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

MEMORANDUM

**SUBJECT:** Isoxaflutole: Review of Eco-Toxicity Studies for  
New Chemical Registration  
DP Barcode D225503, D232445

**FROM:** Elizabeth M. Leovey, Chief *Betsy Gaim for 4/1/97*  
Environmental Risk Characterization Branch  
Environmental Fate and Effects Branch (7505C)

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

The Environmental Risk Characterization Branch has reviewed 3 studies submitted by Phone-Poulenc Ag Co., Research Triangle, NC. These studies were submitted under DP Barcode D225503 and D232445 for section 3 registration of Isoxaflutole.

Review of Submitted Studies

The following is a brief summary of the submitted studies:

● CITATION: Authors: Rodgers, M. H.  
Title: RPA 202248 Subacute Dietary Toxicity (LC<sub>50</sub>)  
to the Bobwhite Quail  
Study Completion Date: December 8, 1995  
Laboratory: Huntingdon Life Sciences Ltd.  
Sponsor: Rhone-Poulenc Ag Company  
Laboratory Report ID: RNP 479  
MRID No.: 43940302

This study is scientifically valid and meets all the guidelines for avian acute dietary study.

**Results Synopsis**

LC<sub>50</sub>: >5230 ppm ai NOEL: 5230 ppm ai

**Classification of study:** Core

●**CITATION:** Authors: Hoberg, J. R.  
Title: RPA 201772- Determination of Effects on Seed Germination, Seedling Emergence and Vegetative Vigor of Ten Plant Species  
Study Completion Date: November 17, 1994  
Laboratory: Springborn Laboratories, Wareham, MA  
Sponsor: Rhone-Poulenc Ag Company, Research Triangle, NC  
Laboratory Report ID: SLI Report # 94-4-5234,  
SLI Study # 10566.0194.6326.610  
MRID No.: 43573242  
DP Barcode: D225503

This study is scientifically valid and meets all the guidelines for seedling emergence study but not for the vegetative vigor study.

### **Results Synopsis**

Germination Study: Most sensitive species: Soybean  
Most sensitive parameter: radicle length  
EC<sub>25</sub>: 0.18 lb ai/A; EC<sub>50</sub>= 0.41 lb ai/A; NOEC = 0.098 lb ai/A

#### Seedling Emergence Study:

Most sensitive monocot: onion  
Most sensitive parameter: shoot length  
EC<sub>25</sub>: 0.01576 lb ai/A EC<sub>50</sub>: 0.03785 lb ai/A  
NOEL: 0.012 lb ai/A Slope= 1.77

Most sensitive dicot: turnip  
Most sensitive parameter: shoot length  
EC<sub>25</sub>: 0.00047 lb ai/A EC<sub>50</sub>: 0.00112 lb ai/A  
NOEL: 0.00011 lb ai/A Slope= 1.79

**Classification of Germination and Seedling Emergence studies: CORE**

#### Vegetative Vigor Study:

Most sensitive monocot: oat  
Most sensitive parameter: shoot weight  
EC<sub>25</sub>: 0.00210 lb ai/A  
NOEL: 0.00048 lb ai/A

Most sensitive dicot: turnip  
Most sensitive parameter: root weight  
EC<sub>25</sub>: 0.00001 lb ai/A  
NOEL: <0.00001 lb ai/A

**Classification of Vegetative Vigor study: SUPPLEMENTAL** for all species due to lack of raw data. Raw data refers to individual plant measurements.

Ryegrass and lettuce species have negative slopes with an irregular dose response. Confidence in the data on these two species is very low. If raw data does not allow an estimation of the EC values, then the **lettuce and ryegrass need to be retested.**

#### GUIDELINE DEVIATIONS

1. No raw data were submitted to run statistical test between reps and derive an  $EC_{25}$  and  $EC_{05}$  values from a continuous data program (Nuthatch).
2. Irregular dose response for lettuce and ryegrass.

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

DP Barcode: D232445

MRID No.: 43940302

DATA EVALUATION RECORD  
§ 71-2(A) -- UPLAND GAME BIRD DIETARY LC<sub>50</sub> TEST

1. CHEMICAL: RPA 202248 (degradate of Isoxaflutole)  
PC Code No.: 123000
2. TEST MATERIAL: Batch No. DJA16-R Purity: 99.9%
3. CITATION Authors: Rodgers, M. H.  
Title: RPA 202248 Subacute Dietary Toxicity  
(LC<sub>50</sub>) to the Bobwhite Quail  
Study Completion Date: December 8, 1995  
Laboratory: Huntingdon Life Sciences Ltd.,  
Sponsor: Rhone-Poulenc Ag Company  
Laboratory Report ID: RNP 479  
MRID No.: 43940302
4. REVIEWED BY: Michael Davy, Agronomist, ERCB, EFED  
Signature: *Michael Davy* Date: 1-16-97
5. PEER REVIEWER: Francis Mastrotta, Biologist, ERCB, EFED  
Signature: *Francis Mastrotta* Date: 1-16-97
6. STUDY PARAMETERS  
Scientific Name of Test Organism: *Colinus virginianus*  
Age of Test Organisms at Test Initiation: 10 days old  
Definitive Study Duration: 8 days
7. CONCLUSIONS: This study is scientifically valid and meets  
all the guidelines for avian acute dietary study.  
Results Synopsis  
LC<sub>50</sub>: >5230 ppm ai NOEL: 5230 ppm ai
8. ADEQUACY OF THE STUDY
  - A. Classification: Core
  - B. Rationale: meets guidelines
  - C. Repairability: n/a
9. GUIDELINE DEVIATIONS None noted
10. SUBMISSION PURPOSE: Section 3 registration

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11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<b>Species:</b> An upland game bird species, preferably the bobwhite ( <i>Colinus virginianus</i> ).	<i>Colinus virginianus</i>
<b>Age at beginning of test:</b> 10-14 days old.	10 days
<b>Supplier</b>	D.R. and R.E. Wise, Monkfield
<b>Chicks appeared healthy and did not have excessive mortality before the test?</b>	Yes
<b>Acclimation period:</b> As long as possible.	9 days

B. Test System

Guideline Criteria	Reported Information
<b>Pen size:</b> about 35 x 100 x 24 cm	80 cm x 50 cm x 60 cm
<b>Brooder temperature:</b> about 35°C (95°F)	not given
<b>Room temperature:</b> 22-27°C (71-81°F)	26-28°C
<b>Relative humidity:</b> 30-80%	63%
<b>Adequate ventilation?</b>	Yes
<b>Photoperiod</b> Minimum of 14 h of light.	14 hour light, 10 hr dark
<b>Diet:</b> A commercial diet for game birds.	Standard Huntingdon Life Sciences chick diet

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**C. Test Design**

Guideline Criteria	Reported Information
Range finding test?	Yes
<b>Definitive Test</b> <b>Nominal concentrations:</b> Four minimum in a geometric scale, unless LC <sub>50</sub> > 5000 ppm.	163, 325, 650, 1300, 2600, 5200 ppm
<b>Controls:</b> Control group tested with diet containing the maximum amount of vehicle used in treated diets?	Yes
<b>Number of birds per group:</b> 10 (strongly recommended)	10
<b>Vehicle:</b> Distilled water, corn oil, propylene glycol, 1% carboxymethylcellulose, or gum arabic.	no vehicle used
<b>Vehicle amount (% of diet by weight):</b> Not more than 2%	not applicable
<b>Test durations:</b> 5 days with treated feed and at least 3 days observation with "clean" feed.	yes
<b>No mortality during last 72 hr of observations?</b>	NONE

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12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Body weights measured at beginning and end of study?	Yes
Estimated consumption per pen reported for pretreatment, treatment, and observation periods?	Yes
Control Mortality: Not more than 10%	none
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes, but no toxicity found

Mortality

Conc. (ppm)		No. of Birds	Cumulative Number of Dead							
Nominal	Mean Measured		Day of Study							
			1	2	3	4	5	6	7	8
Control	0	10	0	0	0	0	0	0	0	0
163	162	10	0	0	0	0	0	0	0	0
325	329	10	0	0	0	0	0	0	0	0
650	655	10	0	0	0	0	0	0	0	0
1300	1330	10	0	0	0	0	0	0	0	0
2600	2670	10	0	0	0	0	0	0	0	0
5200	5230	10	0	0	0	0	0	0	0	0

Other Significant Results: No mortality nor signs of clinical were found. Average body weight, body weight changes and feed consumption were not reduced in any of the test groups.

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DP Barcode: D232445

MRID No.: 43940302

Statistical Results

Statistical Method: observational  
LC<sub>50</sub>: >5200 ppm NOEL= 5200 ppm

13. Verification of Statistical Results

Statistical Method: observational using mean measured  
concentrations LC<sub>50</sub>: >5230 ppm ai NOEL =5230 ppm ai

14. REVIEWER'S COMMENTS: This study is scientifically valid and  
meets all the guidelines for avian acute dietary study.

DATA EVALUATION RECORD  
SEED GERMINATION AND SEEDLING EMERGENCE EC<sub>25</sub> TEST  
§ 123-1(A) (TIER II)

1. CHEMICAL: Isoxaflutole (RPA 201772) PC Code No.:123000

2. TEST MATERIAL: Batch Nos. 39ADM93; Purity:96.8%

3. CITATION Authors: Hoberg, J. R.  
Title: RPA 201772- Determination of Effects on  
Seed Germination, Seedling Emergence and  
Vegetative Vigor of Ten Plant Species

Study Completion Date: November 17, 1994

Laboratory: Springborn Laboratories, Wareham, MA

Sponsor: Rhone-Poulenc Ag Company, Research  
Triangle, NC

Laboratory Report ID: SLI Report # 94-4-5234,  
SLI Study # 10566.0194.6326.610

MRID No.: 43573242

DP Barcode: D225503

4. REVIEWED BY: Michael Davy, Agronomist, ERCB, EFED

Signature: *Michael Davy* Date: 3/4/97

5. APPROVED BY: Nicholas Mastrotta, Biologist, ERCB, EFED

Signature: *Nicholas Mastrotta* Date: 3/4/97

6. STUDY PARAMETERS Definitive Study Duration:  
5-day germination, 14 day seedling emergence

7. CONCLUSIONS:

Germination Study: Most sensitive species: Soybean  
Most sensitive parameter: radicle length  
EC<sub>25</sub>: 0.18 lb ai/A EC<sub>50</sub>= 0.41 lb ai/A NOEC = 0.098 lb ai/A

Seedling Emergence Study:

Most sensitive monocot: onion  
Most sensitive parameter: shoot length  
EC<sub>25</sub>: 0.01576 lb ai/A EC<sub>50</sub>: 0.03785 lb ai/A  
NOEL: 0.012 lb ai/A Slope= 1.77

Most sensitive dicot: turnip  
Most sensitive parameter: shoot length  
EC<sub>25</sub>: 0.00047 lb ai/A EC<sub>50</sub>: 0.00112 lb ai/A  
NOEL: 0.00011 lb ai/A Slope= 1.79

8. ADEQUACY OF THE STUDY

- A. Classification: CORE
- B. Rationale: meets guidelines
- C. Repairability: n/a

9. GUIDELINE DEVIATIONS : Seedling Emergence- Solvent control affected lettuce seedlings

10. SUBMISSION PURPOSE: For EUP

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	<u>Dicots</u> : cabbage, cucumber, lettuce, soybean, tomato, turnip <u>Monocots</u> : corn, oat, onion, ryegrass
<u>Number of seeds per rep</u> : 10.	20 for germination 10 for emergence
<u>Source of Seed and Historical % Germination of Seed</u>	see author's table 1

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	acetone for germination 1% acetone for emergence
<u>Site of test</u>	germination- incubator emergence- growth chamber
<u>Planting method / type of pot</u>	germination- petri dish/rep emergence- 13 cm poly pot/rep
<u>Method of application</u>	germination- 15ml solution emergence- 200 ml solution
<u>Method of watering</u>	subirrigation
<u>Growth stage at application</u>	seed

## C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> : 2x or 3x	3X
<u>Doses</u> : At least 5	5
<u>Controls</u> :Negative and solvent	Negative and solvent
<u>Replicates per dose</u> :At least 3	3
<u>Test Duration</u> :Emergence-14 days Germination-5 days	Emergence- 15 days Germination- 6 days
Were observations made at least weekly?	Yes
Maximum dosage 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 a NOEC observed for each species?	yes
Phytotoxic observations	yes
Were initial chemical concentrations measured? (Optional)	yes
Were adequate raw data included?	Yes

Results for the most sensitive parameter<sup>1</sup> of each species

Species	Parameter	EC <sub>25</sub> (lbs ai/A)	NOEC (lbs ai/A)
Cabbage	germ- radicle length emergence- shoot length	>0.21 0.0018	0.21 0.0011
Corn	germ- radicle length emergence- shoot length	>0.21 >0.19	0.21 0.045
Cucumber	germ- radicle length emergence- shoot length	>0.21 0.0059	0.21 0.0068
Lettuce	germ- radicle length emergence- shoot length	>0.21 0.00057	0.21 0.00049
Oat	germ- radicle length emergence- shoot length	>0.21 0.018	0.21 0.071
Onion	germ- radicle length emergence- shoot length	>0.21 0.013	0.21 0.012
Ryegrass	germ- radicle length emergence- shoot length	>0.21 0.075	0.21 0.043
Soybean	germ- radicle length emergence- shoot length	0.18 0.014	0.098 0.012
Tomato	germ- radicle length emergence- shoot length	>0.21 0.0052	0.21 0.0038
Turnip	germ- radicle length emergence- shoot length	>0.21 0.00039	0.21 0.00027

<sup>1</sup>Determination of the most sensitive parameter is based on EC<sub>25</sub> values.

Observations: No morphological abnormalities were noted in the seed germination study. For information about the seedling emergence study phytotoxic symptoms, please see section 13 in this review.

Statistical Results

Statistical Method: Welch's t-test to compare controls; Dunnett's test for variance; linear regression curves for EC values.

**Author's results:**Germination

Most sensitive dicot: soybean      Parameter: radicle length  
 EC<sub>25</sub>=0.18 lb ai/A      EC<sub>50</sub>= 0.41 lb ai/A      NOEC = 0.098 lb ai/A

Seedling Emergence

Most sensitive dicot: turnip      Parameter: shoot length  
 EC<sub>25</sub>=0.00039 lb ai/A (0.00022-0.00065)  
 EC<sub>50</sub>= 0.0011 lb ai/A (0.00063-0.0018)  
 NOEC = 0.00027 lb ai/A

Most sensitive monocot: Onion      Parameter: Shoot Length  
 EC<sub>25</sub>=0.013 lb ai/A (N/A)      EC<sub>50</sub>= 0.037 lb ai/A (0.012 - 0.13)  
 NOEC = 0.012 lb ai/A

**13. Reviewer's Verification of Statistical Results**

Results for Seedling Emergence Shoot Length and morphological abnormalities of each species

Results are in lb ai/A				LOEL		
Species	EC <sub>25</sub>	EC <sub>50</sub>	NOEC	Chlorosis	Necrosis	Mortality <sup>1</sup>
cabbage	0.00149	0.00310	0.0011	0.0011	n/s	none
Corn	>0.19	>0.19	0.045	0.0230	0.0450	none
cucumber	0.00454	0.01262	<0.0020	<0.0020	0.0120	none
lettuce	0.00066	0.00138	0.00049	0.00029 <sup>2</sup>	0.0010	14
oat	0.02109	0.07863	0.0071	0.0120	0.0430	12
onion	0.01576	0.03785	0.012	0.0230	0.0920	5
ryegrass	0.07967	0.29451	0.021	0.0120	0.0870	8
soybean	0.01857	0.03780	0.0071	0.0071	>0.0870	none
tomato	0.00570	0.06304	0.0038	0.0020	0.0068	3
turnip	0.00047	0.00112	0.00011	0.00027	0.0029	1

<sup>1</sup> The number of plants dead at the top 2 dose levels by day 14.

<sup>2</sup> Solvent control had significant chlorosis (12/30 seedlings).

**14. REVIEWER'S COMMENTS**

This study is scientifically valid and meets all of the guidelines for 123-1(a) germination and seedling emergence for non-target plants. This study is classified as core.

The only deviation from subdivision J and the SEP is that the lettuce seedlings in the solvent control were affected by the solvent. The morphological descriptions of the lettuce seedlings show 12/30 seedlings had chlorosis. The reviewer did a t-test of the negative and solvent controls' shoot height data since the shoot height would be the most sensitive objective measurement. No significant difference was noted. It appears that the solvent control did not affect the shoot height of the lettuce seedlings.

The most sensitive species tested for germination are as follows:

soybean : Most sensitive parameter: radicle length

EC<sub>25</sub>: 0.18 lb ai/A    EC<sub>50</sub> = 0.41 lb ai/A    NOEC = 0.098 lb ai/A

The most sensitive species tested for seedling emergence is as follows:

Most sensitive monocot: onion

EC<sub>25</sub>: 0.01576 lb ai/A

EC<sub>50</sub>: 0.03785 lb ai/A

NOEL: 0.012 lb ai/A

Most sensitive dicot: turnip

Most sensitive parameter: shoot length

EC<sub>25</sub>: 0.00047 lb ai/A

EC<sub>50</sub>: 0.00112 lb ai/A

NOEL: 0.00011 lb ai/A

Although the turnip has the lowest EC<sub>25</sub>, it appears that lettuce may be equally sensitive to isoxaflutole. The EC<sub>25</sub> values of 0.00047 lb ai/A for turnip and 0.00066 lb ai/A for lettuce are very similar. At the 0.0040 lb ai/A dose level, lettuce had 12 dead seedlings and turnip had one dead seedling, however 13 turnip seedlings were necrotic which is preliminary to death. Therefore, over time, the mortality may be similar as well. 13

The reviewer's results are very similar to the author's values for all species. This herbicide shows stress symptoms of chlorosis below and around the EC<sub>25</sub> dose level and necrosis around the EC<sub>50</sub> dose level for most plant species tested. This would suggest that this herbicide would significantly affect the plant around the EC<sub>25</sub> level. The study is too short to determine if the plants would recover from ~~the~~ exposure to isoxaflutole. *in duration*

DATA EVALUATION RECORD  
VEGETATIVE VIGOR EC<sub>25</sub> TEST  
§ 123-1(B) (TIER II)

1. CHEMICAL: Isoxaflutole (RPA 201772) PC Code No.:123000
2. TEST MATERIAL: Batch Nos. 39ADM93; Purity:96.8%
3. CITATION Authors: Hoberg, J. R.  
Title: RPA 201772- Determination of Effects on Seed Germination, Seedling Emergence and Vegetative Vigor of Ten Plant Species

Study Completion Date: November 17, 1994  
Laboratory: Springborn Laboratories, Wareham, MA  
Sponsor: Rhone-Poulenc Ag Company, Research Triangle, NC

Laboratory Report ID: SLI Report # 94-4-5234,  
SLI Study # 10566.0194.6326.610

MRID No.: 43573242

DP Barcode: D225503

4. REVIEWED BY: Michael Davy, Agronomist, ERCB, EFED  
Signature: *Michael Davy* Date: 2/25/97
5. PEER REVIEWED BY: Francis Mastrotta, Biologist, ERCB, EFED  
Signature: *Francis Mastrotta* Date: 2/25/97

6. STUDY PARAMETERS

Definitive Study Duration: 14 days

7. CONCLUSIONS:

Results Synopsis

Most sensitive monocot: oat  
Most sensitive parameter: shoot weight  
EC<sub>25</sub>: 0.00210 lb ai/A  
NOEL: 0.00048 lb ai/A

Most sensitive dicot: turnip  
Most sensitive parameter: root weight  
EC<sub>25</sub>: 0.00001 lb ai/A  
NOEL: <0.00001 lb ai/A

8. ADEQUACY OF THE STUDY

A. Classification: SUPPLEMENTAL for all species due to lack of raw data. Raw data refers to individual plant measurements.

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Ryegrass and lettuce species have negative slopes with an irregular dose response. Confidence in the data for these two species is low since the 95% CL is infinity. If raw data does not allow a reasonable estimation of the EC values, then the lettuce and ryegrass need to be retested.

9. GUIDELINE DEVIATIONS

1. No raw data were submitted to run statistical program between reps and derive an EC<sub>25</sub> and EC<sub>05</sub> values from continuous data program (Nuthatch).
2. Irregular dose response for lettuce and ryegrass.
3. EC<sub>25</sub> values are lower than the lowest dose concentrations.

10. SUBMISSION PURPOSE: Section 3 Registration

11. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> 6 dicots in 4 families, including soybean and a rootcrop; 4 monocots in 2 families, including corn.	<u>Dicots</u> : cabbage, cucumber, lettuce, soybean, tomato, turnip <u>Monocots</u> : corn, oat, onion, ryegrass
<u>Number of plants per rep</u> : 5	5
<u>Source of Seed and Historical % Germination of Seed</u>	see author's table 1

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	10% acetone
<u>Site of test</u>	growth chamber
<u>Planting method / type of pot</u>	13 cm poly pot/rep
<u>Method of application</u>	30 ml from spraymaster hand pump
<u>Method of watering</u>	subirrigation
<u>Growth stage at application</u>	7 days after seedling transplant

## C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> : 2x or 3x	3X
<u>Doses</u> : At least 5	6 and 5
<u>Controls</u> :Negative and solvent	Negative and solvent
<u>Replicates per dose</u> :At least 3	3
<u>Test Duration</u> : 14 days	14 days
Were observations made at least weekly?	Yes
Maximum dosage rate	0.20 lb ai/A

12. REPORTED RESULTS

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Was a NOEC observed for each species?	yes
Phytotoxic observations	yes
Were initial chemical concentrations measured? (Optional)	yes
Were adequate raw data included?	NO

Author's results for the most sensitive parameter<sup>1</sup> of each species

Species	Parameter	EC <sub>25</sub> (lbs ai/A)	NOEC (lbs ai/A)
Cabbage	root weight	0.00004	0.00012
Corn	shoot weight	0.03600	0.07900
Cucumber	root weight	0.00460	0.05300
Lettuce	shoot weight	0.00011	0.00012
Oat	shoot weight	0.00170	0.00048
Onion	shoot weight	0.00230	0.00470
Ryegrass	root weight	0.00160	0.00350
Soybean	shoot weight	0.00160	0.00130
Tomato	root weight	0.00069	0.00048
Turnip	root weight	0.00008	0.00012

<sup>1</sup>Determination of the most sensitive parameter is based on EC<sub>25</sub> values.

Morphological Observations:

Cabbage: significant chlorosis starts at 0.00040 lb ai/A and necrosis at 0.0047 lb ai/A. No deaths noted.

Corn: significant leaf curl starts at 0.0084 lb ai/A and chlorosis at 0.079 lb ai/A. No deaths noted.

Cucumber: significant leaf curl starts at 0.0013 lb ai/A and chlorosis at 0.0013 lb ai/A, and necrosis at 0.053 lb ai/A. No deaths noted.

Lettuce: significant leaf curl and chlorosis starts at 0.00012 lb ai/A and sig. necrosis at 0.0013 lb ai/A. No deaths noted.

Oat: Significant chlorosis starts at 0.0048 lb ai/A. No deaths noted.

Onion: significant necrosis starts at 0.053 lb ai/A and chlorosis at 0.0047 lb ai/A. 0.016 lb ai/A has leaf curl. Four out of 15 plants died at the 0.18 lb ai/A dose.

Ryegrass: Significant chlorosis starts at 0.0064. No sig. deaths noted.

Soybean: Significant chlorosis and leaf curl starts at 0.0013 lb ai/A and necrosis and leaf blotch starts at 0.016 lb ai/A. No deaths noted.

Tomato: Significant chlorosis starts at 0.0086 lb ai/A and necrosis at 0.0035 lb ai/A. No significant deaths noted.

Turnip: Significant leaf curl starts at 0.000034 lb ai/A, chlorosis at 0.00040 lb ai/A and necrosis at 0.0047 lb ai/A. No significant deaths noted.

### Statistical Results

Statistical Method: Welch's t-test to compare controls; Dunnett's test for variance; linear regression curves for EC values

Most sensitive dicot: cabbage                      Parameter: root weight  
 EC<sub>50</sub>= 0.00019 lb ai/A (95% C.L.: 0.000000017 - 0.00058)  
 EC<sub>25</sub>=0.00004 lb ai/A                      NOEC = 0.00012 lb ai/A

Most sensitive monocot: Oat                      Parameter: root weight  
 EC<sub>50</sub>= 0.00350 lb ai/A (95% C.L.: 0.00020 - 0.01300)  
 EC<sub>25</sub>=0.00160 lb ai/A                      NOEC = 0.012 lb ai/A

### 13. Reviewer's Verification of Statistical Results

Results for the most sensitive parameter<sup>1</sup> of each species

Species	Parameter	Lowest Conc. Tested	EC <sub>25</sub> (lbs ai/A)	EC <sub>50</sub> (lbs ai/A)	slope	NOEC (lbs ai/A)
CABBAGE	root weight	0.00003	0.00005	0.00018	1.256	<0.00012
CORN	shoot weight	0.00640	0.04379	0.13473	1.381	<0.0790
CUCUMBER	root weight	0.00040	0.00316	0.02054	0.903	0.0013 <sup>2</sup>
LETTUCE	*	0.00001	*	*	*	*
OAT	shoot weight	0.00048	0.00210	0.00840	1.191	0.00048
ONION	shoot weight	0.00040	0.00274	0.01725	0.844	<0.0047
RYEGRASS	*	0.00086	*	*	*	*

Species	Parameter	Lowest Conc. Tested	EC <sub>25</sub> (lbs ai/A)	EC <sub>50</sub> (lbs ai/A)	slope	NOEC (lbs ai/A)
SOYBEAN	shoot weight	0.00040	0.00183	0.02445	0.603	0.00130
TOMATO	root weight	0.00048	0.00062	0.00127	2.148	0.00048
TURNIP	root weight	0.00001	0.00001	0.000134	0.660	<0.00012

<sup>1</sup> Determination of the most sensitive species is based on EC<sub>25</sub> values.

<sup>2</sup> NOEC based on morphological observations.

\* Lettuce and ryegrass EC<sub>25</sub> and EC<sub>50</sub> values lack any certainty of confidence due to the infinity value for 95% CL.

It should be noted in the absence of NOEC values, EC<sub>05</sub> should be determined. However the lack of raw data prevents the determination of the EC<sub>05</sub> values in NUTHATCH, an EPA continuous data computer program.

#### Results for most sensitive parameter of most sensitive species

	Monocot	Dicot
Species	oat	turnip
Parameter	shoot weight	root weight
EC <sub>25</sub> (lb ai/A)	0.00210 (lb ai/A)	0.00001 lb ai/A
EC <sub>50</sub> (lb ai/A)	0.00840 (lb ai/A)	0.00013 lb ai/A
Probit Slope	1.191	0.6514
NOEC or EC <sub>05</sub> (lb ai/A)	0.00048 (lb ai/A)	<0.00001

**14. REVIEWER'S COMMENTS** The study is scientifically sound and but does not meets all of the guidelines requirements for vegetative vigor study (123-1 b) because of the following reasons:

- There is a lack of raw data. Raw data refers to individual plant measurements. Since no raw data were submitted for any of the species in this study, no EC<sub>05</sub> value could be estimated from the Nuthatch statistical program. It is important for the reviewer to have such data in order to derive an EC<sub>05</sub> value for an endangered plant species risk assessment. In addition, the reviewer should be able to do statistical comparisons between the reps and derive an EC<sub>25</sub> value based on continuous data.

- Ryegrass and lettuce species have negative slopes with an irregular dose response. Confidence in the data on these two species is low due to the infinity value in the 95% CL. If raw data does not allow a reasonable estimation of the EC values, then the lettuce and ryegrass needs to be retested.

Since the author's and reviewer's values are similar for the species tested, the author's  $EC_{25}$  values may be used. However, since the NOEC is much higher than the  $EC_{50}$  value, the author's NOEC for turnip and oat should not be used. Therefore, the raw data becomes more necessary for the  $EC_{05}$  value to be determined.

Some of the tested plants appear to be extremely sensitive to this chemical. Onion was the only species in which significant mortality was noted and that was at 0.18 lb ai/A. Additional mortality may be seen if the study were to continue for another one or two weeks more.

Since isoxaflutole was to be used on corn, one would expect that corn would be more resistant to the herbicide. It appears, however, that corn also exhibits some phytotoxicity to isoxaflutole.

DAVY ISOXAFLUTOLE VIGOR TURNIP ROOT WEIGHT

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.0047	100	92	92	0
.0013	100	74	74	0
.0004	100	49	49	0
.00012	100	37	37	0
.000034		100	57	57
.000011		100	19	19

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 1.683418E-04

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	3.014196E-02		1.494278E-04
1.087503E-04		2.010553E-04	

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H
3	.739389	10.04957

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 = .6514022  
 95 PERCENT CONFIDENCE LIMITS = 9.127629E-02 AND 1.211528

LC50 = 1.318718E-04  
 95 PERCENT CONFIDENCE LIMITS = 1.277558E-06 AND 1.551022E-03

LC10 = 1.480852E-06  
 95 PERCENT CONFIDENCE LIMITS = 1.09386E-19 AND 1.976829E-05

\*\*\*\*\*

SLOPE =  
1.375363E-05

.6514022 LC50 =

.000149 LC25 =