Data Evaluation Report on the 28-day Sub-chronic Toxicity of AE F130060 Technical to the Rainbow Trout (*Oncorhynchus mykiss*).

PMRA Submission Number{.....} EPA MRID Number 45386232

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D284719
OECD Data Point	
EPA MRID	45386232
EPA Guideline	N/A (OECD Guideline No. 204)
OPPTS Guideline	850.1075

1/9/04

Test material: AE F 130060 Technical **Purity:** 94.6%
Common name: Mesosulfuron-methyl
Chemical name: IUPAC: Methyl 2-[3-(4,6-dimethoxyprimidin-2-yl)ureidosulfonyl]-4-methanesulfonamidomethylbenzoate
CAS name: Not reported
CAS No.: Not reported
Synonyms: Code: AE F130060 00 1C95 0001

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Date: 8/22/03

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Date:

Reference/Submission No.:

Company Code:
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EPA PC Code: 122009

Date Evaluation Completed:

CITATION: Sowig, P., *et al.* 2000. Effects on Juvenile Growth of Rainbow Trout (*Oncorhynchus mykiss*) in a 28 Days Static Renewal System, AE F130060; Substance, Technical. Unpublished study performed by Aventis CropScience GmbH, Frankfort, Germany. Laboratory Study Identification CE97/097. Study submitted by Aventis CropScience, Research Triangle Park, NC. Study initiated January 19, 1999 and completed July 3, 2000.

EXECUTIVE SUMMARY:

In a 28-day sub-chronic toxicity study, 2-month old Rainbow trout (*Oncorhynchus mykiss*) were exposed to AE F130060 Technical (Mesosulfuron-methyl) under static renewal conditions at nominal concentrations of 0 (negative control), 0.32, 1.0, 3.2, 10, and 32 ppm. The mean-measured concentrations were 0 (control), 0.32, 0.94, 2.9, 9.3, and 29.6 ppm a.i.

No mortality was observed in the control or treatment groups. The 28-day LC_{50} value was >29.6 ppm a.i. No clinical signs of toxicity or treatment-related effects on terminal length or weight were observed. Based on sub-lethal effects, the NOEC was 26.9 ppm a.i., the highest concentration tested.

The study is scientifically sound; however, it was not designed to fulfill any current U.S. EPA FIFRA guideline. This study is therefore classified SUPPLEMENTAL, as it provides useful information on the 28-day sub-chronic toxicity of AE F130060 Technical (Mesosulfuron-methyl) to the Rainbow trout (*Oncorhynchus mykiss*).

Results Synopsis:

Test Organism Size/Age (mean Weight or Length):	2 months old; means of 4.21-4.28 cm and 1.34-1.38 g
Test Type (Flow-through, Static, Static Renewal):	Static Renewal

28-Day Survival:
LC_{50}: >29.6 ppm a.i.
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

Toxic Effects:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

28-Day Weight:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

28-Day Length:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined in OECD Guideline No. 204 (1984), OECD Draft Guideline (1994), and ISO 10229 (1994). This study was not designed to fulfill any current U.S. EPA FIFRA guideline. General deviations from FIFRA guidance involving acute (§72-1c) and/or early life stage (§72-4a) toxicity studies with Rainbow trout included:

1. The test chemical storage conditions were not reported.

2

2. The pre-test health (including mortality) of the fish were not provided.

3. Test water was aerated from Days 19-28 because the oxygen saturation fell below 60%.

4. The water hardness in terms of mg/L as $CaCO_3$ was not provided.

5. Aside from initiating aeration on Day 19 due to oxygen levels <60% saturation, dissolved oxygen was not provided in terms of percent saturation.

6. The water parameters of total organic carbon, particulate matter, metals, pesticides, chlorine were not reported.

These deviations do not affect the scientific validity of the study. This study was not designed to fulfill any current U.S. EPA FIFRA guideline.

COMPLIANCE: Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. This study was conducted in accordance with OECD principles of GLP (p. 3).

A. MATERIALS:

1. Test Material AE F 130060 Technical (Mesosulfuron-methyl)

Description: Light beige powder

Lot No./Batch No. : Code: AE F130060 00 1C95 0001

Purity: 94.6%

Stability of Compound Under Test Conditions: The test material was stable in the test systems. Mean-measured concentrations in fresh and aged test water ranged from 92.1 to 108.9% of nominal concentrations, with no pattern of decline (Tables 6.2.2 and 6.2.4, pp. 27 and 29).

Storage conditions of test chemicals: Not reported.

OECD requires water solubility, stability in water and light, pK_a, P_{ow} and vapor pressure of the test compound. OECD requirements were not reported.

2. Test organism:

Species: Rainbow trout (*Oncorhynchus mykiss)*

Size/Age: 2-months old, means of 4.21-4.28 cm and 1.34-1.38 g

Source: Charles River Aquatics, Someren, Netherlands

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding Study: No range-finding study was reported.

b. Definitive Study:

Parameter	Details	Remarks *Criteria*
Acclimation period: Conditions (same as test or not): Feeding: Health (any mortality observed):	45 days (from arrival until test initiation) Same as test Standard trout food (Kronen-Fish, Aminostart) was provided 6 times a week at a ration level of 2 x 2% of initial fish weight/day. Not specified	
Duration of the test	28 Days	
Test conditions: static renewal/flow through: Type of dilution system- for flow through method. Renewal rate for static renewal:	Static renewal N/A N/A	
Aeration, if any	Due to declining (to <60% saturation) dissolved oxygen levels, the test water in the tanks were aerated from test Day 19 through test termination.	

Parameter	Details	Remarks *Criteria*
Test vessel Material: Size: Fill volume:	 Stainless steel 50 L (50 x 20 x 60 cm) 50 L fill volume (depths of 16.2 and 17.4 cm)	
Source of dilution water	Tap water and deionized water were combined at a 1:1 ratio, then sand- and charcoal-filtered, and well aerated prior to use.	
Water parameters: Hardness pH Dissolved oxygen Temperature Total Organic Carbon Particulate matter Metals Pesticides Chlorine	 1.61-1.98 as mmol/L (Ca^{2+} + Mg^{2+}) 7.0-8.1 4.6-11.5 mg/L 12.6-13.9°C Not reported Not reported Not reported Not reported Not reported	In acute Rainbow trout studies, (FIFRA §72-1c), EPA requires a water hardness of 40-48 mg/L as $CaCO_3$ and a pH range of 7.2-7.6. The water hardness in terms of mg/L as $CaCO_3$ was not provided. Aside from initiating aeration on Day 19 due to oxygen levels <60% saturation, dissolved oxygen was not provided in terms of percent saturation. Temperature was measured continuously in each aquarium; DO and pH were determined daily in each aquarium; and hardness was determined monthly in the dilution water.

Parameter	Details	Remarks *Criteria*
Concentration of test material: nominal: measured:	0 (negative control), 0.32, 1.0, 3.2, 10, and 32 ppm 0.0 (control), 0.32, 0.94, 2.9, 9.3, and 29.6 ppm a.i.	Fresh and/or aged water samples were collected and analyzed on Days 0, 7, 14, 17, 19, 20, 21, 24, 25, 26, 27, and 28. Mean-measured concentrations were reviewer-calculated from corrected (for purity) fresh and aged analytical data in Tables 6.2.2 and 6.2.4, pp. 27 and 29.
Solvent (type, percentage, if used)	N/A	
Number of fish/replicates: negative control: solvent control: treated:	20 fish, one replicate chamber N/A 20 fish/level: one replicate chamber per concentration	
Biomass loading rate	0.56 g/L (instantaneous)	
Lighting	16 hour light:8 hour dark photoperiod	
Feeding	Daily during the test, fish were fed dried standard trout food (Kronen-Fish, FB-50), about 2% of initial wet body weight per day, divided into two portions.	
Stability of chemical in the test system	Verified. Mean-measured concentrations in fresh and aged test water ranged from 92.1 to 108.9% of nominal concentrations, with no pattern of decline (Tables 6.2.2 and 6.2.4, pp. 27 and 29).	
Recovery of chemical Level of Quantitation Level of Detection	97.6-102.4% of nominal 0.04 ppm 0.02 ppm	Based on matrix spikes analyzed concurrently with the samples at each sampling interval (Tables 6.2.2 and 6.2.4, pp. 27 and 29).
Positive control {if used, indicate the chemical and concentrations}	N/A	

6

Parameter	Details	Remarks *Criteria*
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Parameters measured including the sublethal effects/toxicity symptoms	Survival, sub-lethal effects, and terminal length and weight.	
Observation intervals	Mortality and sub-lethal effects were observed daily. Length and weight were measured at test initiation and test termination.	
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

No mortality was observed in the control or treatment groups. The NOEC was 29.6 ppm a.i., the highest concentration tested.

Table 1: Effect of AE F130060 Technical on Mortality of Rainbow Trout (*Oncorhynchus mykiss*).

Measured and (nominal) concentrations (ppm a.i.)[1]	No. of fish at start of study	0-28 Days	
		No Dead	% mortality
Negative control	20	0	0
0.32 (0.33)	20	0	0
0.94 (1.0)	20	0	0
2.9 (3.2)	20	0	0
9.3 (10.0)	20	0	0
29.6 (32.0)	20	0	0
NOEC	29.6 ppm		
LC_{50}	>29.6 ppm		
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A

[1] Nominal values are in parentheses.

B. NON-LETHAL TOXICITY ENDPOINTS:

No abnormalities were observed in the control or treatment groups during the 28-day study. The NOEC was 29.6 ppm a.i., the highest concentration tested.

No significant effects on length or weight were observed in the treatment groups, compared to the control. The NOEC for both growth endpoints was 29.6 ppm a.i.

Table 2. Effect of AE F130060 Technical on growth of Rainbow Trout (*Oncorhynchus mykiss*).

Measured and (nominal) concentrations (ppm a.i.)[1]	Length (cm)		Weight (g)	
	Day 0	Day 28	Day 0	Day 28
Negative control	4.265	5.8150	1.3540	3.2310
0.33 (0.33)	4.275	5.9800	1.3535	3.5180
0.93 (1.0)	4.210	5.6450	1.3665	3.2530
2.9 (3.2)	4.245	5.8474	1.3350	3.2379
9.3 (10.0)	4.205	5.7400	1.3690	3.4765
29.6 (32.0)	4.265	5.7700	1.3745	3.3695
NOEC, ppm a.i.	29.6		29.6	
LOEC, ppm a.i.	>29.6		>29.6	
MATC, ppm a.i.	Not determined		Not determined	

[1] Nominal values are in parentheses.

C. REPORTED STATISTICS:

The General Linear Models Procedures, DUNCAN'S multiple range test was performed to evaluate the body weight and body length measurements. The LC_{50} value was estimated because no mortalities were observed. The NOEC and LOEC values were determined by a visual inspection of the data.

D. VERIFICATION OF STATISTICAL RESULTS:

There were no mortalities or symptoms of intoxication in this study and the change in fish weight was greater in the treatment groups than the control group. As a result, the NOEC and LOEC for these endpoints were determined visually. Fish length appeared to be unaffected by treatment, showing stimulation in most treatment groups. A Student's T-test was used to compare percent fish growth in the control group to percent fish growth in the treatment group which exhibited the greatest reduction in growth (1.0 mg/L). Results are based on the average of measured fresh and aged test solutions.

28-Day Survival:
LC_{50}: >29.6 ppm a.i.
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

Toxic Effects:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

28-Day Weight:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

28-Day Length:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

E. STUDY DEFICIENCIES:

Currently, there is no U.S. EPA requirement or guidance for a sub-chronic (28 day) freshwater fish toxicity study. The study deficiencies did not affect the scientific validity of the study, and therefore, this study is classified SUPPLEMENTAL because it provides useful information on the 28-day sub-chronic toxicity of Mesosulfuron-methyl to the Rainbow trout (*Oncorhynchus mykiss*).

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were similar to the study authors'. No endpoints were significantly affected by treatment with AE F130060. The study authors based toxicity values on the nominal concentrations, while the reviewer based them on the mean-measured concentrations.

The test concentrations did not exceed 32 mg/L, due to the limited solubility of the test substance in water.

The study authors reported that a flow-through design could not be installed due to the limited water solubility of the test substance (p. 19). Although the dissolved oxygen content fell below 60% on Day 19, and subsequent aeration was employed, this did not have any adverse affect on the concentrations of the test material.

G. CONCLUSIONS:

The study is scientifically sound; however, it was not designed to fulfill any current U.S. EPA FIFRA guideline. This study is therefore classified SUPPLEMENTAL, as it provides useful information on the 28-day sub-chronic toxicity of AE F130060 Technical (Mesosulfuron-methyl) to the Rainbow trout (*Oncorhynchus mykiss*).

28-Day Survival:
LC_{50}: >29.6 ppm a.i.
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

Toxic Effects:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

28-Day Weight:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

10

28-Day Length:
NOEC: 29.6 ppm a.i.
LOEC: >29.6 ppm a.i.

III. REFERENCES:

Organization for Economic Co-operation and Development. 1992. OECD Guideline for Testing of Chemicals; Guideline No. 204: "Fish Prolonged Toxicity Test: 14-day Study" adopted 4 April 1984.

Organization for Economic Co-operation and Development. 1994. OECD Draft guideline "Fish, Juvenile Growth Test - 28 Days" Nov. 1994.

International Organization for Standardization, ISO 10229: Water quality-Determination of the prolonged toxicity of substances to freshwater fish - Method for evaluating the effects of substances on the growth rate of rainbow trout [*Oncorhynchus mykiss* Walbaum (Teleostei, Salmonidae)]

U.S. Environmental Protection Agency (EPA). 1975. Committee on Methods for Toxicity Tests with Aquatic Organisms, Method for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians, EPA-660/3-75-009.

U.S. Environmental Protection Agency (EPA). 1975. Brauhn, J.L., *et al.* Acquisition and Culture Research Fish: Rainbow Trout, Fathead Minnow, Channel Catfish, and Bluegills. EPA-660/3-75-001.

Deutsches Institut fur Normung (DIN). 1989. German standard methods for the examination of water, waste water, and sludge. Normenausschuß Wasserwesen (NAW) im DIN Deutsches Institut fur Normung e.V. Berlin.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

t-Test: Two-Sample Assuming Equal Variances

	Control	*1.0 mg/L*
Mean	36.38439	34.12204
Variance	41.25324	73.49672
Observations	20	20
Pooled Variance	57.37498	
Hypothesized Mean Difference	0	
df	38	
t Stat	0.944493	
P(T<=t) one-tail	0.175442	
t Critical one-tail	1.685953	
P(T<=t) two-tail	0.350885	
t Critical two-tail	2.024394	

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