Record

Data Evaluation ~~Report~~ on the Acute Toxicity of Orthosulfamuron to Aquatic Vascular Plants *Lemna gibba*

PMRA Submission Number {.....} EPA MRID Number 465789-39

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D319377
	OECD Data Point	{.....}
	EPA MRID	465789-39
	EPA Guideline	123-2

Test material: Orthosulfamuron **Purity:** 49.96 a.i.%
Common name
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: IR5878 50WG

Primary Reviewer: Dana Worcester **Signature:** Dana Worcester
Staff Scientist, Cambridge Environmental Inc. **Date:** 2/24/06

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, Cambridge Environmental Inc. **Date:** 3/15/06

Primary Reviewer: Christopher J. Salice **Date:** 6/30/06
EPA/OPP/EFED/ERB IV

Secondary Reviewer(s): Christopher J. Salice **Date:** 7/31/06
EPA/OPP/EFED/ERB IV

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	108209	

Date Evaluation Completed: 31-07-2006

CITATION: Desjardins, D., T.Z. Kendall and H.O. Krueger. 2003. IR5878 50WG ALONE: A 96 hour Toxicity Test with Duckweed (*Lemna gibba*). Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-120 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued September 22, 2003.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

Data Evaluation Report on the Acute Toxicity of Orthosulfamuron to Aquatic Vascular Plants *Lemna gibba*

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EXECUTIVE SUMMARY:

In a 7 day acute toxicity study, the freshwater aquatic vascular plant *Lemna gibba* G3 (duckweed) were exposed to IR5878 50WG (a.i. Orthosulfamuron, 49.96%) at nominal concentrations of 0.074, 0.16, 0.36, 0.81, 1.8 and 4.0 µg/L under static conditions. The measured (mean) concentrations were 0.074, 0.16, 0.52, 0.86, 1.9 and 3.9 µg/L.

After 7 days, frond inhibitions were 6.1, 15, 10, 32, 72 and 83% in the 0.074, 0.16, 0.52, 0.86, 1.9 and 3.9 µg/L treatment groups, respectively, compared to the control. The NOAEC and EC_{50}/IC_{50} values based on frond number were 0.52 and 1.4 µg/L, respectively. The study authors additionally provided toxicity estimates for growth rate (but did not provide replicate data to verify these results); the NOAEC and EC_{50}/IC_{50} values based on growth rate were 0.52 and 2.0 µg/L.

This study is scientifically sound satisfies the guideline requirement for an aquatic vascular plant study with *Lemna gibba*. This study is classified ACCEPTABLE.

Results Synopsis

Test Organism: *Lemna gibba*
Test Type: Static

Frond Number; reviewer-reported:

EC_{05}:	0.28 µg/L (0.14 µg ai/L)	95% C.I.: 0.15-0.53 µg/L (0.07-0.26 µg ai/L)
EC_{50}:	1.4 µg/L (0.7 µg ai/L)	95% C.I.: 1.1-1.8 µg/L (0.55-0.90 µg ai/L)

NOAEC: 0.52 µg/L (0.26 µg ai/L)
Probit Slope: 2.36±0.321

Growth rate; study author-reported:

EC_{05}:	Not determined	95% C.I.: N/A
EC_{50}:	2.0 µg/L (1.0 µg ai/L)	95% C.I.: 1.7-2.5 µg/L (0.85-1.2 µg ai/L)

NOAEC: 0.52 µg/L (0.26 µg ai/L)
Probit Slope: Not reported

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The test protocol was based on the U.S. EPA-FIFRA Pesticide Assessment Guidelines, Subdivision J, Hazard Evaluation: Nontarget Plants Guidelines 122-2 and 123-2. The following deviations from U.S. EPA Guideline 123-2 are noted: The deviations from U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.4400, *Aquatic Plant Toxicity Test Using Lemna spp., Tiers I and II* included:

1. All the test concentrations were not analytically determined. Because the lower nominal concentrations (0.074 and 0.16 µg/L) were less than the LOQ, the analytical results provided for them were questionable. Nominal concentrations were used for these levels in all statistical analyses.

2. Temperature during the study (24.1-24.8°C) ranged higher than recommended 20±2°C.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the UK Good Laboratory Practice standards.

A. MATERIALS:

1. Test material IR5878 50WG (Orthosulfamuron)

Description: Brown granular solid

Lot No./Batch No.: G038/02

Purity: 49.96%

Stability of compound under test conditions: The measured concentrations of orthosulfamuron were 95.5-118% of nominal at Hour 0 and 95.5-169% at 7 days.
(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound) Only the water solubility was reported.

Storage conditions of test chemicals: The test material was stored under ambient conditions.

Physicochemical properties of orthosulfamuron.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Name: Duckweed, *Lemna gibba* *EPA requires a vascular species: Lemna gibba.*
Strain, if provided: G3
Source: In house cultures originally obtained from US Department of Agriculture
Age of inoculum: 2 weeks
Method of cultivation: 20X-AAP

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was not 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 mortality observed)	Continuous culture 20X-AAP Not reported	
Test system Static/static renewal Renewal rate for static renewal	Static N/A	*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	
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/stainless steel)* Size: Fill volume:	Glass beakers 250 mL 100 mL	

Parameter	Details	Remarks *Criteria*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source:	 7.8-7.9 8.7-8.9 disodium EDTA $NaHCO_3$	The pH was adjusted to 7.6 using 10% HCl *EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
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:	 Sterile bottled water Not reported Not reported Not reported Not reported Not detected Not detected 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	

Parameter	Details	Remarks *Criteria*
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	5	*EPA requires 5 plants.*
Number of fronds/plant	3	*EPA requires 3 fronds per plant.*
Test concentrations Nominal: Measured:	0.074, 0.16, 0.36, 0.81, 1.8 and 4.0 µg/L Mean: 0.52, 0.86, 1.9 and 3.9 µg/L; measured concentrations provided for 0.074 and 0.16 ranged from 268-2540% of nominal, so these levels were expressed nominally.	*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	0 and 7 days, samples were analyzed using HPLC	
Test conditions Temperature: Photoperiod: Light intensity and quality:	24.1-24.8°C Continuous 4470-5730 lux with warm white lights	hourly measurement
Reference chemical (if used) name: concentrations:	None	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks/Criteria
Parameters measured (e.g.,: number of fronds, plant dry weight or other toxicity symptoms)	Number of fronds and growth rate	
Measurement technique for frond number and other end points	Direct counts	
Observation intervals	Days 3, 5 and 7	
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes	
Were raw data included?	Replicate data were provided for frond number.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 7 days, frond inhibitions were 6.1, 15, 10, 32, 72 and 83% in the 0.074, 0.16, 0.52, 0.86, 1.9 and 3.9 µg/L. treatment groups, respectively, compared to the control. After 7 days, growth rate inhibitions were 2.3, 6.0, 4.5, 15, 49 and 68% in the 0.074, 0.16, 0.52, 0.86, 1.9 and 3.9 µg/L. treatment groups, respectively, compared to the control.

Table 3: Effect of orthosulfamuron on number of fronds (duckweed, *Lemna gibba*)

Treatment (record measured and nominal concentration (µg/L)	Initial frond number/test solution	frond number at			
		3 days	5 days	7 days	
				frond number	% inhibition
Negative control	15	42	81	201	---
Solvent control (if used)	N/A	N/A	N/A	N/A	N/A
0.074 (0.074)	15	42	81	189	6.1
0.16 (0.16)	15	39	74	171	15
0.36 (0.52)	15	39	71	180	10
0.81 (0.86)	15	34	59	137	32
1.8 (1.9)	15	27	35	56	72
4.0 (3.9)	15	25	27	34	83
Reference chemical (if used)	N/A				

Measured concentrations are in parentheses.

Table 4: Statistical endpoint values.

Statistical Endpoint	frond No.	growth rate	dry weight
NOAEC or EC_{05} (µg/L)	0.52	0.52	NR
LOAEC (µg/L)	NR	NR	NR
IC_{50} or EC_{50} (µg/L) (95% C.I.)	1.3 (0.58-1.7)	2.0 (1.7-2.5)	NR
Other (IC_{25}/EC_{25})	NR	NR	NR
Reference chemical NOAEC IC_{50}/EC_{50}	N/A	N/A	N/A

NR Not reported

B. REPORTED STATISTICS: The 7 day treatment and control response data passed the tests for normality (Shapiro-Wilks) and homogeneity of variance (Bartlett's). The 7 day EC_{50} value was determined by linear interpolation. The reported toxicity values were determined in terms of the mean measured test concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Frond number data were analyzed using the Chi-square and Shapiro-Wilks tests for normality and the Hartley and Bartlett's tests for homogeneity of variances; data satisfied the assumptions of ANOVA. The NOAEC was determined using ANOVA, followed by William's test via TOXSTAT statistical software. The EC_x values were determined using non-linear regression via Nuthatch statistical software. Mean-measured concentrations were used to compute these estimates.

Frond Number:
EC_{05}: 0.28 µg/L (0.14 µg ai/L) 95% C.I.: 0.15-0.53 µg/L (0.07-0.26 µg ai/L)
EC_{50}: 1.4 µg/L (0.7 µg ai/L) 95% C.I.: 1.1-1.8 µg/L (0.55-0.90 µg ai/L)
NOAEC: 0.52 µg/L (0.26 µg ai/L)
Probit Slope: 2.36±0.321

D. STUDY DEFICIENCIES:

There were no study deficiencies.

E. REVIEWER'S COMMENTS:

The reviewer's conclusions regarding frond number (the only endpoint for which replicate data were provided) were similar to the study authors'; because the reviewer's EC_{50} estimate was associated with a narrower 95% confidence interval, it is reported in the Executive Summary and Conclusions sections.

While measured concentrations could not be provided for all test levels (given the limitation set by the LOQ), significant effects only occurred at those levels which could be analyzed.

The experimental start date was June 25, 2003 and the experimental termination date was July 2, 2003.

Due to the extremely high concentrations (114-2540% of nominal) at time 0, the stock solution was retested 4 days later. Four days later the 0.074 and 0.16 µg/L treatment groups were still 268-446% of nominal. Because of the unreliable results, the study authors used the nominal concentrations for statistical analysis.

F. CONCLUSIONS:

This study is scientifically sound and is classified as ACCEPTABLE. Frond number was the more sensitive endpoint, with an EC_{50} of 1.4 µg/L (0.7 µg ai/L); the EC_{05} and NOAEC values were 0.28 µg/L (0.14 µg ai/L) and 0.52 µg/L (0.26 µg ai/L), respectively.

Frond Number; reviewer-reported:
EC_{05}: 0.28 µg/L (0.14 µg ai/L) 95% C.I.: 0.15-0.53 µg/L (0.07-0.26 µg ai/L)
EC_{50}: 1.4 µg/L (0.7 µg ai/L) 95% C.I.: 1.1-1.8 µg/L (0.55-0.90 µg ai/L)
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Growth rate; study author-reported:
EC_{05}: Not determined 95% C.I.: N/A
EC_{50}: 2.0 µg/L (1.0 µg ai/L) 95% C.I.: 1.7-2.5 µg/L (0.85-1.2 µg ai/L)
NOAEC: 0.52 µg/L (0.26 µg ai/L)
Probit Slope: Not reported

III. REFERENCES:

ASTM Standard Guide 1218-90E. 1990. *Standard Guide for Conducting Static 96-Hour Toxicity Tests with Microalgae*. American Society for Testing and Materials. Philadelphia, PA.

Microsoft Coporation. Microsoft Excel 2000. Copyright 1985-1999.

Norgerg-King, T.J. 1993. *A Linear Interpolation Method for Sublethal Toxicity: the Inhibition Concentration (Icp) Approach*. Version 2.0. U.S. Environmental Protection Agency. National Effluent Toxicity Assessment Center. Duluth, MN. Technical Report 03-93.

OECD. 1984. OECD Guidelines for Testing of Chemicals 201. Alga, Growth Inhibition Test.

U.S. Environmental Protection Agency. 1996. Series 850-Ecological Effects Test Guidelines (draft), OPPTS Number 850.5400.: *Algal Toxicity, Tiers I and II.*

West, Inc. and D.D. Gulley. TOXSTAT Version 3.5. Copyright 1996. Western Ecosystems Technology, Inc. Cheyenne, WY.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

frond number (d7)
File: 8939f Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	80941.619	13490.270	30.978
Within (Error)	14	6096.667	435.476	
Total	20	87038.286		

Critical F value = 2.85 (0.05,6,14)
Since F > Critical F REJECT Ho:All groups equal

frond number (d7)
File: 8939f Transform: NO TRANSFORMATION

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

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	201.000	201.000		
2	0.074	188.667	188.667	0.724	
3	0.16	171.333	171.333	1.741	
4	0.52	180.333	180.333	1.213	
5	0.86	136.667	136.667	3.776	*
6	1.9	56.000	56.000	8.510	*
7	3.9	34.000	34.000	9.801	*

Dunnett table value = 2.53 (1 Tailed Value, P=0.05, df=14,6)

frond number (d7)
File: 8939f Transform: NO TRANSFORMATION

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	0.074	3	43.108	21.4	12.333
3	0.16	3	43.108	21.4	29.667
4	0.52	3	43.108	21.4	20.667
5	0.86	3	43.108	21.4	64.333
6	1.9	3	43.108	21.4	145.000
7	3.9	3	43.108	21.4	167.000

frond number (d7)
File: 8939f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	control	3	201.000	201.000	201.000
2	0.074	3	188.667	188.667	188.667
3	0.16	3	171.333	171.333	175.833
4	0.52	3	180.333	180.333	175.833
5	0.86	3	136.667	136.667	136.667
6	1.9	3	56.000	56.000	56.000
7	3.9	3	34.000	34.000	34.000

frond number (d7)
File: 8939f 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	201.000				
0.074	188.667	0.724		1.76	k= 1, v=14
0.16	175.833	1.477		1.85	k= 2, v=14
0.52	175.833	1.477		1.88	k= 3, v=14
0.86	136.667	3.776	*	1.89	k= 4, v=14
1.9	56.000	8.510	*	1.90	k= 5, v=14
3.9	34.000	9.801	*	1.91	k= 6, v=14

s = 20.868
Note: df used for table values are approximate when v > 20.

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.28	0.15	0.53	0.13	0.53
EC10	0.40	0.23	0.69	0.11	0.58
EC25	0.72	0.49	1.1	0.081	0.67
EC50	1.4	1.1	1.8	0.052	0.78

Slope = 2.36 Std.Err. = 0.321

Goodness of fit: p = 0.091 based on DF= 4.0 14.

8939F : frond number (d7)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	3.00	201.	192.	8.92	100.	0.00
0.0740	3.00	189.	192.	-3.17	99.9	0.130
0.160	3.00	171.	190.	-18.2	98.7	1.32
0.520	3.00	180.	162.	18.2	84.4	15.6
0.860	3.00	137.	132.	4.21	69.0	31.0
1.90	3.00	56.0	72.0	-16.0	37.5	62.5
3.90	3.00	34.0	27.9	6.06	14.5	85.5