

Record

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

PMRA Submission Number {.....}

EPA MRID Number 465789-40

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D319377
OECD Data Point	{.....}
EPA MRID	465789-40
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
Staff Scientist, Cambridge Environmental Inc.

Signature: *Dana Worcester*
Date: 2/24/06

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Date: 3/15/06

Primary Reviewer: Christopher J. Salice
EPA/OPP/EFED/ERB IV

Date: 6/30/06

Secondary Reviewer(s): Christopher J. Salice
EPA/OPP/EFED/ERB IV

Date: 7/31/06

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

Company Code {.....} [For PMRA]
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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: A 96 hour Toxicity Test with Duckweed (*Lemna gibba*). Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-114 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued July 6, 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.

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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 0.028, 0.070, 0.18, 0.44, 1.1, 2.7 and 6.8 µg/L under static conditions. The measured (mean) concentrations were 0.45, 0.978, 2.4 and 5.9 µg/L; because the LOQ was 0.200µg/L, lower concentrations were not analytically determined. The mean measured concentrations (µg/L) were corrected for purity of the test material.

After 7 days, frond inhibitions were 5.8, 0.17, -0.17, -0.34, 35, 77 and 86% in the 0.028, 0.070, 0.18, 0.45, 0.978, 2.4 and 5.9 µg/L treatment groups, respectively, compared to the pooled control. The NOAEC and EC₅₀/IC₅₀ values based on frond number, the most sensitive endpoint, were 0.45 and 1.5 µg/L. The study authors additionally provided toxicity estimates for growth rate (but did not provide replicate data to verify these results); the NOAEC and EC₅₀/IC₅₀ values based on growth rate were 0.45 and 2.1 µg/L.

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

Results Synopsis

Test Organism: *Lemna gibba*

Test Type: Static

Frond Number; reviewer-reported:

EC₀₅: 0.24 µg/L (0.12 µg ai/L) 95% C.I.: 0.14-0.41 µg/L (0.07-0.20 µg ai/L)

EC₅₀: 1.5 µg/L (0.75 µg ai/L) 95% C.I.: 1.2-1.8 µg/L (0.60-0.90 µg ai/L)

NOAEC: 0.45 µg/L (0.22 µg ai/L)

Probit Slope: 2.11±0.214

Growth rate; study author-reported:

EC₀₅: Not determined 95% C.I.: N/A

EC₅₀: 2.1 µg/L (1.0 µg ai/L) 95% C.I.: 2.1-2.2 µg/L (1.0-1.1 µg ai/L)

NOAEC: 0.45 µg/L (0.22 µg ai/L)

Probit Slope: Not reported

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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 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 (0.028, 0.070, and 0.18 µg/L) were less than the LOQ, they were not measured. Nominal concentrations were used for these levels in all statistical analyses.
2. Temperature during the study (24-25°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 79.3-98.7% of nominal at Hour 0 and 92.8-108% 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.

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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:	Continuous culture	
Culturing media and conditions: (same as test or not)	20X-AAP Not reported	
Health: (any mortality observed)		
<u>Test system</u>		
Static/static renewal	Static	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).
Renewal rate for static renewal	N/A	
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.

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Parameter	Details	Remarks ----- Criteria
<u>Test vessel</u> Material: (glass/stainless steel) Size: Fill volume:	Glass beakers 250 mL 100 mL	
<u>Details of growth medium name</u> pH at test initiation: pH at test termination: Chelator used: Carbon source:	8.1 8.7-9.0 disodium EDTA NaHCO ₃	The pH was adjusted to 7.6 using 10% HCl ----- <i>EPA recommends the following culture media: Modified Hoagland's E+ or 20X-AAP.</i> <i>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, E1415-91 and D 3978-80 (reapproved 1987).</i>
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Not applicable	
<u>Dilution water</u> source/type: pH: water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	Purified well water Not reported Not reported Not reported Not reported <LOD <LOD Not reported	----- <i>EPA recommends a pH of ~5.0. A solution pH of 7.5 is acceptable if type 20X-AAP nutrient media is used.</i>
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Not reported	

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Parameter	Details	Remarks ----- Criteria
<u>Sediment used (for rooted aquatic vascular plants)</u> Origin: Textural classification (%sand, silt, and clay): Organic carbon (%): Geographic location:	Not applicable	
<u>Number of replicates</u> Control: Solvent control: Treatments:	3 3 3	
Number of plants/replicate	5	----- <i>EPA requires 5 plants.</i>
Number of fronds/plant	3	----- <i>EPA requires 3 fronds per plant.</i>
<u>Test concentrations</u> Nominal: Measured:	0.028, 0.070, 0.18, 0.44, 1.1, 2.7, 6.8 µg/L Mean: 0.45, 0.978, 2.4, 5.9 µg/L; 0.028, 0.070, 0.18 were <LOQ and not measured	----- <i>EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.</i>
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	0 and 7 days, samples were analyzed using HPLC	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:	24.5-25.0°C Continuous 4390-4990 lux with warm white lights	
<u>Reference chemical (if used)</u> name: concentrations:	None	
Other parameters, if any	None	

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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 count.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

After 7 days, frond inhibitions were 5.8, 0.17, -0.17, -0.34, 35, 77 and 86% in the 0.028, 0.070, 0.18, 0.45, 0.978, 2.4 and 5.9 µg/L treatment groups, respectively, compared to the pooled control. After 7 days, growth rate inhibitions were 2.2, -0.17, -0.32, -0.32, 16, 57 and 77% in the 0.028, 0.070, 0.18, 0.45, 0.978, 2.4 and 5.9µg/L treatment groups, respectively, compared to the pooled control.

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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	46	91	208	--
Solvent control (if used)	15	42	86	180	--
0.028 (0.028)	15	41	89	183	5.8
0.070 (0.070)	15	43	90	194	0.17
0.18 (0.18)	15	42	85	195	-0.17
0.44 (0.45)	15	42	85	195	-0.34
1.1 (0.978)	15	35	61	127*	35
2.7 (2.4)	15	27	34	45*	77
6.8 (5.9)	15	24	25	27*	86
Reference chemical (if used)	N/A				

Measured concentrations are in parentheses.

*Significantly reduced compared to the pooled control (Bonferroni's Test).

Table 4: Statistical endpoint values.

Statistical Endpoint	frond No.	growth rate	dry weight
NOAEC or EC ₀₅ (µg/L)	0.45	0.45	NR
LOAEC (µg/L)	NR	NR	NR
IC ₅₀ or EC ₅₀ (µg/L) (95% C.I.)	1.5 (1.3-1.6)	2.1 (2.1-2.2)	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: A t-test was used to compare the dilution water (negative) and solvent control. Results indicated that the controls could be pooled for frond number. The 7 day treatment and control response data passed the tests for normality (Shapiro-Wilks) and homogeneity of variance (Bartlett's). The 7 day EC₅₀ value was determined by linear interpolation. The reported toxicity values were determined in terms of the mean measured test concentrations.

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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 negative and adjuvant control groups were compared for all endpoints and pooled for analysis upon detecting no significant differences using a Student's paired t-test. The NOAEC was determined using ANOVA, followed by Bonferroni'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 : 0.24 µg/L (0.12 µg ai/L)	95% C.I.: 0.14-0.41 µg/L (0.07-0.20 µg ai/L)
EC ₅₀ : 1.5 µg/L (0.75 µg ai/L)	95% C.I.: 1.2-1.8 µg/L (0.60-0.90 µg ai/L)
NOAEC: 0.45 µg/L (0.22 µg ai/L)	
Probit Slope: 2.11±0.214	

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 identical to the study authors'. Furthermore, the results revealed in this study were nearly identical to those reported in MRID 465789-39, which tested the same species at similar levels over a year prior to the definitive study.

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 May 28, 2003 and the experimental termination date was June 4, 2003.

F. CONCLUSIONS:

This study is scientifically sound and is classified ACCEPTABLE. Frond number was the more sensitive endpoint, with an EC₅₀ of 1.5 µg/L (0.75 µg ai/L); the EC₀₅ and NOAEC values were 0.24 µg/L (0.12 µg ai/L) and 0.45 µg/L (0.22 µg ai/L), respectively.

Frond Number; reviewer-reported:

EC ₀₅ : 0.24 µg/L (0.12 µg ai/L)	95% C.I.: 0.14-0.41 µg/L (0.07-0.20 µg ai/L)
EC ₅₀ : 1.5 µg/L (0.75 µg ai/L)	95% C.I.: 1.2-1.8 µg/L (0.60-0.90 µg ai/L)
NOAEC: 0.45 µg/L (0.22 µg ai/L)	
Probit Slope: 2.11±0.214	

Growth rate; study author-reported:

EC ₀₅ : Not determined	95% C.I.: N/A
EC ₅₀ : 2.1 µg/L (1.0 µg ai/L)	95% C.I.: 2.1-2.2 µg/L (1.0-1.1 µg ai/L)
NOAEC: 0.45 µg/L (0.22 µg ai/L)	
Probit Slope: Not reported	

III. REFERENCES:

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

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

frond number (day 7)
File: 8940f Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	7	112935.407	16133.630	73.346
Within (Error)	19	4179.333	219.965	
Total	26	117114.741		

Critical F value = 2.54 (0.05,7,19)
Since F > Critical F REJECT Ho:All groups equal

frond number (day 7)
File: 8940f Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	194.333	194.333		
2	0.028	183.000	183.000	1.081	
3	0.070	194.000	194.000	0.032	
4	0.18	194.667	194.667	-0.032	
5	0.45	195.000	195.000	-0.064	
6	0.98	127.000	127.000	6.420	*
7	2.4	44.667	44.667	14.271	*
8	5.9	27.333	27.333	15.924	*

Bonferroni T table value = 2.70 (1 Tailed Value, P=0.05, df=19,7)

frond number (day 7)
File: 8940f Transform: NO TRANSFORMATION

BONFERRONI T-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	GRPS 1&2 POOLED	6			
2	0.028	3	28.284	14.6	11.333
3	0.070	3	28.284	14.6	0.333
4	0.18	3	28.284	14.6	-0.333
5	0.45	3	28.284	14.6	-0.667

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6	0.98	3	28.284	14.6	67.333
7	2.4	3	28.284	14.6	149.667
8	5.9	3	28.284	14.6	167.000

frond number (day 7)
File: 8940f Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	6	194.333	194.333	194.333
2	0.028	3	183.000	183.000	191.667
3	0.070	3	194.000	194.000	191.667
4	0.18	3	194.667	194.667	191.667
5	0.45	3	195.000	195.000	191.667
6	0.98	3	127.000	127.000	127.000
7	2.4	3	44.667	44.667	44.667
8	5.9	3	27.333	27.333	27.333

frond number (day 7)
File: 8940f 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
GRPS 1&2 POOLED	194.333				
0.028	191.667	0.254		1.73	k= 1, v=19
0.070	191.667	0.254		1.81	k= 2, v=19
0.18	191.667	0.254		1.84	k= 3, v=19
0.45	191.667	0.254		1.85	k= 4, v=19
0.98	127.000	6.420	*	1.86	k= 5, v=19
2.4	44.667	14.271	*	1.87	k= 6, v=19
5.9	27.333	15.924	*	1.87	k= 7, v=19

s = 14.831

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

Estimates of EC%

Parameter	Estimate	95% Bounds		Std.Err.	Lower Bound /Estimate
		Lower	Upper		
EC5	0.24	0.14	0.41	0.11	0.59
EC10	0.36	0.23	0.57	0.096	0.63
EC25	0.70	0.50	0.98	0.070	0.72
EC50	1.5	1.2	1.8	0.046	0.81

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Slope = 2.11 Std.Err. = 0.214

!!!Poor fit: p < 0.001 based on DF= 5.00 19.0

8940F : frond number (day 7)

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	6.00	194.	196.	-2.09	100.	0.00
0.0280	3.00	183.	196.	-13.4	100.	0.0144
0.0700	3.00	194.	196.	-1.90	99.7	0.268
0.180	3.00	195.	191.	3.65	97.2	2.75
0.450	3.00	195.	169.	26.2	86.0	14.0
0.980	3.00	127.	126.	0.862	64.2	35.8
2.40	3.00	44.7	63.6	-18.9	32.4	67.6
5.90	3.00	27.3	19.6	7.72	9.99	90.0