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

1. CHEMICAL: Metsulfuron-methyl
Metabolite of AE F115008
(Iodosulfuron-methyl Sodium)

PC Code No.: 122010

2. TEST MATERIAL: AE F075736 (technical)

Purity: 92.2% (w/w)

3. CITATION:

Author: P. Sowig and O. Weller

Title: AE F075736 (Metasulfuron-methyl) Substance, technical, Metabolite of AE F115008, Code: AE F075736 00 1C92 0001, Duckweed (*Lemna gibba* G3) Growth Inhibition Test

Study Completion Date: November 16, 1998

Laboratory: Hoechst Schering AgrEvo GmbH
Umweltforschung Oekobiologie
D-65926 Frankfurt am Main

Sponsor: Aventis CropScience USA LP (Formerly AgrEvo USA Co.)
Little Falls Centre One/2711 Centerville Road
Wilmington, DE 19808

Laboratory Report ID: CE98/095

MRID No.: 45109112

DP Barcode: D266809

4. REVIEWED BY: Brooke S. Levy, Staff Scientist, Dynamac Corporation

Signature: **Date:**

APPROVED BY: Kathleen Ferguson, Ph.D., Senior Staff Scientist, Dynamac Corporation

Signature: **Date:**

5. APPROVED BY: William Rabert, Biologist, OPP/EFED/ERB III

Signature: *William Rabert*   **Date:** 10/29/01

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6. STUDY PARAMETERS:

Scientific Name of Test Organism: *Lemna gibba* (G3)
Initial Growth Stage: 12 fronds per replicate
Definitive Test Duration: 7 days
Type of Concentrations: Static-renewal; Initial mean measured

7. CONCLUSIONS:

The data suggest that the EC_{50} of AE F075736 to *Lemna gibba* G3 under these test conditions is 0.41 µg/L (mean measured) based on biomass and frond number. The **NOAEC of AE F075736 to *Lemna gibba* G3 under test conditions is 0.190 µg/L** (mean measured) based on biomass. However, the contamination of controls raises questions on the validity of these toxicity values.

There were some minor inconsistencies with standard protocol. In this study the pH, daily observations, maximum labeled rate, and number of plants deviate from the guidelines. Contamination of the controls and unstable test concentrations during the study is unacceptable. Hence, **this study is classified as Supplemental.** This study does not fulfill the guideline requirement.

Results Synopsis:
EC_{50}: 0.41 µg/L   95% C.I.: 0.33 to 0.51 µg ai./L
Probit Slope: N/A   NOAEC: 0.190 µg/L (mean measured)

8. ADEQUACY OF THE STUDY:

A. Classification: Supplemental

B. Rationale: Test controls were contaminated to levels higher than the nominal concentration of the lowest test level.

C. Repairability: Repeat the test with care to prevent chemical contamination.

9. GUIDELINE DEVIATIONS:

1. The number of plants was not specified beyond 3 to 5 plants and 12 fronds per replicate. Each plant should be similar in number of fronds to other plants in all replicates and all treatments. This assumption was not been verified in the report.

2. According to the guideline criteria, test duration should be 14 days. This study lasted 7 days. However, 7 or 14 day *Lemna gibba* studies will be accepted according to the

EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

3. The controls were contaminated with the test substance at levels exceeding the nominal concentration at the lowest test level. The data suggest that all test levels were contaminated as reflected by the increasing concentrations from Day 0 to Day 5.

4. According to the guideline criteria, the pH should be approximately 5.0. In this study, the initial pH was 7.5 and the final pH was 8.7-8.8. However, a pH of 7.5 is acceptable for *Lemna gibba* according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994. The higher pH at the end of the study may have been due to the presence of the organisms.

5. Observations were not made daily.

6. The maximum labeled rate was not provided.

7. According to the guidelines, initial and 14-day frond numbers were measured. In this study, initial and 7-day frond numbers were measured. However, 7- or 14-day *Lemna gibba* studies will be accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

10. **SUBMISSION PURPOSE**: To determine the effect of Metsulfuron-methyl on the growth inhibition of *Lemna gibba*.

11. **MATERIALS AND METHODS**:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Lemna gibba*	*Lemna gibba* G3
Number of plants/fronds: 5 plants, 3 fronds per plant	3 to 5 plans per replicate (unspecified); 12 fronds per replicate
Nutrients: Standard formula, e.g. 20X-AAP	20X-AAP nutrient medium

B. Test System

Guideline Criteria	Reported Information
Solvent:	None
Temperature: 25°C	24°C; within $\pm$ 1°C
Light Intensity: 5.0 Klux (±15%)	60.0 $\mu m * m^{-2} * s^{-1}$ (range: 59.2-60.7 $\pm$ 0.5) = 4.32 Klux
Photoperiod: Continuous	Continuous
pH: Approximately 5.0	7.5 $\pm$ 0.1 (pH of aged water at Day 7: 8.7 - 8.8)
Test System: Static or Renewal	Static renewal; On Days 0, 3, and 5

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	1.8x
Doses: at least 5	0.1, 0.18, 0.32, 0.56, and 1.0 $\mu g/L$
Controls: Negative and/or solvent	Negative control
Replicates per dose: 3 or more	3 reps.
Test duration: 14 days	7 days, acceptable
Daily observations were made?	Observations of frond growth and appearance occurred on Days 3, 5, and 7.
Method of observations:	Frond counts and biomass
Maximum labeled rate:	Not provided

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 14-day frond numbers were measured?	Initial and 7-day frond numbers were measured.
Control frond at 14 days ≥2x initial count?	Yes, 16x
Initial chemical concentrations measured?	Yes

Raw data included?	Yes

Dose Response

Nominal Concentration (ng a.i./L)	Mean Measured Concentration (ng a.i./L)	Terminal Average Frond Number*	% Inhibition*	7-day pH*
Control	128.34	194	---	8.8
92.2	189.72	194	0.0	8.8
165.96	242.83	186	3.3	8.8
295.04	345.76	165	14.2	8.8
516.32	494.83	38	80.4	8.8
922.00	912.37	21	89.1	8.7

     *   Reviewer-calculated mean values.

Other significant results: Biomass increase was inhibited at concentrations ≥ 0.18 µg/L. All fronds were observed to be colored yellow at concentrations ≥ 0.32 µg/L.

Statistical results for frond number: Statistical Method: DUNCAN'S Multiple Range Test for NOAEC. EC_{50} was chosen based on the narrowest 95 confidence intervals between the following three methods: binomial probability, moving average angle, and probit methods for biomass and growth rate EC_{50} values based on measured concentrations.

	Lemna Biomass	*Lemna* Frond Numbers
EC_{50}:	0.440 µg/L	0.511 µg/L
95% C.I.:	0.364 - 0.540 µg/L	0.364 - 0.540 µg/L
Probit Slope:	not reported	not reported
NOAEC:	0.190 µg/L	0.243 µg/L

13. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Williams test for NOAEC determination. EC_{50} was determined using the non-linear regression approach of Bruce and Versteeg (1992) for mean measured concentrations..

	Lemna Biomass	Lemna Frond Number	Lemna Growth Rate
7-Day EC_{50} (95% C.I.):	0.41 (0.33-0.51) µg ai./L	0.41 (0.33-0.51) µg ai./L	0.52 (0.44-0.61) µg ai./L
7-Day EC_{25} (95% C.I.):	0.29 (0.21-0.39) µg ai./L	0.29 (0.21-0.39) µg ai./L	0.35 (0.27-0.45) µg ai./L
7-Day EC_5 (95% C.I.):	0.17* (0.11-0.28) µg ai./L	0.18*(0.11-0.28) µg ai./L	0.20 (0.13-0.30) µg ai./L
Probit Slope (Std. Error):	4.35 (0.718)	4.43 (0.727)	3.97 (0.535)
NOAEC:	0.19 µg ai./L	189.7 µg ai./L	242.8 µg ai./L

*   Toxicity value is not bracketed by the test concentrations.

14. <u>REVIEWER'S COMMENTS</u>:

This reviewer used a non-linear approach as in Bruce and Versteeg (1992), which is appropriate for continuously distributed parameters (like biomass) that were used as endpoints in this study. This study demonstrates that the EC_{50} of AE F075736 to *Lemna gibba* G3 under test conditions is 0.41 µg/L (mean measured) for biomass and frond number. The NOAEC of AE F075736 to *Lemna gibba* G3 under test conditions is 0.19 µg/L (mean measured) for biomass.

The controls in this study were contaminated with metsulfuron-methyl at a mean level of 128.34 ng ai./L. This level of contamination was higher than the lowest nominal concentration (i.e., 92.20 ng ai./L). The levels of contamination in the controls show an increase with the test duration (i.e., 72.96 ng ai./L on Day 3, 153.85 ng ai./L on Day 5 and 158.2 ng ai/L on Day 7). Metsulfuron-methyl levels in the three lowest test levels also appear to be contaminated, as indicated by mean measured concentrations which are higher than their respective nominal levels. All test levels show continuing increases in test concentrations on the Days that the test solution was renewed and subsequently analytically measured on Days 0, 3 and 5.

All test chambers should be covered to prevent cross-contamination as a general rule. In this study, it appears that the contamination may have occurred in the stock solutions.

15. <u>RESULTS FROM VALIDATION OF STATISTICAL ANALYSES</u>

TITLE: AE F075736 (Metsulfuron-methyl) Iodosulfuron-methyl Metabolite - Lemna Biomass

7-Day EC_{50} (95% C.I.): 410 (330 - 510) ng ai./L
7-Day EC_{25} (95% C.I.): 290 (210 - 390) ng ai./L
7-Day EC_5 (95% C.I.): 170* (110 - 280) ng ai./L
Probit Slope (Std. Error): 4.35 (0.718)
NOAEC: 189.7 ng ai./L
* Toxicity value is not bracketed by the test concentrations.

TRANSFORM: NO TRANSFORMATION   NUMBER OF GROUPS: 6

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	20.2000	20.2000
1	Control	2	20.0000	20.0000
1	Control	3	19.9000	19.9000
2	189.72	1	20.2000	20.2000
2	189.72	2	19.7000	19.7000
2	189.72	3	20.0000	20.0000
3	242.83	1	19.1000	19.1000
3	242.83	2	19.3000	19.3000
3	242.83	3	19.4000	19.4000
4	345.76	1	17.3000	17.3000
4	345.76	2	17.0000	17.0000
4	345.76	3	16.8000	16.8000
5	494.83	1	4.0000	4.0000
5	494.83	2	4.4000	4.4000
5	494.83	3	3.2000	3.2000
6	912.37	1	2.3000	2.3000
6	912.37	2	2.1000	2.1000
6	912.37	3	2.4000	2.4000

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	3	19.900	20.200	20.033
2	189.72	3	19.700	20.200	19.967
3	242.83	3	19.100	19.400	19.267
4	345.76	3	16.800	17.300	17.033
5	494.83	3	3.200	4.400	3.867
6	912.37	3	2.100	2.400	2.267

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.023	0.153	0.088
2	189.72	0.063	0.252	0.145
3	242.83	0.023	0.153	0.088
4	345.76	0.063	0.252	0.145
5	494.83	0.373	0.611	0.353
6	912.37	0.023	0.153	0.088

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1046.663	209.333	2203.505
Within (Error)	12	1.140	0.095	
Total	17	1047.803		

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

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	Control	20.033	20.033		
2	189.72	19.967	19.967	0.265	
3	242.83	19.267	19.267	3.046	*
4	345.76	17.033	17.033	11.921	*
5	494.83	3.867	3.867	64.240	*
6	912.37	2.267	2.267	70.598	*

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

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	189.72	3	0.629	3.1	0.067
3	242.83	3	0.629	3.1	0.767
4	345.76	3	0.629	3.1	3.000
5	494.83	3	0.629	3.1	16.167
6	912.37	3	0.629	3.1	17.767

WILLIAMS TEST (Isotonic regression model)   TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	3	20.033	20.033	20.033
2	189.72	3	19.967	19.967	19.967
3	242.83	3	19.267	19.267	19.267
4	345.76	3	17.033	17.033	17.033
5	494.83	3	3.867	3.867	3.867
6	912.37	3	2.267	2.267	2.267

WILLIAMS TEST (Isotonic regression model)   TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	20.033				
189.72	19.967	0.265		1.78	k= 1, v=12
242.83	19.267	3.046	*	1.87	k= 2, v=12
345.76	17.033	11.921	*	1.90	k= 3, v=12
494.83	3.867	64.240	*	1.92	k= 4, v=12
912.37	2.267	70.598	*	1.93	k= 5, v=12

s = 0.308; Note: df used for table values are approximate when v > 20.

TITLE: AE F075736 (Metsulfuron-methyl) Iodosulfuron-methyl Metabolite - Lemna Fronds

7-Day EC_{50} (95% C.I.):   410   (330 - 510)   ng ai./L
7-Day EC_{25} (95% C.I.):   290   (210 - 390)   ng ai./L
7-Day EC_5 (95% C.I.):   180* (110 - 280)   ng ai./L
Probit Slope (Std. Error):   4.43   (0.727)
NOAEC: 189.7   ng ai./L
*   Toxicity value is not bracketed by the test concentrations.

TRANSFORM: NO TRANSFORMATION   NUMBER OF GROUPS: 6

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	196.0000	196.0000
1	Control	2	187.0000	187.0000
1	Control	3	195.0000	195.0000
2	189.72	1	191.0000	191.0000
2	189.72	2	197.0000	197.0000
2	189.72	3	190.0000	190.0000
3	242.83	1	186.0000	186.0000
3	242.83	2	188.0000	188.0000
3	242.83	3	185.0000	185.0000
4	345.76	1	166.0000	166.0000
4	345.76	2	161.0000	161.0000
4	345.76	3	169.0000	169.0000
5	494.83	1	38.0000	38.0000
5	494.83	2	43.0000	43.0000
5	494.83	3	32.0000	32.0000
6	912.37	1	20.0000	20.0000
6	912.37	2	22.0000	22.0000
6	912.37	3	21.0000	21.0000

SUMMARY STATISTICS ON TRANSFORMED DATA   TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	3	187.000	196.000	192.667
2	189.72	3	190.000	197.000	192.667
3	242.83	3	185.000	188.000	186.333
4	345.76	3	161.000	169.000	165.333
5	494.83	3	32.000	43.000	37.667
6	912.37	3	20.000	22.000	21.000

SUMMARY STATISTICS ON TRANSFORMED DATA   TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	24.333	4.933	2.848
2	189.72	14.333	3.786	2.186
3	242.83	2.333	1.528	0.882
4	345.76	16.333	4.041	2.333
5	494.83	30.333	5.508	3.180
6	912.37	1.000	1.000	0.577

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	97924.944	19584.989	1325.280
Within (Error)	12	177.333	14.778	
Total	17	98102.278		

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

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T STAT SIG
1	Control	192.667	192.667		
2	189.72	192.667	192.667	0.000	
3	242.83	186.333	186.333	2.018	
4	345.76	165.333	165.333	8.708	*
5	494.83	37.667	37.667	49.382	*
6	912.37	21.000	21.000	54.692	*

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

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	189.72	3	7.847	4.1	0.000
3	242.83	3	7.847	4.1	6.333
4	345.76	3	7.847	4.1	27.333
5	494.83	3	7.847	4.1	155.000
6	912.37	3	7.847	4.1	171.667

WILLIAMS TEST (Isotonic regression model)   TABLE 1 OF 2

	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	3	192.667	192.667	192.667
2	189.72	3	192.667	192.667	192.667
3	242.83	3	186.333	186.333	186.333
4	345.76	3	165.333	165.333	165.333
5	494.83	3	37.667	37.667	37.667
6	912.37	3	21.000	21.000	21.000

WILLIAMS TEST (Isotonic regression model)   TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	192.667				
189.72	192.667	0.000		1.78	k= 1, v=12
242.83	186.333	2.018	*	1.87	k= 2, v=12
345.76	165.333	8.708	*	1.90	k= 3, v=12
494.83	37.667	49.382	*	1.92	k= 4, v=12
912.37	21.000	54.692	*	1.93	k= 5, v=12

s = 3.844; Note: df used for table values are approximate when v > 20.

TITLE: AE F075736 (Metsulfuron-methyl) Iodosulfuron-methyl Metabolite - Lemna Growth Rate

7-Day EC_{50} (95% C.I.): 520 (440 - 610) ng ai./L
7-Day EC^{25} (95% C.I.): 350 (270 - 450) ng ai./L
7-Day EC_5 (95% C.I.): 200 (130 - 300) ng ai./L
Probit Slope (Std. Error): 3.97 (0.535)
NOAEC: 242.8 ng ai./L

TRANSFORM: NO TRANSFORMATION   NUMBER OF GROUPS: 6

GROUP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	Control	1	0.3990	0.3990
1	Control	2	0.3923	0.3923
1	Control	3	0.3983	0.3983
2	189.72	1	0.3953	0.3953
2	189.72	2	0.3998	0.3998
2	189.72	3	0.3946	0.3946
3	242.83	1	0.3915	0.3915
3	242.83	2	0.3931	0.3931
3	242.83	3	0.3908	0.3908
4	345.76	1	0.3753	0.3753
4	345.76	2	0.3709	0.3709
4	345.76	3	0.3779	0.3779
5	494.83	1	0.1647	0.1647
5	494.83	2	0.1823	0.1823
5	494.83	3	0.1401	0.1401
6	912.37	1	0.0730	0.0730
6	912.37	2	0.0866	0.0866
6	912.37	3	0.0800	0.0800

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GROUP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Control	3	0.392	0.399	0.397
2	189.72	3	0.395	0.400	0.397
3	242.83	3	0.391	0.393	0.392
4	345.76	3	0.371	0.378	0.375
5	494.83	3	0.140	0.182	0.162
6	912.37	3	0.073	0.087	0.080

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GROUP	IDENTIFICATION	VARIANCE	SD	SEM
1	Control	0.000	0.004	0.002
2	189.72	0.000	0.003	0.002
3	242.83	0.000	0.001	0.001
4	345.76	0.000	0.004	0.002
5	494.83	0.000	0.021	0.012
6	912.37	0.000	0.007	0.004

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.3002	0.0600	600.000
Within (Error)	12	0.0011	0.0001	
Total	17	0.3013		

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

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN ORIGINAL	CALCULATED IN UNITS	T TEST SIG
1	Control	0.397	0.397		
2	189.72	0.397	0.397	-0.002	
3	242.83	0.392	0.392	0.581	
4	345.76	0.375	0.375	2.676	*
5	494.83	0.162	0.162	28.680	*
6	912.37	0.080	0.080	38.788	*

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

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	189.72	3	0.020	5.1	-0.000
3	242.83	3	0.020	5.1	0.005
4	345.76	3	0.020	5.1	0.022
5	494.83	3	0.020	5.1	0.234
6	912.37	3	0.020	5.1	0.317

WILLIAMS TEST (Isotonic regression model)   TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	Control	3	0.397	0.397	0.397
2	189.72	3	0.397	0.397	0.397
3	242.83	3	0.392	0.392	0.392
4	345.76	3	0.375	0.375	0.375
5	494.83	3	0.162	0.162	0.162
6	912.37	3	0.080	0.080	0.080

WILLIAMS TEST (Isotonic regression model)   TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
Control	0.397				
189.72	0.397	0.001		1.78	k= 1, v=12
242.83	0.392	0.612		1.87	k= 2, v=12
345.76	0.375	2.821	*	1.90	k= 3, v=12
494.83	0.162	30.232	*	1.92	k= 4, v=12
912.37	0.080	40.887	*	1.93	k= 5, v=12

s =   0.009; Note: df used for table values are approximate when v > 20.

Rice paddy water Concentration (ug/L or ppb)
blue color for input cells
red color for output cell

$Cw = [(11.21)(R)(1000)]/\{[Dw + (Ds)(Ps)] + [(Kd)(Ds)(BD)]\}$

R: application rate (lbs/acre)
Dw: depth of water column (cm)
Ds: depth of sediment zone (cm)
Ps: porosity of sediment (dimensionless fraction)
BD: bulk density of sediment (dry) (grams/cm^3)
Kd: sorption coefficient (mL/gram)
Answer: Cw (ppb)

```
0.050017
  10.16
      1
 0.5095
    1.3
  14.28
19.17973
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Rice paddy water Concentration (ug/L or ppb) $Cw = [(11.21)(R)(1000)]/\{[Dw + (Ds)(Ps)] + [(Kd)(Ds)(BD)]\}$

blue color for input cells

red color for output cell

R: application rate (lbs/acre)   0.050017

Dw: depth of water column (cm)   10.16

Ds: depth of sediment zone (cm)   1

Ps: porosity of sediment (dimensionless fraction)   0.5095

BD: bulk density of sediment (dry) (grams/cm^3)   1.3

Kd: sorption coefficient (mL/gram)   14.28

Answer: Cw (ppb)   19.17973

6. STUDY PARAMETERS:

Definitive Test Duration: 7 days
Type of Concentrations: Mean measured

7. CONCLUSIONS:

Differences between reviewers statistical calculations and those calculated by the testing laboratory are slight and may be attributed to different statistical methods. Reviewer used a non-linear approach as in Bruce and Versteeg (1992), which is appropriate for continuously distributed parameters (like biomass) that were used as endpoints in this study. This study demonstrates that the EC_{50} of AE F075736 to *Lemna gibba* G3 under test conditions is 0.4*l* μg/L (mean measured). The **NOEC of AE F075736 to *Lemna gibba* G3 under test conditions is 0.1*69* μg/L (mean measured).**

The were minor inconsistencies with standard protocol. In this study the pH, temperature, daily observations, maximum labeled rate, and number of fronds per plant deviate from the guidelines. Despite these deviations, **this study is classified as CORE.** ~~*Supplemental*~~ This study fulfills the requirements and can be used in a risk assessment.

Results Synopsis:

EC_{50}: 0.4*l* μg/L   95% C.I.: 0.36 to 0.49 μg/L
Probit Slope: N/A   NOEC: 0.169 μg/L (mean measured)
                                              0.19

8. ADEQUACY OF THE STUDY:

A. Classification: ~~Core~~ *Supplemental*

B. Rationale: N/A

C. Repairability: N/A

9. GUIDELINE DEVIATIONS:

1. The temperature of the study was 24°C at all times. According to the guideline criteria, it should have been 25°C. *∮ 1 °C*

1*2*. According to the guideline criteria, the pH should be approximately 5.0. In this study, the

2. Contamination of controls

2

initial pH was 7.5 and the final pH was 8.7-8.8. However, a pH of 7.5 is acceptable for *Lemna gibba* according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994. The higher pH at the end of the study may have been due to the presence of the organisms.

3. According to the guideline criteria, test duration should be 14 days. This study lasted 7 days. However, 7 or 14 day *Lemna gibba* studies will be accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

4. Observations were not made daily.

5. The maximum labeled rate was not provided.

6. According to the guidelines, initial and 14 day frond numbers were measured. In this study, initial and 7 day frond numbers were measured. However, 7 or 14 day *Lemna gibba* studies will be accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

7. According to the guidelines, control fronds should be measured at 14 days. In this study, control fronds were measured at 7 days. However, 7 or 14 day *Lemna gibba* studies will be accepted according to the EPA Office of Prevention, Pesticides and Toxic Substances memorandum "Closure on Nontarget Plant Phytotoxicity Policy Issues" October 21, 1994.

8. The results indicate the total number of fronds per plant, while the guidelines specify that the total number of <u>new</u> fronds should be reported, as well.

10. <u>SUBMISSION PURPOSE</u>: To determine the effect of Metsulfuron-methyl on the growth inhibition of *Lemna gibba*.

11. MATERIALS AND METHODS:

A. Test Organisms

Guideline Criteria	Reported Information
Species: *Lemna gibba*	*Lemna gibba* G3
Number of plants/fronds: 5 plants, 3 fronds per plant	12 fronds per plant
Nutrients: Standard formula, e.g. 20X-AAP	20X-AAP nutrient medium

B. Test System

Guideline Criteria	Reported Information
Solvent:	None
Temperature: 25°C ± 1°C	24°C
Light Intensity: 5.0 Klux (±15%)	60.0 μm*m^{-2}*s^{-1} (range: 59.2-60.7 ± 0.5) = 4.32 K lux
Photoperiod: Continuous	Continuous
pH: Approximately 5.0	7.5 ± 0.1 (pH of aged water at day 7: 8.7-8.8)
Test System: Static or Renewal	~~7 days semi-static~~ ~~Static~~ Renewal on Days 0, 3 and 5

4

C. Test Design

Guideline Criteria	Reported Information
Dose range: 2x or 3x progression	*2x 1.8x*
Doses: at least 5	0.1, 0.18, 0.32, 0.56, and 1.0 µg/L
Controls: Negative and/or solvent	Negative control
Replicates per dose: 3 or more	3
Test duration: 14 days	7 days
Daily observations were made?	Observance of frond growth and appearance occurred on days 3, 5, and 7.
Method of observations:	Frond counts and biomass *, grow*
Maximum labeled rate:	Not provided

12. REPORTED RESULTS:

Guideline Criteria	Reported Information
Initial and 14 day frond numbers were measured?	Initial and 7 day frond numbers were measured.
Control frond at 14 days ≥2x initial count?	Control frond at 7 days was ≥2x initial count. *YES, 16X*
Initial chemical concentrations measured?	Yes
Raw data included?	Yes

5

Dose Response

Mean Measured Concentration (μg a.i./L)		Terminal Average Frond Number*	% Inhibition*	7-day pH*
Control	0.128	194	---	8.8
0.1	0.190	194	0.0	8.8
0.18	0.24	186	3.3	8.8
0.32	0.35	165	14.2	8.8
0.56	0.49	38	80.4	8.8
1.0	0.91	21	89.1	8.7

* Reviewer-calculated mean values.

Other significant results:
Biomass increase was inhibited at concentrations ≥ 0.18 μg/L. All fronds were observed to be colored yellow at concentrations ≥ 0.32 μg/L.

Statistical results for frond number:

Statistical Method:
DUNCAN'S Multiple Range Test for NOEC. EC_{50} was chosen based on the narrowest 95 confidence intervals between the following three methods: binomial probability, moving average angle, and probit.

EC_{50}: 0.440 μg/L   95% C.I.: 0.364 - 0.540 μg/L
Probit Slope: not reported   NOEC: 0.1 μg/L

13. <u>VERIFICATION OF STATISTICAL RESULTS</u>:

Statistical Method: Williams test for NOEC determination. EC_{50} was determined using the non-linear regression approach of Bruce and Versteeg (1992).

EC_{50}: 0.41 $\mu g/L$ 95% C.I.: 0.38 - 0.57 $\mu g/L$

Probit Slope: ~~N/A~~ 4.35   NOEC: ~~0.169~~ $\mu g/L$ (mean measured) 0.19

                                    0.100 $\mu g/L$ (nominal)

14. <u>REVIEWER'S COMMENTS</u>:

The chemical and physical parameters were given for fresh and aged water at days 0, 3, 5, and 7 (Tables 6.3.1, 6.3.2, 6.3.3, and 6.3.4, pp. 28-31).

Differences between reviewers statistical calculations and those calculated by the testing laboratory are slight and may be attributed to different statistical methods. Reviewer used a non-linear approach as in Bruce and Versteeg (1992), which is appropriate for continuously distributed parameters (like biomass) that were used as endpoints in this study. This study demonstrates that the EC_{50} of AE F075736 to *Lemna gibba* G3 under test conditions is 0.42 $\mu g/L$ (mean measured). The NOEC of AE F075736 to *Lemna gibba* G3 under test conditions is 0.169 $\mu g/L$ (mean measured).

Light intensity units were reported as $\mu m*m^{-2}*s^{-1}$, instead of Klux and no conversion was provided

72 lux = 1 ueinstein

15. RESULTS FROM VALIDATION OF STATISTICAL ANALYSES

BIOMASS NOEC AND EC_{50} FOR 451091-12 LEMNA GIBBA

NOEC

Williams Test

[One-Sided Test for Decrease, alpha =   0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	18.7	.		
0.169	**18.7**	**0.2649**	**N.S.**	
0.244	18	3.046	0.0057	*
0.364	15.7	11.92	<0.005	*
0.54	2.57	64.24	<0.005	*
0.953	0.967	70.6	<0.005	*

 "*"=Significant; "N.S."=Not Significant.

BIOMASS ECx

←[7mEstimates of EC%

←[0m--

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.22	0.15	0.30	0.070	0.71
EC10	0.25	0.19	0.34	0.061	0.74
EC25	0.32	0.25	0.40	0.046	0.80
EC50	**0.42**	**0.36**	**0.49**	**0.032**	**0.85**

          Slope =   5.70 Std.Err. =   0.889

GROWTH RATE NOEC AND EC50 FOR 451091-12 LEMNA GIBBA

NOEC

Williams Test

[One-Sided Test for Decrease, alpha =   0.050000]

Dose	Isotone Means	T-bar	P-value	Significance
0	193	.		
0.169	**193**	**0**	**N.S.**	
0.244	186	2.018	0.039	*
0.364	165	8.708	<0.005	*
0.54	37.7	49.38	<0.005	*
0.953	21	54.69	<0.005	*

"*"=Significant; "N.S."=Not Significant.

ECx FOR FROND NUMBER

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.19	0.13	0.30	0.087	0.65
EC10	0.23	0.16	0.34	0.075	0.69
EC25	0.32	0.24	0.42	0.057	0.76
EC50	**0.44**	**0.37**	**0.54**	**0.039**	**0.83**

Slope =   4.55 Std.Err. =   0.708

16. REFERENCE

Bruce, R.D. and D.J. Versteeg. 1992. "A Statistical Procedure for Modeling Continuous Toxicity Data". *Environmental Toxicology and Chemistry* 11:1485-1494.