Revised

Data Evaluation ~~Report~~ on the Acute Toxicity of Orthosulfamuron (IR5878) to Sheepshead Minnow

PMRA Submission Number {.....} EPA MRID Number 465789-51

Data Requirement:

PMRA Data Code	{.....}
EPA DP Barcode	D319377
OECD Data Point	{.....}
EPA MRID	465789-51
EPA Guideline	72-3

Test material: Orthosulfamuron
Common name: IR5878 Technical **Purity:** 98.56%
Chemical name: IUPAC: 1-(4,6-dimethoxypyrimidin-2-yl)-3-[2-(dimethylcarbamoyl)phenylsulfamoyl]urea
CAS name: 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-*N,N*-dimethylbenzamide
CAS No.: 213464-77-8
Synonyms:

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation
Signature: Rebecca L. Bryan
Date: 2/13/06

Secondary Reviewer: Teri S. Myers
Senior Scientist, Cambridge Environmental Inc.
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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: 6/28/06

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: Palmer, S., Kendall, T., and Krueger, H. 2002. IR5878: A96-Hour Static Acute Toxicity Test with the Sheepshead Minnow. Unpublished study performed by Wildlife International, Ltd., Easton Maryland. Laboratory Report Number: 544A-109. Study submitted by ISAGRO S.p.A., Milano, Italy. Experimental start date was June 24, 2002 and experimental termination date was June 28, 2002. The final report was issued July 31, 2002.

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 fish. 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 (IR5878) to Fish Sheepshead Minnow

PMRA Submission Number {.....}

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EXECUTIVE SUMMARY:

In a 96-h acute toxicity study, sheepshead minnow, *Cyprinodon variegatus*, were exposed to Orthosulfamuron (IR5878) at nominal concentrations of 16, 26, 43, 72, and 120 mg ai/L (mean measured concentrations of 16, 27, 45, 75, and 123 mg ai/L) under static conditions. No mortalities or sublethal effects were observed during the study at any treatment level. The 96-h LC_{50} was >123 mg ai/L. The EC_{50} and NOAEC values, based on mortality/sub-lethal effects, were >123 mg ai/L and 123 mg ai/L, respectively. Based on the results of this study, Orthosulfamuron would be classified as practically nontoxic to sheepshead minnow in accordance with the classification system of the U.S. EPA.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with a marine fish (§72-3a). Although the mean weight of the organisms obtained from ten negative control fish at study termination was 0.40 g, which is less than the recommended initial weight range of 0.5 to 5 g, this deviation does not affect the validity of this study. This study is classified ACCEPTABLE.

Results Synopsis

Test Organism Size/Age (mean weight or length): Juvenile (Age not specified); 3.0 cm and 0.40 g (mean of ten control fish at test termination).
Test Type (Flow-through, Static, Static Renewal): Static

LC_{50}: >123 mg ai/L 95% C.I.: Not applicable
NOAEC: 123 mg ai/L Probit Slope: Not applicable
EC_{50}: >123 mg ai/L
Endpoint(s) Affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in the U.S. EPA Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.1075 (1996); U.S. EPA Standard Evaluation Procedure, Estuarine Fish 96-Hour Acute Toxicity Test, EPA-540/9-85-006 (1985); and ASTM Standard E729-88a, Standard Guide for Conducting Acute Toxicity Test with Fishes, Macroinvertebrates and Amphibians (1994). Deviations from the U.S. EPA Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.1075 (1996) included:

1. The age of the test organism at test initiation was not specified.
2. Mean wet fish weight (0.40 g) was determined from ten negative control fish at study termination, and was less than the recommended initial range of 0.5-5g.
3. Fish were acclimated to actual test conditions for only 51 hours prior to test initiation. Guidelines require a 12-day minimum acclimation period, with 14 days recommended. A minimum of 7 days of the acclimation period must be performed in the test dilution water.
4. The hardness, total organic carbon, particulate matter, and chlorine levels in the seawater were not specified.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Confidentiality statements were provided. This study was conducted in accordance with GLP standards set forth by the U.S. EPA (1989), the OECD (1998), and the Japan MAFF (1999, p. 3).

A. MATERIALS:

1. Test material Orthosulfamuron (IR5878)

Description: White powder

Lot No./Batch No. : G009/02

Purity: 98.56%

Stability of compound under test conditions: The stability of the test substance in the dilution water during the course of the study was demonstrated by analytical determination at 0, 48, and 96 hours. Recoveries at the 16 to 120 mg ai/L (nominal) levels were 101-107% of nominal concentrations in 0-hour samples, 97.5-106% in 48-hour samples, and 100-108% in 96-hour samples, with no pattern of decline.

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

Storage conditions of

test chemicals: 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:

Species: Sheepshead minnow, *Cyprinodon variegatus*
EPA recommends a cold water species (preferably rainbow trout Oncorhynchus mykiss) and a warm water species (preferably bluegill sunfish Lepomis macrochirus). OECD recommends choice of species at discretion of testing laboratory.

Age at test initiation: Juvenile (Age not specified)

Weight at study initiation: 0.40 g and 0.24-0.68 g (mean and range of ten control fish at test termination).
EPA recommends: mean 0.5 - 5 g.

Length at study initiation: 3.0 cm and 2.6-3.5 cm (mean and range of ten control fish at test termination).
EPA recommends: Longest not > 2x shortest; OECD recommends 2.0 ∀ 1.0 cm for bluegill and 5.0 ∀ 1.0 cm for rainbow trout

Source: Aquatic BioSystems, Inc., Fort Collins, Colorado.
EPA recommends that all organisms be from the same source

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study

The nominal definitive test concentrations were based on a range-finding study. However, the results of this range-finding study were not reported.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	51 hours Same as test Commercially-prepared diet and *Artemia nauplii* were provided daily. No mortalities occurred and there were no signs of disease or stress.	The fish were held for at least 14 days prior to the test in water from the same source and at approximately the same temperature as used in the test. *The recommended acclimation period is a minimum of 14 days; OECD guideline recommends a minimum of 12 days. Pretest mortality should be < 3% 48 h. prior to testing. OECD pretest mortality criteria: >10% = rejection of entire batch; ≥ 5 and ≤ 10% = continued acclimation for 7 days; <5% = acceptable.*
Duration of the test	96 hours	*The recommended test duration is 96 hours.*
Test condition Static/flow-through Type of dilution system - for flow-through method. Renewal rate for static renewal	Static Not applicable Not applicable	*A reproducible supply of toxicant is recommended. Consistent flow rate is usually 5-10 vol/24 hours; meter systems should be calibrated before and after study and checked twice daily during test period.*
Aeration, if any	No aeration during testing.	*Aeration is not recommended; OECD guideline recommends aeration. If aeration is necessary, test solutions must be analyzed periodically to verify exposure.*

Parameter	Details	Remarks *Criteria*
Test vessel		
Material: (glass/stainless steel)	Glass aquaria	*Test vessel size is usually 19 L (5 gal) or 30 x 60 x 30 cm.*
Size:	38 L	*Fill volume is usually 15-30 L of solution.*
Fill volume:	15L	
Source of dilution water Quality:	Natural seawater collected at Indian River Inlet, DE was filtered (25 µm) and diluted (to a salinity of approximately 20‰) with well water. Diluted seawater was aerated and filtered (0.45 µm) to remove microorganisms and fine particles.	*Recommended source of dilution water is soft, reconstituted water or water from a natural source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010.pdf) Dilution water should be intensely aerated before the study. OECD permits dechlorinated tap water*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature {Salinity for marine or estuarine species} Intervals of water quality measurement	Not reported 8.0-8.3 5.8-7.5 mg/L (≥ 74% saturation) Not reported Not reported See Appendix 2, p. 22 See Appendix 2, p. 22 Not reported 21.7-24.3°C 20‰ Every 24 hours	*Hardness:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 10 - 250 mg a.i./L)* *pH:* *EPA recommends 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); (OECD recommends pH 6.0 - 8.5)* *Dissolved Oxygen:* *EPA recommends: Static: ∃60% during first 48 hrs and ∃40% during second 48 hrs; flow-through: ∃60%; (OECD guideline recommends at least 80% saturation value).* *Temperature:* *EPA recommends 12EC for coldwater species, 17 or 22EC for warmwater species, and 22 ± 1 EC for estuarine/marine organisms. (OECD recommends 21 - 25°C for bluegill and 13 - 17°C for rainbow trout).* *Salinity:* *EPA recommends 30-34‰ (parts per thousand) for marine, 10-17‰ for estuarine fish, weekly range < 6‰.* *Water quality should be measured at beginning of test and every 48 hours.*
Number of replicates/groups: control: solvent control: treated ones:	2 Not applicable 2	*Recommended number of replicates includes a control and five treatment levels. Each concentration should be 60% of the next highest concentration; concentrations should be in a geometric series.*
Number of organisms per replicate /groups: control: solvent control: treated ones:	10 Not applicable 10	*Number of organisms per replicate should be ∃10/concentration; OECD guideline recommends at least 7 fish/concentration.*

Parameter	Details	Remarks *Criteria*
Biomass loading rate	0.27 g fish/L	*Recommended static conditions are # 0.8 g/L at # 17EC and # 0.5 g/L at > 17EC. Recommended flow-through conditions are # 1 g/L/day. OECD recommends a maximum of 1 g fish/L for static and semi-static, while higher rates are recommended for flow-through.*
Test concentrations: nominal: measured:	16, 26, 43, 72, and 120 mg ai/L 16, 27, 45, 75, and 123 mg ai/L	
Solvent (type, percentage, if used)	Not applicable	*The solvent should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD recommends that the solvent not exceed 100 mg a.i./L.*
Lighting	16-hours light/8-hours dark, with a 30-minute transition period.	*The recommended photo period is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD recommends a photo period of 12 -16 hours.*
Feeding	Animals were not fed during testing.	*Fish should not feed during the study.*
Recovery of chemical Frequency of determination Level of quantization Level of detection	99.8 ± 1.19% of nominal Concurrently with test samples 5.0 mg a.i./L Not reported	Based on matrix spikes (at 10.0, 40.0, and 120 mg a.i./L) analyzed concurrently with the samples.
Positive control {if used, indicate the chemical and concentrations}	Not applicable	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks
		Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	Every 24 hours	
		Observation intervals should be a minimum of every 24 hours.
Were raw data included?	Yes, sufficient	
Other observations, if any	None	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

During the 96-hour test, no mortalities were observed. The NOAEC based on mortality was 123 mg ai/L, the highest concentration tested.

Table 3: Effect of Orthosulfamuron (IR5878) on Mortality of Sheepshead Minnow.

Treatment (mg ai/L) measured and (nominal) concentration used	No. of fish at start of study	Observation period					
		Day 1		Day 2		Day 4	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Control (dilution water only)	20	0	0	0	0	0	0
16 (16)	20	0	0	0	0	0	0
27 (26)	20	0	0	0	0	0	0
45 (43)	20	0	0	0	0	0	0
75 (72)	20	0	0	0	0	0	0
123 (120)	20	0	0	0	0	0	0
NOAEC		123		123		123	
LC_{50}		>123		>123		>123	
Positive control, if used mortality: LC_{50}:		NA		NA		NA	

NA= Not applicable

B. NON-LETHAL TOXICITY ENDPOINTS:

During the 96-hour test, no sublethal effects were observed. The NOAEC based on sublethal effects was 123 mg ai/L, the highest concentration tested.

Table 4: Sub-lethal Effect of Orthosulfamuron (IR5878) on Sheepshead Minnow.

Treatment (mg ai/L) measured and (nominal) concentration used	Observation period		
	Day 1	Day 2	Day 4
	% affected	% affected	% affected
Control (dilution water only)	N	N	N
16 (16)	N	N	N
27 (26)	N	N	N
45 (43)	N	N	N
75 (72)	N	N	N
123 (120)	N	N	N
NOAEC	123	123	123
LOAEC	>123	>123	>123
EC_{50}	>123	>123	>123
Positive control, if used % sublethal effect: EC_{50}:	NA	NA	NA

N= Appears normal
NA= Not applicable

C. REPORTED STATISTICS:

The 96-hour LC_{50} and NOAEC were visually determined, due to the lack of treatment-related mortality or sub-lethal effects at any treatment level.

96-Hour
LC_{50}: >123 mg ai/L 95% C.I.: Not applicable

NOAEC: 123 mg ai/L

Probit Slope: Not applicable 95% C.I.: Not applicable

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} based on mortality and the NOEC and LOEC values based on mortality and sub-lethal effects were determined visually due to a lack of treatment related effects at any level during the definitive exposure period.

LC_{50}: >123 mg ai/L 95% C.I.: Not applicable

NOAEC: 123 mg ai/L

Probit Slope: Not applicable 95% C.I.: Not applicable

E. STUDY DEFICIENCIES:

The mean fish weight of 0.40 g was determined from ten negative control fish at study termination and was less than the required initial weight range of 0.5-5 g.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to the study authors'.

It was not clear if the concentrations were corrected for purity, but the reviewer assumed that they were.

G. CONCLUSIONS:

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with a marine fish (§72-3a). Although the mean weight of the organisms obtained from ten negative control fish at study termination was 0.40 g, which is less than the required initial weight range of 0.5 to 5 g, this deviation does not affect the validity of the study. This study is classified ACCEPTABLE. Based on the results of this study, Orthosulfamuron (IR5878) Technical is categorized as practically nontoxic to juvenile Sheepshead minnow (*Cyprinodon variegatus*) on an acute toxicity basis.

III. REFERENCES:

U.S. Environmental Protection Agency. 1996. Series 850 - Ecological Effects Test Guidelines (draft), OPPTS Number 850.1075: Fish Acute Toxicity Test, Freshwater and Marine.

U.S. Environmental Protection Agency. 1985. Standard Evaluation Procedure, Acute Toxicity Test for Estuarine and Marine Organisms (Estuarine Fish 96-Hour Acute Toxicity Test). Hazard Evaluation Division. Office of Pesticide Programs. EPA-540/9-85-006. Washington, DC.

ASTM Standard E 729-88a. 1994. Standard Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials.