Data Evaluation Report on the acute toxicity of BAS 510 F (TGAI) to rainbow trout (*Oncorhynchus mykiss* WALBAUM 1792).

PMRA Submission Number 2001-1027 **EPA MRID Number {454049-27}**

Data Requirement: PMRA DATA CODE: 9.5.2.1
EPA DP Barcode: D278418
OECD Data Point: IIA 8.2.1 and IIA 8.2.1.2
EPA Guideline: 72-1c

Test material: BAS 510 F **Purity (%):** 95.3%
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 Peter Delorme **Date:** April 30/02
{PMRA}

Secondary Reviewer(s): Thomas M. Steeger, Ph.D **Date:** July 19, 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: Sabine Zok, November 2000. BAS 510 F, Acute Toxicity Study on the Rainbow Trout (*Oncorhynchus mykiss* Walbaum 1792) in a Static System (96 hours). Experimental Toxicology and Ecology BASF Aktiengesellschaft; D-67056 Ludwigshafen,Germany, project #: 12F0179/975131.

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EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, rainbow trout (*Oncorhynchus mykiss*) were exposed to BAS 510 F at nominal concentrations of 0, 0.5, 0.7, 1.2, 1.9 and 3.0 mg a.i/L under static conditions. The 96-hr LC_{50} was2.7 mg a.i/L. The NOEC value, based on mortality and sublethal effects, was 1.9 mg a.i/L. Sublethal effects such as lethargy and tumbling (disorientation) were observed in the groups exposed the highest concentration (3.0 mg ai/L) of BAS 510 F. Based on the results of this study, BAS 510 F would be classified as moderately toxic to rainbow trout in accordance with the classification system of the U.S. EPA.

This toxicity study is classified as supplemental and does not satisfy the guideline requirement for acute fish toxicity study since water samples were not properly analyzed and the amount of soluble material is uncertain. Additionally, EFED is uncertain as to whether the study conditions, *i.e.*, hard water and high pH, affected the solubility and/or toxicity of BAS 510F. However, since mortality occurred at or near the solubility limit of the chemical, EFED is not requiring that this study be repeated at this time.

Results Synopsis

Test Organism Size/Age(mean wet weight or length): 1.7 g, 5 months old.
Test Type: Static

LC_{50}: 2.7 mg a..i./L
NOEL: 1.88 mg a.i./L
Endpoint(s) Effected: Survival and sublethal effects (lethargy, disorientation)

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

EPA guideline "Pesticide Assessment Guidelines, subdivision E, Hazard Evaluation Wildlife and Aquatic Organisms (U.S.) Environmental Protection Agency, Washington DC, para. 72-1, P. 66, October 1982 and the (U.S.) EPA- SEP (Standard Evaluation Procedure) No. 540/9-85-006, June 1985. EEC directive 92/69, Annex V, Cl. OECD guideline No. 203 "Fish Acute Toxicity Test" July 1992.

COMPLIANCE:

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 (Paris 1981).

A. MATERIALS:

1. Test Material BAS 510 F

Description: Solid
Lot No./Batch No. : N26
Purity: 95.3%
Stability of Compound Under Test Conditions: stable
Storage conditions of test chemicals: ambient conditions (room temperature)

Physicochemical properties of BAS 510 F.

Parameter	Values	Comments
Water solubility at 20°C	4.69 mg/L	low solubility (report notes solubility under test conditions as 3 mg/L)
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	-
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: Rainbow trout (Oncorhynchus mykiss WALBAUM 1792).
Age at test initiation: About 5 months
Weight at study initiation: 1.7 (1.1 - 2.4) g
(EPA requires: mean 0.5 - 5 g)
Length at study initiation: 5.8 (5.1 - 6.5) cm
(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: Forellenhof Fredelsloh, Moringen, Germany

B. STUDY DESIGN:

1. Experimental Conditions

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation: Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	14 days similar to test conditions but in flow-through tanks *ad lib* food 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)*
Test condition: Static Type of dilution system- for flow through method Flow rate Renewal rate for static renewal	static	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)*

Parameter	Details	Remarks Criteria
Aeration, if any	None during the first 48 hours thereafter slight aeration.	acceptable *(EPA requires: no aeration; OECD permits aeration)*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	Glass aquaria 48-liter (58.5 x 28 x 29.5 cm); 40-liter fill volume	acceptable; two aquaria for each test group *(EPA requires: size 19 L (5 gal) or 30 x 60 x 30 cm Fill volume: 15-30 L of solution)*
Source of dilution water	Municipal water of the city of Frankenthal, not chlorinated and passed through a charcoal filter, aerated.	It is not clear whether the dilution water is dechlorinated or whether the Frankethal municipal water does not use chlorine at all. *(EPA requires soft reconstituted water or water from a natural source, not dechlorinated tap water); OECD permits dechlorinated tap water)*

Parameter	Details	Remarks Criteria
Water parameters: Hardness pH Dissolved oxygen Total organic carbon Particulate matter Metals Pesticides Chlorine	Total hardness: about 2.5 rmnol/l (= about 250 mg/l $CaCO_3$) Acid capacity : about 5.5 mmol/l Ca content : about 90 mg/l Mg content : about 10 mg/l pH : about 8.0 - 8.6 The test water is regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and the technical services of BASF Aktiengesellschaft as well as for presence of microbes by a contract laboratory.	water hardness exceeds EPA recommended range of 40 - 48 mg/L as $CaCO_3$; pH exceeds EPA recommended range of 7.2 - 7.6
Temperature Intervals of water quality measurement	12 °C Water parameter (pH value, oxygen content and water temperature) were measured at start of exposure (1 hour) and 24,48,72 and 96 hours after start of exposure.	*(Hardness* *EPA : 40 - 48 mg as $CaCO_3$/L* *OECD: 10 -250* mg as $CaCO_3$/L *pH* *(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* Dissolved Oxygen *EPA: Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%)* *OECD: at least 80% saturation value.* *Temperature:* *EPA: estuarine/marine: 22 ± 1 °C* *OECD: 21 - 25°C for bluegill and 13 - 17°C for rainbow trout* *Salinity* *EPA: 30-34 % (parts per thousand) salinity, weekly range < 6 %)* *(EPA water quality: measured at beginning of test and every 48 hours)*
Number of replicates/groups: Control (dilution water): Treatments:	 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)*
Number of organisms per replicate /groups: Control (dilution water): Treatments:	 10 10	acceptable *(EPA: ≥ 10/concentration); OECD requires at least 7 fish/concentration)*

Parameter	Details	Remarks Criteria
Biomass loading rate	0.4 g/L	acceptable *(EPA: static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°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)*
Test concentrations: Nominal: Measured:	0, 0.5, 0.7, 1.2, 1.9, 3, mg/l 0, 0.45, 0.76, 1.23, 1.88, 3.00 mg/L (mean of 1hr and 96 hr measurements) 89-109% of nominal	acceptable
Solvent (type, percentage, if used)	not used	acceptable *(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)*
Lighting	16hrs	acceptable *(EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod)*
Feeding	none during study and 24 hrs prior to study	acceptable *(EPA/OECD requires: no feeding during the study)*
Recovery of chemical: Frequency of determination Level of Detection Level of Quantitation	 at start and at finish of test not stated	
Positive control {if used, indicate the chemical and concentrations}	none	
Other parameters, if any	-	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks / Criteria
Parameters measured including the sublethal effects/toxicity symptoms	mortality	
Observation intervals	1,4,24,48,72 and 96 hours after start of exposure.	*(EPA/OECD requires: minimally every 24 hours)*
Water quality was acceptable (Yes/No)	yes	
Were raw data included?	Yes	
Other observations, if any	-	

II. RESULTS and DISCUSSION:

A. MORTALITY:

Mortality occurred in only the highest test concentration of 3 mg ai/L after 24 hr of exposure, thereafter mortality increased with time to a maximum of 75% at 96 hours.

Table 2: Effect of BAS 510 F on mortality of Rainbow trout.

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
Control (dilution water only), if used	20	0	0	0	0	0	0
0.45	20	0	0	0	0	0	0
0.76	20	0	0	0	0	0	0
1.22	20	0	0	0	0	0	0
1.88	20	0	0	0	0	0	0
2.98	20	1	5	7	35	15	75
NOEC	1.88 mg ai/L						
LC_{50}	2.7 mg ai/L						

B. NON-LETHAL TOXICITY ENDPOINTS:

Sublethal effects such as tumbling (disorientation), lethargy, swimming near the bottom of the tank were observed only at the highest treatment concentration (3.0 mg ai/L). A maximum of 25% of the fish per treatment exhibited these responses. Mortality at this concentration exceeded sublethal effects.

Table 3: Sub-lethal effect of BAS 510 F on Rainbow trout.

Treatment (mg a.i./L) [record measured and nominal concentrations used]	Observation period		
	48 hr	72 hr	96 hr
	(% affected)	% affected	% affected
Control (dilution water only), if used	-	-	-
0.5	-	-	-
0.7	-	-	-
1.2	-	-	-
1.9	-	-	-
3	Tumbling (15) Apathy (5) Convulsions (10)	Tumbling (20) Convulsions (10) Sits on bottom (15)	Apathy (20), Sits on bottom (15)
NOEC	1.9 mgai/L		
LOEC	3.0 mg ai/L		

C. REPORTED STATISTICS:

If possible, the median lethal concentration (LC_{50}) after 1, 4, 24, 48, 72 and 96 hours, based on the nominal concentrations and based on the mean of the analytically determined concentrations is calculated using probit analysis*. If possible the LC_5 and LC_{95} are given as well. Symbols of the model of the dose response relationship F(P) = A + B * LN(K) K = concentration P = relative frequency of dead animals after exposure with K F = inverse function of the cumulative standard normal distribution LN = natural logarithm A, B = model parameters. In case that the data obtained are inadequate for the use of statistical methods for LC_{50} calculation an approximate LC_{50} is calculated as geometric mean of LC_0 and LC_{100}.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

Not required due to lack of partial kills.

E. STUDY DEFICIENCIES: Only one partial kill was achieved at the high concentration resulting in 75% mortality. Without at least two partial kill concentrations, the LC50 estimate has a high uncertainty.

F. REVIEWER'S COMMENTS: All but one of the test concentrations produced no mortality; while the high concentration of 3.0 mg ai/L is near the water solubility of BAS 510 F (4.7 mg ai/L), a

better concentration range should have been used to achieve a more realistic estimate of the LC_{50}. This range could have been: 1.8, 2.2, 2.8, 3.0 and 3.5 mg ai/L. Additionally, since the exposure concentrations used in the study approached the solubility limit and since no col-solvent was used to enhance the solubility, water samples should have been centrifuged and/or filtered prior to analysis. Furthermore, water hardness (250 mg/L as CaCO3) and pH (8.0 - 8.6) exceeded EPA's recommended range of 40 - 48 mg/L as and 7.2 - 7.6, respectively. EFED is uncertain whether these deviations from recommended ranges affects the solubility and/or toxicity on BAS 510F.

G. CONCLUSIONS: The study is supplemental. Water samples were not properly collected for analysis of BAS 510 F in order to accurately determine the amount of chemical in solution. The 96-hr LC_{50} was estimated to be about 2.7 mg ai/L and the NOEC was 1.9 mg ai/L for both mortality and sublethal effects, *i.e.*, lethargy and tumbling.

III. REFERENCES:

Approved 04/01/01 C.K.

3	20	15	75	2.069473
1.9	20	0	0	9.536742E-05
1.2	20	0	0	9.536742E-05
.7	20	0	0	9.536742E-05
.5	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 1.9 AND 3 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 2.653126

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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