US EPA ARCHIVE DOCUMENT

DP Barcode : D210584
PC Code No : 108801
EEB Out : 03/06/95

To: Walter Waldrop/Jane Mitchell
Product Manager 71
Special Review and Reregistration Division (7508W)

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

Attached, please find the EEB review of..

Reg./File # : 108801
Chemical Name : Metolachlor
Type Product : Fungicide
Product Name :
Company Name : Ciba Crop Protection, Greensboro, NC
Purpose : Review guideline studies 72-3a,b,c; 123-1a,b; 123-2
Action Code : 627 Date Due : 04/24/95
Reviewer : William Erickson Date In : 01/11/95

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)			72-7(a)		
71-1(b)			72-2(b)			72-7(b)		
71-2(a)			72-3(a)	434871-01	Y	122-1(a)		
71-2(b)			72-3(b)	434871-02	Y	122-1(b)		
71-3			72-3(c)	434871-03	Y	122-2		
71-4(a)			72-3(d)			123-1(a)	434871-07	Y
71-4(b)			72-3(e)			123-1(b)	434871-08	Y
71-5(a)			72-3(f)			123-2	434871-04 434871-05 434871-06	Y Y Y
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)			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)

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Metolachlor: review of toxicity studies

FROM: Anthony F. Maciorowski, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (7507C) 2/16/95

TO: Walter Waldrop/Jane Mitchell
Product Manager 71
Special Review and Reregistration Division (7508W)

Ciba Crop Protection, Greensboro, NC submitted the following studies to support reregistration of metolachlor:

Machado, M.W. 1994. Metolachlor technical - acute toxicity to sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions. Conducted by Springborn Laboratories, Inc., Wareham, MA. Lab. Report No. 94-7-5378. MRID No. 434871-01.

Dionne, E. 1994. Metolachlor technical - acute toxicity to Eastern oyster (*Crassostrea virginica*) under flow-through conditions. Conducted by Springborn Laboratories, Inc., Wareham, MA. Lab. Report No. 94-7-5365. MRID No. 434871-02.

Machado, M.W. 1994. Metolachlor technical - acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions. Conducted by Springborn Laboratories, Inc., Wareham, MA. Lab. Report No. 94-7-5402. MRID No. 434871-03.

Hoberg, J.R. 1994. Metolachlor technical - 5-day toxicity to the freshwater green alga, *Anabaena flos-aquae*. Conducted by Springborn Laboratories, Inc., Wareham, MA. Lab. Report No. 94-7-5382. MRID No. 434871-04.

Hoberg, J.R. 1994. Metolachlor technical - toxicity to duckweed (*Lemna gibba*). Conducted by Springborn Laboratories, Inc., Wareham, MA. Lab. Report No. 94-8-5404. MRID No. 434871-05.

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Printed with Soy/Canola Ink on paper that contains at least 50% recycled fiber

Hoberg, J.R. 1994. Metolachlor technical - toxicity to the marine diatom, *Skeletonema costatum*. Conducted by Springborn Laboratories, Inc., Wareham, MA. Lab. Report No. 94-7-5382. MRID No. 434871-06.

Chetram, R.S. 1994. Tier 2 seedling emergence nontarget phytotoxicity study using metolachlor. Conducted by Pan-Agricultural Labs, Inc, Madera, CA. Lab. Report # 94349. MRID No. 434871-07.

Chetram, R.S. 1994. Tier 2 vegetative vigor nontarget phytotoxicity study using metolachlor. Conducted by Pan-Agricultural Labs, Inc, Madera, CA. Lab. Report # 94350. MRID No. 434871-08.

Chetram, R.S. 1994. Tier 2 seed germination nontarget phytotoxicity study using metolachlor. Conducted by Pan-Agricultural Labs, Inc, Madera, CA. Lab. Report # 94348. MRID No. 434973-01.

The DERs are attached. The Agency no longer requires seed germination studies; therefore, EEB has not reviewed that study (MRID No. 434973-01). The remainder of the studies submitted are acceptable and fulfill the guideline requirements for 72-3a,b,c and 123-1a,b. Guideline 123-2 (Aquatic Plant Growth) has not been fully satisfied, because the registrant did not submit required phytotoxicity studies for *Selenastrum capricornutum* and for a freshwater diatom.

If you have any questions, please contact Bill Erickson at 305-6212 or Harry Craven at 305-5320.

(3)

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DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE FISH
§ 72-3(A)

1. **CHEMICAL:** Metolachlor
   Shaughnessey No.: 108801

2. **TEST MATERIAL:** Metolachlor technical
   Purity: 97.3%

3. **CITATION**

   Authors: Machado, M.W.
   Title: Metolachlor technical - acute toxicity to sheepshead minnow (*Cyprinodon variegatus*) under flow-through conditions
   Date: 1994
   Laboratory: Springborn Laboratories, Inc., Wareham, MA
   Sponsor: Ciba Crop Protection, Greensboro, NC
   Lab. Report No.: 94-7-5378
   MRID No.: 434871-01

4. **REVIEWED BY:**

   William Erickson
   Biologist
   EEB/EFED

   Signature: W. Erickson
   Date: 1/26/95

5. **APPROVED BY:**

   Harry Craven
   Section Head 4
   EEB/EFED

   Signature: Harry T. Craven
   Date: 2/15/95

6. **CONCLUSIONS:** The study is scientifically sound and satisfies the guideline requirement for an acute marine/estuarine fish toxicity test. The LC_{50} value of 9.8 mg ai/l classifies technical metolachlor as moderately toxic to the sheepshead minnow. The NOEC is 3.6 mg ai/l.

7. **ADEQUACY OF THE STUDY:** Core.

8. **MAJOR GUIDELINE DEVIATIONS:** None.

9. **MATERIALS AND METHODS:**

(4)

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the sheepshead minnow (*Cyprinodon variegatus*) or the Silverside (*Menidia sp.*).	Sheepshead minnow
Mean Wet Weight 0.5 - 5 g	Mean: 0.22 g Range: 0.11-0.35 g
Mean Standard Length Longest not > 2x shortest	Mean: 23 mm Range: 15-27 mm
Supplier	Aquatic Research Organisms, Hampton, NH
All fish from same source?	Yes
All fish from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 14 days	14 days
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
Feeding No feeding during the study	Last fed 48 h prior to test
Pretest Mortality <3% mortality 48 hours prior to testing	No mortality within 48 h of testing

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Filtered seawater
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range < 6‰	31 ‰
Water Temperature 22 ± 1 °C	21-22 °C
pH 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8	8.0
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%	Lowest % DO was 73% at 96 h
Test Aquaria 1. Material: Glass or stainless steel 2. Size: Volume of 19 L (5 gal) or 30 x 60 x 30 cm 3. Fill volume: 15-30 L of solution	**Material:** not described **Size:** 39 x 20 x 25 cm **Fill vol.:** 15 l solution
Type of Dilution System Must provide reproducible supply of toxicant	Mount and Brungs intermittent-flow proportional diluter, temp.-controlled water bath, and set of 14 aquaria
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	6.5 vol/24 hours

Guideline Criteria	Reported Information
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day	0.023 g/L
Photoperiod 16 hours light, 8 hours dark	16 h light
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	**Solvent:** acetone **Maximum conc.:** 0.092 mL/L

D.7 Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 fish, then no definitive test is required.	100% mortality at 20 mg ai/L
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	2.6, 4.3, 7.2, 12, and 20 mg ai/L.
Number of Test Organisms Minimum 10/level, may be divided among containers	20/level 10/rep.
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	Temp. continuously monitored in one rep; measured once daily in both reps of each level DO, pH, and salinity measured once daily in both reps of each level
Chemical Analysis 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	Yes

10. **REPORTED RESULTS**

General Results:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical % of nominal	83-110%
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	2.5% (1 of 40)
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

Mortality:

Concentration (ppm)		Number of Fish	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control		20	0	1	1	1
Solvent Control		20	0	0	0	0
2.6	2.8	20	1	1	1	1
4.3	3.6	20	0	0	0	0
7.2	6.2	20	0	0	1	1
12	11	20	0	0	2	12
20	19	20	1	7	20	20

Other Findings: Sublethal effects, including lethargy and loss of equilibrium, were observed among all surviving fish exposed to the 11 mg ai/l treatment level and to one fish in the 6.2 mg ai/l treatment level.

Statistical Results

Method: **Moving Average** 96-hr LC_{50}: **9.8 mg ai/l**

95% C.I.: **8.5-11 mg ai/l** NOEC: **3.6 mg ai/l**

11. **REVIEWER'S VERIFICATION OF STATISTICAL RESULTS:** (attached)

Method: **Moving Average** 96-hr LC_{50}: **9.8 mg ai/l**

95% C.I.: **8.5-11.4 mg ai/l** NOEC: **3.6 mg ai/l**

12. **REVIEWER'S COMMENTS:** Deviations from guideline procedures include the following:

The size of the test fish (0.11-0.35 g) was smaller than recommended (0.5-5 g).

The report did not mention if any fish were diseased and if any treatment was made.

The study is scientifically sound and satisfies the guideline requirement for an acute marine/estuarine fish toxicity test. The EC_{50} value of 9.8 mg ai/l classifies technical metolachlor as moderately toxic to the sheepshead minnow.

THERE WAS CONTROL MORTALITY, BUT AT LEAST ONE
E LOWER CONCENTRATIONS HAD ZERO MORTALITY.
FORE, ABBOTT'S CORRECTION IS NOT APPLICABLE.

KSON METOLACHLOR SHEEPSHEAD MINNOW ACUTE TEST

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
19	20	20	100	9.536742E-05
11	20	12	60.00001	25.17223
6.2	20	1	5	2.002716E-03
3.6	20	0	0	9.536742E-05
2.8	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 6.2 AND 19 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 10.05811

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	5.026151E-02	9.802751	8.525102	11.47825

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	2.539791	8.462025	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 = 5.375903
95 PERCENT CONFIDENCE LIMITS =-3.191524 AND 13.94333

LC50 = 9.560683
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 5.549446
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

(4)

DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK
§ 72-3(B)

1. **CHEMICAL:** Metolachlor
   Shaughnessey No.: 108801

2. **TEST MATERIAL:** Metolachlor technical
   Purity: 97.3%

3. **STUDY TYPE:** 72-3b - Acute toxicity study to marine/estuarine mollusk.

4. **CITATION:**

   Author: Dionne, E.
   Title: Metolachlor technical -- acute toxicity to Eastern Oyster (*Crassostrea virginica*) under flow-through conditions.
   Date: 1994
   Lab. Report #: 94-7-5365
   Laboratory: Springborn Laboratories, Inc., Wareham, MA
   Sponsor: Ciba Crop Protection, Greensboro, NC
   MRID No.: 434871-02

5. **REVIEWED BY:**

   William Erickson
   Biologist
   EEB/EFED

   Signature: W. Erickson
   Date: 1/26/95

5. **APPROVED BY:**

   Harry Craven
   Section Head 4
   EEB/EFED

   Signature: Harry T. Craven
   Date: 2/15/95

7. **CONCLUSIONS:** This study is scientifically sound and fulfills the guideline requirement for an acute toxicity test with a marine/estuarine mollusk. The 96-hour EC_{50} is 1.7 mg ai/l, which classifies technical metolachlor as moderately toxic to the Eastern oyster. The NOEC is 0.71 mg ai/l.

8. **CLASSIFICATION:** Core.

9. **MAJOR GUIDELINE DEVIATIONS:** None.

10. **MATERIALS AND METHODS:**

(11)

A. Test Organism: Eastern Oyster

Guideline Criteria	Reported Information
Species (Scientific Name)	*Crassostrea virginica*
Mean valve height (25 - 50 mm -- the long axis)	39 mm
Supplier	P. Cummins Oyster Co., Pasadena, MD
All oysters from same source (y/n)	Yes
All oysters from the same year class (y/n)	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period (minimum 10 days)	17 days
Wild caught 7 day quarantine (y/n)	Not reported
Check for signs of disease or injury (y/n; describe)	Yes; checked for mortality and after the shell growth was grounded off, checked for stress.
If diseased it can be treated in 48-hr pretest no sign of the disease remains (Report hours prior to test in which no sign of disease or N/A)	Examined for boring sponges and/or mudworms; several opened to confirm no parasites present
Was peripheral shell growth removed prior to testing? If so how much.	Yes; 3-5 mm
Feeding during the acclimation	Fed a supplemental algal diet of *Isochrysis galbana* Parke, clone T-Iso
<3% mortality 48 hours prior to testing (% mortality, if any)	No mortality within 7 days of testing

C. Test System:

Guideline Criteria	Reported Information
Describe source of dilution water (natural unfiltered seawater)	Natural unfiltered seawater from Cape Cod Canal, Bourne, MA
Does water support test animals without observable signs of stress?	Yes
Salinity of Test water:	31‰ ppt
Water Temperature (between 15°C and 30°C -- but must be consistent)	20 ± 1°C
pH	7.9
Dissolved Oxygen (Static 1st 48 hrs 40%; 2nd 48 hrs 60%; Flow-through 60%) (% of lowest conc. & hour)	Measured daily; 62-88% saturation at 96 h in definitive test
Total Organic Carbon	1.3 mg/l
Test Aquaria 1. Material (glass or stainless steel) 2. a. Static volume (18.9 L (5 gal or 19000 cc) with 15 L solution) b. Static or flow-through volume (300x600x300 = 54000 cc.)	Glass aquaria 18 l 49.5 X 25.5 X 29 cm
Type of Dilution System (Reproducible supply of toxicant)	Harvard Apparatus peristaltic pump was calibrated to deliver 0.1875 ml/min of the 11 mg ai/ml stock solution.
Flow rate Consistent flow rate-meter systems calibrated before study and checked 2*24 hours - 5 to 10 vol/24 hours	6 solution volume replacements per 24 hours
Biomass Loading Rate (all oysters should be able to sit on the bottom with water flowing freely around them).	Yes
Photoperiod (16 L & 8 D)	16 hours light and 8 hours dark

Solvents (Do not exceed 0.5 ml/L for flow-through)	0.5 ml/L acetone

D. Test Design:

Guideline Criteria	Reported Information
Range Finding Test (LC_{50} >100 mg/L with 30 shrimp, no definitive test required.)	Tested concentrations of 0.039 to 6.3 mg ai/l. After 96-hours, growth reduction of 0-97% was observed.
Definitive Test	
Nominal Concentrations (control+5 treatment levels; dosage should be 60% of the next highest concentration; concentrations should be geometric series)	0.71, 1.2, 2.0, 3.3, and 5.5 mg ai/l; also negative and solvent controls
Controls (Minimum control mortality; static 10%; flow-through 5%	No mortality reported
Number of Test Organisms; (Minimum 10/level can be divided among containers)	20 oysters in each test aquarium (40 per treatment level)
All organisms must be randomly assigned to test vessels (y/n)	Impartially assigned
Biological Observations (y/n)	Yes
Water Parameter Measurements 1. Temperature - record every 6 hrs;>1°C. 2. D.O. beginning,48 hrs,end for control high, medium, and low dose. 3. pH beginning,48 hrs, end for control, high, medium, and low dose.	21-22°C Yes; lowest value was 62% at 96 hours Yes; ranged was 7.5-7.9
Chemical Analysis (needed if aeration, volatile, insoluble, precipitate, not steel or glass, known to adsorb, and flow-through) (yes or no)	Yes

11. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Mean Measured Concentrations	0.71, 1.1, 1.7, 2.9, 4.5
Recovery of Chemical (% recovery)	83-100% of nominal
Mortality & Observations (Describe observations & attach mortality tables)	No mortality at any treatment level
Mean shell growth (mm) of control and treatment groups after 96 h exposure	water control 2.2 mm solvent contr 2.4 0.71 mg ai/l 2.6 1.1 1.9 1.7 1.1 2.9 0.36 4.5 0.0
Ratio of mean shell growth of treated to untreated oysters after 96 h exposure	0.71 mg ai/l +12% 1.1 -18% 1.7 -52% 2.9 -84% 4.5 -100% note: control groups pooled
Statistical Results: **EC_{50}:** **95% CL:** **NOEC:**	Regression analysis 1.8 mg ai/l 1.2-2.8 mg ai/l 0.71 mg ai/l

Other findings: Reduced feeding and reduced fecal and pseudofecal production were observed at the 24-, 48-, 72-, and 96-hour intervals at the highest treatment level (4.5 mg ai/l). These effects were not observed at the four lowest treatment levels.

12. **QUALITY ASSURANCE MEASURES:** Yes.

13. **REVIEWER'S DISCUSSION/INTERPRETATION:**

Test Procedures: Test procedures followed guideline criteria.

Statistical Analysis:

Method: **EPA's Nuthatch program** EC_{50}: **1.6 mg ai/l**

95% CL: **1.4-1.9 mg ai/l** NOEC: **0.71 mg ai/l**

Slope: **4.97**

Discussion/Results: The study is scientifically sound and satisfies the guideline requirement for an acute toxicity test with a marine/estuarine mollusk. The EC_{50} value of 1.6 mg ai/l classifies metolachlor technical as moderately toxic to the Eastern oyster.

14. **COMPLETION DATE OF ONE-LINER FOR STUDY:** 1/26/95

Williams Test

!!!Williams: not implemented for 234 degrees of freedom.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.55	0.40	0.77	0.072	0.72
EC5	0.76	0.58	0.99	0.059	0.77
EC25	1.2	1.0	1.4	0.040	0.83
EC50	1.6	1.4	1.9	0.028	0.88

Slope = 4.97 Std.Err. = 0.522

Goodness of fit: p = 0.084 based on DF= 3.0 2.3e+02

OYSTER.DAT : OYSTER SHELL DEPOSITION

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	40.0	2.39	2.48	-0.0871	100.	0.00
0.710	40.0	2.58	2.39	0.193	96.4	3.65
1.10	40.0	1.88	1.98	-0.0990	80.2	19.8
1.40	40.0	1.10	1.15	-0.0566	46.4	53.6
2.90	40.0	0.355	0.266	0.0889	10.7	89.3
4.50	40.0	0.00	0.0355	-0.0355	1.43	98.6

!!!Warning: EC1 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	131.34	237.00	0.55416
Means Lack-of-Fit	3.6654	3.0000	1.2218
Within Groups	127.67	234.00	0.54560

Directly-fitted parameters and Asymptotic Covariances

Parameter	Estimate	Std.Err.	Correlations		
Control Mean =	2.4796	0.14505	1.0000	-0.71602	0.51894
log10 EC50 =	0.21246	0.028414	-0.71602	1.0000	-0.71177
Bruce-V. SIGMA =	0.20140	0.021162	0.51894	-0.71177	1.0000
Starting Values	2.4875	0.20020	0.23583		

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Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

OYSTER.DAT : OYSTER SHELL DEPOSITION

Raw data:

OYSTER SHELL DEPOSITION
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(4)

DATA EVALUATION RECORD
ACUTE LC_{50} TEST WITH AN ESTUARINE/MARINE SHRIMP
§ 72-3(C)

1. **CHEMICAL:** Metolachlor
   Shaughnessey No.: 108801

2. **TEST MATERIAL:** Metolachlor technical
   Purity: 97.3%

3. **CITATION**

   Author: Machado, M.W.
   Title: Metolachlor technical - acute toxicity to mysid shrimp (*Mysidopsis bahia*) under flow-through conditions.
   Date: 1994
   Lab. Report #: 94-7-5402
   Laboratory: Springborn Laboratories, Inc., Wareham, MA
   Sponsor: Ciba Crop Protection, Greensboro, NC
   MRID No.: 434871-03

4. **REVIEWED BY:**

   William Erickson
   Biologist
   EEB/EFED

   Signature: W. Erickson
   Date: 1/26/95

5. **APPROVED BY:**

   Harry Craven
   Section Head 4
   EEB/EFED

   Signature: Henry T. Craven
   Date: 2/15/95

6. **CONCLUSIONS:** The 96-h LC_{50} of 4.9 mg ai/l classifies technical metolachlor as moderately toxic to mysid shrimp. The NOEC is 2.3 mg ai/l.

7. **ADEQUACY OF THE STUDY:** Core.

9. **MAJOR GUIDELINE DEVIATIONS:** None.

10. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are *Mysidopsis bahia*, *Penaeus setiferus*, *P. duorarun*, *P. aztecus* and *Palaemonetes sp.*	*Mysidopsis bahia*
Age Juvenile, mysids should be ≤ 24 hours old	≤24 h old
Supplier	Springborn Laboratories culture facility
All shrimp are from same source?	Yes
All shrimp are from the same year class?	Yes

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period minimum 10 days	14 days
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?	N/A
Feeding No feeding during the study and no feeding for 24 hour before the beginning of the test if organisms are over 0.5 g each.	Time of last feeding was not reported
Pretest Mortality <3% mortality 48 hours prior to testing	% mortality prior to testing was not reported

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Soft reconstituted water or water from a natural source, not dechlorinated tap water	Filtered seawater from Cape Cod Canal, Bourne, MA
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ for marine (stenohaline) shrimp and 10-17 ‰ for estuarine (euryhaline) shrimp, weekly range < 6 ‰	31-32‰
Water Temperature 22 ± 1 °C	25 ± 1 °C
Ph 8.0-8.3 for marine (stenohaline) shrimp, 7.7-8.0 for estuarine (euryhaline) shrimp, monthly range < 0.8	7.9
Dissolved Oxygen Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, Flow-through: ≥ 60%	80-81% at 72 h at highest dosage
Total Organic Carbon	1.4 mg/L
Test Aquaria 1. Material: Glass or stainless steel 2. Size: 19.6 L is acceptable for organisms ≥ 0.5 g (e.g. pink shrimp, white shrimp, and brown shrimp), 3.9 L is acceptable for smaller organisms (e.g. mysids and grass shrimp). 3. Fill volume: 15 L is acceptable for organisms ≥ 0.5 g, 2-3 L is acceptable for smaller organisms.	Glass 39 x 20 x 25 cm 7.0-11 L

Guideline Criteria	Reported Information
Type of Dilution System Must provide reproducible supply of toxicant	Continuous-flow serial diluter calibrated to deliver 50 ml/min of exposure solution to each replicate aquarium
Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period	6.5 vol/24 hours
Biomass Loading Rate Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through. ≤ 1 g/L/day	0.00014 g/L
Photoperiod 16 hours light, 8 hours dark	16 h light, 8 h dark.
Solvents Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests	Solvent: acetone Maximum conc.: 0.1 ml/L.

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If LC_{50} >100 mg/L with 30 shrimp, then no definitive test is required.	3 preliminary tests - 100% mortality at 8.0 mg ai/l
Nominal Concentrations of Definitive Test Control & 5 treatment levels; a geometric series in which each concentration is at least 60% of the next higher one.	0.50, 1.0, 2.0, 4.0, and 8.0 mg ai/l
Number of Test Organisms Minimum 20/level, may be divided among containers	20
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Temperature Measured constantly or, if water baths are used, every 6 hrs, may not vary > 1°C 2. DO and pH Measured at beginning of test and ever 48 h in the high, medium, and low doses and in the control	Temp. continuously monitored in one rep. of control and once daily in all others DO, pH, and salinity measured once daily in both reps of each treatment and control
Chemical Analysis 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	Samples from each replicate analyzed for metolachlor at 0 h and 96 h (except for 2.0 mg ai/l dosage, which was sampled at 24 h and 96 h due to suspected sampling error at 0 h)

11. **REPORTED RESULTS**

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Recovery of Chemical	89-120% of nominal
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	None
Raw data included?	Yes
Signs of toxicity (if any) were described?	Yes

(23)

Mortality:

Concentration (ppm)		Number of Shrimp	Cumulative Number Dead			
			Hour of Study			
Nominal	Mean Measured		24	48	72	96
Control		20	0	0	0	0
Solvent Control		20	0	0	0	0
0.5	0.61	20	0	0	0	0
1.0	1.0	20	0	0	0	0
2.0	2.3	20	0	0	0	0
4.0	4.0	20	0	1	4	7
8.0	7.1	20	0	1	13	16

Other Findings: At the two highest dosages, surviving mysids exhibited dark pigmentation and were lethargic by the end of the study.

B. Statistical Results:

Method: **Probit Analysis** 96-h LC_{50}: **4.9 mg ai/l**

95% C.I.: **4.2-5.9 mg ai/l** NOEC: **2.3 MG AI/l**

Probit Slope: (not reported)

12. **VERIFICATION OF STATISTICAL RESULTS:**

Method: **Probit Analysis** 96-h LC_{50}: **4.9 mg ai/l**

95% CL: **4.2-5.9 mg ai/l** NOEC: **2.3 mg ai/l**

13. **REVIEWER'S COMMENTS:** The study is scientifically sound and fulfills the guideline requirement for an acute toxicity test with mysid shrimp. Technical metolachlor is classified as moderately toxic to marine/estuarine mysid shrimp.

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W. ERICKSON METOLACHLOR MYSID SHRIMP ACUTE TEST

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
7.1	20	16	80	.5908966
4	20	7	35	13.1588
2.3	20	0	0	9.536742E-05
1	20	0	0	9.536742E-05
.61	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 2.3 AND 7.1 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 4.813171

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	9.753802E-02	4.861711	4.172503	5.902251

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.1756815	1	.8140603

SLOPE = 6.06238

95 PERCENT CONFIDENCE LIMITS = 3.521371 AND 8.603389

LC50 = 4.951274

95 PERCENT CONFIDENCE LIMITS = 4.20845 AND 5.944308

LC10 = 3.056527

95 PERCENT CONFIDENCE LIMITS = 2.106609 AND 3.684414

****

(4)

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

1. **CHEMICAL:** Metolachlor
   Shaughnessey No.: 108801

2. **TEST MATERIAL:** Metolachlor technical
   Purity: 97.3%

3. **CITATION:**

   Authors: Hoberg, J.R.
   Title: Metolachlor technical - 5-day toxicity to the freshwater green alga, *Anabaena flos-aquae*
   Date: 1994
   Laboratory: Springborn Laboratories, Inc., Wareham, MA
   Sponsor: Ciba Crop Protection, Greensboro, NC
   Lab. Report No.: 94-7-5382
   MRID No.: 434871-04

4. **REVIEWED BY:**

   William Erickson
   Biologist
   EEB/EFED

   Signature: W. Erickson
   Date: 1/26/95

5. **APPROVED BY:**

   Harry Craven
   Section Head 4
   EEB/EFED

   Signature: Henry T. Craven
   Date: 2/15/95

6. **CONCLUSION:** The study is scientifically sound and fulfills the guideline requirement for a Tier 2 aquatic plant growth study with a blue-green algae. The 120-h EC_{50} value is 1.2 mg ai/l, which classifies technical metolachlor as moderately toxic to *Anabaena flos-aquae*.

7. **ADEQUACY OF THE STUDY:** Core.

8. **MAJOR GUIDELINE DEVIATIONS:** None.

9. **MATERIALS AND METHODS:**

(20)

A. Test Organisms:

Guideline Criteria	Reported Information
Species: *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Anabaena flos-aquae*
Number of Cells: 3,000 - 10,000	10,000
Nutrients:	AAP medium

B. Test System:

Guideline Criteria	Reported Information
Solvent used:	None
Site of test: Growth Chamber	Environmental chamber
Temperature: 20° C for Skeletonema sp.;others are 24-25°C	24 °C
Light Intensity: 2.0 Lux for Anabaena sp.;others are 4-5.0	1.1-3.2
Photoperiod:	continuous illumination
pH: 7.5-8.0	7.5

C. Test Design:

Guideline Criteria	Reported Information
Dose range: 2X or 3X	3X
Doses: at least 5	6
Controls: negative and/or solvent	Negative
Three or more replicates per dose:	3
Duration of test: 120 hours	120 h
Daily Observations?	Yes
Method of Observations:	Compound microscope
Maximum Labeled Rate:	Not reported

10. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Initial and 120 hours Cell Measurements?	Yes
Control cell count at 120 hr from initial increase ≥2X?	Yes
Measured Initial Chemical Concentrations? Optional	Yes
Raw data included? (Y/N)	Excerpted
Quality Assurance Measures?	Yes

Dose Response:

doses→ mg ai/l	control	0.019	0.063	0.19	0.72	2.1	6.8
inhibit %		1	5	16	44	56	82
120 hr pH	9.7	9.5	8.7	8.2	7.9	7.7	7.7

Observations: At test termination, cell fragments were observed in the 0.72, 2.1, and 6.8 mg ai/l concentrations. Bloated cells were observed in the 6.8 mg ai/l concentration. The cells exposed to the remaining concentrations and the control were normal.

Statistical Results:

Method: **Linear Regression (EC_{50}) and Williams' Test (NOEC)**

EC_{50}: **1.1 mg ai/l** 95% C.L.: **0.31-3.9 mg ai/l**

NOEC: **0.063 mg ai/l**

11. **REVIEWER'S DISCUSSION/INTERPRETATION:**

Test Procedures: Test procedures met guideline criteria.

Statistical Analysis:

Method: **EPA Toxanol program (EC_{50}); Williams' Test (NOEC)**

EC_{50}: **1.2 mg ai/l** 95% C.L.: **0.9-1.6 mg ai/l**

Slope: **1.2** NOEC: **0.063 mg ai/l**

Discussion: The study is scientifically sound and fulfills the guideline requirement for a Tier 2 aquatic plant growth study with a blue-green algae.

12. **COMPLETION OF STUDY ONE-LINER:** 1/26/95.

File: A:ANABAENA.DAT Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
0	87.333				
0.019	86.000	0.323		1.76	k= 1, v=14
0.063	82.333	1.210		1.85	k= 2, v=14
0.19	73.333	3.388	*	1.88	k= 3, v=14
0.72	49.000	9.276	*	1.89	k= 4, v=14
2.1	36.667	12.260	*	1.90	k= 5, v=14
6.8	16.000	17.261	*	1.91	k= 6, v=14

s = 5.062

Note: df used for table values are approximate when v > 20

W. ERICKSON METOLACHLOR - ANABAENA TOXICITY

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
6.8	100	82	82	0
2.1	100	56	56	0
.72	100	44	44	0
.19	100	16	16	0
.063	100	5	5	0
.019	100	1	1	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 1.229634

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	3.905641E-02	1.227328	.9407113	1.60024

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	2.118531E-02	1	.6560646

SLOPE = 1.219745

95 PERCENT CONFIDENCE LIMITS = 1.042209 AND 1.397281

LC50 = 1.251928

95 PERCENT CONFIDENCE LIMITS = .9875165 AND 1.619197

LC10 = .1138619

95 PERCENT CONFIDENCE LIMITS = 7.518421E-02 AND .1585785

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ANABAENA CELL DENSITIES
File: A:ANABAENA.DAT Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0	3	83.000	93.000	87.333
2	0.019	3	81.000	89.000	86.000
3	0.063	3	77.000	88.000	82.333
4	0.19	3	65.000	80.000	73.333
5	0.72	3	45.000	55.000	49.000
6	2.1	3	33.000	39.000	36.667
7	6.8	3	14.000	19.000	16.000

ANABAENA CELL DENSITIES
File: A:ANABAENA.DAT Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0	26.333	5.132	2.963
2	0.019	19.000	4.359	2.517
3	0.063	30.333	5.508	3.180
4	0.19	58.333	7.638	4.410
5	0.72	28.000	5.292	3.055
6	2.1	10.333	3.215	1.856
7	6.8	7.000	2.646	1.528

ANABAENA CELL DENSITIES
File: A:ANABAENA.DAT Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0	3	87.333	87.333	87.333
2	0.019	3	86.000	86.000	86.000
3	0.063	3	82.333	82.333	82.333
4	0.19	3	73.333	73.333	73.333
5	0.72	3	49.000	49.000	49.000
6	2.1	3	36.667	36.667	36.667
7	6.8	3	16.000	16.000	16.000

ANABAENA CELL DENSITIES

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

(4)

1. **CHEMICAL:** Metolachlor
   Shaughnessey #: 108801

2. **TEST MATERIAL:** Metolachlor technical
   Purity: 97.3%

3. **CITATION:**

   Authors: Hoberg, J.R.
   Title: Metolachlor technical - toxicity to duckweed (*Lemna gibba*)
   Date: 1994
   Laboratory: Springborn Laboratories, Inc., Wareham, MA
   Sponsor: Ciba Crop Protection, Greensboro, NC
   Lab. Report No.: 94-8-5404
   MRID No.: 434871-05

4. **REVIEWED BY:**

   William Erickson
   Biologist
   EEB/EFED

   Signature: W. Erickson
   Date: 1/26/95

5. **APPROVED BY:**

   Harry Craven
   Section Head 4
   EEB/EFED

   Signature: Harry Craven
   Date: 2/15/95

6. **CONCLUSION:** The study is scientifically sound and fulfills the guideline requirement for a Tier 2 aquatic plant growth study with duckweed. The EC_{50} value is 0.048 mg ai/l, which classifies technical metolachlor as very highly toxic to duckweed. The NOEC is 0.0084 mg ai/l.

7. **ADEQUACY OF THE STUDY:** Core.

8. **MAJOR GUIDELINE DEVIATIONS:** None.

9. **MATERIALS AND METHODS:**

A. Test Organism:

Guideline Criteria	Reported Information
Species: *Lemna gibba*	*Lemna gibba* G3
Number of Plants: 5 Number of fronds per plant: 3	5 3
Nutrients:	Hoagland's medium

B. Test System:

Guideline Criteria	Reported Information
Solvent used:	None
Site of test: Growth Chamber	Environmental chamber
Temperature: 20° C for Skeletonema sp.;others are 24-25°C	24-25 °C
Light Intensity: 2.0 Lux for Anabaena sp.;others are 4-5.0	3.8-4.8
Photoperiod:	Continuous illumination
pH: 5.0	5.0
Static or Renewal System	Static

C. Test Design:

Guideline Criteria	Reported Information
Dose range: 2X or 3X	2X
Doses: at least 5	7
Controls: negative and/or solvent	Negative
Three or more replicates per dose:	3
Duration of test: 14 days	14 days
Daily Observations?	No; 3-day intervals
Method of Observations:	Not specified
Maximum Labeled Rate:	Not reported

10. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Initial and 14 day frond count?	Yes
control frond count at 14 days increase ≥2X?	Yes
Measured Initial Chemical Concentrations? Optional	Yes
Raw data included? (Y/N)	Excerpted
Quality Assurance Measures?	Yes

Dose Response

doses→ mg ai/l	control	0.0011	0.0021	0.0042	0.0084	0.015	0.030	0.058
inhibit %	-	2	11	6	2	18	20	61
pH: 0 /14 day	5.0 6.4	5.0 6.4	5.0 6.4	4.9 6.4	4.9 6.4	4.9 6.2	4.9 6.2	4.9 6.0

Observations: Fronds at the 0.058 mg ai/l level had little root formation and were smaller and chlorotic in comparison to the controls. Fronds at 0.0084-0.030 mg ai/l were slightly chlorotic. Several fronds in control and at dosages of 0.0011-0.0042 mg ai/l were observed to be chlorotic.

Statistical Results:

Method: **Linear Regression (EC_{50}); Williams' Test (NOEC)**

Frond density:

EC_{50}: **0.051 mg ai/l** 95% C.L.: **0.037-0.066 mg ai/l**

NOEC: **0.0084 mg ai/l**

Frond biomass:

EC_{50}: **0.043 mg ai/l** 95% C.L.: **0.018-0.12 mg ai/l**

NOEC: **0.015 mg ai/l**

11. **REVIEWER'S DISCUSSION/INTERPRETATION:**

Test Procedures: Test procedures met guideline criteria.

Statistical Analysis: Frond density

Method: **EPA Toxanol program; Williams' Test**

EC_{50}: **0.048 mg ai/l** 95% C.L.: **0.043-0.056 mg ai/l**

NOEC: **0.0084 mg ai/l**

Discussion: The study is scientifically sound and fulfills the guideline requirement for a Tier 2 aquatic plant growth study with duckweed.

12. **COMPLETION OF STUDY ONE-LINER:** 1/26/95.

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 4.897321E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
1	.1043968	4.897321E-02	4.396628E-02	5.662954E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.7148472	9.371648	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 = 1.169028
95 PERCENT CONFIDENCE LIMITS = .1806308 AND 2.157426

LC50 = 7.721045E-02
95 PERCENT CONFIDENCE LIMITS = 2.679345E-02 AND 704.6784

LC10 = 6.328546E-03
95 PERCENT CONFIDENCE LIMITS = 2.764061E-05 AND 1.640182E-02

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LEMNA GIBBA FROND PRODUCTION
File: A:LEMNA.DAT Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0	3	417.000	481.000	442.667
2	0.0011	3	413.000	474.000	435.667
3	0.0021	3	376.000	404.000	393.333
4	0.0042	3	380.000	478.000	417.333
5	0.0084	3	415.000	444.000	434.000
6	0.015	2	357.000	371.000	364.000
7	0.030	3	323.000	375.000	353.333
8	0.058	3	169.000	180.000	173.667

LEMNA GIBBA FROND PRODUCTION
File: A:LEMNA.DAT Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0	1144.333	33.828	19.531
2	0.0011	1114.333	33.382	19.273
3	0.0021	229.333	15.144	8.743
4	0.0042	2809.333	53.003	30.601
5	0.0084	271.000	16.462	9.504
6	0.015	98.000	9.899	7.000
7	0.030	732.333	27.062	15.624
8	0.058	32.333	5.686	3.283

LEMNA GIBBA FROND PRODUCTION
File: A:LEMNA.DAT Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0	3	442.667	442.667	442.667
2	0.0011	3	435.667	435.667	435.667
3	0.0021	3	393.333	393.333	414.889
4	0.0042	3	417.333	417.333	414.889
5	0.0084	3	434.000	434.000	414.889
6	0.015	2	364.000	364.000	364.000
7	0.030	3	353.333	353.333	353.333
8	0.058	3	173.667	173.667	173.667

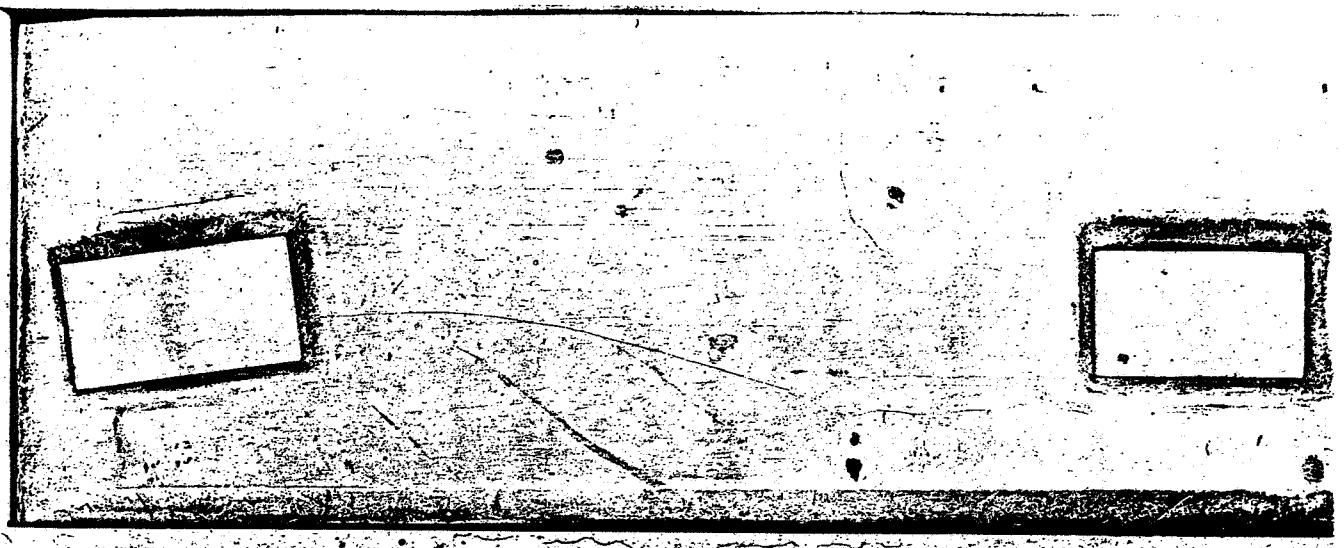
LEMNA GIBBA FROND PRODUCTION
File: A:LEMNA.DAT Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
0	442.667				
0.0011	435.667	0.294		1.75	k= 1, v=15
0.0021	414.889	1.166		1.84	k= 2, v=15
0.0042	414.889	1.166		1.87	k= 3, v=15
0.0084	414.889	1.166		1.88	k= 4, v=15
0.015	364.000	2.954	*	1.89	k= 5, v=15
0.030	353.333	3.751	*	1.90	k= 6, v=15
0.058	173.667	11.294	*	1.90	k= 7, v=15

s = 29.171

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



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

1. **CHEMICAL:** Metolachlor
Shaughnessey No.: 108801

2. **TEST MATERIAL:** Metolachlor technical
Purity: 97.3%

3. **CITATION:**

Authors: Hoberg, J.R.
Title: Metolachlor technical - toxicity to the marine diatom, *Skeletonema costatum*
Date: 1994
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Ciba Crop Protection, Greensboro, NC
Lab. Report No.: 94-7-5382
MRID No.: 434871-06

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED

Signature: W. Erickson
Date: 1/26/95

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED

Signature: Harry Craven
Date: 2/15/95

6. **CONCLUSION:** The study is scientifically sound and fulfills the guideline requirement for a Tier 2 aquatic plant growth study with a marine diatom. The 120-h EC_{50} value is 0.061 mg ai/l, which classifies technical metolachlor as very highly toxic to *Skeletonema costatum*.

7. **ADEQUACY OF THE STUDY:** Core.

8. **MAJOR GUIDELINE DEVIATIONS:** None.

9. **MATERIALS AND METHODS:**

A. Test Organisms:

Guideline Criteria	Reported Information
Species: *Skeletonema costatum* *Anabaena flos-aquae* *Selenastrum capricornutum* *Navicula pelliculosa*	*Skeletonema costatum*
Number of Cells: 3,000 - 10,000	10,000
Nutrients:	AES

B. Test System:

Guideline Criteria	Reported Information
Solvent used:	None
Site of test: Growth Chamber	Environmental chamber
Temperature: 20° C for Skeletonema sp.;others are 24-25°C	19-20 °C
Light Intensity: 2.0 Lux for Anabaena sp.;others are 4-5.0	3.2-4.8
Photoperiod:	continuous illumination
pH: 7.5-8.0	8.1

C. Test Design:

Guideline Criteria	Reported Information
Dose range: 2X or 3X	3X
Doses: at least 5	7
Controls: negative and/or solvent	Negative
Three or more replicates per dose:	3
Duration of test: 120 hours	120 h
Daily Observations?	Yes
Method of Observations:	Compound microscope
Maximum Labeled Rate:	Not reported

10. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
Initial and 120 hours Cell Measurements?	Yes
Control cell count at 120 hr from initial increase ≥2X?	Yes
Measured Initial Chemical Concentrations? Optional	Yes
Raw data included? (Y/N)	Excerpted
Quality Assurance Measures?	Yes

Dose Response:

doses→ mg ai/l	control	0.0017	0.0048	0.014	0.043	0.15	0.56	1.7
inhibit %		-2	20	26	55	56	83	92
120 hr pH	8.5	8.5	8.4	8.4	8.0	8.3	8.0	7.8

Observations: Cell fragments and bloated cells were observed at the three highest concentrations. Thin cell walls also were observed at the two highest test concentrations.

Statistical Results:

Method: **Linear Regression (EC_{50}) and Williams' Test (NOEC)**

EC_{50}: **0.0093 mg ai/l** 95% C.L.: **0.0028-0.030 mg ai/l**

NOEC: **0.0017 mg ai/l**

11. **REVIEWER'S DISCUSSION/INTERPRETATION:**

Test Procedures: Test procedures met guideline criteria.

Statistical Analysis:

Method: **EPA Toxanol program (EC_{50}); Williams' Test (NOEC)**

EC_{50}: **0.061 mg ai/l** 95% C.L.: **0.049-0.076 mg ai/l**

NOEC: **0.0017 mg ai/l**

Discussion: The study is scientifically sound and fulfills the guideline requirement for a Tier 2 aquatic plant growth study with a marine diatom.

12. **COMPLETION OF STUDY ONE-LINER:** 1/26/95.

W. ERICKSON METOLACHLOR SKELETONEMA TOXICITY

****

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1.7	100	92	92	0
.56	100	83	83	0
.15	100	56	56	0
.043	100	55	55	0
.014	100	26	26	0
.0048	100	20	20	0
.0017	100	0	0	0

THE BINOMIAL TEST SHOWS THAT .014 AND .043 CAN BE USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 3.565843E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
6	1.001462E-02	6.123163E-02	4.915391E-02	7.666621E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.1003167	3.651154	2.642334E-03

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

SLOPE = .9827709

95 PERCENT CONFIDENCE LIMITS = .6714999 AND 1.294042

LC50 = 6.097744E-02

95 PERCENT CONFIDENCE LIMITS = 3.142891E-02 AND .1222098

LC10 = 3.111032E-03

95 PERCENT CONFIDENCE LIMITS = 6.803341E-04 AND 7.568381E-03

****

SKELETONEMA CELL DENSITIES
File: A:SKELETON.DAT Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	0	3	91.000	104.000	99.000
2	0.0017	3	95.000	107.000	101.000
3	0.0048	3	76.000	81.000	79.000
4	0.014	3	65.000	78.000	73.333
5	0.043	3	41.000	48.000	44.333
6	0.15	3	37.000	49.000	43.667
7	0.56	3	9.000	21.000	16.667
8	1.7	3	7.000	9.000	8.000

SKELETONEMA CELL DENSITIES
File: A:SKELETON.DAT Transform: NO TRANSFORM

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	0	49.000	7.000	4.041
2	0.0017	36.000	6.000	3.464
3	0.0048	7.000	2.646	1.528
4	0.014	52.333	7.234	4.177
5	0.043	12.333	3.512	2.028
6	0.15	37.333	6.110	3.528
7	0.56	44.333	6.658	3.844
8	1.7	1.000	1.000	0.577

SKELETONEMA CELL DENSITIES
File: A:SKELETON.DAT Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

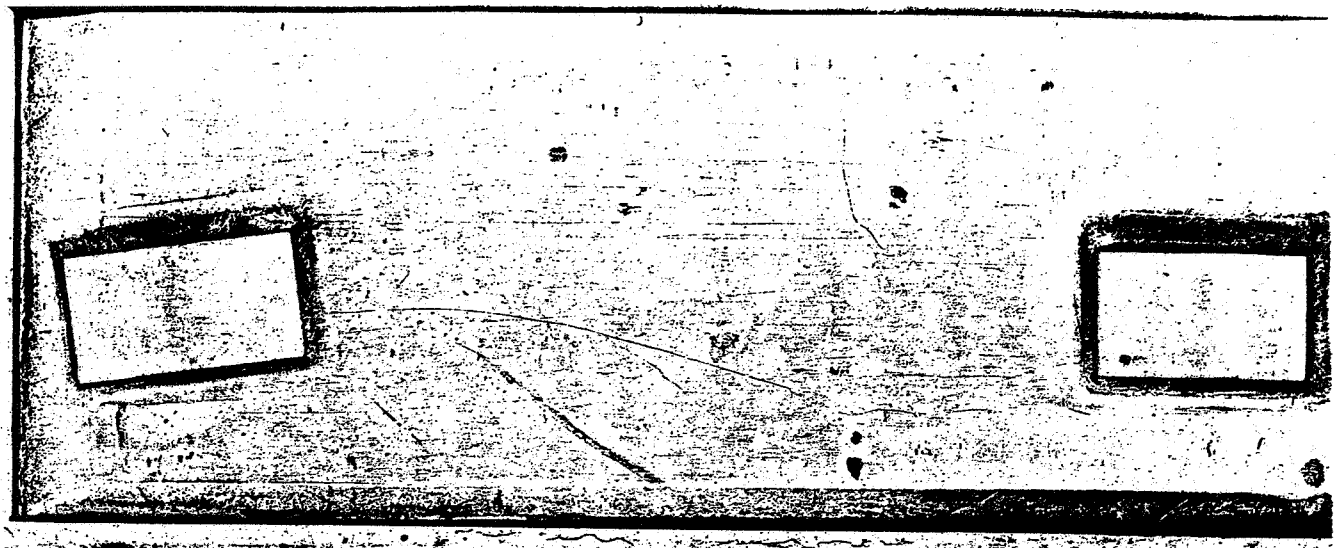
GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	0	3	99.000	99.000	100.000
2	0.0017	3	101.000	101.000	100.000
3	0.0048	3	79.000	79.000	79.000
4	0.014	3	73.333	73.333	73.333
5	0.043	3	44.333	44.333	44.333
6	0.15	3	43.667	43.667	43.667
7	0.56	3	16.667	16.667	16.667
8	1.7	3	8.000	8.000	8.000

SKELETONEMA CELL DENSITIES
File: A:SKELETON.DAT Transform: NO TRANSFORM

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
0	100.000				
0.0017	100.000	0.224		1.75	k= 1, v=16
0.0048	79.000	4.478	*	1.83	k= 2, v=16
0.014	73.333	5.747	*	1.86	k= 3, v=16
0.043	44.333	12.241	*	1.87	k= 4, v=16
0.15	43.667	12.390	*	1.88	k= 5, v=16
0.56	16.667	18.436	*	1.89	k= 6, v=16
1.7	8.000	20.377	*	1.89	k= 7, v=16

s = 5.470
Note: df used for table values are approximate when v > 20.



DATA EVALUATION RECORD
SEEDLING EMERGENCE EC_{25} TEST
GUIDELINE 123-1a (TIER II)

(4)

1. **CHEMICAL:** Metolachlor
   Shaughnessey No.: 108801

2. **TEST MATERIAL:** Metolachlor technical
   Purity: 97.3%

3. **CITATION:**

   Author: Chetram, R.S.
   Date: 1994
   Title: Tier 2 seedling emergence nontarget phytotoxicity study using metolachlor
   Sponsor: Ciba Crop Protection, Greensboro, NC
   Laboratory: Pan-Agricultural Labs, Inc., Madera, CA
   Lab. Report #: 94349
   MRID No.: 434871-07

4. **REVIEWED BY:**

   William Erickson
   Biologist
   EEB/EFED

   Signature: W. Erickson
   Date: 1/26/95

5. **APPROVED BY:**

   Harry Craven
   Section Head 4
   EEB/EFED

   Signature: H.T. Craven
   Date: 2/15/95

6. **CONCLUSION:** The study is scientifically sound and fulfills the guideline requirement for a Tier 2 plant emergence study. Ryegrass, with an EC_{25} of 0.02 lb ai/acre for plant dry weight and height, is the most sensitive of the four monocot species. Lettuce and cucumber, with EC_{25} values of 0.09 lb ai/acre for dry weight, were the most sensitive of the six dicot species.

7. **ADEQUACY OF THE STUDY:** Core.

8. **MAJOR GUIDELINE DEVIATIONS:** None.

9. **MATERIALS AND METHODS:**

A. Test Organisms:

Guideline Criteria	Reported Information
Species: 6 dicots in 4 families including soybean and rootcrop; 4 monocots in 2 families including corn	Monocots: corn, ryegrass, oat, onion Dicots: soybean, lettuce, radish, tomato, cucumber, cabbage
Number of seeds/rep: 10 Number of reps/dose: 3	10 seeds/pot 4 reps/dose
Source & Germination % of Seed:	Yes

B. Test System:

Guideline Criteria	Reported Information
Solvent used:	50% acetone
Site of test:	Greenhouse
Soil type:	Pasteurized sandy loam soil (1.1% organic matter) with perlite added to facilitate drainage
Fertilizer:	17:9:13
Pot size:	7.5 x 7.5 x 6 cm
No. species/pot	1
Method of pesticide application:	Overhead flat-fan nozzle (TeeJet 8001E)
Watering method:	Hand watered immediately after treatment; thereafter, a HydroRain controlled irrigation system was used
Test duration:	21 days
Growth stage at application: (seed)	Seed

C. **Test Design:**

Guideline Criteria	Reported Information
Dose range: 2X or 3X	2X
Doses: at least 5	5
Controls:negative and solvent	Yes
Parameters observed and measured	% emergence, % survival, phytotoxicity, plant height, plant dry weight
Maximum Labeled Rate:	Not stated; highest dose rate was 4.0 lb ai/acre

10. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
NOEC Observed?	Yes
Measured Initial Chemical Concentrations? (optional)	Yes
Raw data included? (Y/N)	Yes
Quality Assurance Measures?	Yes

Emergence, plant dry weight, plant height:

The parameter with the most sensitive EC_{25} value is tabulated below for each of the 10 species.

Species	Parameter	EC_{25}	NOEC
corn	dry weight, height	>0.025	0.025
ryegrass	**dry weight, height**	**0.017 0.018**	**0.012 0.0061**
oat	dry weight	0.11	0.049
onion	height	0.091	0.049
soybean	dry weight	>0.15	0.15
radish	dry weight	1.3	0.44
lettuce	**dry weight, height**	**>0.025**	**0.025**

50

cabbage	dry weight	0.50	0.049
cucumber	dry weight	0.14	0.049
tomato	dry weight	0.33	0.025

Phytotoxicity:

Phytotoxic symptoms included stunting, leaf chlorosis, leaf curling, necrosis leaf desiccation, and plant death. NOEC values (lb ai/acre) are tabulated below.

Species	NOEC (lb ai/acre)
corn	0.025
ryegrass	0.0061
oat	0.15
onion	0.049
soybean	4.0
radish	0.44
lettuce	0.025
cabbage	0.15
cucumber	0.049
tomato	0.44

Mortality:

No mortality occurred for soybean, radish, cucumber, or cabbage. The following mortality was reported for other species:

Lettuce: 100% mortality at 0.44, 1.3, and 4.0 lb ai/acre; 65% at 0.15 lb ai/acre; 8% at 0.049 lb ai/acre.

Tomato: 26% mortality at 4.0 lb ai/acre.

Oat: 14% and 6% mortality at 4.0 and 1.3 lb ai/acre, respectively.

Ryegrass: 47% mortality at 0.44 lb ai/acre.

Corn: 49% mortality at 4.0 lb ai/acre.

Onion: 63% mortality at 4.0 lb ai/acre; 16% and 5% at 1.3 and 0.44 lb ai/acre, respectively.

Statistical Results:

Method: **Regression Analysis**

Most sensitive dicot: **lettuce** Parameter: **dry wt, ht**

Most sensitive monocot: **ryegrass** Parameter: **dry wt, ht**

11. **REVIEWER'S DISCUSSION/INTERPRETATION OF STUDY RESULTS:**

Test Procedures: Test procedures met guideline criteria.

Statistical Analysis:

Methods: **EPA's Toxanol, EC_{25}, and Nuthatch programs**

Most sensitive monocot: **ryegrass** Parameter: **dry wt, ht**

EC_{25}: **0.02 lb ai/acre** 95% C.L.: **0.017-0.033**

Slope: **6.23** NOEC: **0.0031**

Most sensitive dicot: **lettuce, cucumber** Parameter: **dry wt**

EC_{25}: **0.09 lb ai/acre** 95% C.L.: **0.012-0.62 (lettuce); 0.043-0.19 (cucumber)**

Slope: **0.52 (lettuce) 1.01 (cucumber)** NOEC: **0.012 (lettuce) 0.049 (cucumber)**

Discussion: The study is scientifically sound and fulfills the guideline requirement for a Tier 2 plant emergence study.

12. **COMPLETION DATE OF ONE-LINER FOR STUDY:** 1/26/95.

52

Program: NUTHATCH
Path : C:\NUTHATCH
Date : 1/25/95

Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

EMWT-RYE.DAT : EMERGENCE - RYEGRASS WT

Raw data:

```
EMERGENCE - RYEGRASS WT
6
4
4
4
4
4
4
0
0.235
0.146
0.192
0.249
0.0031
0.156
0.161
0.189
0.186
0.0061
0.112
0.122
0.134
0.147
0.012
0.136
0.097
0.131
0.136
0.025
0.18
0.155
0.077
0.055
0.049
0.009
0.024
0.019
0.016
```

EMWT-RYE.DAT : EMERGENCE - RYEGRASS WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance

0	0.205			
0.0031	0.173	1.377	N.S.	
0.0061	0.129	3.251	<0.005	*
0.012	0.125	3.41	<0.005	*
0.025	0.117	3.759	<0.005	*
0.049	0.017	7.984	<0.005	*

"*"=Significant (p-L/E 0.05); "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.013	0.0071	0.024	0.13	0.54
EC5	0.017	0.010	0.028	0.10	0.61
EC25	0.024	0.018	0.033	0.067	0.73
EC50	0.031	0.025	0.039	0.046	0.80

Slope = 6.23 Std.Err. = 1.56

!!!Poor fit: p = 0.021 based on DF= 3.0 18.
EMWT-RYE.DAT : EMERGENCE - RYEGRASS WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.205	0.159	0.0468	100.	0.00
0.00310	4.00	0.173	0.159	0.0143	100.	2.30e-08
0.00610	4.00	0.129	0.159	-0.0300	100.	0.000536
0.0120	4.00	0.125	0.158	-0.0329	99.5	0.505
0.0250	4.00	0.117	0.115	0.00219	72.2	27.8
0.0490	4.00	0.0170	0.0173	-0.000311	10.9	89.1

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.26945	21.000	0.012831
Means Lack-of-Fit	0.11055	3.0000	0.036852
Within Groups	0.15890	18.000	0.0088275

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	0.15873	0.011457	1.0000	-0.44285	0.28427
log10 EC50	=	-1.5076	0.045897	-0.44285	1.0000	-0.63726
Bruce&V. SIGMA	=	0.16062	0.040280	0.28427	-0.63726	1.0000
Starting Values		0.20550	-1.8365	0.62015		

EMHT-ONI.DAT : EMERGENCE - ONION HT

Raw data:

```
EMERGENCE - ONION HT
6
38
37
37
37
24
6
```

0
122
111
172
87
118
130
109
75
124
120
109
115
112
141
87
57
112
38
118
131
146
40
77
123
141
168
110
109
51
166
153
153
147
167
118
153
34
145
0.049
102
76
105
71
57
123
55
64
127
123
139
88
150
99
125
110
88
105
58
155
145
173
133
77
103
34
143
123
117

127
126
154
140
131
153
143
164
0.15
24
138
78
23
67
61
110
51
38
84
127
50
57
12
24
40
67
30
35
25
32
133
55
27
67
112
137
72
50
58
39
64
82
30
70
41
116
0.44
26
42
50
20
36
42
29
6
36
5
20
20
10
35
20
62
31
45
125
64
17
80

EMHT-ONI.DAT : EMERGENCE - ONION HT

Williams Test

!!!Williams: not implemented for 173 degrees of freedom.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.0016	0.00035	0.0074	0.34	0.22
EC5	0.0069	0.0021	0.023	0.26	0.31
EC25	0.055	0.027	0.11	0.16	0.49
EC50	0.23	0.15	0.37	0.099	0.64

Slope = 1.07 Std.Err. = 0.134

!!!Poor fit: p < 0.001 based on DF= 3.00 173.
EMHT-ONI.DAT : EMERGENCE - ONION HT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	38.0	116.	122.	-6.91	100.	0.00
0.0490	37.0	114.	94.0	19.7	76.8	23.2
0.150	37.0	62.9	71.3	-8.45	58.3	41.7
0.440	37.0	41.6	47.1	-5.49	38.4	61.6
1.30	24.0	22.4	25.9	-3.57	21.2	78.8
4.00	6.00	33.7	11.3	22.3	9.27	90.7

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC5 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	3003.5	176.00	17.066
Means Lack-of-Fit	503.85	3.0000	167.95
Within Groups	2499.7	173.00	14.449

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	122.41	7.2771	1.0000	-0.79631	0.45805
log10 EC50	=	-0.62997	0.098948	-0.79631	1.0000	-0.57133
Bruce&V. SIGMA	=	0.93037	0.11632	0.45805	-0.57133	1.0000

Starting Values 115.50 -0.74602 1.2086

EMWT-ONI.DAT : EMERGENCE - ONION WT

Raw data:

EMERGENCE - ONION WT
6
4
4
4
4
4
4
0
0.061
0.048
0.068
0.085
0.049
0.048
0.062
0.082
0.073
0.15
0.042
0.061
0.051
0.055

0.44
0.018
0.017
0.039
0.039
1.3
0.008
0.009
0.004
0.010
4.0
0
0
0.002
0

EMWT-ONI.DAT : EMERGENCE - ONION WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.0659			
0.049	0.0659	-0.04988	N.S.	
0.15	0.0522	1.762	N.S.	
0.44	0.0283	4.954	<0.005	*
1.3	0.00775	7.681	<0.005	*
4	0.0005	8.645	<0.005	*

"**"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.033	0.012	0.087	0.20	0.38
EC5	0.066	0.030	0.15	0.17	0.45
EC25	0.18	0.11	0.31	0.11	0.59
EC50	0.36	0.25	0.53	0.078	0.69

Slope = 2.22 Std.Err. = 0.300

Goodness of fit: p = 0.97 based on DF= 3.0 18.

EMWT-ONI.DAT : EMERGENCE - ONION WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.0655	0.0663	-0.000822	100.	0.00
0.0490	4.00	0.0663	0.0646	0.00168	97.4	2.65
0.150	4.00	0.0522	0.0533	-0.00106	80.4	19.6
0.440	4.00	0.0283	0.0283	-9.35e-05	42.7	57.3
1.30	4.00	0.00775	0.00727	0.000481	11.0	89.0
4.00	4.00	0.000500	0.000686	-0.000186	1.03	99.0

!!!Warning: EC1 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS

Residual	0.048499	21.000	0.0023095
Means Lack-of-Fit	0.00063261	3.0000	0.00021087
Within Groups	0.047866	18.000	0.0026592

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	0.066322	0.0047078	1.0000	-0.69306	0.49121
log10 EC50	=	-0.43894	0.078336	-0.69306	1.0000	-0.71298
Bruce&V. SIGMA	=	0.44998	0.060774	0.49121	-0.71298	1.0000
Starting Values		0.065875	-0.43638	0.42507		

EMHT-RYE.DAT : EMERGENCE - RYEGRASS HT

Raw data:

EMERGENCE - RYEGRASS HT
6
39
39
33
37
40
33
0
192
191
175
129
161
166
170
169
158
142
155
65
115
160
144
132
147
139
163
140
135
165
137
215
164
175
131
197
159
99
136
134
173
132
173
99
153
154
136

0.0031
85
151
165
147
78
130
62
104
116
150
185
169
132
146
156
178
139
93
177
172
138
144
140
187
161
145
123
135
190
187
137
176
141
178
147
128
156
137
130
0.0061
82
147
163
172
95
153
193
136
145
53
145
151
154
111
143
192
162
91
136
138
92
109
168
127
128
65
158
161

185
108
147
138
52
0.012
88
67
139
98
160
167
154
131
173
44
69
173
96
174
143
56
62
104
172
93
125
147
97
146
93
109
139
87
86
145
126
123
75
155
125
140
134
0.025
175
166
175
112
69
171
147
72
139
62
176
157
66
155
162
50
140
115
67
168
45
93
66
79
55

145
116
72
142
76
35
92
73
66
57
58
54
43
100
120
0.049
89
55
22
33
45
36
35
15
44
41
70
38
49
55
52
56
86
78
49
50
27
62
50
37
42
22
65
9
65
36
43
28
22

EMHT-RYE.DAT : EMERGENCE - RYEGRASS HT

Williams Test

!!!Williams: not implemented for 215 degrees of freedom.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err. /Estimate	Lower Bound /Estimate
EC1	0.0058	0.0035	0.0097	0.11	0.60
EC5	0.0099	0.0068	0.014	0.083	0.68
EC25	0.021	0.017	0.026	0.044	0.82
EC50	0.035	0.032	0.040	0.025	0.89

Slope = 2.96 Std.Err. = 0.385

!!!Poor fit: p = 0.028 based on DF= 3.0 2.2e+02

EMHT-RYE.DAT : EMERGENCE - RYEGRASS HT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	39.0	151.	142.	8.71	100.	0.00
0.00310	39.0	144.	142.	2.04	99.9	0.0869
0.00610	33.0	133.	140.	-7.04	98.8	1.19
0.0120	37.0	119.	130.	-11.1	91.8	8.19
0.0250	40.0	103.	95.6	7.63	67.3	32.7
0.0490	33.0	45.6	48.1	-2.49	33.9	66.1

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	2357.8	218.00	10.815
Means Lack-of-Fit	97.151	3.0000	32.384
Within Groups	2260.6	215.00	10.514

Directly-fitted parameters and Asymptotic Covariances

Parameter	Estimate	Std.Err.	Correlations		
Control Mean =	142.05	3.8586	1.0000	-0.58905	0.46596
log10 EC50 =	-1.4503	0.024791	-0.58905	1.0000	-0.31239
Bruce&V. SIGMA =	0.33794	0.043938	0.46596	-0.31239	1.0000
Starting Values	150.77	-1.5026	0.45930		

EMWT-LET.DAT : EMERGENCE - LETTUCE DRY WT

Raw data:

EMERGENCE - LETTUCE DRY WT
6
4
4
4
4
4
4
0
0.419
0.386
0.453
0.361
0.0031
0.348
0.417
0.334
0.308
0.0061
0.391
0.348
0.379
0.34
0.012
0.365

58

0.354
0.417
0.368
0.025
0.314
0.3
0.397
0.294
0.049
0.371
0.301
0.319
0.264

EMWT-LET.DAT : EMERGENCE - LETTUCE DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.405			
0.0031	0.364	1.451	N.S.	
0.0061	0.364	1.451	N.S.	
0.012	0.364	1.451	N.S.	
0.025	0.326	2.802	0.007	*
0.049	0.314	3.248	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	6.2E-05	2.3E-08	0.16	1.6	0.00038
EC5	0.0012	1.3E-05	0.12	0.95	0.011
EC25	0.088	0.012	0.62	0.41	0.14
EC50	1.7	0.016	1.8E+02	0.97	0.0096

Slope = 0.524 Std.Err. = 0.296

Goodness of fit: p = 0.42 based on DF= 3.0 18.

EMWT-LET.DAT : EMERGENCE - LETTUCE DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.405	0.402	0.00280	100.	0.00
0.00310	4.00	0.352	0.372	-0.0198	92.4	7.55
0.00610	4.00	0.364	0.362	0.00274	90.0	10.0
0.0120	4.00	0.376	0.350	0.0262	87.0	13.0
0.0250	4.00	0.326	0.334	-0.00801	83.2	16.8
0.0490	4.00	0.314	0.318	-0.00389	79.0	21.0

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.093592	21.000	0.0044568
Means Lack-of-Fit	0.013206	3.0000	0.0044020
Within Groups	0.080387	18.000	0.0044659

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	0.40195	0.021047	1.0000	0.18364	0.50540
log10 EC50	=	0.23008	0.97128	0.18364	1.0000	0.92459
Bruce&V. SIGMA	=	1.9075	1.0758	0.50540	0.92459	1.0000
Starting Values		0.40475	-0.019712	1.7037		

EMWT-TOM.DAT : EMERGENCE - TOMATO DRY WT

Raw data:

EMERGENCE - TOMATO DRY WT
6
4
4
4
4
4
4
0
0.483
0.691
0.666
0.671
0.049
0.488
0.558
0.54
0.469
0.15
0.519
0.567
0.675
0.477
0.44
0.485
0.489
0.522
0.484
1.3
0.365
0.36
0.306
0.309
4.0
0.092
0.032
0.302
0.1

EMWT-TOM.DAT : EMERGENCE - TOMATO DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.628			
0.049	0.537	1.72	N.S.	
0.15	0.537	1.72	N.S.	
0.44	0.495	2.506	0.013	*
1.3	0.335	5.527	<0.005	*
4	0.132	9.369	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.10	0.022	0.48	0.32	0.21
EC5	0.23	0.071	0.76	0.25	0.31
EC25	0.74	0.37	1.5	0.14	0.50
EC50	1.7	1.1	2.5	0.086	0.66

Slope = 1.92 Std.Err. = 0.422

Goodness of fit: p = 0.60 based on DF= 3.0 18.

EMWT-TOM.DAT : EMERGENCE - TOMATO DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.628	0.572	0.0560	100.	0.00
0.0490	4.00	0.514	0.571	-0.0571	99.8	0.166
0.150	4.00	0.559	0.559	0.000584	97.8	2.25
0.440	4.00	0.495	0.495	-0.000230	86.6	13.4
1.30	4.00	0.335	0.332	0.00254	58.1	41.9
4.00	4.00	0.132	0.133	-0.00139	23.2	76.8

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.46634	21.000	0.022207
Means Lack-of-Fit	0.044888	3.0000	0.014963
Within Groups	0.42146	18.000	0.023414

Directly-fitted parameters and Asymptotic Covariances

Parameter	Estimate	Std.Err.	Correlations		
Control Mean =	0.57177	0.035196	1.0000	-0.65593	0.48439
log10 EC50 =	0.22111	0.086345	-0.65593	1.0000	-0.56317
Bruce&V. SIGMA =	0.52121	0.11477	0.48439	-0.56317	1.0000

Starting Values 0.62775 0.053147 0.81983

EMWT-CRN.DAT : EMERGENCE - CORN DRY WT

Raw data:

EMERGENCE - CORN DRY WT
6

4
4
4
4
4
4
0
1.736
1.407
0.922
1.293
0.049
1.135
0.902
0.205
1.051
0.15
0.957
1.436
1.074
0.801
0.44
1.038
1.396
0.68
0.888
1.3
0.501
0.963
0.471
0.345
4.0
0.097
0.059
0.055
0.188

EMWT-CRN.DAT : EMERGENCE - CORN DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	1.34			
0.049	0.964	1.784	0.046	*
0.15	0.964	1.784	N.S.	
0.44	0.964	1.784	N.S.	
1.3	0.57	3.651	<0.005	*
4	0.0998	5.882	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.21	0.051	0.89	0.30	0.24
EC5	0.37	0.12	1.1	0.24	0.32
EC25	0.80	0.39	1.7	0.15	0.48
EC50	1.4	0.85	2.2	0.10	0.61

Slope = 2.87 Std.Err. = 0.789

Goodness of fit: p = 0.21 based on DF= 3.0 - 18.

EMWT-CRN.DAT : EMERGENCE - CORN DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	1.34	1.08	0.261	100.	0.00
0.0490	4.00	0.823	1.08	-0.255	100.	0.00156
0.150	4.00	1.07	1.08	-0.00872	99.7	0.281
0.440	4.00	1.00	0.996	0.00470	92.3	7.69
1.30	4.00	0.570	0.572	-0.00154	53.0	47.0
4.00	4.00	0.0998	0.0995	0.000286	9.22	90.8

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	2.2838	21.000	0.10875
Means Lack-of-Fit	0.49453	3.0000	0.16484
Within Groups	1.7893	18.000	0.099406

Directly-fitted parameters and Asymptotic Covariances

Parameter	Estimate	Std.Err.	Correlations		
Control Mean =	1.0788	0.095368	1.0000	-0.53815	0.36235
log10 EC50 =	0.13998	0.10160	-0.53815	1.0000	-0.64332
Bruce&V. SIGMA =	0.34813	0.095615	0.36235	-0.64332	1.0000
Starting Values	1.3395	-0.30092	0.98516		

EMWT-CUC.DAT ; EMERGENCE - CUCUMBER DRY WT

Raw data:

```
EMERGENCE - CUCUMBER DRY WT
6
4
4
4
4
4
4
0
2.999
2.861
4.123
3.942
0.049
3.235
3.019
3.536
3.457
0.15
1.748
2.087
2.412
2.520
0.44
1.578
2.039
1.697
1.948
1.3
```



```
0.945
1.088
1.026
1.044
4.0
0.653
0.644
0.522
0.785
```

EMWT-CUC.DAT : EMERGENCE - CUCUMBER DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	3.48			
0.049	3.31	0.7279	N.S.	
0.15	2.19	5.538	<0.005	*
0.44	1.82	7.154	<0.005	*
1.3	1.03	10.55	<0.005	*
4	0.651	12.15	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.0021	0.00045	0.0094	0.32	0.22
EC5	0.0098	0.0030	0.032	0.25	0.30
EC25	0.090	0.043	0.19	0.16	0.47
EC50	0.42	0.26	0.68	0.10	0.62

Slope = 1.01 Std.Err. = 0.105

Goodness of fit: p = 0.070 based on DF= 3.0 - 18.

EMWT-CUC.DAT : EMERGENCE - CUCUMBER DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	3.48	3.60	-0.114	100.	0.00
0.0490	4.00	3.31	2.97	0.339	82.7	17.3
0.150	4.00	2.19	2.43	-0.234	67.5	32.5
0.440	4.00	1.82	1.77	0.0418	49.3	50.7
1.30	4.00	1.03	1.12	-0.0965	31.2	68.8
4.00	4.00	0.651	0.587	0.0635	16.3	83.7

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC5 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	1.0208	21.000	0.048611
Means Lack-of-Fit	0.32427	3.0000	0.10809
Within Groups	0.69655	18.000	0.038697

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	3.5951	0.20087	1.0000	-0.82943	0.55388
log10 EC50	=	-0.37312	0.099776	-0.82943	1.0000	-0.67401
Bruce&V. SIGMA	=	0.99451	0.10422	0.55388	-0.67401	1.0000
Starting Values		3.4813	-0.39239	1.0699		

Program: NUTHATCH
Path : C:\NUTHATCH
Date : 1/25/95

Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

EMHT-ONI.DAT : EMERGENCE - ONION HEIGHT

Raw data:

EMERGENCE - ONION HEIGHT

6				
4				
4				
4				
4				
4				
3				
0				
116				
101				
116				
129				
0.049				
90				
107				
120				
142				
0.15				
66				
53				
74				
62				
0.44				
32				
28				
54				
51				
1.3				
22				
29				
20				
19				
4.0				
19				
56				
27				

EMHT-ONI.DAT : EMERGENCE - ONION HEIGHT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	116			

0.049	115	0.07498	N.S.	
0.15	63.8	5.173	<0.005	*
0.44	41.3	7.423	<0.005	*
1.3	27.4	8.804	<0.005	*
4	27.4	8.151	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.00039	6.1E-06	0.025	0.87	0.016
EC5	0.0026	0.00010	0.067	0.68	0.039
EC25	0.038	0.0053	0.28	0.41	0.14
EC50	0.25	0.072	0.87	0.26	0.29

Slope = 0.829 Std.Err. = 0.200

!!!Poor fit: p = 0.0028 based on DF= 3.0 17.

EMHT-ONI.DAT : EMERGENCE - ONION HEIGHT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	116.	123.	-7.75	100.	0.00
0.0490	4.00	115.	88.9	25.8	72.1	27.9
0.150	4.00	63.8	70.6	-6.87	57.3	42.7
0.440	4.00	41.3	51.7	-10.4	41.9	58.1
1.30	4.00	22.5	34.1	-11.6	27.6	72.4
4.00	3.00	34.0	19.6	14.4	15.9	84.1

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	163.63	20.000	8.1813
Means Lack-of-Fit	90.499	3.0000	30.166
Within Groups	73.127	17.000	4.3016

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	123.25	15.686	1.0000	-0.81402	0.45469
log10 EC50	=	-0.60192	0.25942	-0.81402	1.0000	-0.64231
Bruce&V. SIGMA	=	1.2064	0.29119	0.45469	-0.64231	1.0000
Starting Values		115.50	-0.82082	1.6257		

EMHT-RYE.DAT : EMERGENCE - RYEGRASS HEIGHT

Raw data:

EMERGENCE - RYEGRASS HEIGHT

6				
4				

4
4
4
4
4
0
165
136
158
143
0.0031
119
153
154
152
0.0061
144
130
135
127
0.012
131
102
121
125
0.025
129
126
89
70
0.049
41
55
49
36

EMHT-RYE.DAT : EMERGENCE - RYEGRASS HEIGHT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	151			
0.0031	145	0.5239	N.S.	
0.0061	134	1.441	N.S.	
0.012	119	2.728	0.008	*
0.025	104	4.104	<0.005	*
0.049	45.3	9.189	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.0059	0.0026	0.013	0.17	0.45
EC5	0.010	0.0054	0.018	0.13	0.55
EC25	0.021	0.015	0.029	0.067	0.72
EC50	0.035	0.030	0.042	0.037	0.84

Slope = 2.99 Std.Err. = 0.581

Goodness of fit: p = 0.28 based on DF= 3.0 18.

EMHT-RYE.DAT : EMERGENCE - RYEGRASS HEIGHT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	151.	142.	8.67	100.	0.00
0.00310	4.00	145.	142.	2.78	99.9	0.0785
0.00610	4.00	134.	140.	-6.23	98.9	1.13
0.0120	4.00	119.	130.	-11.2	91.9	8.05
0.0250	4.00	104.	95.4	8.11	67.3	32.7
0.0490	4.00	45.3	47.4	-2.16	33.4	66.6

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	55.542	21.000	2.6449
Means Lack-of-Fit	10.417	3.0000	3.4724
Within Groups	45.125	18.000	2.5069

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	141.83	5.8205	1.0000	-0.60867	0.47579
log10 EC50	=	-1.4528	0.037047	-0.60867	1.0000	-0.39291
Bruce&V. SIGMA	=	0.33397	0.064787	0.47579	-0.39291	1.0000
Starting Values		150.50	-1.5074	0.44907		

EMHT-LET.DAT : EMERGENCE - LETTUCE HEIGHT

Raw data:

EMERGENCE - LETTUCE HEIGHT
6
4
4
4
4
4
4
0
102
94
99
86
0.0031
90
93
86
79
0.0061
88
88
91
92
0.012
92
90
101
89
0.025
90

64

89
97
82
0.049
89
78
87
82

EMHT-LET.DAT : EMERGENCE - LETTUCE HEIGHT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	95.3			
0.0031	89.9	1.369	N.S.	
0.0061	89.9	1.369	N.S.	
0.012	89.9	1.369	N.S.	
0.025	89.5	1.476	N.S.	
0.049	84	2.887	0.0058	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	6.5E-06	1.9E-14	2.2E+03	4.1	2.9E-09
EC5	0.0035	4.4E-06	2.8	1.4	0.0012
EC25	28.	3.8E-06	2.0E+08	3.3	1.4E-07
EC50	1.4E+04	2.1E-09	9.2E+16	6.2	1.5E-13

Slope = 0.249 Std.Err. = 0.269

Goodness of fit: p = 0.19 based on DF= 3.0 18.

EMHT-LET.DAT ; EMERGENCE - LETTUCE HEIGHT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	95.3	95.0	0.219	100.	0.00
0.00310	4.00	87.0	90.4	-3.41	95.1	-4.86
0.00610	4.00	89.8	89.7	0.0868	94.4	5.65
0.0120	4.00	93.0	88.8	4.17	93.5	6.53
0.0250	4.00	89.5	87.8	1.69	92.4	7.60
0.0490	4.00	84.0	86.8	-2.76	91.3	8.70

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	7.8402	21.000	0.37335
Means Lack-of-Fit	1.7841	3.0000	0.59470
Within Groups	6.0561	18.000	0.33645

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	95.031	2.9765	1.0000	0.21464	0.34516
log10 EC50	=	4.1466	6.1627	0.21464	1.0000	0.98856
Bruce&V. SIGMA	=	4.0135	4.3380	0.34516	0.98856	1.0000
Starting Values		95.250	1.4190	2.0964		

Dose	Isotone Means	T-bar	P-value	Significance
)	3.96			
).049	3.96	0.01294	N.S.	
).15	3.52	1.432	N.S.	
).44	3.52	1.432	N.S.	
.3	2.86	3.553	<0.005	*
	1.49	7.977	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

arameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
C1	0.19	0.047	0.75	0.29	0.25
C5	0.42	0.15	1.2	0.21	0.36
C25	1.3	0.79	2.2	0.11	0.60
C50	2.9	2.2	3.9	0.060	0.75

Slope = 1.95 Std.Err. = 0.443

oodness of fit: p = 0.36 based on DF= 3.0 18.

GWT-SOY.DAT : VEGETATIVE VIGOR - PLANT DRY WEIGHT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	3.96	3.79	0.167	100.	0.00
0.0490	4.00	3.96	3.79	0.164	100.	0.0262
0.150	4.00	3.35	3.77	-0.419	99.4	0.588
0.440	4.00	3.68	3.59	0.0948	94.6	5.41
1.30	4.00	2.86	2.86	0.000866	75.4	24.6
4.00	4.00	1.49	1.50	-0.00543	39.5	60.5

Analysis of Weighted Variance

ource	SS	DF	MS
esidual	1.5839	21.000	0.075425
Means Lack-of-Fit	0.25413	3.0000	0.084709
Within Groups	1.3298	18.000	0.073877

Directly-fitted parameters and Asymptotic Covariances

arameter	Estimate	Std.Err.	Correlations		
ontrol Mean =	3.7931	0.15558	1.0000	-0.54037	0.46536
og10 EC50 =	0.46603	0.059574	-0.54037	1.0000	-0.21642
ruce&V. SIGMA =	0.51200	0.11626	0.46536	-0.21642	1.0000
tarting Values	3.9598	0.41425	0.72442		

GWT-OAT.DAT : VEG VIG - OAT DRY WT

aw data:

EG VIG - OAT DRY WT

4
4
4
0
1.640
2.114
2.145
1.763
0.049
2.024
2.052
1.753
1.761
0.15
1.885
2.206
1.441
1.22
0.44
1.488
1.09
0.973
0.979
1.3
0.491
0.652
0.374
0.596
4.0
0.408
0.597
0.228
0.597

VGWT-OAT.DAT : VEG VIG - OAT DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	1.92			
0.049	1.9	0.09967	N.S.	
0.15	1.69	1.26	N.S.	
0.44	1.13	4.336	<0.005	*
1.3	0.528	7.682	<0.005	*
4	0.457	8.073	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.0059	0.00066	0.053	0.46	0.11
EC5	0.023	0.0042	0.13	0.36	0.18
EC25	0.16	0.056	0.47	0.22	0.35
EC50	0.63	0.32	1.2	0.14	0.51

Slope = 1.15 Std.Err. = 0.200

Goodness of fit: p = 0.093 based on DF= 3.0 18.

VGWT-OAT.DAT : VEG VIG - OAT DRY WT

Program: NUTHATCH
Path : C:\NUTHATCH
Date : 1/16/95

Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

VGWT-CAB.DAT : VEG VIG - CABBAGE DRY WT

Raw data:

VEG VIG - CABBAGE DRY WT

6				
4				
4				
4				
4				
4				
4				
0				
1.116				
1.654				
1.550				
2.579				
0.049				
1.506				
1.565				
2.502				
2.261				
0.15				
2.056				
2.79				
2.184				
2.134				
0.44				
2.031				
2.785				
1.776				
2.154				
1.3				
1.939				
2.007				
1.952				
2.836				
4.0				
0.358				
0.481				
0.282				
0.455				

VGWT-CAB.DAT : VEG VIG - CABBAGE DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	2.07			
0.049	2.07	-1.125		N.S.
0.15	2.07	-1.125		N.S.
0.44	2.07	-1.125		N.S.
1.3	2.07	-1.125		N.S.
4	0.394	4.349	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

!!!Failure #3: Data not suitable for probit model fit.

Criterion is 3 or more distinct isotone means.

VGWT-SOY.DAT : VEGETATIVE VIGOR - PLANT DRY WEIGHT

Raw data:

VEGETATIVE VIGOR - PLANT DRY WEIGHT

6				
4				
4				
4				
4				
4				
4				
0				
4.359				
4.017				
4.297				
3.166				
0.049				
3.865				
4.563				
3.616				
3.779				
0.15				
3.493				
3.781				
2.7				
3.432				
0.44				
3.615				
3.733				
3.783				
3.6				
1.3				
3.142				
2.857				
2.355				
3.092				
4.0				
2.226				
0.969				
1.136				
1.644				

VGWT-SOY.DAT : VEGETATIVE VIGOR - PLANT DRY WEIGHT

Williams Test

[One-Sided Test for Decrease]

Statistical Analysis:

Method: **Regression Analysis**

Most sensitive dicot: **cucumber** Parameter: **dry weight**

Most sensitive monocot: **ryegrass** Parameter: **dry weight**

Phytotoxicity:

Symptoms: stunting, leaf chlorosis, leaf necrosis, leaf deformity, leaf curling, restriction of leaf expansion, and leaf desiccation

NOECs (lb ai/acre): soybean, lettuce, cabbage = 1.3; radish, corn, onion = 0.44; tomato, oat = 0.15; cucumber = 0.049; ryegrass = 0.012

Mortality: Significant mortality (65%) occurred in ryegrass at a treatment rate of 1.3 lb ai/acre and in radish, tomato, and cabbage (20-85%) at 4.0 lb ai/acre.

11. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

Test Procedures: Test procedures met guideline criteria.

Statistical Analysis:

Method: **EPA Nuthatch program**

Most sensitive monocot: **ryegrass** Parameter: **dry weight**

EC_{25}: **0.016 lb ai/acre** 5% C.L.: **0.012-0.020 lb ai/acre**

NOEC: **0.0031 lb ai/acre**

Most sensitive dicot: **cucumber** Parameter: **dry weight**

EC_{25}: **0.030 lb ai/acre** 95% C.L.: **0.020-0.046 lb ai/acre**

NOEC: **0.025 lb ai/acre**

Discussion: The study is scientifically sound and fulfills the guideline requirement for a Tier 2 vegetative vigor phytotoxicity study.

13. **COMPLETION DATE OF STUDY ONE-LINER:** 1/26/95.

Guideline Criteria	Reported Information
Controls: negative and solvent (y/n)	Yes
Replicates per dose: (3)	4
Maximum Labeled Rate:	Not specified
Weekly Observations (y/n)	Yes

10. **REPORTED RESULTS:**

Guideline Criteria	Reported Information
NOEC Observed?	Yes
Measured Initial Chemical Concentrations? (optional)	Yes
Raw data included?	Yes
Quality Assurance Measures?	Yes

Plant dry weight and height EC_{25} values: The most sensitive EC_{25} value (lb ai/acre) is tabulated for each plant species. Values were recorded 21 days posttreatment.

species	Parameter	EC_{25}	NOEC
corn	dry weight	0.65	0.44
oat	dry weight	0.31	0.15
ryegrass	**dry weight**	**0.02**	**0.012**
onion	dry weight	0.58	0.44
soybean	dry weight	1.7	0.44
lettuce	dry weight	1.2	0.44
radish	dry weight, height	1.5	0.44
tomato	dry weight	1.2	0.44
cucumber	**dry weight**	**0.055**	**0.025**
cabbage	dry weight	2.1	1.3

69

A. Test Organisms:

Guideline Criteria	Reported Information
Species: 6 dicots in 4 families including soybean and rootcrop; 4 monocots in 2 families including corn	Monocots: corn, ryegrass, oat, onion Dicots: soybean, lettuce, radish, tomato, cucumber, cabbage
Number of plants/rep: (5)	5
Source of Seed: (y/n)	Yes

B. Test System:

Guideline Criteria	Reported Information
Solvent used:	50% acetone
Site of test:	Greenhouse
Soil type:	Sterilized sandy loam (1.1% organic matter), containing perlite (1:4) to facilitate drainage
Pot size:	7.5 x 7.5 x 6.0 cm
Fertilizer:	17:9:13
Planting Method: species/pot	1 seed/pot
Method of Application:	Spray booth equipped with an overhead nozzle (TeeJet 8001-E)
Watering Method:under foliage	By hand to prevent wetting of leaves during first 48 h; thereafter, by low volume mist nozzles
No. Days After Application: (14)	21
Growth Stage/Application: Past first true leaf stage (y/n)	Yes

C. Test Design:

Guideline Criteria	Reported Information
Dose range: (2X-3X)	3X
Doses: (≥5)	5

DATA EVALUATION RECORD
VEGETATIVE VIGOR EC_{25} TEST
GUIDELINE 123-1b (TIER II)

1. **CHEMICAL:** Metolachlor
Shaughnessey #: 108801

2. **TEST MATERIAL:** Metolachlor
Purity: 97.3%

3. **CITATION:**

Author: Chetram, R.S.
Date: 1994
Title: Tier 2 vegetative vigor nontarget phytotoxicity study using metolachlor
Sponsor: Ciba Crop Protection, Greensboro, NC
Laboratory: Pan-Agricultural Labs, Inc., Madera, CA
Lab. Report #: 94350
MRID No.: 434871-08

4. **REVIEWED BY:**

William Erickson
Biologist
EEB/EFED

Signature: W. Erickson
Date: 1/26/95

5. **APPROVED BY:**

Harry Craven
Section Head 4
EEB/EFED

Signature: Harry T. Craven
Date: 2/15/95

6. **CONCLUSION:** This study is scientifically sound and satisfies the guideline requirement for a Tier 2 vegetative vigor test. Ryegrass, with an EC_{25} value of 0.016 lb ai/acre for dry plant weight was the most sensitive monocot. Cucumber was the most sensitive dicot; the EC_{25} value was 0.030 lb ai/acre for dry plant weight. The NOEC values were 0.0031 and 0.025 lb ai/acre for ryegrass and cucumber, respectively.

7. **ADEQUACY OF THE STUDY:** Core.

8. **MAJOR GUIDELINE DEVIATIONS:** None.

9. **MATERIALS AND METHODS:**

Residual	1.3007	21.000	0.061940
Means Lack-of-Fit	0.41357	3.0000	0.13786
Within Groups	0.88718	18.000	0.049288

Directly-fitted parameters and Asymptotic Covariances

Parameter	Estimate	Std.Err.	Correlations		
Control Mean =	2.8639	0.16377	1.0000	-0.65315	0.62128
log10 EC50 =	0.39739	0.099409	-0.65315	1.0000	-0.24457
Bruce&V. SIGMA =	0.82466	0.19444	0.62128	-0.24457	1.0000
Starting Values	2.8443	0.39654	1.0751		

VGWT-CU2.DAT : VEG VIG - CUCUMBER DRY WT (2ND TEST)

Raw data:

VEG VIG - CUCUMBER DRY WT (2ND TEST)
6
4
4
4
4
4
4
)
2.891
3.060
3.850
3.781
).0031
3.338
2.958
3.541
3.818
).0061
3.121
.22
3.177
3.393
).012
2.591
2.814
3.254
2.908
).025
.22
2.793
3.173
2.484
).049
2.316
.985
.342
.114

GWT-CU2.DAT : VEG VIG - CUCUMBER DRY WT (2ND TEST)

Williams Test

One-Sided Test for Decrease]

ose	Isotone	T-bar	P-value	Significance

Means

0	3.43			
0.0031	3.43	-0.1158	N.S.	
0.0061	3.43	-0.1158	N.S.	
0.012	2.9	1.697	N.S.	
0.025	2.9	1.697	N.S.	
0.049	1.94	5.036	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.0053	0.0011	0.025	0.33	0.21
EC5	0.011	0.0038	0.032	0.22	0.34
EC25	0.030	0.020	0.046	0.086	0.66
EC50	0.062	0.043	0.089	0.076	0.69

Slope = 2.19 Std.Err. = 0.755

Goodness of fit: p = 0.37 based on DF= 3.0 18.
VGWT-CU2.DAT : VEG VIG - CUCUMBER DRY WT (2ND TEST)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	3.40	3.39	0.00134	100.	0.00
0.00310	4.00	3.41	3.39	0.0273	99.8	0.226
0.00610	4.00	3.48	3.35	0.131	98.6	1.40
0.0120	4.00	2.89	3.19	-0.298	94.0	6.01
0.0250	4.00	2.92	2.73	0.188	80.4	19.6
0.0490	4.00	1.94	1.99	-0.0500	58.6	41.4

!!!Warning: EC50 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	1.2211	21.000	0.058146
Means Lack-of-Fit	0.18988	3.0000	0.063294
Within Groups	1.0312	18.000	0.057288

Directly-fitted parameters and Asymptotic Covariances

Parameter	Estimate	Std.Err.	Correlations		
Control Mean =	3.3942	0.14404	1.0000	-0.055538	0.55972
log10 EC50 =	-1.2104	0.076100	-0.055538	1.0000	0.59987
Bruce&V. SIGMA =	0.45715	0.15774	0.55972	0.59987	1.0000
Starting Values	3.4290	-1.1926	0.59490		

VGWT-ONI.DAT : VEG VIG - ONION DRY WT

Raw data:

VEG VIG - ONION DRY WT
6
4
4

EC25	0.96	0.48	1.9	0.14	0.50
EC50	3.1	2.1	4.6	0.080	0.68

Slope = 1.31 Std.Err. = 0.291

Goodness of fit: p = 0.65 based on DF= 3.0 18.
VGWT-LET.DAT : VEG VIG - LETTUCE DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	2.82	2.76	0.0563	100.	0.00
0.0490	4.00	2.75	2.74	0.00959	99.1	0.904
0.150	4.00	2.60	2.65	-0.0432	95.8	4.20
0.440	4.00	2.28	2.40	-0.118	86.8	13.2
1.30	4.00	2.05	1.91	0.138	69.1	30.9
4.00	4.00	1.19	1.23	-0.0421	44.4	55.6

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.91062	21.000	0.043363
Means Lack-of-Fit	0.076779	3.0000	0.025593
Within Groups	0.83384	18.000	0.046324

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	2.7644	0.12450	1.0000	-0.53451	0.59632
log10 EC50	=	0.49537	0.079932	-0.53451	1.0000	-0.052308
Bruce&V. SIGMA	=	0.76360	0.16964	0.59632	-0.052308	1.0000
Starting Values		2.8207	0.46854	0.80996		

VGWT-CRN.DAT : VEG VIG - CORN DRY WT

Raw data:

VEG VIG - CORN DRY WT
6
4
4
4
4
4
4
4
0
2.636
2.865
2.947
2.266
0.049
2.751
3.115
3.041
2.209
0.15
2.63
3.078
3.405
3.188

0.44
2.537
2.133
1.689
2.646
1.3
2.133
1.56
1.358
1.492
4.0
1.344
1.154
1.072
1.368

VGWT-CRN.DAT : VEG VIG - CORN DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	2.84			
0.049	2.84	-0.6884	N.S.	
0.15	2.84	-0.6884	N.S.	
0.44	2.25	1.775	N.S.	
1.3	1.64	4.331	<0.005	*
4	1.23	5.998	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.030	0.0029	0.31	0.49	0.097
EC5	0.11	0.020	0.61	0.36	0.18
EC25	0.69	0.29	1.7	0.18	0.42
EC50	2.5	1.6	4.0	0.099	0.62

Slope = 1.21 Std.Err. = 0.286

Goodness of fit: p = 0.070 based on DF= 3.0 18.
VGWT-CRN.DAT : VEG VIG - CORN DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	2.68	2.86	-0.185	100.	0.00
0.0490	4.00	2.78	2.81	-0.0299	98.1	1.92
0.150	4.00	3.08	2.67	0.410	93.1	6.93
0.440	4.00	2.25	2.35	-0.0963	82.0	18.0
1.30	4.00	1.64	1.82	-0.181	63.4	36.6
4.00	4.00	1.23	1.15	0.0832	40.2	59.8

!!!Warning: EC1 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS

13

.69
.3
.538
.588
.331
.636
.0
.355
.473

GWT-TOM.DAT : VEG VIG - TOMATO DRY WT

Williams Test

One-Sided Test for Decrease]

ose	Isotone Means	T-bar	P-value	Significance
	4.44			
.049	4.04	0.7573	N.S.	
.15	4.04	0.7573	N.S.	
.44	3.48	1.83	N.S.	
.3	2.52	3.668	<0.005	*
	0.207	8.103	<0.005	*

'*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

arameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
:1	0.38	0.19	0.75	0.14	0.50
:5	0.57	0.33	0.98	0.11	0.58
:25	1.0	0.71	1.5	0.076	0.69
:50	1.5	1.2	2.0	0.054	0.77

Slope = 3.83 Std.Err. = 0.657

oodness of fit: p = 0.54 based on DF= 3.0 18.

GWT-TOM.DAT : VEG VIG - TOMATO DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	4.44	4.04	0.402	100.	0.00
0.0490	4.00	3.92	4.04	-0.115	100.	5.32e-07
0.150	4.00	4.17	4.04	0.128	100.	0.00568
0.440	4.00	3.48	3.96	-0.476	98.1	1.92
1.30	4.00	2.52	2.44	0.0786	60.6	39.4
4.00	4.00	0.207	0.221	-0.0135	5.46	94.5

Analysis of Weighted Variance

ource	SS	DF	MS
esidual	3.9294	21.000	0.18711
Means Lack-of-Fit	0.43168	3.0000	0.14389
Within Groups	3.4977	18.000	0.19432

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	4.0368	0.22541	1.0000	-0.44608	0.27289
log10 EC50	=	0.18388	0.054471	-0.44608	1.0000	-0.58871
Bruce&V. SIGMA	=	0.26112	0.044823	0.27289	-0.58871	1.0000
Starting Values		4.4390	0.018526	0.49675		

```
4
4
4
4
0
0.473
0.468
0.580
0.536
0.049
0.497
0.538
0.45
0.554
0.15
0.409
0.522
0.519
0.489
0.44
0.555
0.407
0.432
0.468
1.3
0.336
0.409
0.355
0.309
4.0
0.194
0.238
0.372
0.271
```

VGWT-ONI.DAT : VEG VIG - ONION DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.514	.		
0.049	0.51	0.1116	N.S.	
0.15	0.485	0.7314	N.S.	
0.44	0.465	1.209	N.S.	
1.3	0.352	4.017	<0.005	*
4	0.269	6.087	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.034	0.0028	0.41	0.52	0.083
EC5	0.14	0.024	0.81	0.37	0.17
EC25	1.0	0.47	2.3	0.17	0.45
EC50	4.2	2.6	6.9	0.10	0.61

Slope = 1.11 Std.Err. = 0.283

Goodness of fit: p = 0.83 based on DF= 3.0 18.

VGWT-ONI.DAT : VEG VIG - ONION DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.514	0.517	-0.00295	100.	0.00
0.0490	4.00	0.510	0.509	0.000706	98.4	1.58
0.150	4.00	0.485	0.489	-0.00468	94.6	5.37
0.440	4.00	0.465	0.446	0.0195	86.2	13.8
1.30	4.00	0.352	0.370	-0.0175	71.5	28.5
4.00	4.00	0.269	0.264	0.00492	51.0	49.0

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.16165	21.000	0.0076974
Means Lack-of-Fit	0.0073505	3.0000	0.0024502
Within Groups	0.15430	18.000	0.0085720

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	0.51720	0.024586	1.0000	-0.36731	0.63555
log10 EC50	=	0.62487	0.10281	-0.36731	1.0000	0.21710
Bruce&V. SIGMA	=	0.89982	0.22896	0.63555	0.21710	1.0000
Starting Values		0.51425	0.62791	0.90753		

VGWT-TOM.DAT : VEG VIG - TOMATO DRY WT

Raw data:

```
VEG VIG - TOMATO DRY WT
6
4
4
4
4
4
4
0
3.778
5.286
4.090
4.602
0.049
3.629
5.326
3.012
3.721
0.15
3.603
3.112
4.419
5.526
0.44
3.715
3.163
3.366
```

Program: NUTHATCH
Path : C:\NUTHATCH
Date : 1/16/95

Toxicity measurement for continuous endpoints, using weighted nonlinear regression, weighting proportional to predicted means.

Reference

R.D. Bruce and D.J. Versteeg. 1992. A statistical procedure for modeling continuous toxicity data. Env. Tox. and Chem. 11:1485-1494.

VGHT-SOY.DAT : VEG VIG - SOYBEAN HT

Raw data:

VEG VIG - SOYBEAN HT
5
20
20
20
20
20
20
0
400
434
476
474
493
451
458
401
410
422
445
420
485
464
471
405
496
431
404
490
0.049
461
490
489
420
475
465
401
421
460
479
425
460
489
495
406
452
444
476
411
426

.15
350
412
358
395
376
315
406
375
382
345
492
335
501
495
490
380
419
475
569
410
0.44
351
320
394
470
415
345
412
392
366
424
381
419
370
385
326
370
421
390
365
401
1.3
385
342
290
430
409
280
395
470
390
381
335
440
345
420
445
401
375
450
365
335
4.0
320
381
209
290
385

350
0
341
320
334
205
210
404
384
291
280
351
329
319
300

VGHT-SOY.DAT : VEG VIG - SOYBEAN HT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	407			
0.049	406	0.04694		N.S.
0.15	406	0.04694		N.S.
0.44	386	1.108		N.S.
1.3	384	1.199		N.S.
4	300	5.705	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC1	0.54	0.12	2.3	0.32	0.23
EC5	1.2	0.51	3.0	0.19	0.41
EC25	4.0	3.1	5.1	0.054	0.78
EC50	9.0	4.7	17.	0.14	0.52

Slope = 1.90 Std.Err. = 0.698

Goodness of fit: p = 0.58 based on DF= 3.0 1.1e+02

VGHT-SOY.DAT : VEG VIG - SOYBEAN HT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	20.0	407.	402.	4.06	100.	0.00
0.0490	20.0	397.	402.	-5.18	100.	0.000842
0.150	20.0	414.	402.	11.7	100.	0.0364
0.440	20.0	386.	400.	-14.0	99.4	0.639
1.30	20.0	384.	380.	4.01	94.5	5.54
4.00	20.0	300.	301.	-0.577	74.7	25.3

!!!Warning: EC50 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS

Residual	1144.5	117.00	9.7820
Means Lack-of-Fit	19.664	3.0000	6.5546
Within Groups	1124.8	114.00	9.8670

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	402.44	7.5795	1.0000	0.25759	0.45736
log10 EC50	=	0.95209	0.14212	0.25759	1.0000	0.92457
Bruce&V. SIGMA	=	0.52565	0.19285	0.45736	0.92457	1.0000
Starting Values		406.50	1.0501	0.69183		

VGHT-LET.DAT : VEG VIG - LETTUCE HT

Raw data:

VEG VIG - LETTUCE HT
6
20
20
20
20
20
20
0
156
165
203
166
202
125
130
130
129
120
125
133
144
165
151
138
152
130
134
133
0.049
130
135
130
134
158
184
165
198
149
148
120
149
145
146
150
135
134
140

58
42
.15
58
45
40
65
46
35
46
48
55
47
40
49
75
41
30
54
32
51
70
46
.44
50
50
47
51
55
30
60
70
35
15
50
50
80
27
52
39
24
35
28
10
.3
27
50
42
43
70
25
35
40
19
45
32
15
21
60
28
30
34
22
55
31
.0
02
08
17

97
121
149
130
142
135
136
132
145
143
99
0
100
78
121
122
101

VGHT-LET.DAT : VEG VIG - LETTUCE HT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	148			
0.049	148	-0.1517	N.S.	
0.15	148	-0.1517	N.S.	
0.44	143	0.5445	N.S.	
1.3	137	1.47	N.S.	
4	114	4.871	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.29	0.045	1.9	0.41	0.15
EC5	0.91	0.30	2.7	0.24	0.33
EC25	4.5	3.1	6.6	0.082	0.69
EC50	14.	5.2	37.	0.22	0.37

Slope = 1.39 Std.Err. = 0.506

Goodness of fit: p = 0.96 based on DF= 3.0 1.1e+02

VGHT-LET.DAT : VEG VIG - LETTUCE HT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	20.0	147.	147.	-0.821	100.	0.00
0.0490	20.0	148.	147.	0.177	100.	0.0325
0.150	20.0	149.	147.	1.74	99.7	0.313
0.440	20.0	143.	145.	-1.73	98.1	1.86
1.30	20.0	137.	136.	0.587	92.4	7.64
4.00	20.0	114.	114.	-0.115	77.4	22.6

!!!Warning: EC25 not bracketed by doses evaluated.

!!!Warning: EC50 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	393.21	117.00	3.3608
Means Lack-of-Fit	0.97628	3.0000	0.32543
Within Groups	392.24	114.00	3.4407

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	147.37	2.9787	1.0000	0.32903	0.53406
log10 EC50	=	1.1420	0.21682	0.32903	1.0000	0.93340
Bruce&V. SIGMA	=	0.71906	0.26144	0.53406	0.93340	1.0000
Starting Values		147.57	1.0890	0.64821		

VGHT-TOM.DAT : VEG VIG - TOMATO HT

Raw data:

VEG VIG - TOMATO HT
6
20
20
20
20
20
20
0
320
325
320
310
319
290
315
320
280
321
250
301
296
298
285
315
306
261
260
301
0.049
275
270
278
269
285
285
330
319
291
305
230
291
256
251

310
260
245
275
274
305
0.15
290
285
274
282
295
297
290
262
261
251
269
284
280
261
281
280
321
251
330
290
0.44
270
268
255
285
265
275
270
268
205
239
296
240
281
295
274
252
275
291
256
234
1.3
265
282
290
235
291
262
285
181
201
239
284
201
231
250
239
158
181
189
210
174

4.0
0
0
0
162
154
159
160
152
157
161
0
0
0
0
0
0
0
0
0
0
0

VGHT-TOM.DAT : VEG VIG - TOMATO HT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
)	300			
).049	281	1.454	N.S.	
).15	281	1.454	N.S.	
).44	265	2.718	<0.005	*
1.3	232	5.229	<0.005	*
4	55.3	19	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.53	0.31	0.89	0.12	0.59
EC5	0.81	0.54	1.2	0.090	0.66
EC25	1.5	1.2	2.0	0.056	0.78
EC50	2.3	2.0	2.7	0.035	0.85

Slope = 3.60 Std.Err. = 0.493

Goodness of fit: p = 0.57 based on DF= 3.0 1.1e+02

VGHT-TOM.DAT : VEG VIG - TOMATO HT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	20.0	300.	282.	18.0	100.	0.00
0.0490	20.0	280.	282.	-1.49	100.	7.80e-08
0.150	20.0	282.	282.	0.0121	100.	0.000897
0.440	20.0	265.	280.	-15.7	99.5	0.457
1.30	20.0	232.	231.	1.60	81.9	18.1
4.00	20.0	55.3	56.1	-0.877	19.9	80.1

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	2357.3	117.00	20.148
Means Lack-of-Fit	41.141	3.0000	13.714
Within Groups	2316.1	114.00	20.317

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	281.69	8.5878	1.0000	-0.48161	0.34440
log10 EC50	=	0.36752	0.034985	-0.48161	1.0000	-0.67542
Bruce&V. SIGMA	=	0.27779	0.038055	0.34440	-0.67542	1.0000
Starting Values		299.65	0.21373	0.49928		

VGHT-RAD.DAT : VEG VIG - RADISH HT

Raw data:

VEG VIG - RADISH HT
6
20
20
20
20
20
20
0
120
126
134
130
110
110
144
131
110
120
110
105
119
120
149
151
136
121
139
137
0.049
110
115
120
141
136
140
136
137
131
120
120
129
135
163

144
155
104
112
128
120
0.15
138
130
129
115
151
120
132
101
119
125
138
125
124
134
136
116
135
128
132
99
0.44
99
112
118
125
154
135
131
137
101
136
126
92
106
130
104
129
119
104
118
105
1.3
120
110
109
85
114
105
91
84
89
68
118
84
130
85
98
112
81
104
96
106

4.0
124
0
0
0
0
0
0
0
0
0
0
0
0
0
0
75
0
0
108
0

VGHT-RAD.DAT : VEG VIG - RADISH HT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	128			
0.049	128	-0.2838	N.S.	
0.15	126	-0.03835	N.S.	
0.44	119	1.081	N.S.	
1.3	99.5	4.088	<0.005	*
4	15.3	16.99	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.54	0.31	0.96	0.12	0.57
EC5	0.80	0.51	1.3	0.10	0.63
EC25	1.4	1.0	1.9	0.065	0.74
EC50	2.1	1.7	2.5	0.045	0.82

Slope = 4.02 Std.Err. = 0.626

Goodness of fit: p = 0.92 based on DF= 3.0 1.1e+02

VGHT-RAD.DAT : VEG VIG - RADISH HT

Observed vs. Predicted Treatment Group Means

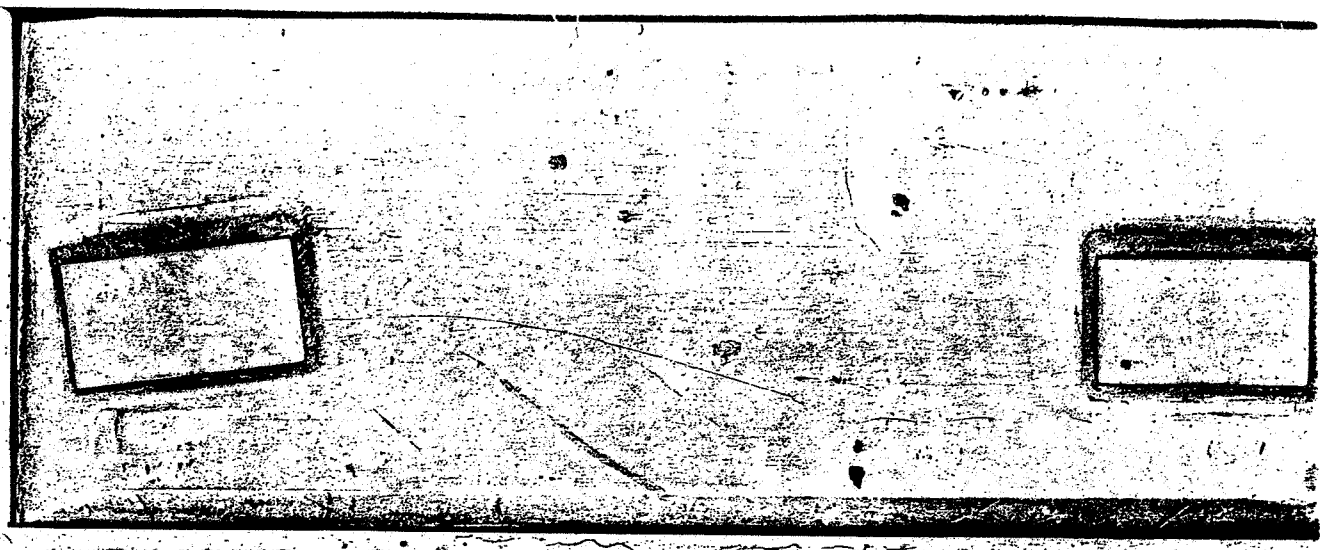
Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	20.0	126.	126.	0.587	100.	0.00
0.0490	20.0	130.	126.	4.29	100.	3.38e-09
0.150	20.0	126.	126.	0.838	100.	0.000241
0.440	20.0	119.	125.	-6.02	99.6	0.354
1.30	20.0	99.5	99.0	0.459	78.9	21.1
4.00	20.0	15.3	15.4	-0.0795	12.3	87.7

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	1994.2	117.00	17.045
Means Lack-of-Fit	8.9394	3.0000	2.9798
Within Groups	1985.3	114.00	17.415

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	125.51	5.2414	1.0000	-0.45442	0.31944
Log10 EC50	=	0.31341	0.044746	-0.45442	1.0000	-0.68103
Bruce&V. SIGMA	=	0.24874	0.038728	0.31944	-0.68103	1.0000
Starting Values		127.95	0.27598	0.27501		



Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	1.92	2.02	-0.107	100.	0.00
0.0490	4.00	1.90	1.82	0.0801	89.9	10.1
0.150	4.00	1.69	1.54	0.146	76.3	23.7
0.440	4.00	1.13	1.15	-0.0211	57.0	43.0
1.30	4.00	0.528	0.724	-0.196	35.8	64.2
4.00	4.00	0.457	0.359	0.0985	17.8	82.2

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC5 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	1.4101	21.000	0.067146
Means Lack-of-Fit	0.41340	3.0000	0.13780
Within Groups	0.99667	18.000	0.055370

Directly-fitted parameters and Asymptotic Covariances

Parameter	Estimate	Std.Err.	Correlations		
Control Mean =	2.0226	0.16857	1.0000	-0.82432	0.59862
log10 EC50 =	-0.20235	0.13934	-0.82432	1.0000	-0.67487
Bruce&V. SIGMA =	0.86975	0.15161	0.59862	-0.67487	1.0000
Starting Values	1.9155	-0.21160	0.94123		

VGWT-RAD.DAT : VEG VIG -RADISH DRY WT

Raw data:

VEG VIG -RADISH DRY WT
6
4
4
4
4
4
4
0
1.308
1.279
1.216
1.217
0.049
1.089
1.294
1.324
1.161
0.15
1.372
1.278
1.257
1.288
0.44
1.405
1.287
1.131
1.188

1.3
1.118
1.036
1.294
1.223
4.0
0.277
0
0
0.166

VGWT-RAD.DAT : VEG VIG -RADISH DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	1.26			
0.049	1.26	-0.0265	N.S.	
0.15	1.26	-0.0265	N.S.	
0.44	1.25	0.03111	N.S.	
1.3	1.17	1.206	N.S.	
4	0.111	15.82	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.93	0.48	1.8	0.14	0.52
EC5	1.2	0.71	2.1	0.11	0.59
EC25	1.8	1.2	2.6	0.076	0.69
EC50	2.3	1.8	3.0	0.053	0.78

Slope = 5.79 Std.Err. = 1.26

Goodness of fit: p = 0.96 based on DF= 3.0 18.

VGWT-RAD.DAT : VEG VIG -RADISH DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	1.25	1.26	-0.000880	100.	0.00
0.0490	4.00	1.22	1.26	-0.0389	100.	1.77e-14
0.150	4.00	1.30	1.26	0.0429	100.	2.51e-10
0.440	4.00	1.25	1.26	-0.00311	100.	0.00134
1.30	4.00	1.17	1.17	2.58e-06	93.0	7.02
4.00	4.00	0.111	0.111	-1.21e-07	8.82	91.2

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.61764	21.000	0.029411
Means Lack-of-Fit	0.010702	3.0000	0.0035672
Within Groups	0.60694	18.000	0.033719

Directly-fitted parameters and Asymptotic Covariances

Ryegrass

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	1.2559	0.048057	1.0000	-0.45066	0.39503
log10 EC50	=	0.36858	0.052737	-0.45066	1.0000	-0.88612
Bruce&V. SIGMA	=	0.17269	0.037680	0.39503	-0.88612	1.0000
Starting Values		1.2569	0.36936	0.16228		

VGWT-RYE.DAT : VEG VIG - RYEGRASS DRY WT

Raw data:

VEG VIG - RYEGRASS DRY WT
5
4
4
4
4
4
0
0.621
0.426
0.506
0.486
0.049
0.147
0.401
0.431
0.128
0.15
0.139
0.123
0.072
0.203
0.44
0.124
0.098
0.071
0.017
1.3
0
0.02
0
0.013

VGWT-RYE.DAT : VEG VIG - RYEGRASS DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.51			
0.049	0.277	3.788	<0.005	*
0.15	0.134	6.105	<0.005	*
0.44	0.0775	7.028	<0.005	*
1.3	0.00825	8.154	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound
		Lower	Upper		
EC1	0.0013	0.00014	0.012	0.46	0.11
EC5	0.0040	0.00066	0.024	0.37	0.17
EC25	0.020	0.0060	0.065	0.25	0.30
EC50	0.060	0.026	0.14	0.17	0.44

Slope = 1.39 Std.Err. = 0.265

Goodness of fit: p = 0.52 based on DF= 2.0 15.

VGWT-RYE.DAT : VEG VIG - RYEGRASS DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.510	0.508	0.00223	100.	0.00
0.0490	4.00	0.277	0.278	-0.00163	54.9	45.1
0.150	4.00	0.134	0.147	-0.0126	28.9	71.1
0.440	4.00	0.0775	0.0578	0.0197	11.4	88.6
1.30	4.00	0.00825	0.0159	-0.00765	3.13	96.9

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC25 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.55322	17.000	0.032542
Means Lack-of-Fit	0.046018	2.0000	0.023009
Within Groups	0.50720	15.000	0.033813

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	0.50752	0.063914	1.0000	-0.65290	0.32473
log10 EC50	=	-1.2223	0.16868	-0.65290	1.0000	-0.75260
Bruce&V. SIGMA	=	0.71781	0.13634	0.32473	-0.75260	1.0000
Starting Values		0.50975	-1.2305	0.71113		

VGWT-CUC.DAT : VEG VIG - CUCUMBER DRY WT

Raw data:

VEG VIG - CUCUMBER DRY WT
6
4
4
4
4
4
4
0
5.241
4.708
5.087
5.690
0.049
3.804

4.27
3.781
3.672
0.15
2.736
3.36
3.447
2.933
0.44
2.69
2.776
2.949
3.538
1.3
2.007
1.965
2.716
2.846
4.0
1.269
1.801
0.841
1.143

VGWT-CUC.DAT : VEG VIG - CUCUMBER DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	5.18			
0.049	3.88	4.823	<0.005	*
0.15	3.12	7.654	<0.005	*
0.44	2.99	8.139	<0.005	*
1.3	2.38	10.38	<0.005	*
4	1.26	14.54	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.00027	2.0E-05	0.0036	0.54	0.074
EC5	0.0026	0.00036	0.019	0.41	0.14
EC25	0.066	0.021	0.20	0.23	0.32
EC50	0.62	0.32	1.2	0.14	0.52

Slope = 0.693 Std.Err. = 0.0949

Goodness of fit: p = 0.090 based on DF= 3.0 18.
VGWT-CUC.DAT : VEG VIG - CUCUMBER DRY WT

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	5.18	5.11	0.0689	100.	0.00
0.0490	4.00	3.88	3.97	-0.0910	77.7	22.3
0.150	4.00	3.12	3.40	-0.280	66.5	33.5
0.440	4.00	2.99	2.76	0.224	54.1	45.9
1.30	4.00	2.38	2.10	0.280	41.1	58.9

4.00	4.00	1.26	1.47	-0.204	28.7	71.3

!!!Warning: EC1 not bracketed by doses evaluated.

!!!Warning: EC5 not bracketed by doses evaluated.

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	1.4838	21.000	0.070658
Means Lack-of-Fit	0.43995	3.0000	0.14665
Within Groups	1.0439	18.000	0.057993

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	5.1126	0.29672	1.0000	-0.84553	0.49420
log10 EC50	=	-0.20948	0.13770	-0.84553	1.0000	-0.50270
Bruce&V. SIGMA	=	1.4435	0.19774	0.49420	-0.50270	1.0000
Starting Values		5.1815	-0.24948	1.4942		

VGWT-RY2.DAT : VEG VIG - RYEGRASS DRY WT (TEST 2)

Raw data:

VEG VIG - RYEGRASS DRY WT (TEST 2)
6
4
4
4
4
4
4
0
0.582
0.506
0.526
0.593
0.0031
0.517
0.489
0.452
0.583
0.0061
0.409
0.534
0.436
0.533
0.012
0.453
0.525
0.404
0.474
0.025
0.227
0.206
0.37
0.256
0.049
0.115
0.086
0.1
0.125

VGWT-RY2.DAT : VEG VIG - RYEGRASS DRY WT (TEST 2)

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	0.552			
0.0031	0.51	1.095	N.S.	
0.0061	0.478	1.946	0.039	*
0.012	0.464	2.316	0.019	*
0.025	0.265	7.574	<0.005	*
0.049	0.107	11.75	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.0045	0.0025	0.0080	0.12	0.56
EC5	0.0075	0.0048	0.012	0.094	0.64
EC25	0.016	0.012	0.020	0.056	0.77
EC50	0.026	0.022	0.030	0.034	0.85

Slope = 3.04 Std.Err. = 0.388

Goodness of fit: p = 0.48 based on DF= 3.0 18.

VGWT-RY2.DAT : VEG VIG - RYEGRASS DRY WT (TEST 2)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	4.00	0.552	0.524	0.0281	100.	0.00
0.00310	4.00	0.510	0.522	-0.0121	99.7	0.252
0.00610	4.00	0.478	0.509	-0.0310	97.2	2.80
0.0120	4.00	0.464	0.443	0.0211	84.6	15.4
0.0250	4.00	0.265	0.272	-0.00740	52.0	48.0
0.0490	4.00	0.107	0.105	0.00135	20.1	79.9

Analysis of Weighted Variance

Source	SS	DF	MS
Residual	0.15665	21.000	0.0074593
Means Lack-of-Fit	0.019611	3.0000	0.0065369
Within Groups	0.13703	18.000	0.0076130

Directly-fitted parameters and Asymptotic Covariances

Parameter		Estimate	Std.Err.	Correlations		
Control Mean	=	0.52369	0.019858	1.0000	-0.66639	0.49115
log10 EC50	=	-1.5858	0.033646	-0.66639	1.0000	-0.61993
Bruce&V. SIGMA	=	0.32906	0.041976	0.49115	-0.61993	1.0000
Starting Values		0.55175	-1.6453	0.41290		

VGWT-LET.DAT : VEG VIG - LETTUCE DRY WT

Raw data:

VEG VIG - LETTUCE DRY WT

6
4
4
4
4
4
4
0
2.722
2.698
3.192
2.671
0.049
2.65
3.171
2.275
2.9
0.15
2.661
2.426
2.808
2.525
0.44
2.311
2.259
2.301
2.251
1.3
1.968
1.806
2.194
2.23
4.0
1.752
1.267
0.645
1.082

VGWT-LET.DAT : VEG VIG - LETTUCE DRY WT

Williams Test

[One-Sided Test for Decrease]

Dose	Isotone Means	T-bar	P-value	Significance
0	2.82			
0.049	2.75	0.3568	N.S.	
0.15	2.6	1.073	N.S.	
0.44	2.28	2.687	0.0088	*
1.3	2.05	3.835	<0.005	*
4	1.19	8.127	<0.005	*

"*"=Significant (p L/E 0.05) "N.S."=Not Significant.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC1	0.052	0.0075	0.37	0.41	0.14
EC5	0.17	0.042	0.71	0.29	0.24