Record

Data Evaluation ~~Report~~ on the Acute Toxicity of Orthosulfamuron to freshwater diatom *Navicula pelliculosa*

PMRA Submission Number {.....} EPA MRID Number 465789-32

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D319377
OECD Data Point	{.....}
EPA MRID	465789-32
EPA Guideline	123-2

Test material: Orthosulfamuron
Common name
Chemical name: IUPAC: Not reported
CAS name: Not reported
CAS No.: Not reported
Synonyms: IR5878 50WG

Purity: 49.96 a.i.%

Primary Reviewer: Dana Worcester
Staff Scientist, Cambridge Environmental Inc.

Signature: Dana Worcester
Date: 2/24/06

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.

Signature: Teri S Myers
Date: 3/11/06

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]
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 50 WG Alone: A 96 hour Toxicity Test with the Freshwater Diatom (*Navicula pelliculosa*). Unpublished study performed by Wildlife International, Ltd, Easton, MD, Project No. 544A-119 and submitted by ISAGRO S.p.A., Milano, Italy. Final report issued March 26, 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 nonvascular 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.

EXECUTIVE SUMMARY:

In a 96 hour acute toxicity study, cultures of the freshwater diatom, *Navicula pelliculosa* were exposed to IR5878 50WG (a.i. Orthosulfamuron, 49.96%) at nominal concentrations of 7.5, 15, 30, 60 and 120 mg/L under static conditions. The measured (mean) concentrations were 7.7, 15, 29, 56 and 108 mg/L.

By 96 hours, cell density percent inhibitions were –4.5, -4.9, -1.8, 0.41, and -11% for the 7.7, 15, 29, 56 and 108 mg/L treatment groups, respectively, compared to the control. The cell density EC_{50} was >108 mg/L and the NOAEC was 108 mg/L. By 96 hours, biomass (area under the curve) inhibitions were –14, -13, -0.32, -1.2 and 4.2% for the 7.7, 15, 29, 56 and 108 mg/L treatment groups, respectively, compared to the control. The biomass EC_{50} was >108 mg/L and the NOAEC was 108 mg/L. By 96 hours growth rate inhibitions were –1.1, -1.1, -0.39, 0.091 and –2.5% for the 7.7, 15, 29, 56 and 108 mg/L treatment groups, respectively, compared to the control. The growth rate EC_{50} was >108 mg/L and the NOAEC was 108 mg/L.

There were no compound related phytotoxic effects.

The study is scientifically sound and satisfies guideline requirement for an aquatic nonvascular plant study with *Navicula pelliculosa*. This study is classified ACCEPTABLE.

Results Synopsis

Test Organism: *Navicula pelliculosa*
Test Type (Flow-through, Static, Static Renewal): Static

Cell density (96 Hours):

EC_{05}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
EC_{50}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
NOAEC:	108 mg/L (54 mg ai/L)	
Probit Slope:	N/A	

Growth rate (0-96 hours):

EC_{05}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
EC_{50}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
NOAEC:	108 mg/L (54 mg ai/L)	
Probit Slope:	N/A	

Biomass (0-96 hours):

EC_{05}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
EC_{50}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
NOAEC:	108 mg/L (54 mg ai/L)	
Probit Slope:	N/A	

Endpoint(s) Affected: None
Most sensitive endpoint(s): None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study followed OECD Guideline 201 and U.S. Environmental Protection Agency Series 850-Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.5400, *Algal Toxicity, Tiers I and II*. The following deviations from these guidelines are:

1. There were only three replicates used per treatment group; EPA recommends four replicates per treatment group for this species, as there is high intraspecies variability.
2. The dilution water characteristics of TOC, particulate matter, and chlorine content were not reported.

COMPLIANCE: Signed and dated GLP, Quality Assurance and No Data Confidentiality statements were provided. The study followed the U.S. EPA (40 CFR, Part 160) Good Laboratory Practice.

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 95.1-113% of nominal at Hour 0 and 84.8-93.8% at 96 hours.
(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: Freshwater diatom *Navicula pelliculosa*

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: UTEX 667
Source: Current in-house laboratory cultures originally obtained from Culture Collection of Algae at the University of Austin.
Age of inoculum: Two weeks old
Method of cultivation: Algal Assay Procedure (AAP) medium

B. STUDY DESIGN:

1. Experimental Conditions

a. A range-finding study was not reported.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period:	Continuous	*EPA recommends two week acclimation period.*
Culturing media and conditions: (same as test or not)	Algal Assay Procedure (AAP) medium; same as test.	*OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Health: (any mortality observed)	Not reported	

Parameter	Details	Remarks *Criteria*
Test system Static/static renewal Renewal rate for 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).*
Incubation facility	Environmental chamber	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Erlenmeyer flasks 250 mL 100 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	 6.7-7.2 7.4-7.6 disodium EDTA $NaHCO_3$ N/A	*OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *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)	N/A	

Parameter	Details	Remarks *Criteria*
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 well water Not reported Not reported Not reported Not reported <LOD <LOD Not reported	*EPA pH: Skeletonema costatum = ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Stock solution	
Aeration or agitation	Agitation, 100 rpm	
Initial cells density	10,000	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates Control: Solvent control: Treatments:	 3 N/A 3	*EPA requires a negative and/or solvent control with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations		

Parameter	Details	Remarks *Criteria*
Nominal: Measured:	7.5, 15, 30, 60 and 120 mg/L 7.7, 15, 29, 56 and 108 mg/L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A	
Method and interval of analytical verification	At 0 and 96 hours samples were analyzed by HPLC	
Test conditions Temperature: Photoperiod: Light intensity and quality:	 23.7-24.5°C continuous 3890-4700 lux, cool white light	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	None	
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	Cell density, biomass (area under the curve), growth rate	*EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Hemacytometer and microscope	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	24, 48, 72 and 96 hours	*EPA and OECD: every 24 hours.*
Other observations, if any	None	
Indicate whether there was an exponential growth in the control	Yes	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Replicate data were provided	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

By 96 hours, cell density inhibitions were –4.5, -4.9, -1.8, 0.41 and -11% for the mean measured 7.7, 15, 29, 56 and 108 mg/L treatment groups, respectively, compared to the control. By 96 hours biomass inhibitions were –14, -13, -0.32, -1.2 and 4,2% for the mean measured 7.7, 15, 29, 56 and 108 mg/L treatment groups, respectively, compared to the control. By 96 hours growth rate inhibitions were –1.1, -1.1, -0.39, 0.091 and –2.5% for the mean measured 7.7, 15, 29, 56 and 108 mg/L treatment groups, respectively, compared to the control.

There were no compound related phytotoxic effects.

Table 3: Effect of Orthosulfamuron on freshwater diatom *Navicula pelliculosa*

Treatment (record measured and nominal concentration (mg/L)	Initial cell density	Cell density at			
		24 hours	48 hours	96 hours	
				cell count	% inhibition
Negative control	10,000	39,250	285,750	910,000	--
Solvent control (if used)	N/A	N/A	N/A	N/A	N/A
7.5 (7.7)	10,000	27,750	465,000	951,250	
15 (15)	10,000	25,750	542,500	955,000	
30 (29)	10,000	30,500	270,760	926,250	
60 (56)	10,000	32,500	194,250	906,250	
120 (108)	10,000	32,250	55,000	1,013,750	
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

Table 4: Statistical endpoint values.

Statistical Endpoint	biomass	growth rate	cell density
NOAEC or EC_{05} (mg/L)	108	108	108
EC_{50} (mg/L)	>108	>108	>108
IC_{50} or EC_{50} (mg/L) (95% C.I.)	>108	>108	>108
Other (IC_{25}/EC_{25})	NR	NR	NR
Reference chemical, if used NOAEC IC_{50}/EC_{50}	N/A	N/A	NA

NR Not reported

B. REPORTED STATISTICS:

The 96-Hour treatment and control response data passed the tests for normality (Shapiro-Wilks) and homogeneity of variance (Levene's). The 96-Hour EC_{50} value was determined by non-linear regression. The reported toxicity values were determined in terms of the mean measured test concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Statistical analyses were not conducted, as percent inhibition did not exceed 4% for any parameter. Results could be visually verified.

Cell density (96 Hours):

EC_{05}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
EC_{50}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
NOAEC:	108 mg/L (54 mg ai/L)	
Probit Slope:	N/A	

Growth rate (0-96 hours):

EC_{05}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
EC_{50}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
NOAEC:	108 mg/L (54 mg ai/L)	
Probit Slope:	N/A	

Biomass (0-96 hours):

EC_{05}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
EC_{50}:	>108 mg/L (>54 mg ai/L)	95% C.I.: N/A
NOAEC:	108 mg/L (54 mg ai/L)	
Probit Slope:	N/A	

Endpoint(s) Affected: None
Most sensitive endpoint(s): None

D. STUDY DEFICIENCIES:

No significant deviations were noted.

E. REVIEWER'S COMMENTS:

The experimental start date was February 20, 2003 and the experimental termination date was February 25, 2003.

F. CONCLUSIONS:

The study is scientifically sound and is classified ACCEPTABLE. No endpoint was sensitive to treatment. The EC_{50} was >108 mg/L (>54 mg ai/L); the EC_{05} and NOAEC values were >108 mg/L (>54 mg ai/L) and 108 mg/L (54 mg/L), respectively.

Cell density (96 Hours):
EC_{05}: >108 mg/L (>54 mg ai/L) 95% C.I.: N/A
EC_{50}: >108 mg/L (>54 mg ai/L) 95% C.I.: N/A
NOAEC: 108 mg/L (54 mg ai/L)
Probit Slope: N/A

Growth rate (0-96 hours):
EC_{05}: >108 mg/L (>54 mg ai/L) 95% C.I.: N/A
EC_{50}: >108 mg/L (>54 mg ai/L) 95% C.I.: N/A
NOAEC: 108 mg/L (54 mg ai/L)
Probit Slope: N/A

Biomass (0-96 hours):
EC_{05}: >108 mg/L (>54 mg ai/L) 95% C.I.: N/A
EC_{50}: >108 mg/L (>54 mg ai/L) 95% C.I.: N/A
NOAEC: 108 mg/L (54 mg ai/L)
Probit Slope: N/A

Endpoint(s) Affected: None
Most sensitive endpoint(s): None

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.

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

Cohen, J. 1977. Statistical Power Analysis for the Behavioral Sciences. Academic Press, New York.

Official Journal of the European Communities. 1992. No. L383. Method C.3.: *Algal Inhibition Test.*

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

The SAS System for Windows. 1999. Version 8.02. SAS Institute Inc. Cary, NC.

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