5	0.4	8	1.0000	1.0000
5	0.4	9	3.0000	3.0000
5	0.4	10	3.0000	3.0000
6	0.8	1	3.0000	3.0000
6	0.8	2	2.0000	2.0000
6	0.8	3	3.0000	3.0000
6	0.8	4	1.0000	1.0000
6	0.8	5	4.0000	4.0000
6	0.8	6	4.0000	4.0000
6	0.8	7	1.0000	1.0000
6	0.8	8	4.0000	4.0000
6	0.8	9	3.0000	3.0000
6	0.8	10	3.0000	3.0000
7	1.6	1	1.0000	1.0000
7	1.6	2	5.0000	5.0000
7	1.6	3	2.0000	2.0000
7	1.6	4	2.0000	2.0000
7	1.6	5	7.0000	7.0000
7	1.6	6	1.0000	1.0000
7	1.6	7	2.0000	2.0000
7	1.6	8	1.0000	1.0000
7	1.6	9	3.0000	3.0000
7	1.6	10	2.0000	2.0000
8	3.2	1	4.0000	4.0000
8	3.2	2	0.0000	0.0000
8	3.2	3	2.0000	2.0000
8	3.2	4	3.0000	3.0000
8	3.2	5	2.0000	2.0000

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8	3.2	6	1.0000	1.0000
8	3.2	7	0.0000	0.0000
8	3.2	8	3.0000	3.0000
8	3.2	9	4.0000	4.0000
8	3.2	10	3.0000	3.0000

Chronic Daphnid Life Cycle Using BAS 510F
 File: c:\DAP_C Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP IDENTIFICATION	N	MIN	MAX	MEAN
1	0 10	1.000	3.000	1.600
2	0.05 10	0.000	3.000	0.900
3	0.1 10	0.000	5.000	1.800
4	0.2 10	1.000	4.000	2.100
5	0.4 10	0.000	3.000	1.100
6	0.8 10	1.000	4.000	2.800
7	1.6 10	1.000	7.000	2.600
8	3.2 10	0.000	4.000	2.200

Chronic Daphnid Life Cycle Using BAS 510F
 File: c:\DAP_C Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP IDENTIFICATION	VARIANCE	SD	SEM	
1	0	0.489	0.699	0.221
2	0.05	1.656	1.287	0.407
3	0.1	2.178	1.476	0.467
4	0.2	0.989	0.994	0.314
5	0.4	1.433	1.197	0.379
6	0.8	1.289	1.135	0.359

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7	1.6	3.822	1.955	0.618
8	3.2	2.178	1.476	0.467

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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	31.687	4.527	2.581
Within (Error)	72	126.300	1.754	
Total	79	157.988		

Critical F value = 2.17 (0.05,7,60)
 Since $F > \text{Critical F}$ REJECT H_0 : All groups equal

Chronic Daphnid Life Cycle Using BAS 510F
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BONFERRONI T-TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN CALCULATED IN ORIGINAL UNITS			T STAT	SIG
		MEAN	MEAN	MEAN		
1	0	1.600	1.600			
2	0.05	0.900	0.900	1.182		
3	0.1	1.800	1.800	-0.338		
4	0.2	2.100	2.100	-0.844		
5	0.4	1.100	1.100	0.844		
6	0.8	2.800	2.800	-2.026		
7	1.6	2.600	2.600	-1.688		
8	3.2	2.200	2.200	-1.013		

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Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=70,7)

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BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum (IN ORIG. UNITS)	Sig Diff	% of DIFFERENCE CONTROL FROM CONTROL
1	0 10				
2	0.05 10	1.488	93.0	0.700	
3	0.1 10	1.488	93.0	-0.200	
4	0.2 10	1.488	93.0	-0.500	
5	0.4 10	1.488	93.0	0.500	
6	0.8 10	1.488	93.0	-1.200	
7	1.6 10	1.488	93.0	-1.000	
8	3.2 10	1.488	93.0	-0.600	

Chronic Daphnid Life Cycle Using BAS 510F
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	ORIGINAL N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0 10	1.600	1.600	1.250	
2	0.05 10	0.900	0.900	1.250	
3	0.1 10	1.800	1.800	1.667	
4	0.2 10	2.100	2.100	1.667	
5	0.4 10	1.100	1.100	1.667	
6	0.8 10	2.800	2.800	2.533	
7	1.6 10	2.600	2.600	2.533	
8	3.2 10	2.200	2.200	2.533	

Chronic Daphnid Life Cycle Using BAS 510F
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

ISOTONIZED CALC. SIG TABLE DEGREES OF
IDENTIFICATION MEAN WILLIAMS P=.05 WILLIAMS FREEDOM

0	1.250			
0.05	1.250	0.591	1.67	k= 1, v=72
0.1	1.667	0.113	1.75	k= 2, v=72
0.2	1.667	0.113	1.77	k= 3, v=72
0.4	1.667	0.113	1.78	k= 4, v=72
0.8	2.533	1.576	1.79	k= 5, v=72
1.6	2.533	1.576	1.79	k= 6, v=72
3.2	2.533	1.576	1.80	k= 7, v=72

s = 1.324

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

Chronic Daphnid Life Cycle Using BAS 510F

File: c:\DAP_C Transform: NO TRANSFORM

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	0	1.600	1.600	370.000
2	0.05	0.900	0.900	238.500
3	0.1	1.800	1.800	382.000
4	0.2	2.100	2.100	457.500
5	0.4	1.100	1.100	274.500
6	0.8	2.800	2.800	571.000
7	1.6	2.600	2.600	478.500
8	3.2	2.200	2.200	468.000

Calculated H Value = 5.247 Critical H Value Table = 14.070
 Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

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DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP IDENTIFICATION	GROUP		
	TRANSFORMED MEAN	ORIGINAL MEAN	00000000
			25134876
2	0.05	0.900	0.900 \
5	0.4	1.100	1.100 .\
1	0	1.600	1.600 ..\
3	0.1	1.800	1.800 ... \
4	0.2	2.100	2.100 \
8	3.2	2.200	2.200 \
7	1.6	2.600	2.600 \
6	0.8	2.800	2.800 * \

* = significant difference (p=0.05)

. = no significant difference

Table q value (0.05,8) = 3.124

SE = 10.133