

File

12-20-96

DP Barcode : D232182
 PC Code No : 128810
 EEB Out : DEC 20 1996

To: Cynthia Giles-Parker/John Bazuin
 Product Manager 22
 Registration Division (7505C)

From: Anthony F. Maciorowski, Chief
 Ecological Effects Branch/EFED (7507C)

Attached, please find the EEB review of...

Reg./File # : 10182-UNI
 Chemical Name : Azoxystrobin (degradates)
 Type Product : Fungicide
 Product Name :
 Company Name : Zeneca Ag Products
 Purpose : Degradate toxicity to aquatic organisms
 Action Code : 101 Date Due : 04/17/97
 Reviewer : William Erickson Date In : 12/17/96

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.	Gdln No.	MRID No.	Cat.
71-1(a)			72-2(a)	441588-04 441588-01 441588-02	Y S S	72-7(a)		
71-1(b)			72-2(b)			72-7(b)		
71-2(a)			72-3(a)			122-1(a)		
71-2(b)			72-3(b)			122-1(b)		
71-3			72-3(c)			122-2		
71-4(a)			72-3(d)			123-1(a)		
71-4(b)			72-3(e)			123-1(b)		
71-5(a)			72-3(f)			123-2		
71-5(b)			72-4(a)			124-1		
72-1(a)			72-4(b)			124-2		
72-1(b)			72-5			141-1		
72-1(c)	441588-03	Y	72-6			141-2		
72-1(d)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur

P=Partial (Study partially fulfilled Guideline but additional information is needed)

S=Supplemental (Study provided useful information but Guideline was not satisfied)

①

DP BARCODE: D232182

CASE: 005533
SUBMISSION: S515795

DATA PACKAGE RECORD
BEAN SHEET

DATE: 12/20/96
Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REGISTRATION ACTION: 101 RESB NC-FOOD/FEED USE
RANKING : 0 POINTS ()
CHEMICALS: 128810 Azoxystrobin (BSI proposed name) (CAS Reg. No. 131 50.0000%
ID#: 010182-UNI ICIA5504 50WG
COMPANY: 010182 ZENECA AG PRODUCTS
PRODUCT MANAGER: 22 CYNTHIA GILES-PARKER 703-305-5540 ROOM: CM2 229
PM TEAM REVIEWER: JOHN BAZUIN 703-305-7381 ROOM: CM2 261
RECEIVED DATE: 11/15/96 DUE OUT DATE: 05/24/97

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 232182 EXPEDITE: Y DATE SENT: 12/18/96 DATE RET.: / /
CHEMICAL: 128810 Azoxystrobin (BSI proposed name) (CAS Reg. No. 131860-33-8)
DP TYPE: 001 Submission Related Data Package
CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT	ADMIN DUE DATE: 04/17/97
DIV : EFED	12/18/96	/ /	NEGOT DATE: / /
BRAN: EEB	12/19/96	12/20/96	PROJ DATE: / /
SECT: RS4	12/19/96	12/19/96	
REVR : WERICKSO	12/19/96	12/19/96	
CONTR:	/ /	/ /	

* * * DATA REVIEW INSTRUCTIONS * * *

Bill,

Here are four supplementary aquatic toxicology (Rainbow Trout and Daphnia magna) that Zeneca has provided to help with the finalization of the EEB work. Unlike the copies I gave you before, I just discovered that these have actual M-R-I-D Numbers (441588-01, -02, -03, and -04)! Thus, your request is answered. Please use these in your completion of the EEB reviews and evaluations and please be sure that EFED logs this Bean out when it is through.

Thanks,

John Bazuin

* * * DATA PACKAGE EVALUATION * * *

No evaluation is written for this data package

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

DEC 20 1996

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Azoxystrobin (128810): review of degrade aquatic toxicity data for new chemical; D232182; S515795; Zeneca Ag Products

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C)

TO: Cynthia Giles-Parker/John Bazuin
Product Manager 22
Registration Division (7505C)

Handwritten notes: *Sumner*, *12.20.96*, *Calk*

Zeneca Ag Products submitted the following degrade toxicity studies to support registration of the new chemical azoxystrobin:

Kent, S.J., S.A. Sankey, S.K. Cornish, and J.E. Caunter. 1993. R234886: Acute Toxicity to rainbow trout (*Oncorhynchus mykiss*). Conducted by Brixham Environmental Laboratory, Zeneca Limited, Brixham, Devon, UK. Lab. Report No. BL5010/B.

Kent, S.J., S.A. Sankey, A.J. Banner, and P.A. Johnson. 1993. R234886: Acute Toxicity to *Daphnia magna*. Conducted by Brixham Environmental Laboratory, Zeneca Limited, Brixham, Devon, UK. Lab. Report No. BL5008/B.

Hamer, M.J. 1996. R401553: Toxicity to first instar *Daphnia magna*. Conducted by Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, U.K. Lab. Report No. TMJ3703B.

Hamer, M.J. 1996. R402173: Toxicity to first instar *Daphnia magna*. Conducted by Zeneca Agrochemicals, Jealott's Hill Research Station, Bracknell, Berkshire, U.K. Lab. Report No. TMJ3704B.

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The DERs are attached. A summary of the findings are tabulated below.

Degradate	Test species	Toxicity (ppm)	Toxicity category	MRID No.	Classification
R234886	Rainbow trout	LC50 > 150	practically nontoxic	441588-03	core
R234886	<i>Daphnia magna</i>	EC50 > 190	practically nontoxic	441588-04	core
R401553	<i>Daphnia magna</i>	EC50 > 50	slightly toxic	441588-01	supplemental
R402173	<i>Daphnia magna</i>	EC50 > 50	slightly toxic	441588-02	supplemental

The studies were not required but were submitted to provide additional information to assist EFED in conducting the risk assessment for use of azoxystrobin on turf. Because the toxicity values for degradate R234886 exceed 100 ppm, it is considered practically nontoxic to freshwater organisms. The toxicity of degradates R401553 and R402173 exceeded 50 ppm (the only concentration tested) but was not shown to exceed 100 ppm; therefore, these two degradates are considered slightly toxic to freshwater invertebrates.

Contact Bill Erickson at 305-6212 or Harry Craven at 305-5320 if you have any questions about these reviews.

DATA EVALUATION RECORD
S 72-2 -- ACUTE TESTS WITH A FRESHWATER INVERTEBRATE

1. CHEMICAL: Azoxystrobin PC Code No.: 128810
2. TEST MATERIALS: R401553 (degradate; Compound 28); 99% w/w
R402173 (degradate; Compound 30); 98% w/w

3. CITATIONS

Author: Hamer, M.J.
Title: R401553: Toxicity to first instar
Daphnia magna
Date: 1996
Laboratory: Zeneca Agrochemicals, Jealott's Hill
Research Station, Bracknell, Berkshire,
U.K.
Sponsor: ZENECA Ag Products, ZENECA Inc.,
Wilmington, Delaware
Lab. Report ID: TMJ3703B
MRID No.: 441588-01

Author: Hamer, M.J.
Title: R402173: Toxicity to first instar
Daphnia magna
Date: 1996
Laboratory: Zeneca Agrochemicals, Jealott's Hill
Research Station, Bracknell, Berkshire,
U.K.
Sponsor: ZENECA Ag Products, ZENECA Inc.,
Wilmington, Delaware
Lab. Report ID: TMJ3704B
MRID No.: 441588-02

4. REVIEWED BY:

William Erickson
Biologist
EEB/EFED/EPA

Signature: *W. Erickson*

Date: *12/18/96*

5. APPROVED BY:

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: *Harry T. Craven*

Date: *12/19/96*

6. STUDY PARAMETERS/RESULTS SYNOPSIS:

Age of Test Organisms: <24 hours
Definitive Test Durations: 48 hours
Study Methods: Static
Type of Concentrations: Nominal

R401553 EC50: >50 mg/l
 R401553 NOEC: 50 mg/l
 R402173 EC50: >50 mg/l
 R402173 NOEC: 50 mg/l

7. **CONCLUSIONS:** Both studies are scientifically sound but do not fulfill any guideline requirement for azoxystrobin. Because the EC50s are >50 mg/l, the only concentration tested, the two degradates are considered to be slightly toxic to *Daphnia magna*.
8. **ADEQUACY OF THE STUDIES:** Supplemental.
9. **Guideline Deviations:** The following deviated from guideline requirements and recommendations:
 1. Only one concentration was tested; an EC50 was not established nor was it shown to exceed 100 mg/l.
 2. Raw data were not submitted.
 3. Only 10 daphnids were tested per level; 20 per level are required.
 4. Dechlorinated tap water should not be used as a source of dilution water; pH, temperatures, and DO levels were not reported; hardness was higher than recommended.
 5. Plastic beakers were used as test vessels; glass or stainless steel vessels are recommended.
 6. It was not reported whether the daphnids were randomly or impartially assigned to test vessels.
10. **SUBMISSION PURPOSE:** New chemical. R401553 (Compound 28) and R402173 (Compound 30) are reported to be major degradates of azoxystrobin under field conditions.
11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>

Guideline Criteria	Reported Information
<u>Life Stage</u> Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 rd instar.	<24 h old
<u>Supplier</u>	In-house cultures
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	N/A
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	Not reported
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	Not reported
<u>Feeding</u> No feeding during the study.	Not fed during testing
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing.	Not reported

C. Test System:

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water.	dechlorinated mains water
Does water support test ani- mals without observable signs of stress?	Yes

Guideline Criteria	Reported Information
<u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20°C nominal
<u>pH</u> Prefer 7.2 to 7.6.	Not reported
<u>Dissolved Oxygen</u> Static: $\geq 60\%$ during 1 st 48 h and $\geq 40\%$ during 2 nd 48 h, flow-through: $\geq 60\%$.	Not reported
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO ₃ .	160-180 mg/l as CaCO ₃
<u>Test Aquaria</u> 1. <u>Material:</u> Glass or stainless steel. 2. <u>Size:</u> 250 mL (daphnids and midges) or 3.9 L (1 gal). 3. <u>Fill volume:</u> 200 mL (daphnids and midges) or 2-3 L.	Plastic beakers 200 ml 140 ml
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant.	N/A
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^\circ\text{C}$, ≤ 0.5 g/L at $> 17^\circ\text{C}$; flow- through: ≤ 1 g/L/day.	10 daphnids per beaker
<u>Photoperiod</u> 16 hours light, 8 hours dark.	16 hours light 8 hours dark
<u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.	None

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $LC_{50} > 100$ mg/L, then no definitive test is required.	None reported
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	50 mg/l and a dilution water control
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers.	10 each in the treatment and control beakers
Test organisms randomly or impartially assigned to test vessels?	Not reported
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary $> 1^{\circ}C$. 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	Temperature in the water bath reportedly was maintained at $20^{\circ}C$ Not reported
<u>Chemical Analysis</u> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was use	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	No QA statement

<u>Control Mortality</u> Static: ≤10% Flow-through: ≤5%	None reported
<u>Percent Recovery of Chemical</u>	Not reported
<u>Raw data included?</u>	No

No. Daphnids Dead or Immobilized: None.

13. REVIEWER'S COMMENTS: The study provides only supplemental data for both degradates, due to the guideline deviations noted under point "9". Because the EC50s exceed 50 mg/l (the only concentration tested) but were not shown to exceed 100 mg/l, both the R401553 and R402173 degradates must be considered slightly toxic to *Daphnia magna*.

No. Daphnids Effected:

Concentration (ppm)		No. Organisms	Cumulative No. Immobilized	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	<0.027	20	0	0
18	19	20	0	0
32	35	20	0	0
56	59	20	0	2
100	110	20	0	0
180	190	20	0	6

Statistical Results: No analysis conducted; an EC50 reportedly could not be calculated from the data. The 48-h EC50 was reported to be >180 mg/l.

13. VERIFICATION OF STATISTICAL RESULTS: Because immobilization of daphnids at all test concentrations was less than 50%, the EC50 is determined to be >190 mg/l, the highest concentration tested.
14. REVIEWER'S COMMENTS: The source of the dilution water was not reported. However, it is probably acceptable because no signs of toxicity or mortality were observed in the control daphnids. Other deviations from guideline requirements occurred (see "9.") The study is scientifically sound, but degradate testing is not required for azoxystrobin. Because the EC50 exceeded 190 mg/l, the R234886 degradate is considered practically nontoxic to *Daphnia magna*.

DATA EVALUATION RECORD
S 72-1(B) -- ACUTE LC50 TEST WITH A COLDWATER FISH

1. CHEMICAL: Azoxystrobin PC Code No.: 128810
2. TEST MATERIAL: R234886 (degradate); 98% w/w
3. CITATION

Authors: Kent, S.J., S.A. Sankey, S.K. Cornish,
and J.E. Caunter
Title: R234886: Acute Toxicity to rainbow trout
(*Oncorhynchus mykiss*)
Study Completion Date: October 22, 1993.
Laboratory: Brixham Environmental Laboratory, ZENECA
Limited, Brixham, Devon, UK
Sponsor: ZENECA Ag Products, ZENECA Inc.,
Wilmington, Delaware
Laboratory Report ID: BL5010/B
MRID No.: 441588-03

4. REVIEWED BY:

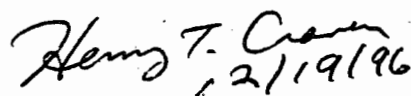
William Erickson
Biologist
EEB/EFED/EPA

Signature: 

Date: 12/18/96

5. APPROVED BY:

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature: 

Date: 12/19/96

6. STUDY PARAMETERS/RESULTS SYNOPSIS:

Size of Test Organism: 1.77 g (mean wt.)
Definitive Test Duration: 96 hours
Study Method: Static
Type of Concentrations: Mean measured
LC50: >150 mg/l ai
NOEC: 150 mg/l ai
95% CI: N/A
Slope: N/A

7. CONCLUSIONS: This study is scientifically sound but does not fulfill any guideline requirement. An acute freshwater fish toxicity test with azoxystrobin degradate R234886 is not required. Because the LC50 is >150 ppm, the degradate is considered practically nontoxic to rainbow trout.
8. ADEQUACY OF THE STUDY: Core.

9. GUIDELINE DEVIATIONS

1. Test temperatures were approximately 3° higher than recommended (12°C).
2. Dechlorinated tap water was used as a source of dilution water; the guidelines do not recommend using tap water.

10. SUBMISSION PURPOSE: New chemical; degradate data.

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is the rainbow trout (<i>Oncorhynchus mykiss</i>)	<i>Oncorhynchus mykiss</i>
<u>Mean Weight</u> 0.5-5 g	Mean: 1.77 g Range: 1.20-2.51 g
<u>Mean Standard Length</u> Longest not > 2x shortest	Mean: 50 mm Range: 46-55 mm
<u>Supplier</u>	Zeals Fish Farm, Zeals, Wiltshire, U.K.
All fish from same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 14 days	41 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A

Guideline Criteria	Reported Information
<u>Feeding</u> No feeding during the study	The fish were not fed for 24 hours prior to test initiation or during the testing
<u>Pretest Mortality</u> < 3% mortality 48 hours prior to testing	<1% mortality during the 2 weeks prior to testing

C. Test System

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water	Dechlorinated tap water passed through activated carbon, filtered, and dechlorinated with sodium thiosulphate; also UV sterilized
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> 12°C	14.6-15.4°C
<u>pH</u> Prefer 7.2 to 7.6	5.8-7.5
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 st 48 hrs and ≥ 40% during 2 nd 48 hrs, flow-through: ≥ 60%	9.2-9.8 mg/l
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO ₃	Dilution water hardness was 29.3 mg/l as CaCO ₃
<u>Test Aquaria</u> 1. <u>Material</u> : Glass or stainless steel 2. <u>Size</u> : Volume of 18.9 L (5 gal) or 30 x 60 x 30 cm 3. <u>Fill volume</u> : 15-30 L of solution	Glass vessels 27.5 l (40 x 28 x 28 cm) 20 l
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant	N/A

Guideline Criteria	Reported Information
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	N/A
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^{\circ}\text{C}$, ≤ 0.5 g/L at $> 17^{\circ}\text{C}$; flow-through: ≤ 1 g/L/day	0.89 g/l
<u>Photoperiod</u> 16 hours light, 8 hours dark	16 hours light 8 hours dark
<u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests	No solvent; prior to dilution, test solutions were given 5 min. ultrasonic treatment and stirred

D. Test Design

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $\text{LC}_{50} > 100$ mg/L with 30 fish, then no definitive test is required.	None reported
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be in a geometric series	18, 32, 56, 100, and 180 mg/l and a dilution water control
<u>Number of Test Organisms</u> Minimum 10/level, may be divided among containers	10 per level
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temperature was monitored hourly within test vessels DO and pH were measured daily
<u>Chemical Analysis</u> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used	Concentrations of R234886 technical in test solutions were sampled and measured at 0, 48, and 96 h

12. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Recovery of Chemical</u>	83-103%
<u>Control Mortality</u> Not more than 10% control organisms may die or show abnormal behavior.	None
Raw data included?	Yes
Signs of toxicity (if any) were described?	None observed

Mortality:

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
Nominal (mg ai/L)	Mean Measured (mg ai/L)		Hour of Study			
			24	48	72	96
Control	<0.03	10	0	0	0	0
18	18	10	0	0	0	0
32	33	10	0	0	0	0
56	53	10	0	0	0	0
100	94	10	0	0	0	0
180	150	10	0	0	0	0

Other Findings: No symptoms of toxicity were observed at any test concentration.

13. VERIFICATION OF STATISTICAL RESULTS: No mortality or symptoms of toxicity occurred during the study. The 96-h LC50 is >150 mg/l, the highest dose tested.
14. REVIEWER'S COMMENTS: The test was conducted at a temperature approximately 3°C higher than recommended for rainbow trout, and dechlorinated water was used as the dilution water. However, because no mortality occurred in the controls or in any of the five test concentrations, the higher test temperature and dilution water had no apparent adverse effects. Total residual chlorine measured during the test was below the detection limit (<4 µg/l CL₂). The study is scientifically sound, but a freshwater fish acute test using azoxystrobin degradate R234886 is not required.

DATA EVALUATION RECORD
S 72-2 -- ACUTE LC₅₀ TEST WITH A FRESHWATER INVERTEBRATE

1. CHEMICAL: Azoxystrobin PC Code No.: 128810

2. TEST MATERIALS: R234886 (degrade); 98% w/w

3. CITATION

Authors: Kent, S.J., S.A. Sankey, A.J. Banner, and
P.A. Johnson
Title: R234886: Acute Toxicity to *Daphnia magna*
Study Completion Date: November 7, 1993
Laboratory: Brixham Environmental Laboratory, ZENECA
Limited, Brixham, Devon, UK
Sponsor: ZENECA Ag Products, ZENECA Inc.,
Wilmington, Delaware
Laboratory Report ID: BL5008/B
MRID No.: 441588-04

4. REVIEWED BY:

William Erickson
Biologist
EEB/EFED/EPA

Signature:

Date:

W. Erickson
12/18/96

5. APPROVED BY:

Harry Craven
Section Head 4
EEB/EFED/EPA

Signature:

Date:

Henry T. Craven
12/19/96

6. STUDY PARAMETERS/RESULTS SYNOPSIS:

Age of Test Organism: <24 hours
Definitive Test Duration: 48 hours
Study Method: Static
Type of Concentrations: Mean measured
EC50: >190 mg/l
95% CI: N/A
Slope: N/A

7. CONCLUSIONS: This study is scientifically sound but does not fulfill any guideline requirement. An acute freshwater invertebrate toxicity test with azoxystrobin degrade R234886 is not required. Because the EC50 is >190 mg/l, the degrade is considered practically nontoxic to *Daphnia magna*.

8. ADEQUACY OF THE STUDY: Core.

9. Guideline Deviations: The following deviated from guideline requirements and recommendations:

1. Pretest mortality was not reported; guidelines specify $\leq 3\%$ mortality during the 48 h prior to testing.
2. The source of dilution water was not reported.
3. pH ranged up to 8.5; guidelines prefer that pH remain in the range of 7.2 to 7.6.
4. Dilution water hardness (171 mg/l as CaCO_3) was higher than recommended (40-48).
5. It was not reported whether the daphnids were randomly or impartially assigned to test vessels.

10. SUBMISSION PURPOSE: New Chemical; degradate data.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> Preferred species is <i>Daphnia magna</i>	<i>Daphnia magna</i>
<u>Life Stage</u> Daphnids: 1 st instar (<24 h). Amphipods, stoneflies, and mayflies: 2 nd instar. Midges: 2 nd & 3 rd instar.	1 st instar (<24 h)
<u>Supplier</u>	In-house cultures
All organisms from the same source?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
<u>Acclimation Period</u> Minimum 7 days	N/A
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	None

Guideline Criteria	Reported Information
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
<u>Feeding</u> No feeding during the study.	Not fed during testing
<u>Pretest Mortality</u> No more than 3% mortality 48 hours prior to testing.	Not reported

C. Test System:

Guideline Criteria	Reported Information
<u>Source of dilution water</u> Soft reconstituted water or water from a natural source, not dechlorinated tap water.	Not reported
Does water support test animals without observable signs of stress?	Yes
<u>Water Temperature</u> Daphnia: 20°C Amphipods and mayflies: 17°C Midges and mayflies: 22°C Stoneflies: 12°C	20.1-20.3°C
<u>pH</u> Prefer 7.2 to 7.6.	7.3-8.5
<u>Dissolved Oxygen</u> Static: ≥ 60% during 1 st 48 h and ≥ 40% during 2 nd 48 h, flow-through: ≥ 60%.	8.6-9.2 mg/l
<u>Total Hardness</u> Prefer 40 to 48 mg/L as CaCO ₃ .	171 mg/l as CaCO ₃
<u>Test Aquaria</u> 1. <u>Material</u> : Glass or stainless steel. 2. <u>Size</u> : 250 mL (daphnids and midges) or 3.9 L (1 gal). 3. <u>Fill volume</u> : 200 mL (daphnids and midges) or 2-3 L.	Glass beakers 250 ml 200 ml

Guideline Criteria	Reported Information
<u>Type of Dilution System</u> Must provide reproducible supply of toxicant.	N/A
<u>Flow Rate</u> Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.	N/A
<u>Biomass Loading Rate</u> Static: ≤ 0.8 g/L at $\leq 17^{\circ}\text{C}$, ≤ 0.5 g/L at $> 17^{\circ}\text{C}$; flow-through: ≤ 1 g/L/day.	5 daphnids per beaker
<u>Photoperiod</u> 16 hours light, 8 hours dark.	16 hours light 8 hours dark
<u>Solvents</u> Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests.	No solvent; stock solutions were stirred overnight and given 15 min. ultrasonic treatment prior to use

D. Test Design:

Guideline Criteria	Reported Information
<u>Range Finding Test</u> If $\text{LC}_{50} > 100$ mg/L, then no definitive test is required.	None reported
<u>Nominal Concentrations of Definitive Test</u> Control & 5 treatment levels; a geometric series with each concentration being at least 60% of the next higher one.	18, 32, 56, 100, 180, and a dilution water control
<u>Number of Test Organisms</u> Minimum 20/level, may be divided among containers.	20 per level, 5 per replicate
Test organisms randomly or impartially assigned to test vessels?	Not reported

<u>Water Parameter Measurements</u> 1. <u>Temperature</u> Measured continuously or, if water baths are used, every 6 h, may not vary > 1°C. 2. <u>DO and pH</u> Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control.	Temperature was monitored hourly in an extra beaker next to the control DO and pH were measured on two replicates of each test solution and control at 0 and 48 hours
<u>Chemical Analysis</u> Needed if solutions were aerated, if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was use.	Chemical analysis was performed on test solutions at 0 and 48 hours

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
<u>Control Mortality</u> Static: ≤10% Flow-through: ≤5%	None
<u>Percent Recovery of Chemical</u>	105-110% of nominal
Raw data included?	Yes

No. Daphnids Effected:

Concentration (ppm)		No. Organisms	Cumulative No. Immobilized	
Nominal	Mean Measured		Hour of Study	
			24	48
Control	<0.027	20	0	0
18	19	20	0	0
32	35	20	0	0
56	59	20	0	2
100	110	20	0	0
180	190	20	0	6

Statistical Results: No analysis conducted; an EC50 reportedly could not be calculated from the data. The 48-h EC50 was reported to be >180 mg/l.

13. VERIFICATION OF STATISTICAL RESULTS: Because immobilization of daphnids at all test concentrations was less than 50%, the EC50 is determined to be >190 mg/l, the highest concentration tested.
14. REVIEWER'S COMMENTS: The source of the dilution water was not reported. However, it is probably acceptable because no signs of toxicity or mortality were observed in the control daphnids. Other deviations from guideline requirements occurred (see "9.") The study is scientifically sound but is not a guideline requirement. Because the EC50 exceeded 190 mg/l, the R234886 degradate is considered practically nontoxic to *Daphnia magna*.