01/09/04

Data Evaluation Report on the 7Day acute toxicity of AE F160460 to aquatic vascular plants *Lemna gibba*

PMRA Submission #: {.....}

EPA MRID#: 45386312

Data Requirement:

PMRA Data Code:	{.....}
EPA DP Barcode:	D295614
OECD Data Point:	{.....}
EPA MRID:	45386312
EPA Guideline:	123-2

Test material: AE F160460 Pure (Mesosulfuron-methyl metabolite) Purity: 96.1%

Common name: AE F160460

Chemical name: IUPAC: 2-[3-(4-hydroxy-6-methoxyprimidin-2-yl) ureidosulfonyl]-4-methanesulfonamidomethyl-benzoic acid

CAS name: Not reported

CAS No.: Not reported

Synonyms: metabolite of AE F130060

Primary Reviewer: Dana Worcester
Staff Scientist, Dynamac Corporation

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Date: 11/7/03

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{EPA/OECD/PMRA}

Date: {.....}

Company Code {.....} [For PMRA]
Active Code {.....} [For PMRA]
EPA PC Code 122009

Date Evaluation Completed: {dd-mmm-yyyy}

CITATION: Sowig, P. and Weller, O. 2000. Duckweed (Lemna gibba G3) Growth Inhibition Test, AE F160460, Substance, pure (Metabolite of AE F130060). Unpublished study performed by Aventis CropScience GmbH, Frankfort, Germany. Laboratory Study Identification No. CE00/059. Study submitted by Aventis CropScience, Research Triangle Park, NC. Experimental start date August 25, 2000 and experimental termination date September 1, 2000. The final report issued October 13, 2000.

EXECUTIVE SUMMARY:

In a 7-day acute toxicity study, freshwater floating aquatic vascular plants Duckweed, *Lemna gibba* G3, were exposed to AE F160460 at mean measured concentrations of 9.38, 16.92, 29.19, 50.71, and 94.71 mg a.i./L under static-renewal conditions. Nominal concentrations were 10, 18, 32, 56, and 100 mg/L (9.61, 17.30, 30.75, 53.82, and 96.10 mg a.i./L). Mean frond number, dry weight, and growth rate (frond number and biomass) were not adversely affected at any treatment level. Therefore, the EC_{50} was >94.71 mg a.i./L and the NOEC was 94.71 mg a.i./L.

This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Core.

Results Synopsis

Test Organism: *Lemna gibba* G3
Test Type: Static-renewal

Number of fronds:
NOEC: 94.71 mg a.i./L
EC_{05}: >94.71 mg a.i./L 95% C.I.: N/A
EC_{50}: >94.71 mg a.i./L 95% C.I.: N/A
Slope: N/A

Dry Weight:
NOEC: 94.71 mg a.i./L
EC_{05}: >94.71 mg a.i./L 95% C.I.: N/A
EC_{50}: >94.71 mg a.i./L 95% C.I.: N/A
Slope: N/A

Growth rate (frond number and biomass):
NOEC: 94.71 mg a.i./L
EC_{05}: >94.71 mg a.i./L 95% C.I.: N/A
EC_{50}: >94.71 mg a.i./L 95% C.I.: N/A
Slope: N/A

Endpoint(s) Affected: None.

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test was based on the following guidelines: OECD Guideline *Lemna* Growth Inhibition Test, June 1998, US-EPA Subdivision J. §123-2, and American Society for Testing and Materials Guide E 1415-91. The following deviations from U.S. EPA Guideline 123-2 are noted:

1. The pretest health of the test organism was not reported.
2. The number of plants tested (3-5 plants) ranged lower than the required 5 plants; therefore, there were 12 fronds per replicate, instead of the 15 fronds per replicate that is recommended.
3. The storage conditions of the test chemical, carbon source of the growth medium, and some dilution water characteristics were not reported.

These deviations were considered to be minor, having no effect on the results, so they did not affect the acceptability or the validity of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material AE F160460 (mesosulfuron-methyl metabolite)

Description: White powder

Lot No./Batch No. : AE F 160460 00 1B96 0001

Purity: 96.1%

Stability of Compound
Under Test Conditions: Measured concentrations (days 0, 3, and 5) for new test solutions ranged from 91.9 to 107.4% of nominal a.i. concentrations and measured concentrations (days 3, 5, and 7) of old test concentrations ranged from 94.8 to 107.0% of nominal concentrations, showing that the test material was stable under test conditions. OECD requirements were not reported.

(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: Not reported.

2. Test organism:

Name: Duckweed, *Lemna gibba* *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: G3
Source: Maintained at Laboratory of Ecotoxicology at Aventis CropScience, FRG (original supplier: Plant Hormone Laboratory, USDA, Beltsville, MD)
Age of inoculum: 6 weeks

Method of cultivation: 20X AAP culture medium

B. STUDY DESIGN:

a) Range-finding Study: No range-finding study was reported.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: culturing media and conditions: (same as test or not) health: (any toxicity observed)	Approximately 6 weeks 20X AAP medium: same as test. Not reported.	
Test system static/static renewal/ renewal rate for static renewal:	Static-renewal	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Environmental chamber-water bath	
Duration of the test	7 days	*EPA requires a duration of 14 days. Seven day studies will be accepted for review by the Agency.*
Test vessel material: (glass/polystyrene) size: fill volume:	Glass Erlenmeyer-flasks 300 mL 150 mL	
Details of growth medium name: pH at test initiation: pH at test termination: Chelator used: Carbon source:	20X AAP medium 7.4 8.6-8.8 $Na_2EDTA \cdot 2H_2O$ $NaHCO_3$	*EPA recommend the following culture media: Modified hoagland's E+ or 20X-AAP. Chelators are not recommended.*

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Parameter	Details	Remarks *Criteria*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
Dilution water source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Reagent grade water 7.5 ± 0.1 Deionized water which is additionally filtered by an ultrafiltration, ion exchange and a charcoal unit. Not reported Not reported Not reported Not reported Not reported	*EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Not reported	
Sediment used **(for rooted aquatic vascular plants)** origin: textural classification (% sand, silt and clay): organic carbon (%): geographic location:	Not applicable	
Number of replicates control: solvent control: treatments:	 3 N/A 3	
Number of plants/replicate	3-5 plants per replicate	There were probably three fronds per plant. *EPA requires 3 fronds per plant.*
Number of fronds/plant	12 fronds per replicate at test initiation	The number of plants (3-5 plants) ranged lower than the required 5 plants. *EPA requires 5 plants.*

Parameter	Details	Remarks *Criteria*
Test concentrations nominal: measured:	10, 18, 32, 56, and 100 mg/L 9.61, 17.30, 30.75, 53.82, and 96.10 mg a.i./L 9.38, 16.92, 29.19, 50.71, and 94.71 mg a.i./L	Mean measured concentrations were reviewer-calculated from mean fresh water and mean aged water values. *EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	HPLC; new test solutions at 0, 3, and 5 days and old test solutions at 3, 5 and 7 days.	
Test conditions temperature: photoperiod: light intensity and quality:	24.0-24.5°C continuous light 100-106 $\mu E*m^{-2}*s^{-1}$, white fluorescent lighting	*EPA temperature: 25°C* *EPA photoperiod: continuous* *EPA light: 5.0 Klux (±15%)*
Reference chemical (if used) name: concentrations:	None	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (eg: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds, dry weights, growth rates, and toxicity symptoms (yellow-colored fronds)	
Measurement technique for frond number and other end points	Direct counts	
Observation intervals	3, 5, and 7 days.	
Other observations, if any	None	

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Indicate whether there was an exponential growth in the control	Yes, average frond number at test initiation was 15x the average frond number at test initiation in the control group.	
Were raw data included?	Replicate data provided	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Mean frond number did not decrease as test concentrations increased, when compared to the solvent control. Mean percent inhibition was -2.10, -0.48, -0.97, -1.13, and -2.26% in the 9.38, 16.92, 29.19, 50.71, and 94.71 mg a.i./L treatment groups, respectively. By day 7, the mean dry weights were 22.4, 22.1, 22.2, 22.2, and 22.5 mg in the 9.38, 16.92, 29.19, 50.71, and 94.71 mg/L treatment groups, respectively.

The mean doubling times were 1.776, 1.774, 1.764, 1.796, and 1.755 days in the 9.38, 16.92, 29.19, 50.71, and 94.71 mg a.i./L treatment groups, respectively. The mean biomass growth rates were 21.06, 20.73, 20.83, 20.86, and 21.1 in the 9.38, 16.92, 29.19, 50.71, and 94.71 mg/L treatment groups, respectively.

Table 3: Effect of AE F160460 on frond number of Duckweed, *Lemna gibba*

Treatment[1] (measured and nominal concentration) mg a.i./L	Initial frond number/test solution	Mean frond number at[2]				Mean Growth Rate at Day 7	Mean Dry Weight of Fronds (biomass) (mg)[2]
		3 days	5 days	7 days	% inhibition at 7 days[3]		
Negative control (dilution water)	12	42	89	183	---	0.38888	20.63
9.38 (9.61)	12	42	92	184	-1	0.39025	21.06
16.92 (17.30)	12	39	92	185	-1	0.39073	20.73
29.19 (30.75)	12	43	92	188	-3	0.39302	20.83
50.71 (53.82)	12	40	93	179	3	0.38597	20.86
94.71 (96.10)	12	39	92	191	-5	0.39506	21.10
Reference chemical (if used)	Not applicable						

[1] Mean measured concentrations of AE F160460 were reviewer-calculated. Nominal (a.i.) concentrations are in parentheses.
[2] Mean frond number and dry weights were reviewer-calculated from replicate data.
[3] % inhibition was determined by comparing the treatment groups to the dilution water control.

Table 4: Statistical endpoint values.

Statistical Endpoint[a]	frond No.	growth rate	dry weight (biomass)
NOEC or EC_{05} (mg/L)	100	100	100
LOEC (mg/L)	>100	>100	>100
IC_{50} or EC_{50} (mg/L) (95% C.I.)	Not reported	>100	>100
other (IC_{25}/EC_{25})	Not reported	Not reported	Not reported
Reference chemical NOAEC IC_{50}/EC_{50}	Not applicable	Not applicable	Not reported

[a] Toxicity values reported by the study authors are based on nominal test concentrations.

B. REPORTED STATISTICS: The formulas used for growth rates, doubling time, and mean percent inhibitions on found on pages 18 and 19. The NOEC was verified using Analysis of Variance, General Linear Models with DUNCAN's Multiple Range Test Procedures (SAS 1989).

Biomass:
NOEC: 100 mg/L
EC_{50}: >100 mg/L 95% C.I.: N/A
Slope: N/A

Growth Rate:
NOEC: 100 mg/L
EC_{50}: >100 mg/L 95% C.I.: N/A
Slope: N/A

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Statistical analyses were not required, as it could be visually determined that there were no effects of treatment on any endpoint.

Number of fronds:
NOEC: 94.71 mg a.i./L
EC_{05}: >94.71 mg a.i./L 95% C.I.: N/A
EC_{50}: >94.71 mg a.i./L 95% C.I.: N/A
Slope: N/A

Dry Weight:
NOEC: 94.71 mg a.i./L
EC_{05}: >94.71 mg a.i./L 95% C.I.: N/A
EC_{50}: >94.71 mg a.i./L 95% C.I.: N/A
Slope: N/A

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Growth rate (frond number and biomass):
NOEC: 94.71 mg a.i./L
EC_{05}: >94.71 mg a.i./L 95% C.I.: N/A
EC_{50}: >94.71 mg a.i./L 95% C.I.: N/A
Slope: N/A

D. STUDY DEFICIENCIES:

The deviations, including the reduced replicate size, were not considered to have impacted the study results, so they did not affect the acceptability or validity of the study.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions agreed with the study authors'; there were no effects of AE F160460 on any endpoint.

F. CONCLUSIONS: This toxicity study is scientifically sound and satisfies the U.S. EPA Guideline Subdivision J, §123-2 for an aquatic vascular plant study with *Lemna gibba*. As a result, this study is classified as Core.

Number of fronds:
NOEC: 94.71 mg a.i./L
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EC_{50}: >94.71 mg a.i./L 95% C.I.: N/A
Slope: N/A

Endpoint(s) Affected: None.

III. REFERENCES:

Organization of Economic Co-operation and Development. Draft OECD Guideline for Testing of Chemicals Guideline: *Lemna*, Growth Inhibition Test, April 1997.

U.S. Environmental Protection Agency (EPA), 1982 Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants; Tier 2 of nontarget area testing; §123-2 Growth and reproduction of aquatic plants.

U.S. Environmental Protection Agency (EPA), April 1996. Ecological Effects Test Guidelines: OPPTS 850.4400 Aquatic Plant Toxicity Test Using Lemna spp., Tiers I and II; EPA 712-C-96-156, Public Draft.

ASTM (1991). Standard Guide for Conducting Static Toxicity Test With Lemna gibba G3. American Society for Testing and Materials. E 1415-91

U.S. Environmental Protection Agency (EPA). 1983. Toxic Substances Control: Good Laboratory Practice Standards; Final Rule (40 CFR Part 792) Fed. Reg., Vol. 48, No. 230, Nov. 23, 1983, pp. 53922-53944.

SAS Institute Inc., 1989. Release 6.08 TS 407. Cary, North Carolina 27511.

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