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by Bill Evans
2001

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MRID No. 442951-01

DATA EVALUATION RECORD
EC₅₀ TEST WITH *LEMNA GIBBA*
GUIDELINE 123-2 (TIER II)1. CHEMICAL: 2,4-D PC Code No.: 0300012. TEST MATERIAL: 2,4-D Purity: 96.2%3. CITATION:Authors: J.S. Hughes, T.L. Williams, and L.A. ConderTitle: Effect of 2,4-Dichlorophenoxyacetic Acid on the Growth and Reproduction of *Lemna gibba* G3Study Completion Date: June 5, 1997Laboratory: Carolina Ecotox, Inc., Durham, NCSponsor: Industry Task Force II on 2,4-D Research
Data c/o DowElanco, Indianapolis, INLaboratory Report ID: 10-05-1DP Barcode: Not availableMRID No.: 442951-014. REVIEWED BY: Max Feken, M.S., Environmental Toxicologist,
Golder Associates Inc.Signature:Date:APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist,
Golder Associates Inc.Signature:Date:5. APPROVED BY:Signature:

Santhini Ramsamy

Date: Jan 04, 01

SANTHINI RAMSAMY, ERB I

6. STUDY PARAMETERS:Definitive Test Duration: 14 daysType of Concentrations: Mean measured

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**DATA EVALUATION RECORD
EC₅₀ TEST WITH LEMNA GIBBA
GUIDELINE 123-2 (TIER II)**

1. **CHEMICAL:** 2,4-D PC Code No.: 030001

2. **TEST MATERIAL:** 2,4-D Purity: 96.2%

3. **CITATION:**

Authors: J.S. Hughes, T.L. Williams, and L.A. Conder

Title: Effect of 2,4-Dichlorophenoxyacetic Acid on the Growth and Reproduction of *Lemna gibba* G3

Study Completion Date: June 5, 1997

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Sponsor: Industry Task Force II on 2,4-D Research
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Laboratory Report ID: 10-05-1

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4. **REVIEWED BY:** Max Feken, M.S., Environmental Toxicologist,
Golder Associates Inc.

Signature: 

Date: 6/26/98

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

Signature: P. Kosalwat

Date: 6/26/98

5. **APPROVED BY:**

Signature:

Date:

6. **STUDY PARAMETERS:**

Definitive Test Duration: 14 days

Type of Concentrations: Mean measured

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

Results Synopsis

EC₅₀: 695 ppb ai

95% C.I.: 603 - 802 ppb ai

NOEC: 58.1 ppb ai

Probit Slope: N/A

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Results Synopsis

EC₅₀: 695 ppb ai

95% C.I.: 603 - 802 ppb ai

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Probit Slope: N/A

8. ADEQUACY OF THE STUDY:

A. Classification: Core

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. Three plants with 4 fronds each were used in this study; however, 5 plants with 3 fronds each are recommended.

2. The pH of the test solutions at initiation (7.77 - 7.85) was higher than recommended (5.0).

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
<u>Species</u> <i>Lemna gibba</i>	<i>Lemna gibba</i>
<u>Number of Plants/Fronds</u> 5 plants, 3 fronds per plant.	3 plants; 4 fronds per plant.
<u>Nutrients</u> Standard formula, e.g. 20X-AAP	20X-AAP

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	DMF (0.5 mL/L) (N,N-dimethyl formamide)
<u>Temperature</u> 25°C	24.15 - 24.81°C
<u>Light Intensity</u> 5.0 Klux ($\pm 15\%$)	4.42 - 5.80 Klux
<u>Photoperiod</u> Continuous	Continuous
<u>pH</u> Approximately 5.0	Initial: 7.77 - 7.85 Final: 8.77 - 9.46
<u>Test System</u> Static or renewal	Static

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	62.5, 125, 249, 500, 1000, and 2000 $\mu\text{g ai/L}$
<u>Controls</u> negative and/or solvent	Negative and solvent control
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 14 days	14 days
Daily observations were made?	Observations made on Days 3, 5, 7, 10, 12 and 14.

Guideline Criteria	Reported Information
<u>Method of Observations</u>	Frond counts and dry weights. Frond counts were more sensitive than frond dry weight
<u>Maximum Labeled Rate</u>	38 lb acid equivalent/A

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 14 day frond count?	Yes
Control frond count at 14 day $\geq 2X$ initial count?	Yes
Initial chemical concentrations measured? (Optional)	Yes
Raw data included?	Yes

Dose Response - Frond Count

Mean Measured Concentration ($\mu\text{g ai/L}$)	Mean Frond Number	% Inhibition*	14-Day pH
Control	658	--	9.46
Solvent Control	628	--	9.42
58.1	578	8	9.42
122	553	12	9.42
250	520	17	9.40

Mean Measured Concentration ($\mu\text{g ai/L}$)	Mean Frond Number	% Inhibition*	14-Day pH
494	392	38	9.37
983	233	63	9.08
2029	112	82	8.77

*Versus the solvent control

Other Significant Results: Fronds from the 983 and 2029 $\mu\text{g ai/L}$ test concentrations were curled. Roots on fronds from the highest test concentration (2029 $\mu\text{g ai/L}$) were much shorter than those in the controls.

Statistical Results

Statistical Method: Weighted least squares nonlinear regression and Dunnett's test for means comparisons versus the pooled control. Results based on mean measured concentrations.

EC₅₀: 695 $\mu\text{g ai/L}$ 95% C.I.: 603 - 802 $\mu\text{g ai/L}$

Probit Slope: N/A NOEC: 58.1 $\mu\text{g ai/L}$

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Weighted nonlinear model (PROC NLIN) and Dunnett's (one tail) test for comparing means versus the solvent control. Results based on mean measured concentrations.

EC₅₀: 739 ppb ai 95% C.I.: 639 - 854 ppb ai

Probit Slope: N/A NOEC: 58.1 ppb ai

14. REVIEWER'S COMMENTS: This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity study. Based on mean measured concentrations, the 14-day EC₅₀ and NOEC for *Lemna gibba* exposed to 2,4-D were 695 and 58.1 ppb ai, respectively. This study is classified as Core.

2,4-D - LEMNA
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Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	SOLVENT	3	627.667	627.667	627.667
2	58.1	3	578.333	578.333	578.333
3	122	3	553.000	553.000	553.000
4	250	3	520.333	520.333	520.333
5	494	3	392.000	392.000	392.000
6	983	3	233.000	233.000	233.000
7	2029	3	111.667	111.667	111.667

2,4-D - LEMNA
File: 44295101

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
SOLVENT	627.667				
58.1	578.333	1.524		1.76	k= 1, v=14
122	553.000	2.306	*	1.85	k= 2, v=14
250	520.333	3.315	*	1.88	k= 3, v=14
494	392.000	7.278	*	1.89	k= 4, v=14
983	233.000	12.189	*	1.90	k= 5, v=14
2029	111.667	15.936	*	1.91	k= 6, v=14

s = 39.656

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

NOEC = 58.1 ppb ai

MAX FEKEN LEMNA 06-15-98

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
2029	100	82	82	0
983	100	63	63	0
494	100	38	38	0
250	100	17	17	0
122	100	12	12	0
58.1	100	8	8	0

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

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 687.1251

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	.0390846	699.3131	597.3889 818.3768

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	2.406772E-02	1	9.142119E-02

SLOPE = 1.630966
95 PERCENT CONFIDENCE LIMITS = 1.377941 AND 1.88399

LC50 = 663.9358
95 PERCENT CONFIDENCE LIMITS = 562.1798 AND 797.9798

LC10 = 110.5214
95 PERCENT CONFIDENCE LIMITS = 80.43564 AND 141.466

2,4-D - LEMNA

14:15 Monday, June 15, 1998

1

OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.0		572	642	669	.	.	.
2	58.1	1.76418	591	508	636	.	.	.
3	122.0	2.08636	519	591	549	.	.	.
4	250.0	2.39794	573	514	474	.	.	.
5	494.0	2.69373	400	399	377	.	.	.
6	983.0	2.99255	251	218	230	.	.	.
7	2029.0	3.30728	119	111	105	.	.	.

2,4-D - LEMNA

2

MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
WEIGHTED REGRESSION 14:15 Monday, June 15, 1998

Non-Linear Least Squares Iterative Phase

Iter	LOG_EC50	SIGMA	CO	Weighted SS
0	2.822000	0.613000	628.000000	76.168538
1	2.870239	0.480826	603.126487	49.992575
2	2.868671	0.482277	603.938134	49.958497
3	2.868594	0.482397	603.974100	49.957915
4	2.868589	0.482406	603.976740	49.957879
5	2.868588	0.482407	603.976941	49.957876
6	2.868588	0.482407	603.976956	49.957876

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Dependent Variable COUNT

Source	DF	Weighted SS	Weighted MS
Regression	3	9048.0000000	3016.0000000
Residual	18	49.9578759	2.7754375
Uncorrected Total	21	9097.9578759	
(Corrected Total)	20	2677.0708347	

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval	
			Lower	Upper
LOG_EC50	2.8685883	0.029947238	2.80567190	2.93150470
SIGMA	0.4824066	0.035950461	0.40687800	0.55793524
CO	603.9769558	16.231978134	569.87506051	638.07885108

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.633929431	-0.749012391
SIGMA	-0.633929431	1	0.5672593927
CO	-0.749012391	0.5672593927	1

2,4-D - LEMNA

3

MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
SUMMARY OF NONLINEAR REGRESSION
14:15 Monday, June 15, 1998

OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	2.86859	0.48241	603.977	49.9579	738.904

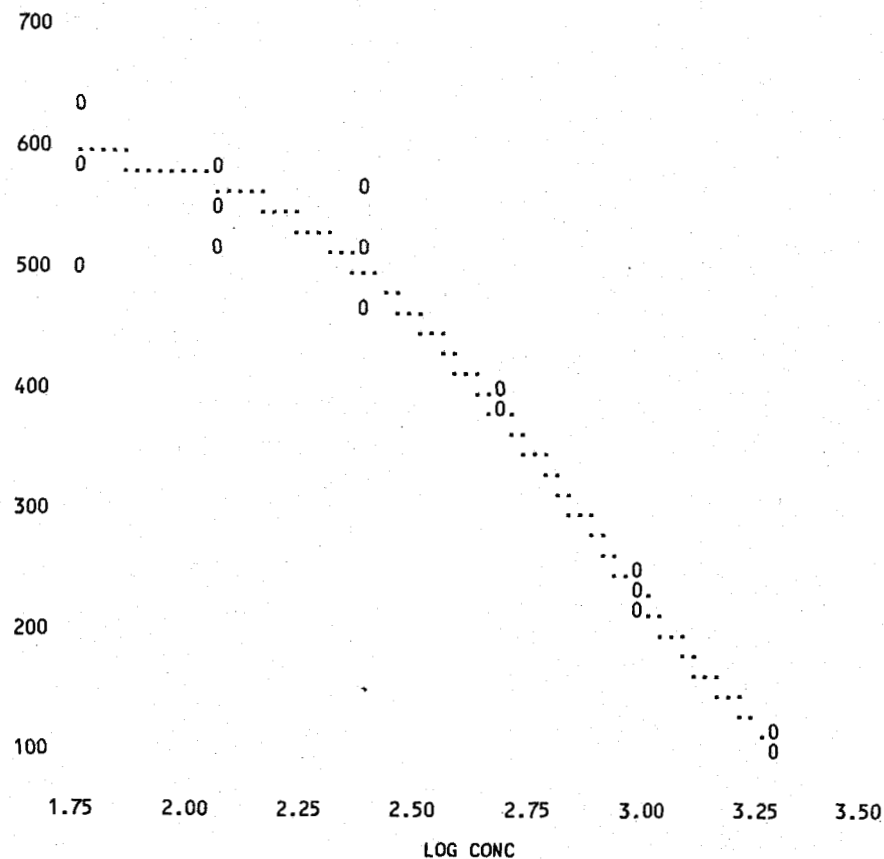
2,4-D - LEMNA

4

MODEL: COUNT = CO * PROBNORM ((LOG_EC50 - LOG_CONC) / SIGMA)
14:15 Monday, June 15, 1998

Plot of COUNT*LOG_CONC. Symbol used is 'O'.
Plot of PRED*LOG_CONC. Symbol used is 'I'.

COUNT



NOTE: 1574 obs had missing values. 1498 obs hidden.

2,4-D - LEMNA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL
14:15 Monday, June 15, 1998

5

General Linear Models Procedure
Class Level Information

Class	Levels	Values
DOSE	7	0 122 250 494 983 2029 58.1

Number of observations in data set = 42

NOTE: Due to missing values, only 21 observations can be used in this analysis.

2,4-D - LEMNA
COMPARISON OF MEANS FOR NOEL DETERMINATION
TEST IF TREATMENT IS LESS THAN CONTROL

6

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General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	677843.9048	112973.9841	71.84	0.0001
Error	14	22016.6667	1572.6190		
Corrected Total	20	699860.5714			

R-Square	C.V.	Root MSE	RESPONSE Mean
0.968541	9.204039	39.65626	430.8571

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	6	677843.9048	112973.9841	71.84	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	6	677843.9048	112973.9841	71.84	0.0001

2,4-D - LEMNA 7
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 14:15 Monday, June 15, 1998

General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	3	627.666667	50.0632933
122	3	553.000000	36.1662826
250	3	520.333333	49.8029450
494	3	392.000000	13.0000000
983	3	233.000000	16.7032931
2029	3	111.666667	7.0237692
58.1	3	578.333333	64.9332991

2,4-D - LEMNA 8
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
 14:15 Monday, June 15, 1998

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= 14 MSE= 1572.619
 Critical Value of Dunnett's T= 2.532
 Minimum Significant Difference= 81.991

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
-----------------	-------------------------------------	--------------------------	-------------------------------------

58.1 - 0	-131.32	-49.33	32.66	
122 - 0	-156.66	-74.67	7.32	
250 - 0	-189.32	-107.33	-25.34	***
494 - 0	-317.66	-235.67	-153.68	***
983 - 0	-476.66	-394.67	-312.68	***
2029 - 0	-597.99	-516.00	-434.01	***