

DATA EVALUATION RECORD
AQUATIC PLANT EC₅₀ TEST
GUIDELINE 123-2 (TIER II)

1. **CHEMICAL:** Mesotrione **PC Code No.:** 122990

2. **TEST MATERIAL:** ZA1296 **Purity:** 97.6%

3. **CITATION:**

Authors: D.V. Smyth, S.J. Kent, and N. Shillabeer

Title: ZA1296: Toxicity to Duckweed (*Lemna gibba*)

Study Completion Date: October 23, 1996

Laboratory: Brixham Environmental Laboratory, Brixham
Devon, UK

Sponsor: ZENECA Ag Products, Wilmington, DE

Laboratory Report ID: BL5849/B

DP Barcode: D245475

MRID No.: 445051-23

4. **REVIEWED BY:** Karl Bullock, M.S., Environmental Scientist,
Golder Associates Inc.

Signature: *Karl Bullock* **Date:** 7/30/98

APPROVED BY: Max Feken, M.S., Environmental Toxicologist,
Golder Associates Inc.

Signature: *Max Feken* **Date:** 7/30/98

5. **APPROVED BY:**

Signature: *Goodyear* **Date:** 6/13/00

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 a Tier II aquatic plant toxicity study.

Results Synopsis

EC₅₀: 17.7 ppb

95% C.I.: 11.9 - 26.4 ppb

Probit Slope: N/A

NOEC: 7.5 ppb

8. ADEQUACY OF THE STUDY:**A. Classification:** Core.**B. Rationale:** N/A.**C. Repairability:** N/A.**9. GUIDELINE DEVIATIONS:** Three plants were used for each replicate; five are recommended.**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 each	3 plants, 4 fronds per plant, total of 12 fronds per replicate
<u>Nutrients</u> Standard formula, e.g. 20XAAP	M-Hoagland's medium

B. Test System

Guideline Criteria	Reported Information
<u>Solvent</u>	None
<u>Temperature</u> 25°C	24.4 - 25.2°C
<u>Light Intensity</u> 5.0 KLux ($\pm 15\%$)	Approximately 4.87 - 4.98 Klux
<u>Photoperiod</u> Continuous	Continuous
<u>Test System</u> Static or Renewal	Renewal (on Days 5 and 9)
<u>pH</u> Approx. 5.0	New: 4.5 - 5.0 Old: 5.1 - 5.9

C. Test Design

Guideline Criteria	Reported Information
<u>Dose range</u> 2X or 3X progression	2X
<u>Doses</u> at least 5	0.50, 1.0, 2.0, 4.0, 8.0, 16, 32 and 64 µg/L, not corrected for percent active ingredient
<u>Controls</u> negative and/or solvent	Negative control
<u>Replicates per dose</u> 3 or more	3
<u>Duration of test</u> 14 days	14 days
Daily observations were made?	Counts and observations made every two or three days
<u>Method of Observations</u>	Number of fronds
<u>Maximum Labeled Rate</u>	Not reported

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 14 day frond numbers were measured?	Yes
Control frond at 14 days $\geq 2X$ initial count?	Yes
Initial chemical concentrations measured? (Optional)	Chemical concentrations were determined on days 0, 5, 9, and 14 from "new" solutions. Mean recoveries ranged from 93 - 103% of nominal.
Raw data included?	Yes

Dose Response

Toxicant concentration ($\mu\text{g/L}$)		Average Frond Number	% Inhibition ^c	14-day pH
Nominal	Mean Measured ^a			
Control	<0.51	309	-	5.8
0.50	0.48 ^b	340	0	5.9
1.0	0.93	285	8	5.9
2.0	1.9	306	1	5.8
4.0	3.8	369	0	5.7
8.0	7.5	308	0	5.6
16	15	111	64	5.5
32	32	86	72	5.5
64	66	71	77	5.4

^a Averages of Day 0 (new), 5 (old and new), 9 (old and new), and 14 (old) test solutions.

^b Four of the six analyses were below the detection limit, therefore the reported measured value (0.48 ppb) was calculated using the 96% overall mean recovery rate of the other nominal concentrations.

^c Compared to the negative control.

Other Significant Results: Signs of toxicity, including reduced root growth, stunted new frond growth, and pale coloration were observed in all treatments ≥ 4.0 ppb nominal.

Statistical Results

Statistical Method: Moving average angle for EC_{50} and ANOVA followed by Dunnett's test for NOEC. Analyses were conducted using nominal concentrations.

Frond Density:

EC_{50} : 22 $\mu\text{g/L}$

95% C.I.: 20 - 26 $\mu\text{g/L}$

Probit Slope: N/A

NOEC: 8 $\mu\text{g/L}$

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Non-linear regression analysis for EC_{50} and Williams' test for NOEC. Results based on frond density and mean measured concentrations.

EC_{50} : 17.7 ppb

95% C.I.: 11.9 - 26.4 ppb

Probit Slope: N/A

NOEC: 7.5 ppb

- 14. REVIEWER'S COMMENTS:** This study is scientifically sound and fulfills the guideline requirements for an aquatic plant toxicity test. Based on mean measured concentrations, the 14-day EC_{50} and NOEC for *L. gibba* exposed to ZA1296 were 17.7 and 7.5 ppb, respectively. This study can be classified as **Core**.

ZA1296: Toxicity to Lemna - Frond Growth
 File: 44505123 Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	3	309.333	309.333	324.500
2	0.48	3	339.667	339.667	324.500
3	0.93	3	285.000	285.000	320.000
4	1.9	3	306.000	306.000	320.000
5	3.8	3	369.000	369.000	320.000
6	7.5	3	308.333	308.333	308.333
7	15	3	111.333	111.333	111.333
8	32	3	85.667	85.667	85.667
9	66	3	70.667	70.667	70.667

ZA1296: Toxicity to Lemna - Frond Growth
 File: 44505123 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
Control	324.500				
0.48	324.500	0.708		1.73	k= 1, v=18
0.93	320.000	0.498		1.82	k= 2, v=18
1.9	320.000	0.498		1.85	k= 3, v=18
3.8	320.000	0.498		1.86	k= 4, v=18
7.5	308.333	0.047		1.87	k= 5, v=18
15	111.333	9.248	*	1.87	k= 6, v=18
32	85.667	10.447	*	1.88	k= 7, v=18
66	70.667	11.148	*	1.88	k= 8, v=18

s = 26.221

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

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OBS	CONC	LOG_CONC	Y1	Y2	Y3	Y4	Y5	Y6
1	0.00		288	339	301			
2	0.48	-0.31876	303	312	404			
3	0.93	-0.03152	303	264	288			
4	1.90	0.27875	291	285	342			
5	3.80	0.57978	362	392	353			
6	7.50	0.87506	292	304	329			
7	15.00	1.17609	96	118	120			
8	32.00	1.50515	92	79	86			
9	66.00	1.81954	71	70	71			

MODEL: COUNT = CO * PROB NORM ((LOG_EC50 - LOG_CONC) / SIGMA)

WEIGHTED REGRESSION 14:25 Friday, July 17, 1998

Non-Linear Least Squares Iterative Phase

Method: Gauss-Newton

Iter	Dependent Variable	Count	Weighted SS	Weighted MS
0	LOG_EC50	309.000000	313.388670	
1	LOG_EC50	331.551887	288.854307	
2	LOG_EC50	329.382556	286.743032	
3	LOG_EC50	330.980146	287.310541	
4	LOG_EC50	330.089113	286.907661	
5	LOG_EC50	330.604976	287.111017	
6	LOG_EC50	330.313131	286.986336	
7	LOG_EC50	330.480375	287.054633	
8	LOG_EC50	330.385244	287.014763	
9	LOG_EC50	330.439584	287.037204	
10	LOG_EC50	330.408619	287.024308	
11	LOG_EC50	330.426288	287.031632	
12	LOG_EC50	330.416213	287.027445	
13	LOG_EC50	330.421960	287.029829	
14	LOG_EC50	330.418683	287.028468	
15	LOG_EC50	330.420552	287.029244	
16	LOG_EC50	330.419486	287.028801	
17	LOG_EC50	330.420094	287.029054	
18	LOG_EC50	330.419747	287.028910	
19	LOG_EC50	330.419945	287.028992	
20	LOG_EC50	330.419832	287.028945	
21	LOG_EC50	330.419897	287.028972	
22	LOG_EC50	330.419860	287.028957	
23	LOG_EC50	330.419881	287.028965	
24	LOG_EC50	330.419869	287.028960	
25	LOG_EC50	330.419876	287.028963	

NOTE: Convergence criterion met.

Non-Linear Least Squares Summary Statistics

Source	DF	Weighted SS	Dependent Variable	Count
Regression	3	6555.000000		
Residual	24	287.0289632		
Uncorrected Total	27	6842.0289632		
(Corrected Total)	26	2273.0442681		

Parameter	Estimate	Asymptotic Std. Error	Asymptotic 95 % Confidence Interval
LOG_EC50	1.2483193	0.083980108	1.07499426 1.42164431
SIGMA	0.5453358	0.097327982	0.34446233 0.74620925
CO	330.4198758	20.045432068	289.04847054 371.79128104

MODEL: COUNT = CO * PROB NORM ((LOG_EC50 - LOG_CONC) / SIGMA)

EC₅₀ = 17.7 ppb (11.88 - 26.4)

Asymptotic Correlation Matrix

Corr	LOG_EC50	SIGMA	CO
LOG_EC50	1	-0.625324612	-0.701725587
SIGMA	-0.625324612	1	0.5237672063
CO	-0.701725587	0.5237672063	1

MODEL: COUNT = CO * PROB NORM ((LOG_EC50 - LOG_CONC) / SIGMA)

SUMMARY OF NONLINEAR REGRESSION

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OBS	CONC	LOG_EC50	SIGMA	CO	RESID_SS	EC50
1	0	1.24832	0.54534	330.420	287.029	17.7141

MODEL: COUNT = CO * PROB NORM ((LOG_EC50 - LOG_CONC) / SIGMA)

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Plot of COUNT*LOG_CONC. Symbol used is 'o'.

Plot of PRED*LOG_CONC.

COUNT

400

0

0

0

0

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350

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-0.5

0.0

0.5

1.0

1.5

2.0

LOG_CONC

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0

0

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0

0

NOTE: 58 obs had missing values. 51 obs hidden.

General Linear Models Procedure
 Class Level Information

Class	Levels	Values
DOSE	9	0 15 32 66 1.9 3.8 7.5 0.48 0.93

Number of observations in data set = 54

NOTE: Due to missing values, only 27 observations can be used in this analysis.

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 ZA1296 Toxicity to Lemna
 COMPARISON OF MEANS FOR NOEL DETERMINATION
 TEST IF TREATMENT IS LESS THAN CONTROL
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General Linear Models Procedure

Dependent Variable: RESPONSE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	334230.6667	41778.8333	60.76	0.0001
Error	18	12376.0000	687.5556		

Corrected Total	26	346606.6667			
R-Square		C.V.	Root MSE	RESPONSE Mean	
0.964294		10.80053	26.22128	242.7778	

Source	DF	Type I SS	Mean Square	F Value	Pr > F
DOSE	8	334230.6667	41778.8333	60.76	0.0001
Source	DF	Type III SS	Mean Square	F Value	Pr > F
DOSE	8	334230.6667	41778.8333	60.76	0.0001

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 ZA1296 Toxicity to Lemna
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General Linear Models Procedure

Level of DOSE	N	Mean	SD
0	3	309.333333	26.5015723
15	3	111.333333	13.3166562
32	3	85.666667	6.5064071
66	3	70.666667	0.5773503
1.9	3	306.000000	31.3209195
3.8	3	369.000000	20.4205779
7.5	3	308.333333	18.8767935
0.48	3	339.666667	55.8957363

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= 18 MSE= 687.5556
 Critical Value of Dunnett's T= 2.580
 Minimum Significant Difference= 55.247

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

DOSE Comparison	Simultaneous Lower Confidence Limit	Difference Between Means	Simultaneous Upper Confidence Limit
3.8 - 0	4.42	59.67	114.91
0.48 - 0	-24.91	30.33	85.58
7.5 - 0	-56.25	-1.00	54.25
1.9 - 0	-58.58	-3.33	51.91
0.93 - 0	-79.58	-24.33	30.91
15 - 0	-253.25	-198.00	-142.75
32 - 0	-278.91	-223.67	-168.42
66 - 0	-293.91	-238.67	-183.42