7-1-91

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

July 1991

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

SUBJECT: Review Studies Conducted Amitraz Degradates DP
Barcode: 163178
ID No: 04569-RUA

FROM: Douglas J. Urban, Acting Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Dennis Edwards, PM 12
Insecticide-Rodenticide Branch
Registration Division (H7505C)

BACKGROUND

Because of the persistence in the environment of Amitraz degradates BTS 27919 and BTS 27271, degradate toxicology studies were requested. In response, Nor-Am Chemical Company sent the following studies:

72-1 96 hour LC_{50} with Rainbow trout

Study Identification: Schupner, J. K. 1991. The Acute Toxicity of BTS 27271 to the Rainbow trout, Oncorhynchus mykiss in a flow through system. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-03. ID No. 45639-RUA (Amitraz 45639-51). STUDY No. 512L.

72-1 96 hour LC_{50} with Rainbow trout.

Study Identification: Schupner, J. K. 1991. The Static Acute Toxicity of BTS 27919 to the Rainbow trout, Oncorhynchus mykiss. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-06. ID No. 045639-RUA (Amitraz 45639-51). STUDY No. 501L.

72-1 96 hour LC_{50} with Bluegill sunfish.

Study Identification: Schupner, J. K. and Stachura, B. J. 1991. The Acute Toxicity of BTS 27271 to the Bluegill sunfish, Lepomis macrochirus in a flow through system. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-02. ID No. 45639-RUA (Amitraz 45639-51). Study No. 513L.

72-1 96 hour LC_{50} with Bluegill sunfish.

Study Identification: Schupner, J. K. 1991. The Static Acute Toxicity of BTS 27919 to the Bluegill sunfish, Lepomis macrochirus. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-05. ID No. 045639-RUA (Amitraz 04569-51). Study No. 500L.

72-2 Freshwater invertebrate 48 hour EC_{50} with Daphnia magna.

Study Identification: Schupner, J. K. and Stachura, B. J. 1991. The Static Acute Toxicity of BTS 27271 to Daphnia magna. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-04. ID No. 45639-RUA (Amitraz 45639-51). STUDY No. 511L.

72-2 48 hour EC_{50} with Daphnia.

Study Identification: Schupner, J. K. and Young, B. M. 1991. The Static Acute Toxicity of BTS 27919 to Daphnia magna. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-07. ID No. 45639-RUA (Amitraz 45639-51). Study No. 507L.

REVIEW SUMMARY

SPECIES	TEST MATERIAL	RESULTS	MRID#	AUTHOR	FULFILLS REQMNTS.
Trout	BTS27271, Degradate	96 Hour LC_{50} = 28.36 ppm	418272-03	Schupner	No[1]
Trout	BTS27919, Degradate	96 Hour LC_{50} = 66.23 ppm	418272-06	Schupner	Yes
Bluegill	BTS27271, Degradate	96 Hour LC_{50} = 29.33ppm	418272-02	Schupner & Stachura	No[1]
Bluegill	BTS27919, Degradate	96 Hour LC_{50} > 100 ppm	418272-05	Schupner	Yes
Daphnia	BTS27271, Degradate	48 Hour EC_{50} = 2.59 ppm	418272-04	Schupner	No[1]
Daphnia	BTS27919, Degradate	48 Hour EC_{50} > 100 ppm	418272-07	Schupner & Young	Yes

[1] More information is needed on BTS 27271 and its adduct BTS 27271-HCl. EEB needs to have data on how BTS 27271 and BTS 27271-HCL compare, toxicologically. Specifically, data on the dissociation constant and water solubility of BTS 27271 and BTS 27271-HCl is desired in order to ascertain if BTS 27271-HCl is adequate for investigating the toxicity of BTS 27271. The studies can be re-evaluated after this additional information has been supplied.

REQUIREMENTS FOR REGISTRATION

The following studies with these degradates are still required. Species in parenthesis are preferred:

71-2 Avian dietary LC_{50} with an upland game bird (Bobwhite quail) and a waterfowl species (mallard).

72-3 Estuarine and marine acute LC_{50} with fish (Sheepshead minnow), shrimp (Mysid shrimp), and mollusks (Eastern oyster).

Pending the evaluation of the degradate acute studies and environmental fate data, the following studies are reserved:

72-4 Avian reproduction with an upland game (Bobwhite quail) and a waterfowl species (mallard).

72-4 Freshwater fish early life-stage (Rainbow trout) and freshwater invertebrate life-cycle with Daphnia magna.

72-4 Estuarine fish early life stage (Sheepshead minnow) and estuarine invertebrate life-cycle (Mysid shrimp).

72-5 Fish full life-cycle.

72-6 Aquatic organism accumulation.

The following data requirement with technical grade Amitraz is not fulfilled:

71-4 Avian reproduction with bobwhite and mallard.

The following field testing may be required depending on the results of the above and environmental fate data:

71-5 Simulated or actual field testing --mammals and birds.

72-7 Simulated or actual field testing--aquatic organisms.

If you have any questions, please contact Heather Mansfield.

DATA EVALUATION REPORT

1. Chemical: Amitraz/106201

2. Test Material: BTS 27919, degradate

3. Study Type: 48 hour EC_{50} with Daphnia.

4. Study Identification: Schupner, J. K. and Young, B. M. 1991. The Static Acute Toxicity of BTS 27919 to Daphnia magna. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-07. ID No. 45639-RUA (Amitraz 45639-51). Study No. 507L.

5. Reviewed By:
Heather N. Mansfield, Zoologist
Ecological Effects Branch
Environmental Fate and Effects Division
Signature: Heather Mansfield
Date: 7/19/91

6. Approved by:
for Norman J. Cook, Head, Section 2
Ecological effects Branch
Environmental Fate and Effects Division
Signature: Allen W. Vaughan
Date: 7.17.91

7. Conclusions: The study is scientifically sound and meets the requirements for an acute toxicity test with daphnia. The reported results indicate that BTS 27919 is "practically non-toxic" to Daphnia magna. The 48 hour EC_{50} is >100 ppm.

8. Recommendations: N/A

9. Background: This study was reviewed as a part of the registration of Amitraz.

10. Discussion of individual studies: N/A

11. Materials and Methods:

Test Material: BTS 27919, degradate of Amitraz
Test Organism: Daphnia magna
Source: Parental stock purchased from Aquatic Research Organisms, Hampton, New Hampshire (origin: USEPA laboratories in Cincinnati, Ohio). Young reared at Nor-Am Chemical Company.
Age: ≤ 24 hours
Acclimation: 4 hours under test conditions
Container: 250 mL glass beaker
Aerated: No
Concentration: Measured
Number of Organisms per container: 10
Replicates: 3 per test level
Temperature: 19.6 C± .46
Photoperiod: 16:8 light:dark
Control(s): Synthetic, soft freshwater
DMF
Treatment of stock solution: ultrasonicated for one hour
Feeding: Unfed during test.

12. Reported Results:

DO at start ≥ 96% at 24 hours and ≥ 99% at 48 hours

pH 7.0 at start and 7.2 at end.

Nominal (Treatment-Replicate) Concentration (ppm)	Measured 0 Hour Concentration (ppm)	Measured 48 Hour Concentration (ppm)
Control-1	0	0
Control-2	0	0
Control-3	0	0
Solvent Control-1	0	0
Solvent Control-2	0	0
Solvent Control-3	0	0
100-1	108	94
100-2	106	100
100-3	108	105

* Test Conc. (ppm)	# Tested	Cumulative # of Abnormalities 24hrs	48hrs
(measured at end of study)			
Control-1	10	0	0
Control-2	10	0	0
Control-3	10	0	0
Solvent Control-1	10	0	0
Solvent Control-2	10	0	0
Solvent Control-3	10	0	0
94	10	10[1]	4
100	10	10[1,2,3]	8
105	10	10[1]	5

* The 3 replicates of nominal concentration 100 ppm are listed in the above table as their measured 48 hour concentration.

[1] Lethargic
[2] Surfacing
[3] Erratic

13. Study Author's Conclusions:
48 hour EC_{50} >100 ppm

There were no mortalities in any of the test tanks during the study.

NOEC: Not obtained

17 out of 30 daphnids displayed abnormal behavior at 48 hours.

14. Reviewer's Discussion:

A. **Test Procedure**-The test procedures were in accordance with Subdivision E and SEP guidelines with the following exceptions:

The report does not specify if the daphnids used are of at least the fourth brood of the parents.

No precise NOEC value was reported.

B. **Statistical Analysis**-No statistical analysis was performed.

C. **Discussion of Results:**-The EC_{50} is > 100 ppm.

The NOEC was not reported.

All daphnids showed abnormal behavior at 24 hours. 13 (out of 30) daphnids recovered by 48 hours. No deaths occurred.

The results indicate that the degradate of Amitraz is "practically non-toxic" to Daphnia magna .

D. **Category of Study**-Core, BTS 27919, degradate.

15. Completion of One Liner: Completed, 5/2/91.

16. CBI Attachments: N/A

ONE-LINER FORMAT FOR ENTERING DATA

Shaughnessy No. 106201 Chemical Name: (Amitraz) BTS 27919, degradate

Chemical Class: I

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
48-hour EC50 Species: daphnia magna Lab: Nor-Am Chemical Company Acc#: MRID No. 418272-07		EC50> 100 ppm Slope= 95% CL (N/A) Animals/level= 30 Control Mort.= 0 Temperature= 19°C ± .46 Sol Cont Mort= 0 48-hour dose level ppm (% mortality) % mortalities 100 (0), 100 (57), ( ), ( ), ( ), ( ) Comments: 9% abnormalities	[illegible] 5/2/91	Core

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
48-hour LC50 Species: Lab: Acc#:		LC50= pp Slope= 95% CL ( ) Animals/level= Control Mort.= Temperature= Sol Cont Mort= 48-hour dose level pp (% mortality) ( ), ( ), ( ), ( ), ( ), ( ) Comments:		

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
48-hour LC50 Species: Lab: Acc#:		LC50= pp Slope= 95% CL ( ) Animals/level= Control Mort.= Temperature= Sol Cont Mort= 48-hour dose level pp (% mortality) ( ), ( ), ( ), ( ), ( ), ( ) Comments:		

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
48-hour LC50 Species: Lab: Acc#:		LC50= pp Slope= 95% CL ( ) Animals/level= Control Mort.= Temperature= Sol Cont Mort= 48-hour dose level pp (% mortality) ( ), ( ), ( ), ( ), ( ), ( ) Comments:		

DATA EVALUATION REPORT

1. Chemical: Amitraz/106201

2. Test Material: BTS 27919, degradate

3. Study Type: 96 hour LC_{50} with Bluegill sunfish.

4. Study Identification: Schupner, J. K. 1991. The Static Acute Toxicity of BTS 27919 to the Bluegill sunfish, Lepomis macrochirus. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-05. ID No. 045639-RUA (Amitraz 04569-51). Study No. 500L.

5. Reviewed By:
Heather N. Mansfield, Zoologist
Ecological Effects Branch
Environmental Fate and Effects Division
Signature: Heather Mansfield
Date: 7/19/91

6. Approved by:
for Norman J. Cook, Head, Section 2
Ecological effects Branch
Environmental Fate and Effects Division
Signature: Allen W. Vaughan
Date: 7.17.91

7. Conclusions: The study is scientifically sound and meets the requirements for an acute toxicity test with freshwater fish. The reported results indicate that BTS 27919 is "practically non-toxic" to Bluegill sunfish. The 96 hour LC_{50} is >100 ppm.

The NOEC was not obtained. All treated fish showed abnormal behavior.

8. Recommendations: N/A

9. Background: This study was reviewed as a part of the registration of Amitraz.

10. Discussion of individual studies: N/A

11. Materials and Methods:

Test Material: BTS 27919, degradate of Amitraz
Test Organism: Bluegill sunfish
Source: Aquatic Research Organisms, Hampton, New Hampshire
Age: 3-4 months
Acclimation: 48 hours under test conditions, without food
Container: 19 L glass aquarium
(Volume of test solution: 10 L)
Aerated: No
Concentration: Measured
Number of Organisms per container: 10
Replicates: 3 containers
Temperature: 22.5 C± 1
Photoperiod: 16:8 light:dark
Control(s): Synthetic, soft freshwater
DMF
Treatment of test solution: ultrasonicated for 1 hour to dissolve compound
Feeding: Unfed during test.
Loading: 0.69 g/L

12. Reported Results:

DO at start 60% at end 40%

pH at start 7.1 at end 6.5.

Nominal (Treatment-Replicate) Concentration (ppm)	Measured 0 Hour Concentration (ppm)	Measured 96 Hour Concentration (ppm)
Control-1	0	0
Control-2	0	0
Control-3	0	0
Solvent Control-1	0	0
Solvent Control-2	0	0
Solvent Control-3	0	0
100-1	97	96
100-2	104	99
100-3	103	100

* Test Conc. (ppm)	# Tested	Cumulative # of Mortalities 24hrs	48hrs	72hrs	96hrs
(measured at end of study)					
Control-1	10	0	0	0	0
Control-2	10	0	0	0	0
Control-3	10	0	0	0	0
Solvent Control-1	10	0	0	0	0
Solvent Control-2	10	0	0	0	0
Solvent Control-3	10	0	0	0	0
99	10	0	0	0	0
96	10	0	0	0	0
100	10	0	0	0	0

*The 3 replicates of nominal concentration 100 ppm are listed in the above table as their measured 96 hour concentration.

13. Study Author's Conclusions:
96 hour LC_{50} >100 ppm

NOEC: Not reported. All test organisms showed some abnormal behavior.

14. Reviewer's Discussion:

A. **Test Procedure**-The test procedures were in accordance with Subdivision E and SEP guidelines with the following exceptions:

No precise NOEC value was reported.

As indicated by study reviewer, the loading factor was .69 g/L, higher than the .5 g/L suggested by SEP guidelines.

B. **Statistical Analysis**-No statistical analysis was performed.

C. **Discussion of Results**-All test organisms showed abnormalities throughout the entire study. The NOEC value was not obtained.

The loading factor was higher than recommended by SEP guidelines. The loading factor may affect the dissolved oxygen content. However, this reviewer agrees with the study author's conclusion that dissolved oxygen levels were high enough to compensate for this discrepancy. An increased loading factor may also affect the test concentration. In this case, the test concentration was measured and the minimal decline in concentration indicates this was not a problem.

The LC_{50} is > 100 ppm.

The results indicate that the degradate of Amitraz is "practically non-toxic" to the Bluegill sunfish.

D. **Category of Study**-Core, BTS 27919, degradate.

15. Completion of One Liner: Completed, 5/2/91.

16. CBI Attachments: N/A

ONE-LINER FORMAT FOR ENTERING DATA

Shaughnessy No. 106201 Chemical Name: (Amitraz) BTS 27919, degradate

Chemical Class: I

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
96-hour LC50		LC50= 7100 ppm Slope=	Heather Mansfield 5/2/91	Core
Species: Bluegill sunfish Lepomis macrochirus		95% CL ( ) Animals/level= 30		
		Control Mort.= 0		
Lab: NOR-AM Chemical Company		Temperature= 22.5°C±1 Sol Cont Mort= 0		
Acc#: MRID No. 418272-05		96-hour dose level ppm (% mortality)		
		100 (0), ( ), ( ), ( ), ( ), ( )		
		Comments: All test organisms displayed abnormalities throughout the entire study		

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Accession Number	a.i.	Results	Reviewer Date	Valid. Status
96-hour LC50		LC50= pp Slope=		
Species:		95% CL ( ) Animals/level=		
		Control Mort.=		
Lab:		Temperature= Sol Cont Mort=		
		96-hour dose level pp (% mortality)		
Acc#:		( ), ( ), ( ), ( ), ( ), (		
		Comments:		

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
96-hour LC50		LC50= pp Slope=		
Species:		95% CL ( ) Animals/level=		
		Control Mort.=		
Lab:		Temperature= Sol Cont Mort=		
		96-hour dose level pp (% mortality)		
Acc#:		( ), ( ), ( ), ( ), ( ), (		
		Comments:		

DATA EVALUATION REPORT

1. Chemical: Amitraz/106201

2. Test Material: BTS 27919, degradate

3. Study Type: 96 hour LC_{50} with Rainbow trout.

4. Study Identification: Schupner, J. K. 1991. The Static Acute Toxicity of BTS 27919 to the Rainbow trout, Oncorhynchus mykiss. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-06. ID No. 045639-RUA (Amitraz 45639-51). STUDY No. 501L.

5. Reviewed By:
Heather N. Mansfield, Zoologist
Ecological Effects Branch
Environmental Fate and Effects Division
Signature: Heather Mansfield
Date: 7/19/91

6. Approved by:
for Norman J. Cook, Head, Section 2
Ecological effects Branch
Environmental Fate and Effects Division
Signature: Allen W. Vaughan
Date: 7.17.91

7. Conclusions: The study is scientifically sound and meets the requirements for an acute toxicity test with freshwater fish. The reported results indicate that BTS 27919 is slightly toxic to Rainbow trout. The reported LC_{50}s are listed:

24 hour...98.02 ppm
48 hour...92.80 ppm
72 hour...77.14 ppm
96 hour...74.02 ppm

The NOEC is 31.4 ppm.

8. Recommendations: N/A

9. Background: This study was reviewed as a part of the registration of Amitraz.

10. Discussion of individual studies: N/A

11. Materials and Methods:

Test Material: BTS 27919, degradate of Amitraz
Test Organism: Rainbow trout
Source: Aquatic Research Organisms, Hampton, New Hampshire
Age: 22 days
Acclimation: 10 days in test chamber
Unfed 48 hours prior to testing
Container: 38 L glass aquarium
(30 L of test solution)
Aerated: No
Concentration: Measured
No. of Organisms per container: 5
Replicates: 2 containers per test level
Temperature: 12.4 C±1
Photoperiod: 16:8 light:dark
Control(s): Synthetic soft water
DMF
Treatment of test solution: stock solution was utrasonicated for one hour to dissolve test material
Feeding: Unfed during test.

12. Reported Results:

DO at start 94% at end 40%

pH at start 7.4 at end 6.9.

(Treatment-Replicate) Nominal Concentration (ppm)	Measured 0 Hour Concentration (ppm)	Measured 96 Hour Concentration(ppm)
Control	0	0
Control	0	0
Solvent Control-1	0	0
Solvent Control-2	0	0
17-1	19.5	19.6
17-22	18.0	18.7
28-1	31.1	30.5
28-2	31.4	30.5
47-1	52.9	52.5
47.2	53.5	54.5
78-1	82.0	92.8
78-2	84.9	84.5
130-1	118	131
130-3	104	128

* Test Conc. (ppm)	# Tested	Cumulative # of Mortalities 24hrs	48hrs	72hrs	96hrs
(measured at end of study)					
Control	5	0	0	0	0
Control	5	0	0	0	0
Solvent Control	5	0	0	0	0
Solvent Control	5	0	0	0	0
18.7	5	0	0	0	0
19.6	5	0	0	0	0
30.5	5	0	0	0	0
32.7	5	0	0	0	0
52.5	5	0	0	0	0
54.5	5	0	0	0	0
84.5	5	0	0	2	3
92.8	5	1	3	2	3
128	5	5	5	5	5
131	5	5	5	5	5

* Although only 5 initial test concentrations (other than control) were employed, the replicates varied slightly in their measured concentrations. For increased accuracy, the measured concentrations were used in calculations and are noted here.

13. Study Author's Conclusions:

96 hour LC_{50} 74.02 ppm (angle transformation and linear interpolation)
95% Confidence Limits: 53.43 and 120 ppm
Slope: 2.90
NOEC: 31.4 ppm

14. Reviewer's Discussion:

A. **Test Procedure**-The test procedures were in accordance with Subdivision E and SEP guidelines.

B. **Statistical Analysis**-The LC_{50} was calculated (attached) by the binomial test using EPA's Toxanal computer program.

C. **Discussion of Results**-The LC_{50} was 66.23 ppm, with a 95% confidence interval of 0 and +infinity ppm. The slight discrepancy between the reviewer and study author LC_{50} (74.02) may be attributed to both the reviewer's use of measured replicate concentration as opposed to study author's use of average measured concentration and to the different statistical methods used. The NOEC was 31.44 ppm.

The results indicate that the degradate of Amitraz is slightly toxic to the Rainbow trout.

D. **Category of Study**-Core, BTS 27919, degradate.

15. Completion of One Liner: Completed 5/2/91.

16. CBI Attachments: N/A

bts27919 amitraz rainbow trout

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
118	5	5	100	3.125
104	5	5	100	3.125
84.9	5	3	60.00001	50
82	5	5	100	3.125
53.5	5	0	0	3.125
52.9	5	0	0	3.125
31.4	5	0	0	3.125
31.1	5	0	0	3.125
19.5	5	0	0	3.125
18	5	0	0	3.125

THE BINOMIAL TEST SHOWS THAT 0 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 66.23444

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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ONE-LINER FORMAT FOR ENTERING DATA

Shaughnessy No. 106201 Chemical Name: (Amitraz) BTS 27919, degradate

Chemical Class: I

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
96-hour LC50		LC50= 66.23 pp m Slope= 2.90	Heather Macfarlane 5/2/91	Core
Species: Rainbow Trout Oncorhynchus mykiss	____	95% CL (0 to + infinity) Animals/level= 10 Control Mort.= 0		
Lab: Nor-Am Chemical Company		Temperature= Sol Cont Mort= 0		
Acc#: MRID # 418272-01		96-hour dose level pp (% mortality) 17 (0), 28 (0), 47 (0), 78 (20), 130 (100), ( ) Comments:		

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
96-hour LC50		LC50= pp____ Slope=		
Species:	____	95% CL ( ) Animals/level=		
		Control Mort.=		
Lab:		Temperature= Sol Cont Mort=		
		96-hour dose level pp (% mortality)		
Acc#:		( ), ( ), ( ), ( ), ( ), ( ) Comments:		

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Study/Species/Lab Accession Number	Chem a.i.	Results	Reviewer Date	Valid. Status
96-hour LC50		LC50= pp____ Slope=		
Species:	____	95% CL ( ) Animals/level=		
		Control Mort.=		
Lab:		Temperature= Sol Cont Mort=		
		96-hour dose level pp (% mortality)		
Acc#:		( ), ( ), ( ), ( ), ( ), ( ) Comments:		

DATA EVALUATION REPORT

1. Chemical: Amitraz/106201

2. Test Material: BTS 27271-HCl, degradate

3. Study Type: 48 hour EC_{50} with Daphnia.

4. Study Identification: Schupner, J. K. and Stachura, B. J. 1991. The Static Acute Toxicity of BTS 27271 to Daphnia magna. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-04. ID No. 45639-RUA (Amitraz 45639-51). STUDY No. 511L.

5. Reviewed By:
Heather N. Mansfield, Zoologist
Ecological Effects Branch
Environmental Fate and Effects Division
Signature: Heather Mansfield
Date: 7/19/91

6. Approved by:
for Norman J. Cook, Head, Section 2
Ecological effects Branch
Environmental Fate and Effects Division
Signature: Allen W. Vaughan
Date: 7.18.91

7. Conclusions: This study has been reviewed and has been found to be scientifically sound. It does not fulfill the requirements for an acute toxicity test for daphnia because the test material was not the degradate requested, but rather an adduct.

The 48 hour EC_{50} is 3.28 ppm.

NOEC=1.34 ppm.

Slope= 5.54

The reported results indicate that BTS 27271-HCl is moderately toxic to Daphnia magna.

8. Recommendations: The study author's report indicates that the instability of BTS 27271 necessitated the formulation of BTS 27271-HCl as the test substance. EEB would like a more thorough explanation as to why it was necessary to formulate the test compound as a hydrochloride and how this adduct compares, toxicologically, to BTS 27271. The dissociation constant and water solubility of BTS 27271 and BTS 27271-HCl are needed.

9. Background: This study was reviewed as a part of the registration of Amitraz.

10. Discussion of individual studies: N/A

11. Materials and Methods:

Test Material: BTS 27271-HCl
Test Organism: Daphnia magna
Source: Parental stock purchased from Aquatic Research Organisms, Hampton, New Hampshire (originated from USEPA laboratories in Cincinnati Ohio). Young reared at Nor-Am Chemical Company.
Age: ≤ 24 hours
Acclimation: 4 hours under test conditions
Container: 250 mL glass beaker
Aerated: No
Concentration: Measured
Number of Organisms per container: 10
Replicates: 2 per concentration
Temperature: 21.4 C± .10
Photoperiod: 16:8 light:dark
Control(s): Synthetic, soft freshwater
Feeding: Unfed during test.

12. Reported Results:

DO at start ≥ 97% at 24 hours and ≥ 95% at 48 hours

pH 6.6 at start and 6.5 at end.

Nominal (Treatment-Replicate) Concentration (ppm)	Measured 0 Hour Concentration (ppm)	Measured 48 Hour Concentration (ppm)
Control-1	0	0
Control-2	0	0
1.9-1	1.61	1.06
1.9-2		1.10
3.2-1	3.17	1.86
3.2-2		1.92
5.4-1	4.98	3.65
5.4-2		3.45
9-1	8.44	5.70
9-2		5.89
15-1	13.6	8.77
15-2		8.45
25-1	22.6	16.4
25-2		15.0

* Test Conc. (ppm)	# Tested	Cumulative # of Abnormalities and Mortalities 24hrs	48hrs
(measured at end of study)			
Control-1	10	0	0
Control-2	10	0	0
1.06	10	0	0
1.10	10	0	0
1.86	10	2	2
1.92	10	1	4
3.65	10	0	7
3.45	10	0	8
5.70	10	9	9
5.89	10	3	10
8.77	10	4	10
8.45	10	4	10
16.4	10	4	10
15.0	10	3	10

* Although only 6 initial test concentrations were employed, the replicates varied slightly in their measured concentrations. For increased accuracy, the measured concentrations were used in calculations and are noted here.

13. Study Author's Conclusions:

48 hour EC_{50} =3.28 ppm. 95% Confidence Limits: 3.03 to 3.54.
Slope: 5.54
NOEC: 1.34 ppm.

14. Reviewer's Discussion:

A. **Test Procedure**-The test procedure was acceptable. Use of the modified degradate precludes this test from fulfilling the guideline requirements.

B. **Statistical Analysis**- The LC_{50} was calculated by probit analysis using EPA's Toxanal program.

C. **Discussion of Results**-The LC_{50} was found to be 2.59 ppm, with 95% confidence limits of 2.16 and 3.06.

The EEB's calculated LC_{50} is slightly lower than that of the study author as EEB employed measured concentrations at 48 hours in calculations rather than the average concentration. For the same reason, EEB's NOEC is 1.08 ppm, rather than the reported 1.34 ppm of the study author.

The results indicate that the BTS 27271-HCl is moderately toxic to Daphnia magna.

D. **Category of Study**--Supplemental, because the test material was a modified product: BTS 27271-HCl.

15. Completion of One Liner: Not completed.

16. CBI Attachments: N/A

11.

BT27271 amitraz daphnia magna

****

CONC. (mg/L)	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
16.4	10	10	100	9.765625E-02
15	10	10	100	9.765625E-02
8.770001		10	10	100
9.765625E-02				
8.45	10	10	100	9.765625E-02
5.89	10	10	100	9.765625E-02
5.7	10	9	90	1.074219
3.65	10	7	70	17.1875
3.45	10	8	80	5.46875
1.92	10	4	40	37.69531
1.86	10	2	20	5.46875
1.1	10	0	0	9.765625E-02
1.06	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 1.1 AND 5.7 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 2.210242

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
11	3.736556E-02		2.966951	2.407952
3.550873				

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	.1027303	1	.9574267

SLOPE = 5.182737

95 PERCENT CONFIDENCE LIMITS = 3.521588 AND 6.843885

LC50 = 2.588027

95 PERCENT CONFIDENCE LIMITS = 2.163954 AND 3.065466

LC10 = 1.472057

95 PERCENT CONFIDENCE LIMITS = 1.040513 AND 1.812579

****

DATA EVALUATION REPORT

1. Chemical: Amitraz/106201

2. Test Material: BTS 27271-HCl

3. Study Type: 96 hour LC_{50} with Rainbow trout

4. Study Identification: Schupner, J. K. 1991. The Acute Toxicity of BTS 27271 to the Rainbow trout, Oncorhynchus mykiss in a flow through system. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-03. ID No. 45639-RUA (Amitraz 45639-51). STUDY No. 512L.

5. Reviewed By:
Heather N. Mansfield, Zoologist
Ecological Effects Branch
Environmental Fate and Effects Division
Signature: Heather Mansfield
Date: 7/19/91

6. Approved by:
for Norman J. Cook, Head, Section 2
Ecological effects Branch
Environmental Fate and Effects Division
Signature: Allen W. Vaughan
Date: 7.18.91

7. Conclusions: This study has been reviewed and has been found to be scientifically sound. It does not, however, fulfill the requirements for an acute toxicity test for coldwater fish because the test material was not the degradate requested, but rather an adduct.

The reported results indicate that BTS 27271-HCl is slightly toxic to Rainbow trout. The reported LC_{50}s are listed:

24 hour...31.41 ppm (probit analysis)
48 hour...27.92 ppm (moving
72 hour...27.92 ppm average
96 hour...27.92 ppm method)

The NOEC is 13.4 ppm.

8. Recommendations: The study author's report indicates that the instability of BTS 27271 necessitated the formulation of BTS 27271-HCl as the test substance. EEB would like a more thorough explanation as to why it was necessary to formulate the test compound as a hydrochloride and how this adduct compares, toxicologically, to BTS 27271. The dissociation constant and water solubility of BTS 27271 and BTS 27271-HCl are needed.

9. Background: This study was reviewed as a part of the registration of Amitraz.

10. Discussion of individual studies: N/A

11. Materials and Methods:

Test Material: BTS 27271-HCl
Test Organism: Rainbow trout
Source: Aquatic Research Organisms
Age: 9 weeks
Acclimation: 3 days in test chamber, without food
Container: 12 L glass aquarium
(Volume of test solution: 10 L)
Aerated: No
Concentration: Measured
Replicates: 2 per treatment level
Temperature: 12 C±1
Photoperiod: 16:8 light:dark
Control(s): Synthetic soft freshwater
Feeding: Unfed during test.

12. Reported Results:

DO at start 92% at end 86%

pH at start 7.0 at end 6.5.

Nominal (Treatment-Replicate) Concentration (ppm)	Measured 0 Hour Concentration (ppm)	Measured 96 Hour Concentration (ppm)
Control	0	0
Control	0	0
6.5-1	5.4	5.3
6.5-2	5.0	4.9
11-1	8.5	8.1
11-2	9.0	8.5
18-1	14.0	13.1
18-2	13.4	13.1
30-1	23.9	23.6
30-2	23.5	23.1
50-1	34.4	38.5
50-2	40.1	38.7

* Test Conc. (ppm)	# Tested	Cumulative # of Mortalities 24hrs	48hrs	72hrs	96hrs
(measured at end of study)					
Control	10	1	0	0	0
Control	10	0	0	0	0
4.9	10	0	0	0	0
5.3	10	0	0	0	0
8.1	10	0	0	0	0
8.5	10	0	0	0	0
13.1	10	0	0	0	0
13.1	10	0	0	0	0
23.1	10	1	1	1	1
23.6	10	2	2	2	2
38.5	10	7	9	9	9
38.7	10	8	10	10	10

* Although only 6 initial test concentrations were employed, the replicates varied slightly in their measured concentrations. For increased accuracy, the measured concentrations were used in calculations and are noted here.

13. Study Author's Conclusions:
96 hour LC_{50} 27.92 ppm (moving average)
95% Confidence Limits: 24.83 and 32.26 ppm
Slope: 2.06
NOEC: 13.4 ppm

14. Reviewer's Discussion:

A. **Test Procedure**-The test procedure was acceptable. Use of the modified degradate, however, precludes this test from fulfilling the guideline requirements.

B. **Statistical Analysis**-The LC_{50} (attached) was calculated by the probit test using EPA's Toxanal computer program.

C. **Discussion of Results**-The LC_{50} was 28.36 ppm, with a 95% confidence interval of 25.46 and 31.91 ppm. This value varies only 0.44 ppm from the study author's conclusion.

Contrary to the study author's NOEL of 13.4 ppm, EEB finds the NOEL to be < 4.9 ppm based on the effects that are seen at every test level throughout the study.

The results indicate that BTS 27271-HCl is slightly toxic to the Rainbow trout.

D. **Category of Study**-Supplemental, because the test material was a modified product: BTS 27271-HCl.

15. Completion of One Liner: Not completed.

16. CBI Attachments: N/A

NOTE: THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
OF THE LOWER CONCENTRATIONS HAD ZERO MORTALITY.
THEREFORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

BTS2721-HCl Amitraz Rainbow trout 96 hour LC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
38.7	10	10	100	9.765625E-02
38.5	10	9	90	1.074219
23.6	10	2	20	5.46875
23.1	10	1	10	1.074219
13.11	10	0	0	9.765625E-02
13.1	10	0	0	9.765625E-02
8.5	10	0	0	9.765625E-02
8.100001		10	0	0
9.765625E-02				
5.3	10	0	0	9.765625E-02
4.9	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT 23.1 AND 38.5 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 28.89277

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
9	7.085946E-02	24.90572	19.99428	
33.9735				

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	
GOODNESS OF FIT PROBABILITY			
14	.1807618	1	
.9963281			

SLOPE = 12.37472
95 PERCENT CONFIDENCE LIMITS = 7.113474 AND 17.63597

LC50 = 28.36218
95 PERCENT CONFIDENCE LIMITS = 25.46042 AND 31.90793

LC10 = 22.39294
95 PERCENT CONFIDENCE LIMITS = 18.24035 AND 25.01407

****

DATA EVALUATION REPORT

1. Chemical: Amitraz/106201

2. Test Material: BTS 27271-HCl

3. Study Type: 96 hour LC_{50} with Bluegill sunfish.

4. Study Identification: Schupner, J. K. and Stachura, B. J. 1991. The Acute Toxicity of BTS 27271 to the Bluegill sunfish, Lepomis macrochirus in a flow through system. Study performed by Nor-Am Chemical Company, Nor-Am Research Center, Pikesville, NC 27863. MRID No. 418272-02. ID No. 45639-RUA (Amitraz 45639-51). Study No. 513L.

5. Reviewed By:
Heather N. Mansfield, Zoologist
Ecological Effects Branch
Environmental Fate and Effects Division
Signature: Heather Mansfield
Date: 7/19/91

6. Approved by:
for Norman J. Cook, Head, Section 2
Ecological effects Branch
Environmental Fate and Effects Division
Signature: Allen W. Vaughan
Date: 7.18.91

7. Conclusions: This study has been reviewed and has been found to be scientifically sound. It does not, however, fulfill the requirements for an acute toxicity test for warmwater fish because the test material was not the degradate requested, but rather an adduct.

The reported results indicate that BTS 27271-HCl is slightly toxic to Bluegill sunfish. The 96 hour LC_{50} is 29.98 ppm.

The NOEC is < 6.8 ppm. No precise NOEC was obtained.

8. Recommendations: The study author's report indicates that the instability of BTS 27271 necessitated the formulation of BTS 27271-HCl as the test substance. EEB would like a more thorough explanation as to why it was necessary to formulate the test compound as a hydrochloride and how this adduct compares, toxicologically, to BTS 27271. The dissociation constant and water solubility of BTS 27271 and BTS 27271-HCl are needed.

9. Background: This study was reviewed as a part of the registration of Amitraz.

10. Discussion of individual studies: N/A

11. Materials and Methods:

Test Material: BTS 27271-HCl
Test Organism: Bluegill sunfish
Source: Sea Plantations, Inc.
Age: 5 months
Acclimation: 48 hours under test conditions, without food
Container: 19 L glass aquarium
(Volume of test solution: 10 L)
Aerated: No
Concentration: Measured
Number of Organisms per container: 10
Replicates: 2 for test level
Temperature: 21.5 C±.39
Photoperiod: 16:8 light:dark
Control(s): Synthetic, soft freshwater
Feeding: Unfed during test.

12. Reported Results:

DO at start 100% at end 92%

pH at start 7.1 at end 6.8.

Nominal (Treatment-Replicate) Concentration (ppm)	Measured 0 Hour Concentration (ppm)	Measured 96 Hour Concentration (ppm)
Control-1	0	0
Control-2	0	0
10-1	7.0	6.8
10-2	6.8	6.8
17-1	12	12
17-2	12	12
29-1	19	18
29-2	20	20
48-1	32	32
48-2	33	31
80-1	57	52
80-2	55	52

* Test Conc. (ppm)	# Tested	Cumulative # of Mortalities 24hrs	48hrs	72hrs	96hrs
(measured at end of study)					
Control	10	0	0	0	0
Control	10	0	0	0	0
6.8	10	0	0	0	0
6.8	10	0	0	0	0
12	10	0	0	0	0
12	10	0	0	0	0
18	10	2	2	2	2
19	10	2	2	2	2
32	10	3	3	3	3
31	10	5	5	5	5
52	10	10	10	10	10
52	10	10	10	10	10

* Although only 6 initial test concentrations were employed, the replicates varied slightly in their measured concentrations. For increased accuracy, the measured concentrations were used in calculations and are noted here.

13. Study Author's Conclusions:
96 hour LC_{50} 29.98 ppm (probit)
95% Confidence Limits: 22.76 and 40.48 ppm
Slope: 1.5
NOEC: < 6.8 ppm

14. Reviewer's Discussion:

A. **Test Procedure**-The test procedure was acceptable. Use of the modified degradate precludes this test from fulfilling the guideline requirements.

B. **Statistical Analysis**-The LC_{50} (attached) was calculated by the probit analysis using EPA's Toxanal computer program.

C. **Discussion of Results**-The LC_{50} was 29.33 ppm, with a 95% confidence interval of 25.23 and 34.32 ppm.

The results indicate that BTS 27271-HCl is slightly toxic to the Bluegill sunfish.

D. **Category of Study**-Supplemental, because the test material was a modified product: BTS 27271-HCl.

15. Completion of One Liner: Not completed.

16. CBI Attachments: N/A

' LC50 for 96 hours - cumulative deaths

bts27271-hcl amitraz bluegill sunfish

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
52	20	20	100	9.536742E-05
31.5	20	8	40	25.17223
18.5	20	4	20	.5908966
12	20	0	0	9.536742E-05
6.8	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 18.5 AND 52 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 33.52637

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.161344E-02	28.15049	24.837	32.43627

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	.1241088	1	.1280705

SLOPE = 6.109026
95 PERCENT CONFIDENCE LIMITS = 3.956872 AND 8.26118

LC50 = 29.32562
95 PERCENT CONFIDENCE LIMITS = 25.22722 AND 34.32111

LC10 = 18.17013
95 PERCENT CONFIDENCE LIMITS = 13.41606 AND 21.63287

SCHERING BERLIN

March 27, 1991

NOR-AM Chemical Company

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ATTN: DENNIS H. EDWARDS, JR.
PRODUCT MANAGER (12)

Dear Mr. Edwards:

SUBJ: **OVASYN (EPA FILE SYMBOL 45639-RUA)**
AMITRAZ (EPA REG. NO. 45639-51, TECHNICAL);
SUBMISSION OF STUDIES AS REQUESTED IN
AGENCY REVIEW OF AUGUST 15, 1990

NOR-AM Chemical Company is herein submitting four copies of the following study reports:

72-1 "The Acute Toxicity of BTS 27271 to the Bluegill Sunfish, Lepomis macrochirus, in a Flow Through System"

72-1 "The Acute Toxicity of BTS 27271 to the Rainbow Trout, Oncorhynchus mykiss, in a Flow Through System"

72-2 "The Static Acute Toxicity of BTS 27271 to Daphnia magna"

72-1 "The Static Acute Toxicity of BTS 27919 to the Bluegill Sunfish, Lepomis macrochirus"

72-1 "The Static Acute Toxicity of BTS 27919 to the Rainbow Trout, Oncorhynchus mykiss"

72-2 "The Static Acute Toxicity of BTS 27919 to Daphnia magna"

This submission is organized according to PR Notice, 86-5, and includes a transmittal document. Please note that Volume 2 of this submission presents an overview of the submitted data and a comparison to similar data on amitraz technical.

These data show that the metabolites BTS 27271 and BTS 27919 are much less toxic to the tested species as compared to amitraz technical. BTS 27271 ranges in magnitude from 37 to 93 times less toxic than amitraz while BTS 27919 ranges in magnitude from 100 to > 2800 times less toxic than amitraz.

This submission is made to address some of the data requirements set forth in the EEB Review dated August 15, 1990; record number 265301 (copy attached). Additional data are being generated and will be submitted as soon as available. If warranted, please make the appropriate reviewers of the pending OVASYN EUP applications aware of the availability of these data.

If you have any questions, please call me at 302-575-2048. I look forward to an expeditious review of the enclosed study reports. Best regards.

Sincerely,

Jacob J. Vukich
Registration Project Manager

mmg-p
121.1A
Attach.