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

1. **CHEMICAL:** Metolachlor PC Code No.: 108801

2. **TEST MATERIAL:** CGA-354743 (metolachlor metabolite)- 99% pure

3. **CITATION:** Authors: T.J. Ward, J.P. Magazu, and R.L. Boeri
   Title: Toxicity of CGA-354743 to the Duckweed, *Lemna gibba* G3
   Study Completion Date: February 1, 1999
   Laboratory: T.R. Wilbury Laboratories, Inc., Marblehead, MA
   Sponsor: Novartis Crop Protection, Inc., Greensboro, NC
   Laboratory Report ID: 1688-NO
   DP Barcode: D260420
   MRID No.: 449317-20

4. **REVIEWED BY:** Mark Mossler, M.S., Environmental Toxicologist,
   Golder Associates Inc.
   Signature: **Date:**4/13/2000

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

5. **APPROVED BY:** Brian Montague, Fisheries Biologist
   ERB I, Environmental Fate and Effects Division
   Signature: Brian Montague **Date: 5/15/2000**

6. **STUDY PARAMETERS:**
   Definitive Test Duration:14 days
   Type of Concentrations: Initial measured

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

   Results Synopsis

7-Day EC_{50}: 43 mg ai/L	95% C.I.: 30-61 mg ai/L
NOEC(EC_5): 4.0 mg ai/L	Probit Slope: 1.6

8. **ADEQUACY OF THE STUDY:**

A. **Classification:** Core
B. **Rationale:** N/A
C. **Repairability:** N/A

9. **GUIDELINE DEVIATIONS:** The initial frond number (11, 12, or 13) was not identical among each test replicate.

10. **SUBMISSION PURPOSE:** Submitted to support reregistration of Metolachlor products.

11. **MATERIALS AND METHODS:**

A. Test Organisms

Guideline Criteria	Reported Information
Species *Lemna gibba*	*Lemna gibba*
Number of Plants/Fronds 5 plants, 3 fronds per plant.	3 to 5 plants/3 or 4 fronds per plant
Nutrients Standard formula, e.g. 20X-AAP	M-Hoagland's medium without sucrose or EDTA

B. Test System

Guideline Criteria	Reported Information
Solvent	None
Temperature 25°C	23.4 - 25.4°C
Light Intensity 5.0 Klux (±15%)	5.5 Klux
Photoperiod Continuous	Continuous
pH Approximately 5.0	5.0 - 5.2
Test System Static or static renewal	Static

C. Test Design

Guideline Criteria	Reported Information
Dose range 2X or 3X progression	2X
Doses at least 5	6.5, 13, 25, 50, and 100 mg active ingredient (ai)/L
Controls negative and/or solvent	Negative control
Replicates per dose 3 or more	Three replicates
Duration of test 14 days	14 days
Daily observations were made?	Observations made on Days 2, 4, 7, 9, 11, and 14.
Method of Observations	Frond counts
Maximum Labeled Rate	Test material is a metabolite

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 14 day frond count?	Yes
Control frond count at 14 day ≥2X initial count?	Yes
Initial chemical concentrations measured? (Optional)	Samples of each treatment solution were taken at 0 and 14 days. Initial values ranged from 94 to 100% of nominal. Recovery of spiked samples averaged 102%.
Raw data included?	Yes

Measured Concentrations

Nominal Conc. (mg ai/L)	Measured Concentration (mg ai/L)	
	Day 0	Day 14
Control	<2.0	<2.0
6.5	6.1	<2.0
13	13	5.6
25	24	15
50	49	40
100	98	90

Dose Response - Frond Count

Initial Concentration (mg ai/L)	Day 7 Mean Frond Number	% Inhibition	Final pH values
Control	199	--	5.2
6.1	160	20	5.2
13	170	15	5.3
24	120	40	5.2
49	87	56	5.1
98	67	67	5.0

Other Significant Results: Small and shriveled fronds were noted at the three highest-concentration treatment levels and approximately half of the fronds at the highest treatment concentration were chlorotic.

Statistical Results

Statistical Method: Non-linear regression was used to estimate the EC value and Dunnett's test was used for NOEC determination. Results are based on Day 14 non-chlorotic frond counts and initial measured concentrations.

EC_{50}: 32 mg ai/L 95% C.I.: 15-65 mg ai/L

Probit Slope: N/A NOEC: <6.1 mg ai/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The EC value was estimated using non-linear regression. The NOEC was determined using Williams' test/probit analysis. Results are based on total Day 7 frond counts and initial measured concentrations.

EC_{50}: 43 mg ai/L	95% C.I.: 30-61 mg ai/L
Probit Slope: 1.6	NOEC(EC_5): 4.0 mg ai/L

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity study. Based on initial measured concentrations, the 7-day EC_{50} for *Lemna gibba* exposed to CGA-354743 was 43 mg ai/L. The NOEC was estimated to be 4.0 mg ai/L. This study is classified as **Core**.

Lemna total frond number (Day 7)
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WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	3	199.000	199.000	199.000
2	6.1 ppm ai	3	160.000	160.000	165.000
3	13 ppm ai	3	170.000	170.000	165.000
4	24 ppm ai	3	120.333	120.333	120.333
5	49 ppm ai	3	87.333	87.333	87.333
6	98 ppm ai	3	66.667	66.667	66.667

Lemna Day 7 total frond counts
File: lem Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG	TABLE P=.05 WILLIAMS	DEGREES OF FREEDOM
Control	199.000				
6.1 ppm ai	165.000	3.033	*	1.78	k= 1, v=12
13 ppm ai	165.000	3.033	*	1.87	k= 2, v=12
24 ppm ai	120.333	7.017	*	1.90	k= 3, v=12
49 ppm ai	87.333	9.961	*	1.92	k= 4, v=12
98 ppm ai	66.667	11.805	*	1.93	k= 5, v=12

s = 13.730
Note: df used for table values are approximate when v > 20.

Metolachlor CGA 354743 Acute-Lemna gibba 14 Day frond counts
File: a:\metollem.gib Transform: NO TRANSFORM

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	316976.667	63395.333	9.858
Within (Error)	12	77173.333	6431.111	
Total	17	394150.000		

Critical F value = 3.11 (0.05,5,12)
Since F > Critical F REJECT Ho:All groups equal

Metolachlor CGA 354743 Acute-Lemna gibba
File: a:\metollem.gib Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	Control	600.667	600.667		
2	6.1	393.333	393.333	3.166	*
3	13	437.000	437.000	2.500	
4	24	308.000	308.000	4.470	*
5	49	262.000	262.000	5.172	*
6	98	191.000	191.000	6.257	*

Dunnett table value = 2.50 (1 Tailed Value, P=0.05, df=12,5)

Metolachlor CGA 354743 Acute-Lemna gibba
File: a:\metollem.gib Transform: NO TRANSFORM

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	Control	3			
2	6.1	3	163.696	27.3	207.333
3	13	3	163.696	27.3	163.667
4	24	3	163.696	27.3	292.667
5	49	3	163.696	27.3	338.667
6	98	3	163.696	27.3	409.667

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Estimated EC Values and Confidence Limits

Point	Conc.	Lower 95% Confidence Limits	Upper 95% Confidence Limits
EC 1.00	1.4978	0.4888	2.9327
EC 5.00	4.0347	1.8349	6.5149
EC10.00	6.8434	3.6995	10.0114
EC15.00	9.7755	5.9180	13.4259
EC50.00	44.1288	36.5569	54.7282
EC85.00	199.2075	135.4670	371.8864
EC90.00	284.5592	181.0884	596.7994
EC95.00	482.6480	277.5564	1206.4149
EC99.00	1300.1655	615.2667	4538.7842

$y = 2.4 + 1.58(x)$

Day 7 Lemna Counts 1
10:53 Tuesday, April 4, 2000

OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0	.	206	176	215	.	.	.
2	13	1.11394	189	163	158	.	.	.
3	24	1.38021	115	127	119	.	.	.
4	49	1.69020	82	100	80	.	.	.
5	98	1.99123	80	56	64	.	.	.

Day 7 Lemna Counts 2
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION 10:53 Tuesday, April 4, 2000

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

Iter	LOG_EC50	SIGMA	C0	Weighted SS
0	1.645000	0.633000	199.000000	26.295555
1	1.628105	0.711631	203.078391	24.564623
2	1.632625	0.704693	202.186618	24.575232
3	1.632114	0.705885	202.266237	24.574346
4	1.632194	0.705695	202.253333	24.574495
5	1.632181	0.705725	202.255386	24.574471
6	1.632183	0.705721	202.255057	24.574475
7	1.632183	0.705721	202.255110	24.574474
8	1.632183	0.705721	202.255101	24.574475

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	1930.0000000	643.3333333
Residual	12	24.5744745	2.0478729
Uncorrected Total	15	1954.5744745	
(Corrected Total)	14	312.5158902	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC50	1.6321831	0.069221150	1.48136294	1.78300325
SIGMA	0.7057212	0.104612175	0.47779050	0.93365187
C0	202.2551013	11.644321377	176.88426712	227.62593549

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	C0
LOG_EC50	1	-0.450500075	-0.81773526
SIGMA	-0.450500075	1	0.4628153262
C0	-0.81773526	0.4628153262	1

Day 7 Lemna Counts 3
MODEL: COUNT = C0 * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
10:53 Tuesday, April 4, 2000

OBS	CONC	LOG_EC50	SIGMA	C0	RESID_SS	EC50
1	0	1.63218	0.70572	202.255	24.5745	42.8729

Day 7 Lemna Counts 4
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
WEIGHTED REGRESSION 10:53 Tuesday, April 4, 2000

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

Iter	LOG_EC25	SIGMA	C0	Weighted SS
0	1.224000	0.633000	199.000000	26.069889
1	1.148030	0.712243	203.098922	24.556190
2	1.157396	0.704620	202.182172	24.575295
3	1.155989	0.705897	202.267026	24.574336
4	1.156212	0.705693	202.253208	24.574496
5	1.156176	0.705726	202.255406	24.574471
6	1.156182	0.705720	202.255054	24.574475
7	1.156181	0.705721	202.255110	24.574474
8	1.156181	0.705721	202.255101	24.574475

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	1930.0000000	643.3333333
Residual	12	24.5744745	2.0478729
Uncorrected Total	15	1954.5744745	
(Corrected Total)	14	312.5158903	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval Lower	Upper
LOG_EC25	1.1561812	0.119041837	0.89681095	1.41555148
SIGMA	0.7057212	0.104612175	0.47779050	0.93365187
C0	202.2551012	11.644321374	176.88426705	227.62593540

Asymptotic Correlation Matrix

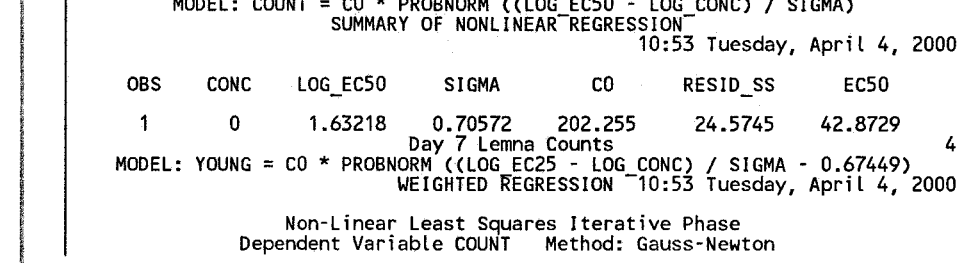
Corr	LOG_EC25	SIGMA	C0
LOG_EC25	1	-0.854691109	-0.749826828
SIGMA	-0.854691109	1	0.462815327
C0	-0.749826828	0.462815327	1

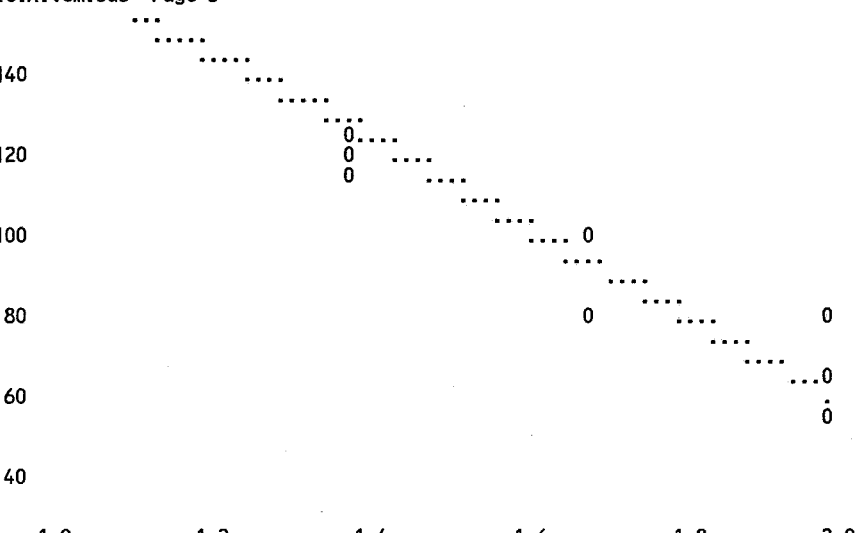
Day 7 Lemna Counts 5
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
SUMMARY OF NONLINEAR REGRESSION
10:53 Tuesday, April 4, 2000

OBS	CONC	LOG_EC25	SIGMA	C0	RESID_SS	EC25
1	0	1.15618	0.70572	202.255	24.5745	14.3279

Day 7 Lemna Counts 6
MODEL: YOUNG = C0 * PROBNORM ((LOG_EC25 - LOG_CONC) / SIGMA - 0.67449)
10:53 Tuesday, April 4, 2000

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





NOTE: 902 obs had missing values. 825 obs hidden.

Day 7 Lemna Counts 7
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
10:53 Tuesday, April 4, 2000

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	5	0 13 24 49 98

Number of observations in data set = 30

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

Day 7 Lemna Counts 8
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
10:53 Tuesday, April 4, 2000

General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	36831.33333	9207.83333	45.95	0.0001
Error	10	2004.00000	200.40000		
Corrected Total	14	38835.33333			

R-Square	C.V.	Root MSE	RESPONSE Mean

R-Square	C.V.	Root MSE	RESPONSE Mean
0.948398	11.00228	14.15627	128.6667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	4	36831.33333	9207.83333	45.95	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	4	36831.33333	9207.83333	45.95	0.0001

Day 7 Lemna Counts 9
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
10:53 Tuesday, April 4, 2000

General Linear Models Procedure

Level of DOSE	N	RESPONSE Mean	RESPONSE SD
0	3	199.000000	20.4205779
13	3	170.000000	16.6433170
24	3	120.333333	6.1101009
49	3	87.333333	11.0151411
98	3	66.666667	12.2202019

Day 7 Lemna Counts 10
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
10:53 Tuesday, April 4, 2000

General Linear Models Procedure

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

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

Alpha= 0.05 Confidence= 0.95 df= 10 MSE= 200.4
Critical Value of Dunnett's T= 2.466
Minimum Significant Difference= 28.498

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit	
13 - 0	-57.50	-29.00	-0.50	***
24 - 0	-107.17	-78.67	-50.17	***
49 - 0	-140.17	-111.67	-83.17	***
98 - 0	-160.83	-132.33	-103.83	***