Data Evaluation ~~Report~~ Record on the Acute Toxicity of IR5878 (Orthosulfamuron) to Freshwater Invertebrates - *Daphnia magna*

PMRA Submission Number{.....} EPA MRID Number 46219044

Data Requirement:

PMRA DATA CODE	
EPA DP Barcode	D304186
OECD Data Point	
EPA MRID	46219044
EPA Guideline	§72-2a

Test material: IR5878 **Purity:** 98.54 ± 0.51%
Common name: Orthosulfamuron
Chemical name: IUPAC: 2-[[[[[(4,6-dimethoxy-2-pyrimidinyl)amino]carbonyl]amino]sulfonyl]amino]-N,N-dimethylbenzamide
CAS name: Not reported
CAS No.: 213464-77-8
Synonyms: N/A

Primary Reviewer: John Marton
Staff Scientist, Dynamac Corporation
Signature:
Date: 12/14/04

QC Reviewer: Gregory S. Hess
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Date: 7/31/06

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Company Code:
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CITATION: Hertl, J. 2000. Final Report: Acute toxicity of IR2878 to *Daphnia magna* in a 48-hour immobilization test. Unpublished study performed by Institut für Biologische Analytik und Consulting IBACON GmbH, Arheilger Weg, Rossdorf, Germany. Laboratory Project Identification No. 8222220. Study sponsored by ISAGRO SpA, 20153 Centro Uffici San Siro - Fabbricato D, Milano, Italy. Study initiated May 10, 2000 and completed June 27, 2000.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of Orthosulfamuron to the freshwater invertebrate, *Daphnia magna*, was studied under static conditions. Daphnids were exposed to the test material at nominal concentrations of 0 (negative control) 6.25, 12.5, 25, 50 and 100 ppm a.i.. Mean-measured concentrations for the control and the two highest treatment levels (the only two levels analytically verified) were <1.7 (<LOQ, negative control), 47.0 and 97.3 ppm a.i., resulting in 94 and 97.3% of nominal recoveries, respectively.

After 48 hours of exposure, 20% mortality was observed at the 97.3 ppm a.i. level, the highest treatment level tested. No mortality was observed at the control or lower treatment levels. The 48-hour EC_{50} was >97.3 ppm a.i., which categorizes IR5878 (Orthosulfamuron) as practically non-toxic to Daphnids (*Daphnia magna*) on an acute toxicity basis. The NOAEC for mortality/immobility was 47.0 ppm a.i., the second highest treatment level tested.

This study is scientifically sound and satisfies the guideline requirements for an acute toxicity study with freshwater invertebrate (§72-2a), *Daphnia magna*. Consequently, this study is classified as ACCEPTABLE.

Results Synopsis

Test Organism Age (eg. 1st instar): <24 Hours
Test Type (Flow-through, Static, Static Renewal): Static

48-Hour
EC_{50}: >97.3 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOAEC: 47.0 ppm a.i.
LOAEC: 97.3 ppm a.i.

Endpoints Affected: Mortality/immobility

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the Commision Directive 92/69/EEC, Annex Part C, C.2: "Acute Toxicity for *Daphnia*", Official Journal of the European Communities No. L 383 A, December 29, 1992; OECD Guideline for Testing of Chemicals, Section 2, No 202: "*Daphnia sp.*, Acute Immobilization Test and Reproduction Test," Part I adopted April 04, 1984; and EPA Guideline 712-C-96-114: OPPTS 850.1010, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids", April 1996. Deviations from §72-2a included:

1. The pre-test health and feeding regime of the daphnid laboratory cultures were not reported.
2. It was not reported whether or not the test vessels were aerated during the definitive exposure period.
3. The fill volume of test medium (80 mL) was less than recommended (200 mL).

4. The hardness (250 mg as $CaCO_3$/L) of the dilution water was higher than recommended (40 - 48 mg/L as $CaCO_3$).

5. The pH range (6.9-7.9) exceeded recommendations (7.2-7.6).

6. The results of the EPA recommended periodic screening of the dilution water for contaminants such as metals, pesticides, residual chlorine, TOC, and particulate matter were not reported.

7. The temperature (21°C) during testing was slightly higher than recommended and was not measured continuously or every 6 hours (if a water bath was used to control the temperatures within the test vessels).

8. The loading rate was not provided.

9. The study author did not report whether or not the test organisms were fed during the definitive test.

10. Not all treatment concentrations were analytically verified at test initiation and termination.

The above deviations were considered minor and did not affect the validity or acceptability this study, note section **E. STUDY DEFICIENCIES:** below for further details.

COMPLIANCE: Signed and dated GLP, No Data Confidentiality, and Quality Assurance statements were provided. The study followed the OECD Principles of Good Laboratory Practice (1997) and Chemikaliengesetz (Chemical Act) der Bundesrepublik Deutschland (Chem G), Anhang 1, 1994/97.

A. MATERIALS:

1. Test Material IR5878 (Orthosulfamuron)

Description: White solid

Lot No./Batch No. : FCF/T/168-00 (ex 20525-03-9)

Purity: 98.54 ± 0.51%

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 determinations at 0 and 48 hours for the two highest treatment levels (nominal 50 and 100 ppm a.i.). The measured recoveries for the nominal 50 and 100 ppm a.i. treatment levels at 0-hours were 93-99% of nominal concentrations and the 48-hours were 89-99% of nominal, with no evidence of instability.

Storage conditions of test chemicals: The test chemical was stored at room temperature in the dark.

OECD requires water solubility, stability in water and light, pK_a, P_{ow}, and vapor pressure of the test compound. The following OECD requirements were reported:

Solubility: Approx. 200 ppm in water
Stability: Stable in water for at least 96 hours.

2. **Test organism**:

Species: *Daphnia magna* (Straus), clone 5

Age at test initiation: 7-23.5 hours

Source: In-house laboratory cultures originally supplied in 1997 by the Umweltbundesamt, Institut für Wasser-, Boden- und Lufthygiene, Berlin, Germany.

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: The concentrations for the definitive test were based on the results of a range-finding test. The results of the 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)	Continuous laboratory cultures were maintained. Same as test conditions Not reported Not reported	*EPA requires 7 day minimum acclimation period.*
Duration of the test	48 hours	*EPA requires 48 hours*
Test condition - static/flow through Type of dilution system (for flow through method) Renewal rate (for static renewal)	Static N/A N/A	*EPA requires consistent flow rate of 5 - 10 volumes/24 hours, meter systems calibrated before study and checked twice daily during test period.*
Aeration, if any	Not reported	
<u>Test vessel</u> Material: *(glass/stainless steel)* Size: Fill volume:	Glass beaker 250 ml 80	The fill volume of test medium (80 mL) was less than recommended (200 mL). *EPA requires: size 250 ml or 3.9 L fill 200 ml*
Source of dilution water	The dilution water was deionized water to which analytical grade salts were added.	Original source of water prior to deionization was not reported and a screening for contaminants was apparently not performed. *EPA requires soft reconstituted water or water from a natural source, not*

Parameter	Details	Remarks *Criteria*
		dechlorinated tap water.
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine Interval of water quality measurements	250 mg $CaCO_3$/L 6.9-7.9 9.4-10.8 mg/L (>60% saturation) 21°C Not reported Not reported Not reported Not reported Not reported DO and pH were measured in all test media of all treatment levels and the control at test initiation and termination. Temperature was measured at the same time as DO and pH but was only measured in the test medium of one control beaker. Hardness was determined prior to test initiation.	The hardness (250 mg $CaCO_3$/L) was higher than recommended. The pH range (6.9-7.9) exceeded recommendations (7.2-7.6). Screening for contaminants such as metals, pesticides, chlorine, and particulate matter were apparently not performed. The temperature (21°C) was slightly higher than recommended and was not measured every 6 hours.
		EPA requires: *hardness: 40 - 48 mg/L as $CaCO_3$* *pH: 7.2 - 7.6* *-Temperature: 20°C (measured continuously or if water baths are used, every 6 hr, may not vary > 1°C* *Dissolved oxygen:* *Static: ≥ 60% during 1st 48 hr and ≥ 40% during 2nd 48 hr* *Flow-through: ≥60%*
Number of organisms per replicate Solvent control: Negative control: Treatments:	N/A 10 10	The loading rate was not provided.
		EPA requires 5 treatment levels plus control with a minimum of 20 daphnid per treatment. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17 °C, ≤ 0.5 g/L at > 17 °C; flow-through: ≤ 1 g/L/day.
Number of replicates Solvent control: Negative control: Treatments:	N/A 2 2	
Treatment concentrations nominal: measured:	0 (negative control), 6.25, 12.5, 25, 50 and 100 ppm a.i.. <1.7 (<LOQ, negative control),	0- and 48-Hour measured treatment concentrations were only reported for the two highest treatment levels (nominal 50 and 100 ppm a.i.)

Parameter	Details	Remarks
	47.0 and 97.3 ppm a.i..	***Criteria***
		EPA requires a geometric series with each concentration being at least 60% of the next higher one.
Solvent (type, percentage, if used)	None used	
		EPA requires solvents not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-though tests.
Lighting	16 hours light:8 hours dark, sudden transitions from light to dark were avoided.	Light intensity was 540 Lux.
		EPA requires 16 hours light, 8 hours dark.
Feeding	Not Reported.	
		EPA/OECD requires: No feeding during the study
Stability of chemical in the test system	Verified by analytical determination at 0- and 48-Hours for the two highest treatment levels only.	
Recovery of chemical Frequency of measurement: Level of Quantitation Level of Detection	86-107% of nominal 0 and 48 hours 1.7 ppm a.i. 0.5 ppm a.i.	Based on fortified samples at 10, 20 and 100 ppm a.i., analyzed concurrently with the samples from the test media.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
		Criteria

Parameters measured including the sub-lethal effects	Mortality	
Observation intervals	24 and 48 hours	
Were raw data included?	Yes, sufficient	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY

After 48 hours of exposure, mortality was 20% at the mean-measured 97.3 ppm a.i. treatment level. No mortality or adverse effects were observed in the negative control group or mean-measured ≤47.0 ppm a.i. treatment groups. The 48-Hour EC_{50} was >97.3 ppm a.i.. The NOAEC and LOAEC values for mortality were 47.0 and >97.3 ppm a.i., respectively. Mortality and immobility were considered synonymous by the reviewer in this particular study (MRID # 46219044). The above toxicity values were determined by the study author in terms of the nominal treatment concentrations, however, the reviewer converted these values to reflect the mean-measured treatment concentrations (see attached Excel spreadsheet for calculations).

Table 3: Effects of IR5878 (Orthosulfamuron) on Mortality of *Daphnia magna*.

Treatment, ppm a.i. Mean-Measured and (Nominal) Concn.	Observation Period			
	24-Hours		48-Hours	
	No. Dead	% Mortality	No. Dead	% Mortality
Dilution water control	0/20	0	0/20	0
*(6.25)	0/20	0	0/20	0
* (12.5)	0/20	0	0/20	0
* (25)	0/20	0	0/20	0
47.0 (50)	0/20	0	0/20	0
97.3 (100)	4/20	20	4/20	20
NOEC, ppm a.i.	47.0		47.0	
LOEC, ppm a.i.	97.3		97.3	
LC_{50}/EC_{50} (with 95% C.I.), ppm a.i.	>97.3		>97.3	

* The nominal 6.25, 12.5, and 25 ppm a.i. treatment levels were not analytically verified at any time during the definitive test because these treatment levels were considered to be below the 48-hour NOEC value, determined in this study (p. 18).

B. SUB-LETHAL TOXICITY ENDPOINTS:

After 48 hours, no sub-lethal effects were observed in the control group or any of the treatment groups.

C. REPORTED STATISTICS:

The 48-hour EC_{50}, NOAEC and LOAEC values were visually determined from the mortality/immobility data due to the low toxicity of the test material up to the highest concentration tested. All toxicity values were determined and reported in terms of the nominal treatment concentrations by the study author. However, the reviewer converted these values to reflect the mean-measured treatment concentrations (see attached Excel spreadsheet for calculations).

48-Hour
EC_{50}: >97.3 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 47.0 ppm a.i.
LOEC: 97.3 ppm a.i.

Endpoints Affected: Mortality/immobility

D. VERIFICATION OF STATISTICAL RESULTS:

The 48-hour EC_{50}, NOAEC , and LOAEC values were visually determined due to the lack of $\geq$50% mortality/immobility at any of the treatment levels and because mean-measured treatment concentrations could not be calculated by the reviewer due to the lack of analytical data for all nominal treatment levels <50 ppm a.i.. All toxicity values were determined in terms of the reviewer-determined mean-measured treatment concentrations.

48-Hour
Mortality:
EC_{50}: >97.3 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 47.0 ppm a.i.
LOEC: 97.3 ppm a.i.

Endpoints Affected: Mortality/immobility

E. STUDY DEFICIENCIES:

Only the two highest nominal concentrations (50 and 100 ppm a.i.) were analytically verified during the study period. The study author noted that the nominal 6.25 to 25 ppm a.i. treatment concentrations were not analytically verified because they were below the 48-hour NOAEC determined for this test. The reviewer considers the fact that not all treatment levels were analytically verified a significant deviation. However, since treatment-related effects were limited to the highest treatment level and the NOAEC was determined to be the second highest treatment level by the reviewer and study author, the reviewer considers this deviation minor and does not affect the validity or acceptability of this study.

All other deficiencies/deviations were also considered minor and did not affect the validity or acceptability of the definitive test.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study author, i.e. the study author's conclusions based

on the nominal treatment concentrations corresponded exactly to the reviewer's conclusions based on the reviewer-determined mean-measured treatment concentrations.

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-2a, and is classified as ACCEPTABLE. The 48-hour EC_{50} was >97.3 ppm a.i., which categorizes IR5878 (Orthosulfamuron) as practically non-toxic to the daphnid, *Daphnia magna*, on an acute toxicity basis. The NOAEC for mortality and sub-lethal effects (same conclusions) was 47.0 ppm a.i.

48-Hour
Mortality:
EC_{50}: >97.3 ppm a.i. 95% C.I.: N/A
Slope: N/A
NOEC: 47.0 ppm a.i.
LOEC: 97.3 ppm a.i.

Endpoints Affected: Mortality/immobility

III. REFERENCES:

Chemikaliengesetz der Bundesrepublik Deutschland (ChemG). Anhang 1, in der Fassung der Bekanntmachung vom 25. Juli 1994 (BGB1. I S. 1703) mit Änderungen vom 27. September 1994 (BGB1. I S 2705) und 14. Mai 1997 (BGB1 I S 1060).

Commission Directive 92/69/EEC, Annex Part C, C.2: "Acute Toxicity for Daphnia," Official Journal of the European Communities No. L 383 A, dated December 29, 1992.

EPA Guideline 712-C-96-114: OPPTS 850.1010, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" April 1996.

OECD Guideline for Testing of Chemicals, Section 2, No. 202: " *Daphnia sp.*, Acute Immobilization Test and Reproduction Rest", Part 1, adopted April 04, 1984.

The OECD Principles of Good Laboratory Practice, adopted by Council on 26th November 1997 [C(97)186/Final], Environment Directorate, Organization for Economic Co-operation and Development, Paris 1998).