1-27-98

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

MEMORANDUM

SUBJECT: Isoxaflutole: Review of Phytotoxicity Studies for New Chemical Registration, DP Barcode D240106

FROM: Michael Davy, Agronomist
Environmental Risk Branch II
Environmental Fate and Effects Branch (7505C)

Thru: Elizabeth M. Leovey, Chief
Environmental Risk Branch II
Environmental Fate and Effects Branch (7505C)

TO: Joanne Miller, PM-23
Registration Division (H7506)

ERB II has reviewed six studies submitted by Phone-Poulenc Ag Co., Research Triangle, NC. These studies were submitted under DP Barcode D240106 for section 3 registration of Isoxaflutole.

Review of Submitted Studies
The following is a brief summary of the submitted studies:

- **CITATION:**
  Author: D. Teixeira
  Title: RPA 201772 - Determination of Effects on Vegetative Vigor of Three Plant Species
  Study Completion Date: September 17, 1997
  Laboratory: Springborn Laboratories, Inc., Wareham, MA
  Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC
  Laboratory Report ID: 97-9-7076
  MRID No.: 443999-05

This study is scientifically sound and fulfills the guideline requirement for a tier II vegetative vigor study with terrestrial plants.

Results Synopsis

Most sensitive monocot: Ryegrass; Most sensitive parameter: Root weight
EC_{25}: 0.0086 lb. ai/A NOEL: 0.0076 lb. ai/A

Most sensitive dicot: Lettuce; Most sensitive parameter: Root weight
EC_{25}: 0.00006 lb. ai/A NOEL: 0.00004 lb. ai/A

•CITATION:

Author: D. Teixeira
Title: RPA 203328 - Determination of Effects on Vegetative Vigor of Ten Plant Species
Study Completion Date: September 19, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID: 97-9-7082
MRID No.: 443999-06

This study is scientifically sound and fulfills the guideline requirements for a Tier I vegetative vigor study with terrestrial plants. None of the parameters measured were affected by 25% or more for all ten test species when treated at the maximum label rate of 0.14 lb. ai/A (0.13 lb. ai/A measured rate).

•CITATION:

Author: D. Teixeira
Title: RPA 203328 - Determination of Effects on Seedling Emergence of Ten Plant Species
Study Completion Date: September 18, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor:Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID: 97-9-7068
MRID No.: 443999-07
DP Barcode: D240106

This study is scientifically sound and fulfills the guideline requirements for a Tier I seedling emergence study with terrestrial plants. None of the parameters measured were affected by 25% or more for all ten test species when treated at the maximum label rate of 0.14 lb. ai/A.

•**CITATION:**

Author: J.R. Hoberg
Title: RPA 202248 Technical - Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*
Study Completion Date: September 17, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID: 97-9-7073
MRID No.: 443999-08

This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test.

Results Synopsis: EC_{50}: 5.0 ppm ai (95% C.I.: 4.6 - 5.4 ppm ai)
NOEC: 0.08 ppm ai Probit Slope: N/A

•**CITATION:**

Author: J.R. Hoberg
Title: RPA 202248 Technical - Toxicity to Duckweed, *Lemna gibba*
Study Completion Date: September 16, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID: 97-9-7066
MRID No.: 443999-09

This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test.

Results Synopsis: EC_{50}: 0.075 ppm ai (95% C.I.: 0.065 - 0.088 ppm ai)
Probit Slope: N/A NOEC: 0.009 ppm ai

•**CITATION:**

Author: J.R. Hoberg
Title: RPA 203328 Technical - Toxicity to Duckweed, *Lemna gibba*
Study Completion Date: September 17, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID: 97-9-7067
MRID No.: 443999-10

This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. RPA 203328 did not adversely affect the growth of *L. gibba* by 25% or more at a concentration of 9.8 ppm ai (equivalent to ten times the maximum application rate).

Non-Target Plant Risk Discussion

The data from the above studies show that the terminal degradate, RPA 203328, is not phytotoxic. **However, this does not change EFED's conclusion about the phytotoxicity of isoxaflutole and its primary degradate, RPA 202248, of 22 ppt in water.** EFED continues to have concerns that irrigation water from surface or ground water containing 22 ppt or higher may adversely affect non-target plants. RPA 202248 is persistent and potentially mobile in the laboratory and in the field. **EFED predicts that RPA 202248 will persist and accumulate in surface water and shallow ground water resources surrounding treated areas.** GENEEC and PRZM-EXAMS modeling support this conclusion. For RPA 202248, the 56-day EEC from GENEEC is 6100 ppt and the one-in-ten year EEC from PRZM-EXAMS is 2400 ppt. **These predicted concentrations exceed RPA 202248's phytotoxic level of concern (22 ppt) by several hundreds of times for non-target terrestrial plants including crops.**

If you have any questions, please do not hesitate to contact Mike Davy at 305-7081.

DATA EVALUATION RECORD
VEGETATIVE VIGOR TEST
§ 123-1 (TIER II)

1. **CHEMICAL:** Isoxaflutole PC Code No.: 123000

2. **TEST MATERIAL:** RPA 201772 technical Purity: 96.3%

3. **CITATION:**

Author: D. Teixeira
Title: RPA 201772 - Determination of Effects on Vegetative Vigor of Three Plant Species
Study Completion Date: September 17, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID: 97-9-7076
MRID No.: 443999-05
DP Barcode: D240106

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist, Golder Associates, Inc.

Signature: Mark Mossler **Date:** 1/12/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist Golder Associates, Inc.

Signature: P. Kosalwat **Date:** 1/12/98

5. **APPROVED BY:**

Signature: Michael Davy **Date:** 1/21/98

6. **STUDY PARAMETERS:**

Definitive Study Duration: 14 days

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier II vegetative vigor study with terrestrial plants.

Results Synopsis:

Most sensitive monocot: Ryegrass
Most sensitive parameter: Root weight
EC_{25}: 0.0086 lb ai/A
NOEL: 0.0076 lb ai/A

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Most sensitive dicot: Lettuce
Most sensitive parameter: Root weight
EC_{25}: 0.00006 lb ai/A
NOEL: 0.00004 lb ai/A

8. **ADEQUACY OF THE STUDY:**

A. Classification: When this core data are added to core data for the other seven required species, the entire study can be placed in the core category.

B. Rationale: N/A.

C. Repairability: N/A.

9. **GUIDELINE DEVIATIONS:** No guideline deviations were noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	Dicots: lettuce, turnip Monocots: ryegrass
Number of plants per rep 5	5
Source of Seed	Untreated seed obtained from various commercial suppliers

B. Test System

Guideline Criteria	Reported Information
Solvent	10% acetone solution
Site of test	Growth chamber

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Guideline Criteria	Reported Information
Planting method / type of pot	Three- to four-day-old seedlings planted into polypropylene pots (13-cm top diameter) and allowed to grow for 7 days
Method of application	Belt sprayer
Method of watering	Subirrigation
Growth stage at application 1-3 true leaf stage.	1-3 true leaf stage

C. **Test Design**

Guideline Criteria	Reported Information
Dose range 2x or 3x	Either 2x or 4x
Doses At least 5	5 or 6 - rates ranging from 0.00001 to 0.025 lb of active ingredient (ai)/A
Controls Negative and solvent	Negative and solvent (10% acetone) control groups
Replicates per dose At least 3	3
Duration of test 14 days	14 days
Were observations made at least weekly?	Observations made on days 7 and 14
Maximum labeled rate	0.18 lb ai/A

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was an NOEL observed for each species?	Yes

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Guideline Criteria	Reported Information
Phytotoxic observations	Yes
Were initial chemical concentrations measured? (Optional)	The measured concentrations ranged from 98 to 120% of nominal.
Were adequate raw data included?	Raw data for phytotoxicity observations were not included in the report.

Results for the most sensitive parameter* of each species

Species	Parameter	EC_{25} (lb ai/A)	NOEL (lb ai/A)
Lettuce	root weight	0.000056	0.000035
Turnip	root weight	0.00010	0.000035
Ryegrass	root weight	0.0086	0.0076

*Determination of the most sensitive parameter is based on EC_{25} values.

Observations: Symptoms of test material toxicity included chlorosis and necrosis.

Statistical Method: Analyses were based on measured application rates with comparison to the pooled control data. Bonferroni's test was used for mean separation and regression analysis (with or without various transformations) was used for EC value determination.

Most sensitive dicot: lettuce Parameter: root weight
EC_{25} 95% C.L.: 0.0000026 - 0.00091 lb ai/A

Most sensitive monocot: ryegrass Parameter: root weight
EC_{25} 95% C.L.: 0.0006 - 0.034 lb ai/A

13. **VERIFICATION OF STATISTICAL RESULTS:** Williams' test was used for mean separation. Probit analysis was used for EC_{25} estimation. Where appropriate, responses for the most sensitive parameter for each species were remodeled using non-linear regression.

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Species	Parameter	EC_{25} (lb ai/A)	NOEL (lb ai/A)
Lettuce	root weight	0.00007	0.00001*
Turnip	root weight	0.00046	0.00010*
Ryegrass	root weight	0.0095	0.0042*

*The probit EC_5 result.

Results for most sensitive parameter of most sensitive species

	Monocot	Dicot
Species	ryegrass	lettuce
Parameter	root weight	root weight
EC_{25} (lb ai/A)	0.0086	0.00006
95% C.I. (lb ai/A)	0.0006 - 0.0340	0.000003 - 0.00091
Probit Slope	N/A	N/A
NOEL (lb ai/A)	0.0076	0.00004

14. **REVIEWER'S COMMENTS:** Each pot of plants received 9.4 mL of spray solution. This volume translates to a field rate of 966 gallons per acre. This is an unusually large volume of diluent. Future studies should try to apply treatment solutions near the intended diluent volume.

This study is scientifically sound and fulfills the guideline requirements when added to the core data from the other seven required test species. The study is classified as **Core.**

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lettuce root weight
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION (lb ai/A)	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	0.031	0.031	0.033
2	0.000011	3	0.036	0.036	0.033
3	0.000035	3	0.027	0.027	0.027
4	0.00014	3	0.019	0.019	0.019
5	0.00054	3	0.010	0.010	0.010
6	0.0022	3	0.004	0.004	0.004

lettuce root weight
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WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	0.033				
0.000011	0.033	0.306		1.78	k= 1, v=12
0.000035	0.027	0.529		1.87	k= 2, v=12
0.00014	0.019	1.549		1.90	k= 3, v=12
0.00054	0.010	2.664	*	1.92	k= 4, v=12
0.0022	0.004	3.451	*	1.93	k= 5, v=12

s = 0.009
Note: df used for table values are approximate when v > 20.

$0.00014 > EC_{25}$ ∴

NOEL = 1.2×10^{-5} lb ai/A (probit EC_5)

lettuce root weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limit	Upper 95% Confidence Limit
EC 1.00	0.3466	0.1271	0.7031
EC 5.00	1.2170	0.5808	2.0660
EC10.00	2.3774	1.2989	3.6904
EC15.00	3.7356	2.2269	5.4814
EC50.00	25.2304	19.4298	32.6555
EC85.00	170.4065	117.4563	280.7902
EC90.00	267.7632	174.6234	480.9517
EC95.00	523.0533	312.1872	1074.6261
EC99.00	1836.3567	918.0020	4908.3374

$EC_{25} = 7.32 \times 10^{-5}$

lettuce root weight 1
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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0	.	0.0346	0.0155	0.0423	.	.	.
2	3.5	0.54407	0.0369	0.0141	0.0291	.	.	.
3	14.0	1.14613	0.0247	0.0156	0.0161	.	.	.
4	54.0	1.73239	0.0063	0.0221	0.0021	.	.	.
5	220.0	2.34242	0.0059	0.0049	0.0014	.	.	.

lettuce root weight 2
MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION
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Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	CO	Weighted SS
0	1.402000	0.800000	0.030800	0.049697
1	1.377216	0.842231	0.031124	0.049465
2	1.378730	0.840679	0.031096	0.049473
3	1.378641	0.840778	0.031097	0.049473
4	1.378646	0.840772	0.031097	0.049473

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	0.27160000000	0.09053333333
Residual	12	0.04947295593	0.00412274633
Uncorrected Total	15	0.32107295593	
(Corrected Total)	14	0.16195428824	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	1.378646371	0.32961689132	0.66047180155	2.0968209407
SIGMA	0.840771814	0.31562997642	0.15307215881	1.5284714689
CO	0.031097199	0.00622556981	0.01753282775	0.0446615705

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.693608416	-0.787953123
SIGMA	-0.693608416	1	0.5323074719
CO	-0.787953123	0.5323074719	1

lettuce root weight 3
MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	1.37865	0.84077	0.031097	0.049473	23.9137

lettuce root weight 4
MODEL: YOUNG = CO * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
WEIGHTED REGRESSION
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Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC25	SIGMA	CO	Weighted SS
0	0.885000	0.800000	0.030800	0.049591

1	0.809154	0.842274	0.031124	0.049463
2	0.811702	0.840678	0.031096	0.049473
3	0.811544	0.840778	0.031097	0.049473
4	0.811554	0.840772	0.031097	0.049473

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	0.27160000000	0.09053333333
Residual	12	0.04947295593	0.00412274633
Uncorrected Total	15	0.32107295593	
(Corrected Total)	14	0.16195428841	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC25	0.8115541980	0.50131104928	-.28071035674	1.9038187527
SIGMA	0.8407718090	0.31562997507	0.15307215693	1.5284714611
CO	0.0310971991	0.00622556980	0.01753282772	0.0446615704

Asymptotic Correlation Matrix

Corr	LOG_EC25	SIGMA	CO
LOG_EC25	1	-0.88071929	-0.7441392
SIGMA	-0.88071929	1	0.5323074734
CO	-0.7441392	0.5323074734	1

lettuce root weight 5
MODEL: YOUNG = CO * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
SUMMARY OF NONLINEAR REGRESSION
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OBS	CONC	LOG_EC25	SIGMA	CO	RESID_SS	EC25
1	0	0.81155	0.84077	0.031097	0.049473	6.47969 $\times 10^{-5}$

lettuce root weight 6
MODEL: YOUNG = CO * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
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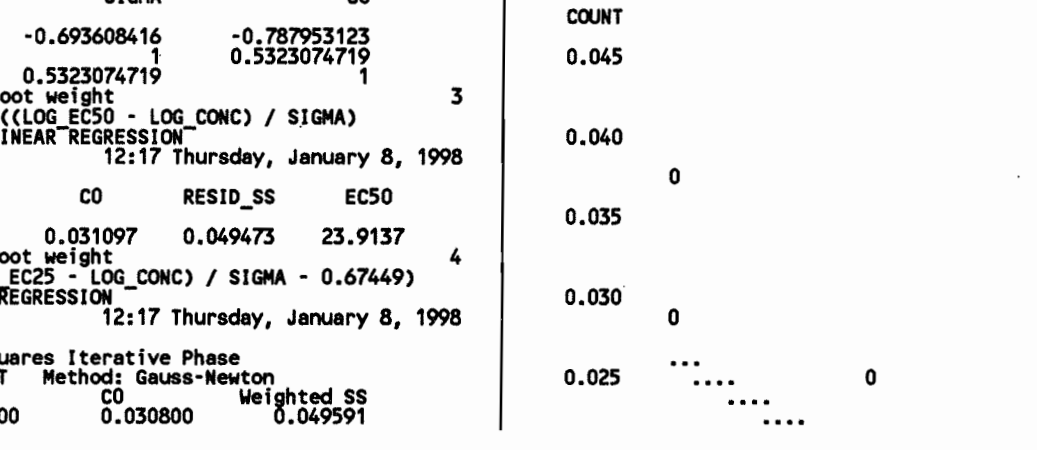
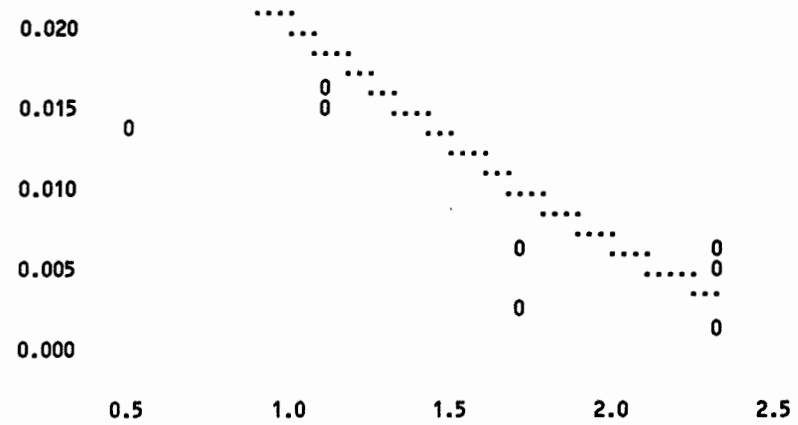
Plot of COUNT*LOG_CONC. Symbol used is '0'.
Plot of PRED*LOG_CONC. Symbol used is '.'.





NOTE: 1823 obs had missing values. 1753 obs hidden.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	5	0 14 54 220 3.5

Number of observations in data set = 30

NOTE: Due to missing values, only 15 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	0.00148684	0.00037171	3.97	0.0349
Error	10	0.00093527	0.00009353		
Corrected Total	14	0.00242211			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.613860	53.41101	0.009671	0.018107

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	4	0.00148684	0.00037171	3.97	0.0349

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	4	0.00148684	0.00037171	3.97	0.0349

General Linear Models Procedure

Level of DOSE	N	RESPONSE Mean	RESPONSE SD
0	3	0.03080000	0.01379819
14	3	0.01880000	0.00511566
54	3	0.01016667	0.01054577
220	3	0.00406667	0.00236291
3.5	3	0.02670000	0.01158792

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 10 MSE= 0.000094
Critical Value of Dunnett's T= 2.466
Minimum Significant Difference= 0.0195

Comparisons significant at the 0.05 level are indicated by '***'.

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
3.5 - 0	-0.023569	-0.004100	0.015369	
14 - 0	-0.031469	-0.012000	0.007469	
54 - 0	-0.040102	-0.020633	-0.001164	***
220 - 0	-0.046202	-0.026733	-0.007264	***

turnip root weight
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION (lb ai/A)	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	0.108	0.108	0.111
2	0.000011	3	0.114	0.114	0.111
3	0.000035	3	0.093	0.093	0.093
4	0.00014	3	0.082	0.082	0.083
5	0.00054	3	0.084	0.084	0.083
6	0.0022	3	0.026	0.026	0.026
7	0.0076	3	0.019	0.019	0.019

turnip root weight
File: tur Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	0.111				
0.000011	0.111	0.221		1.76	k= 1, v=14
0.000035	0.093	1.071		1.85	k= 2, v=14
0.00014	0.083	1.843		1.88	k= 3, v=14
0.00054	0.083	1.843		1.89	k= 4, v=14
0.0022	0.026	6.034	*	1.90	k= 5, v=14
0.0076	0.019	6.514	*	1.91	k= 6, v=14

s = 0.017
Note: df used for table values are approximate when v > 20.

0.00054 > EC_{25}, ∴
NOEL = 0.00010 lb ai/A (probit EC_5)

turnip root weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	0.3603		
EC 5.00	1.0265		
EC10.00	1.7937		
EC15.00	2.6144		
EC50.00	12.8508		
EC85.00	63.1665		
EC90.00	92.0664		
EC95.00	160.8866		
EC99.00	458.3497		

$EC_{75} = 4.57 \times 10^{-4}$ lb ai/A = 0.00046 lb ai/A

ryegrass root weight
File: rye Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION (lb ai/A)	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	0.055	0.055	0.065
2	0.00095	3	0.074	0.074	0.065
3	0.0022	3	0.066	0.066	0.065
4	0.0043	3	0.044	0.044	0.044
5	0.0076	3	0.044	0.044	0.044
6	0.014	3	0.031	0.031	0.031
7	0.026	3	0.012	0.012	0.012

ryegrass root weight
File: rye Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	0.065				
0.00095	0.065	0.571		1.76	k= 1, v=14
0.0022	0.065	0.571		1.85	k= 2, v=14
0.0043	0.044	0.630		1.88	k= 3, v=14
0.0076	0.044	0.630		1.89	k= 4, v=14
0.014	0.031	1.323		1.90	k= 5, v=14
0.026	0.012	2.396	*	1.91	k= 6, v=14

s = 0.022
Note: df used for table values are approximate when v > 20.

$0.014 > EC_{25}$, ∴
NOEL = 0.0042 lb ai/A (probit EC_5)

ryegrass root weight

Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limit	Upper 95% Confidence Limit
EC 1.00	0.0024	0.0013	0.0035
EC 5.00	0.0042	0.0027	0.0054
EC10.00	0.0055	0.0039	0.0068
EC15.00	0.0067	0.0050	0.0080
EC50.00	0.0149	0.0132	0.0169
EC85.00	0.0334	0.0273	0.0455
EC90.00	0.0404	0.0320	0.0583
EC95.00	0.0536	0.0404	0.0844
EC99.00	0.0910	0.0621	0.1696

EC_{25} = 0.0088 lb a.i/A

ryegrass root weight 11
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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0000	.	0.0639	0.0588	0.0424	.	.	.
2	0.0076	-2.11919	0.0228	0.0420	0.0670	.	.	.
3	0.0140	-1.85387	0.0378	0.0279	0.0284	.	.	.
4	0.0260	-1.58503	0.0149	0.0138	0.0078	.	.	.

ryegrass root weight 12
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION
12:17 Thursday, January 8, 1998

Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC50	SIGMA	C0	Weighted SS
0	-1.827000	0.338000	0.055000	0.031758
1	-1.814283	0.311008	0.054469	0.031013
2	-1.813239	0.309568	0.054364	0.031015
3	-1.813133	0.309403	0.054357	0.031013
4	-1.813121	0.309384	0.054356	0.031013
5	-1.813120	0.309382	0.054356	0.031013
6	-1.813120	0.309382	0.054356	0.031013
7	-1.813120	0.309382	0.054356	0.031013

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	0.42750000000	0.14250000000
Residual	9	0.03101260135	0.00344584459
Uncorrected Total	12	0.45851260135	
(Corrected Total)	11	0.14698278782	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	-1.813119529	0.08828003006	-2.0128245302	-1.6134145272
SIGMA	0.309382110	0.10552411483	0.0706679469	0.5480962736
C0	0.054355700	0.00765754993	0.0370329716	0.0716784292

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.612654944	-0.76893536
SIGMA	-0.612654944	1	0.5114210181
C0	-0.76893536	0.5114210181	1

ryegrass root weight 13
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
12:17 Thursday, January 8, 1998

OBS	CONC	LOG_EC50	SIGMA	C0	RESID_SS	EC50
1	0	-1.81312	0.30938	0.054356	0.031013	0.015377

ryegrass root weight 14
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
WEIGHTED REGRESSION
12:17 Thursday, January 8, 1998

Non-Linear Least Squares Iterative Phase
Dependent Variable COUNT Method: Gauss-Newton

Iter	LOG_EC25	SIGMA	C0	Weighted SS
0	-2.054000	0.338000	0.055000	0.031717
1	-2.024049	0.310954	0.054468	0.031013
2	-2.022035	0.309565	0.054364	0.031015
3	-2.021822	0.309403	0.054357	0.031013
4	-2.021798	0.309384	0.054356	0.031013
5	-2.021795	0.309382	0.054356	0.031013
6	-2.021795	0.309382	0.054356	0.031013
7	-2.021795	0.309382	0.054356	0.031013

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	0.42750000000	0.14250000000
Residual	9	0.03101260135	0.00344584459
Uncorrected Total	12	0.45851260135	
(Corrected Total)	11	0.14698278783	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC25	-2.021794668	0.14338152648	-2.3461489751	-1.6974403613
SIGMA	0.309382110	0.10552411482	0.0706679469	0.5480962735
C0	0.054355700	0.00765754993	0.0370329716	0.0716784292

Asymptotic Correlation Matrix

Corr	LOG_EC25	SIGMA	C0
LOG_EC25	1	-0.873614336	-0.727304346
SIGMA	-0.873614336	1	0.5114210182
C0	-0.727304346	0.5114210182	1

ryegrass root weight 15
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
SUMMARY OF NONLINEAR REGRESSION
12:17 Thursday, January 8, 1998

OBS	CONC	LOG_EC25	SIGMA	C0	RESID_SS	EC25
1	0	-2.02179	0.30938	0.054356	0.031013	.0095105

ryegrass root weight 16
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
12:17 Thursday, January 8, 1998

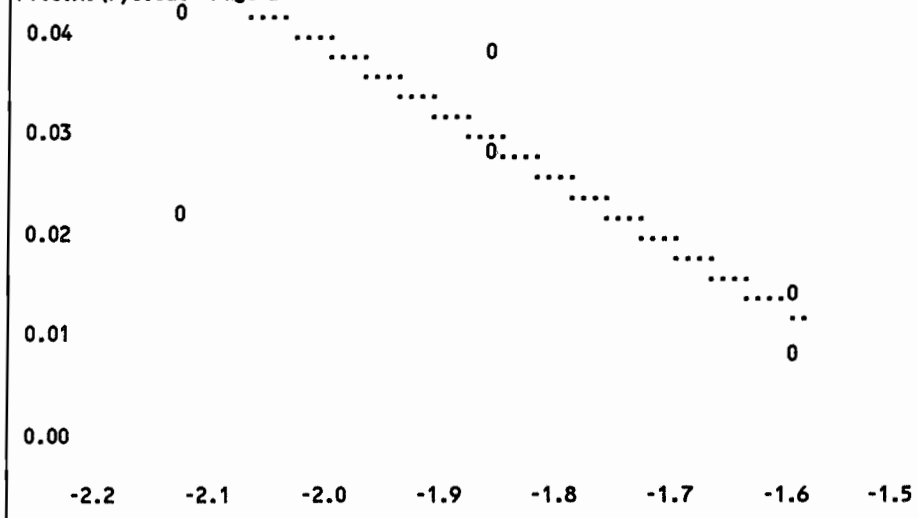
Plot of COUNT*LOG_CONC. Symbol used is '0'.
Plot of PRED*LOG_CONC. Symbol used is '.'.

```
COUNT
 0.07
                0


 0.06


 0.05

                ..
                  .....
```



NOTE: 556 obs had missing values. 486 obs hidden.

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	4	0 0.014 0.026 0.0076

Number of observations in data set = 24

NOTE: Due to missing values, only 12 observations can be used in this analysis.

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	0.00304242	0.00101414	6.12	0.0182
Error	8	0.00132625	0.00016578		
Corrected Total	11	0.00436866			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.696418	36.14201	0.012876	0.035625

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	3	0.00304242	0.00101414	6.12	0.0182

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	3	0.00304242	0.00101414	6.12	0.0182

General Linear Models Procedure

Level of DOSE	N	RESPONSE Mean	RESPONSE SD
0	3	0.05503333	0.01123403
0.014	3	0.03136667	0.00557704
0.026	3	0.01216667	0.00382143
0.0076	3	0.04393333	0.02216333

General Linear Models Procedure

Dunnett's One-tailed T tests for variable: RESPONSE

NOTE: This tests controls the type I experimentwise error for comparisons of all treatments against a control.

Alpha= 0.05 Confidence= 0.95 df= 8 MSE= 0.000166
Critical Value of Dunnett's T= 2.416
Minimum Significant Difference= 0.0254

Comparisons significant at the 0.05 level are indicated by '***'.

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
0.0076 - 0	-0.03650	-0.01110	0.01430	
0.014 - 0	-0.04907	-0.02367	0.00174	
0.026 - 0	-0.06827	-0.04287	-0.01746	***

DATA EVALUATION RECORD
VEGETATIVE VIGOR TEST
§ 122-1 (TIER I)

1. **CHEMICAL:** Isoxaflutole — PC Code No.: 123000

2. **TEST MATERIAL:** RPA 203328 — Purity: 99%

3. **CITATION:**

Author:	D. Teixeira
Title:	RPA 203328 - Determination of Effects on Vegetative Vigor of Ten Plant Species
Study Completion Date:	September 19, 1997
Laboratory:	Springborn Laboratories, Inc., Wareham, MA
Sponsor:	Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID:	97-9-7082
MRID No.:	443999-06
DP Barcode:	D240106

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist, Golder Associates, Inc.

Signature: for MAM **Date:** 1/12/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist Golder Associates, Inc.

Signature: P. Kosalwat **Date:** 1/12/98

5. **APPROVED BY:**

Signature: **Date:** 1/28/98

6. **STUDY PARAMETERS:**

Definitive Study Duration: 14 days

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier I vegetative vigor study with terrestrial plants. None of the parameters measured were affected by 25% or more for all ten test species when treated at the maximum label rate of 0.14 lb ai/A (0.13 lb ai/A measured rate).

8. **ADEQUACY OF THE STUDY:**

A. Classification: Core.

B. Rationale: N/A.

C. **Repairability:** N/A.

9. **GUIDELINE DEVIATIONS:** No guideline deviations of consequence were noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	Dicots: cabbage, cucumber, lettuce, soybean, tomato, turnip Monocots: corn, oat, onion, ryegrass
Number of plants per rep 5	5
Source of Seed	Untreated seed obtained from various commercial suppliers

B. **Test System**

Guideline Criteria	Reported Information
Solvent	10% acetone solution
Site of test	Growth chamber
Planting method / type of pot	Two- to six-day-old seedlings planted into polypropylene pots (13-cm top diameter) and allowed to grow for 7 days
Method of application	140 µL of solution applied to each pot by overhead atomizer
Method of watering	Subirrigation
Growth stage at application 1-3 true leaf stage.	1-2 true leaf stage

C. Test Design

Guideline Criteria	Reported Information
Dose range 2x or 3x	N/A
Doses At least 5	1 (0.14 lb ai/A)
Controls Negative and solvent	Negative and solvent control groups
Replicates per dose At least 3	3
Duration of test 14 days	14 days
Were observations made at least weekly?	Observations made on days 7 and 14
Maximum labeled rate	0.14 lb ai/A

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was an NOEL observed for each species?	N/A
Phytotoxic observations	Yes
Were initial chemical concentrations measured? (Optional)	The measured concentration averaged 93% of nominal.
Were adequate raw data included?	Raw data for phytotoxicity observations were not included in the report.

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Results for the most sensitive parameter of each species

Species	Parameter	EC_{25} (lb ai/A)	NOEL (lb ai/A)
Cabbage	shoot length = shoot weight	>0.13	0.13
Cucumber	shoot length = shoot weight	>0.13	0.13
Lettuce	shoot length = shoot weight	>0.13	0.13
Soybean	shoot length = shoot weight	>0.13	0.13
Tomato	shoot length = shoot weight	>0.13	0.13
Turnip	shoot length = shoot weight	>0.13	0.13
Corn	shoot length = shoot weight	>0.13	0.13
Oat	shoot length = shoot weight	>0.13	0.13
Onion	shoot length = shoot weight	>0.13	0.13
Ryegrass	shoot length = shoot weight	>0.13	0.13

Observations: No symptoms of test material toxicity were noted during the test.

Statistical Method: No statistical analyses were conducted.

13. **VERIFICATION OF STATISTICAL RESULTS:** Tier I results indicated that none of the ten test species were injured by 25% or more for either parameter evaluated when compared to the solvent control data.

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for a Tier I vegetative vigor test. The study is classified as **Core.**

DATA EVALUATION RECORD
SEEDLING EMERGENCE TEST
§ 122-1 (TIER I)

1. **CHEMICAL:** Isoxaflutole PC Code No.: 123000

2. **TEST MATERIAL:** RPA 203328 Purity: 99%

3. **CITATION:**

Author:	D. Teixeira
Title:	RPA 203328 - Determination of Effects on Seedling Emergence of Ten Plant Species
Study Completion Date:	September 18, 1997
Laboratory:	Springborn Laboratories, Inc., Wareham, MA
Sponsor:	Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID:	97-9-7068
MRID No.:	443999-07
DP Barcode:	D240106

4. **REVIEWED BY:** Mark Mossler, M.S., Toxicologist, Golder Associates, Inc.

Signature: for MAM **Date:** 1/12/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist Golder Associates, Inc.

Signature: P. Kosalwat **Date:** 1/12/98

5. **APPROVED BY:**

Signature: Michael Davy **Date:** 1/28/98

6. **STUDY PARAMETERS:**

Definitive Study Duration: 14 days

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for a Tier I seedling emergence study with terrestrial plants. None of the parameters measured were affected by 25% or more for all ten test species when treated at the maximum label rate of 0.14 lb ai/A.

8. **ADEQUACY OF THE STUDY:**

A. Classification: Core.

B. Rationale: N/A.

C. **Repairability:** N/A.

9. **GUIDELINE DEVIATIONS:** No guideline deviations of consequence were noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. **Test Organisms**

Guideline Criteria	Reported Information
Species 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	Dicots: cabbage, cucumber, lettuce, soybean, tomato, turnip Monocots: corn, oat, onion, ryegrass
Number of seeds per rep 10	10
Source of Seed	Untreated seed obtained from various commercial suppliers
Historical % Germination of Seed	≥85%

B. **Test System**

Guideline Criteria	Reported Information
Solvent	10% acetone solution
Site of test	Growth chamber
Planting method / type of pot	Planted at 1.0-cm depth/ polypropylene pots (13-cm top diameter)
Method of application	130 µL of solution applied to each pot by overhead atomizer
Method of watering	Subirrigation
Growth stage at application Seed or plant.	Seed

C. Test Design

Guideline Criteria	Reported Information
Dose range 2x or 3x	N/A
Doses At least 5	1 (0.14 lb ai/A)
Controls Negative and solvent	Negative and solvent control groups
Replicates per dose At least 3	3
Duration of test 14 days	14 days
Were observations made at least weekly?	Observations made on days 10 and 14
Maximum labeled rate	0.14 lb ai/A

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was an NOEL observed for each species?	N/A
Phytotoxic observations	Yes
Were initial chemical concentrations measured? (Optional)	The measured concentration averaged 100% of nominal.
Were adequate raw data included?	Raw data for phytotoxicity observations were not included in the report.

Results for the most sensitive parameter of each species

Species	Parameter	EC_{25} (lb ai/A)	NOEL (lb ai/A)
Cabbage	emergence = shoot length	>0.14	0.14
Cucumber	emergence = shoot length	>0.14	0.14
Lettuce	emergence = shoot length	>0.14	0.14
Soybean	emergence = shoot length	>0.14	0.14
Tomato	emergence = shoot length	>0.14	0.14
Turnip	emergence = shoot length	>0.14	0.14
Corn	emergence = shoot length	>0.14	0.14
Oat	emergence = shoot length	>0.14	0.14
Onion	emergence = shoot length	>0.14	0.14
Ryegrass	emergence = shoot length	>0.14	0.14

Observations: No symptoms of test material toxicity were noted during the test.

Statistical Method: No statistical analyses were conducted.

13. **VERIFICATION OF STATISTICAL RESULTS:** Tier I results indicated that none of the ten test species were injured by 25% or more for either parameter evaluated when compared to the solvent control data.

14. **REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for a Tier I seedling emergence test. The study is classified as **Core.**

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DATA EVALUATION RECORD
ALGAE OR DIATOM EC_{50} TEST
GUIDELINE 123-2 (TIER II)

1. **CHEMICAL:** Isoxaflutole — PC Code No.: 123000

2. **TEST MATERIAL:** RPA 202248 technical — Purity: 99.9%

3. **CITATION:**

Author: J.R. Hoberg
Title: RPA 202248 Technical - Toxicity to the Freshwater Green Alga, *Selenastrum capricornutum*
Study Completion Date: September 17, 1997
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID: 97-9-7073
DP Barcode: D240106
MRID No.: 443999-08

4. **REVIEWED BY:** Mark A. Mossler, M.S., Toxicologist, Golder Associates, Inc.

Signature: Mark Mossler **Date:** 1/13/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates, Inc.

Signature: P. Kosalwat **Date:** 1/13/98

5. **APPROVED BY:**

Signature: Michael Davy **Date:** 1/26/98

6. **STUDY PARAMETERS:**

Definitive Test Duration: 120 hours
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test.

Results Synopsis

EC_{50}: 5.0 ppm ai — 95% C.I.: 4.6 - 5.4 ppm ai
NOEC: 0.08 ppm ai — Probit Slope: N/A

8. **ADEQUACY OF THE STUDY:**

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:** None noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Selenastrum capricornutum*
Initial Number of Cells 3,000 - 10,000 cells/ml	3,000 cells/ml
Nutrients Standard formula, e.g. 20XAAP	AAP medium

B. Test System

Guideline Criteria	Reported Information
Solvent	Acetone (0.1 mL/L)
Temperature Skeletonema: 20°C Others: 24-25°C	24°C
Light Intensity Anabaena: 2.0 KLux (±15%) Others: 4.0-5.0 KLux (±15%)	3.4 - 4.3 KLux
Photoperiod Skeletonema: 14 h light, 10 h dark or 16 h light, 8 h dark Others: Continuous	Continuous

Guideline Criteria	Reported Information
pH Skeletonema: approx. 8.0 Others: approx. 7.5	Initial 7.1 - 7.5 Final 7.3 - 8.9

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	3X
Doses at least 5	0.024, 0.081, 0.27, 0.90, 3.0, and 10 mg active ingredient (ai)/L
Controls negative and/or solvent	Negative and solvent
Replicates per dose 3 or more	3
Duration of test 120 hours	120 hours
Daily observations were made?	Yes
Method of Observations	Cellular counts
Maximum Labeled Rate	0.14 lb ai/acre

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Initial and 120 h cell densities were measured?	Yes
Control cell count at 120 hr ≥2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

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Dose Response

Mean Measured Concentration (mg ai/L)	Terminal Avg. Cell Density (x 10^4 cells/ml)	% Inhibition*	120-Hour pH
Control	150	N/A	8.9
Solvent Control	154	N/A	8.9
0.024	154	-1.2	8.9
0.077	152	0.6	8.9
0.29	149	2.1	8.9
0.86	146	4.0	8.7
2.9	142	6.6	8.6
9.4	6	96	7.3

*Compared to the pooled control.

Other Significant Results: At termination, cells exposed at the four highest-concentration treatment levels appeared bloated. Some cells in the 2.9 and 9.4 mg ai/L groups were also reported as fragmented. Cells in the two lowest-concentration treatment groups and in the control groups were normal in appearance.

Statistical Results

Statistical Method: linear regression and Williams' test

EC_{50}: 5.5 ppm ai 95% C.I.: 3.3 - 7.8 ppm ai
Probit Slope: N/A NOEC: 0.29 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS

Statistical Method: Moving average angle for the EC value and Williams' test for mean separation. Comparison to the solvent control.

EC_{50}: 5.0 ppm ai 95% C.I.: 4.6 - 5.4 ppm ai
Probit Slope: N/A NOEC: 0.08 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an algal toxicity test. This study is categorized as **Core.**

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Selenastrum cell density
File: sel Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	154.667	154.667	154.667
2	0.024 ppm ai	3	154.333	154.333	154.333
3	0.077 ppm ai	3	151.667	151.667	151.667
4	0.29 ppm ai	3	149.333	149.333	149.333
5	0.86 ppm ai	3	146.667	146.667	146.667
6	2.9 ppm ai	3	142.333	142.333	142.333
7	9.4 ppm ai	3	6.033	6.033	6.033

Selenastrum cell density
File: sel Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	154.667				
0.024 ppm ai	154.333	0.114		1.76	k= 1, v=14
0.077 ppm ai	151.667	1.024		1.85	k= 2, v=14
0.29 ppm ai	149.333	1.820		1.88	k= 3, v=14
0.86 ppm ai	146.667	2.730	*	1.89	k= 4, v=14
2.9 ppm ai	142.333	4.209	*	1.90	k= 5, v=14
9.4 ppm ai	6.033	50.722	*	1.91	k= 6, v=14

s = 3.589
Note: df used for table values are approximate when v > 20.

Since bloated cells were observed in the 0.29 ppm ai group, the NOEC will be reported as:

0.08 ppm ai

Mossler isoxaflutole deg Selenastrum capricornutum 1-12-98

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
9.399999		100	96	96
0				
2.9	100	8	8	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.990245

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	1.687528E-02	4.990245	4.615594	5.386937

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
2		3.279296E-02	1

(CANNOT BE CALCULATED)

SLOPE = 6.178913

95 PERCENT CONFIDENCE LIMITS = 5.059984 AND 7.297842

LC50 = 4.895485

95 PERCENT CONFIDENCE LIMITS = 4.410958 AND 5.456474

LC10 = 3.049706

95 PERCENT CONFIDENCE LIMITS = 2.639166 AND 3.427404

****

DATA EVALUATION RECORD
AQUATIC PLANT EC_{50} TEST
GUIDELINE 123-2 (TIER II)

1. **CHEMICAL:** Isoxaflutole PC Code No.: 123000

2. **TEST MATERIAL:** RPA 202248 technical Purity: 99.9%

3. **CITATION:**

Author:	J.R. Hoberg
Title:	RPA 202248 Technical - Toxicity to Duckweed, *Lemna gibba*
Study Completion Date:	September 16, 1997
Laboratory:	Springborn Laboratories, Inc., Wareham, MA
Sponsor:	Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID:	97-9-7066
DP Barcode:	D240106
MRID No.:	443999-09

4. **REVIEWED BY:** Mark A. Mossler, M.S., Toxicologist, Golder Associates, Inc.

Signature: Mark A. Mossler **Date:** 1/13/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates, Inc.

Signature: P. Kosalwat **Date:** 1/13/98

5. **APPROVED BY:**

Signature: Michael Davy **Date:** 1/26/98

6. **STUDY PARAMETERS:**

Definitive Test Duration: 14 days
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test.

Results Synopsis:

EC_{50}: 0.075 ppm ai 95% C.I.: 0.065 - 0.088 ppm ai
Probit Slope: N/A NOEC: 0.009 ppm ai

8. **ADEQUACY OF THE STUDY:**

   A. Classification: Core

   B. Rationale: N/A

   C. Repairability: N/A

9. **GUIDELINE DEVIATIONS:** None noted.

10. **SUBMISSION PURPOSE:**

11. **MATERIALS AND METHODS:**

   A. Test Organisms

Guideline Criteria	Reported Information
Species *Lemna gibba*	*Lemna gibba*
Number of Plants/Fronds 5 plants, 3 fronds each	15 fronds/replicate (5 plants with 3 fronds each)
Nutrients Standard formula, e.g. 20XAAP	Hoagland's medium

   B. Test System

Guideline Criteria	Reported Information
Solvent	Acetone (0.1 mL/L)
Temperature 25°C	24-25°C
Light Intensity 5.0 KLux (±15%)	3.4-4.1 KLux
Photoperiod Continuous	Continuous
Test System Static or Renewal	Renewal at 3-day intervals
pH Approx. 5.0	5.1-6.5

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C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses at least 5	0.0093, 0.019, 0.039, 0.075, 0.15, and 0.30 mg active ingredient (ai)/L
Controls negative and/or solvent	Negative and solvent control
Replicates per dose 3 or more	3
Duration of test 14 days	14 days
Daily observations were made?	Frond counts were made on days 3, 6, 9, 12, and 14 of the study
Method of Observations	Number of fronds and dry weight
Maximum Labeled Rate	0.14 lb ai/A

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Initial and 14 day frond numbers were measured?	Yes
Control frond at 14 days ≥2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Results are based on mean measured concentrations computed from day 0 and 3 measurements
Raw data included?	Yes

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Dose Response

Mean Measured concentration (mg ai/L)	Terminal Avg. Frond Number	% Inhibition*	14-day pH
Control	416	N/A	6.2
Solvent Control	432	N/A	6.5
0.0091	457	-7.9	6.5
0.022	408	3.6	6.3
0.039	263	38	6.3
0.075	168	60	6.1
0.15	146	65	6.0
0.27	117	72	5.9

*Based on comparison to the pooled control.

Other Significant Results: Fronds on plants in the four highest-concentration groups appeared chlorotic, smaller, curled, and had less root formation in comparison to the control plants, which appeared normal. The fronds of plants in the 0.022 treatment group also appeared chlorotic and were smaller and had less root formation than the control plants.

Statistical Results for Frond Number

Statistical Method: linear regression and Williams' test

EC_{50}: 0.083 ppm ai 95% C.I.: 0.031 - 0.23 ppm ai
Probit Slope: N/A NOEC: 0.022 ppm ai

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The dose response could not be adequately modeled using probit analysis with subsequent non-linear remodeling. Consequently, the moving average method was used to determine the EC_{50} value. Williams' test was used to determine the NOEC.

EC_{50}: 0.075 ppm ai 95% C.I.: 0.065 - 0.088 ppm ai
Probit Slope: N/A NOEC: 0.009 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically sound, fulfills the guideline requirements for an aquatic plant toxicity test, and is categorized as **Core**.

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Lemna frond number
File: lem Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Sol. Con.	3	431.667	431.667	444.333
2	0.0091 ppm ai	3	457.000	457.000	444.333
3	0.022 ppm ai	3	408.333	408.333	408.333
4	0.039 ppm ai	3	263.333	263.333	263.333
5	0.075 ppm ai	3	167.667	167.667	167.667
6	0.15 ppm ai	3	146.333	146.333	146.333
7	0.27 ppm ai	3	117.333	117.333	117.333

Lemna frond number
File: lem Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Sol. Con.	444.333				
0.0091 ppm ai	444.333	0.418		1.76	k= 1, v=14
0.022 ppm ai	408.333	0.769		1.85	k= 2, v=14
0.039 ppm ai	263.333	5.550	*	1.88	k= 3, v=14
0.075 ppm ai	167.667	8.704	*	1.89	k= 4, v=14
0.15 ppm ai	146.333	9.408	*	1.90	k= 5, v=14
0.27 ppm ai	117.333	10.364	*	1.91	k= 6, v=14

s = 37.146
Note: df used for table values are approximate when v > 20.

Since non-lethal effects were noted at the 0.022 ppm ai level, NOEC will be reported as:

0.009 ppm ai

Mossler isoxaflutole lemna gibba 1-12-98

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.27	100	73	73	0
.15	100	66	66	0
.075	100	61	61	0
.039	100	39	39	0
.022	100	6	6	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 5.408327E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	3.261201E-02	.0753329	6.466601E-02	8.768728E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.7883758	7.796878	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.617662

95 PERCENT CONFIDENCE LIMITS = .1813314 AND 3.053993

LC50 = 8.089726E-02

95 PERCENT CONFIDENCE LIMITS = 1.803023E-02 AND .5046692

LC10 = 1.326936E-02

95 PERCENT CONFIDENCE LIMITS = 9.482018E-09 AND 3.651794E-02

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DATA EVALUATION RECORD
AQUATIC PLANT EC_{50} TEST
GUIDELINE 122-2 (TIER I)

1. **CHEMICAL:** Isoxaflutole — PC Code No.: 123000

2. **TEST MATERIAL:** RPA 203328 — Purity: 99%

3. **CITATION:**

Author:	J.R. Hoberg
Title:	RPA 203328 Technical - Toxicity to Duckweed, *Lemna gibba*
Study Completion Date:	September 17, 1997
Laboratory:	Springborn Laboratories, Inc., Wareham, MA
Sponsor:	Rhone-Poulenc Ag Company, Research Triangle Park, NC
Laboratory Report ID:	97-9-7067
DP Barcode:	D240106
MRID No.:	443999-10

4. **REVIEWED BY:** Mark A. Mossler, M.S., Toxicologist, Golder Associates, Inc.

Signature: Mark A. Mossler **Date:** 1/13/98

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, Golder Associates, Inc.

Signature: P. Kosalwat **Date:** 1/13/98

5. **APPROVED BY:** Michael Davy

Signature: **Date:** 1/26/98

6. **STUDY PARAMETERS:**

Definitive Test Duration: 14 days
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. RPA 203328 did not adversely affect the growth of *L. gibba* by 25% or more at a concentration of 9.8 ppm ai (equivalent to ten times the maximum application rate).

8. **ADEQUACY OF THE STUDY:**

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS: None noted.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species *Lemna gibba*	*Lemna gibba*
Number of Plants/Fronds 5 plants, 3 fronds each	15 fronds/replicate (5 plants with 3 fronds each)
Nutrients Standard formula, e.g. 20XAAP	Hoagland's medium

B. Test System

Guideline Criteria	Reported Information
Solvent	Acetone (0.1 mL/L)
Temperature 25°C	24-25°C
Light Intensity 5.0 KLux (±15%)	3.2-4.2 KLux
Photoperiod Continuous	Continuous
Test System Static or Renewal	Renewal at 3-day intervals
pH Approx. 5.0	5.1-6.3

C. **Test Design**

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	N/A
Doses at least 5	0.1 and 10 mg active ingredient (ai)/L
Controls negative and/or solvent	Negative and solvent control
Replicates per dose 3 or more	3
Duration of test 14 days	14 days
Daily observations were made?	Frond counts were made on days 3, 6, 9, 12, and 14 of the study
Method of Observations	Number of fronds and dry weight
Maximum Labeled Rate	0.14 lb ai/A

12. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Initial and 14 day frond numbers were measured?	Yes
Control frond at 14 days ≥2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Results are based on mean measured concentrations computed from day 0 and 3 measurements
Raw data included?	Yes

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Dose Response

Mean Measured concentration (mg ai/L)	Terminal Avg. Frond Number	% Inhibition*	14-day pH
Control	448	N/A	6.1
Solvent Control	394	N/A	6.3
0.10	412	-4.6	6.3
9.8	392	0.4	6.3

*Based on comparison to the solvent control.

Other Significant Results: None noted.
Statistical Results for Frond Number

Statistical Method: Dunnett's test

EC_{50}: >9.8 ppm ai 95% C.I.: N/A
Probit Slope: N/A NOEC: 9.8 ppm ai

13. **VERIFICATION OF STATISTICAL RESULTS:** It is apparent from the results that the test material did not adversely affect the growth or reproduction of duckweed. Consequently, no statistical analyses were conducted.

14. **REVIEWER'S COMMENTS:** This study is scientifically sound, fulfills the guideline requirements for an aquatic plant toxicity test, and is categorized as **Core.**

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