Data Evaluation Report on the Acute Toxicity of Florasulam to Terrestrial Vascular Plants: Vegetative Vigor and Seedling Emergence

PMRA Submission Number {.....} **EPA MRID Number 46808324**

Data Requirement:
PMRA Data Code: 9.8.6 (EP)
EPA DP Barcode:
OECD Data Point: IIA 8.12 (TGAI) and IIIA 10.8.1.1 (EP)
EPA Guideline:

Test material: XDE-570 **Purity:** TEP
Common name florasulam
Chemical name: IUPAC 2',6',8-trifluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonanilide
CAS name *N*-(2,6-difluorophenyl)-8-fluoro-5-methoxy[1,2,4]triazolo[1,5-*c*]pyrimidine-2-sulfonamide
CAS No. 145701-23-1
Synonyms

Primary Reviewer: Peter Takacs
PMRA **Date:** 10.10.2000

Primary Reviewer: Brian D. Kiernan, Biologist
EPA **Date:** 3.21.2007 10/8/07

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

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	129108	

Date Evaluation Completed: 3.06.2007

CITATION: Cordes, R. C. 1998. The Effects of DE-570 on the Emergence and Vegetative Vigor of Non-Target Terrestrial Plants (Tier II Study). Global Environmental Chemistry Laboratory, Indianapolis, Indiana. Testing Lab Name ENV98072. Study ID No. November 20, 1998. Full Date Dow AgroSciences Canada Inc. ENV98072. Volume No. 6. 216 Pages. Calgary, Canada. Unpublished.

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 terrestrial 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.

Data Evaluation Report on the Acute Toxicity of Florasulam to Terrestrial Vascular Plants: Vegetative Vigor and Seedling Emergence

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EXECUTIVE SUMMARY:

In a 21-day vegetative vigor and seedling emergence laboratory toxicity test, a total of four monocotyledonous [corn (*Zea mays*), oat (*Avena sativa*), onion (*Allium cepa*) and ryegrass (*Lolium spp.*)] and six dicotyledonous species [carrot (*Daucus carota*), cucumber (*Cucumis sativus*) radish (*Raphanus satirus*), soybean (*Glycina max*), sunflower (*Helianthus annus*) and tomato (*Lycopersicon esculentum*)] were exposed to a single application of EF-1343, a formulation containing XDE-570, to pre-planted pots in the seedling emergence test and foliar applied to established plants in the vegetative vigor test. The rates of EF-1343 applied to all species in both tests ranged from 0.014 g a.i./ha to10.0 g a.i./ha in a natural soil. The number seedlings emerged, total number of alive/dead plants, percent visual phytotoxicity, measurements of shoot length and shoot weight were examined. The discoloration, stunting and stand loss were evaluated in the visual phytotoxicity tests. This study was conducted in accordance with the US EPA Ecological Effects Test Guidelines, OPPTS 850.4225 and OPPTS 850.4250 and the EPA GLP standards.

The Agency's selected endpoints are slightly higher than those selected by the PMRA. The Agency only uses visual phytotoxic symptoms for qualitative characterization. The study is classified as supplemental due the high degree of variability in the seedling emergence test, but sufficient to allow a risk assessment for acute toxicity of florasulam to non-target terrestrial plants. EFED accepts the PMRA DER in lieu of the generation of a new DER., although the endpoints below were used in the Agency's risk assessment.

Results Synopsis

	Seedling Emergence	**Vegetative vigor**
Monocot		
EC_{25}/IC_{25}:	>0.0089 lb a.i/A	0.0006 lb a.i/A
NOEC:	0.089 lb a.i/A	0.0001 lb a.i/A
Most sensitive monocot:	none	onion
Most sensitive parameter	none	biomass
Dicot		
EC_{25}/IC_{25}:	>0.0089 lb a.i/A	0.00005 lb a.i/A
NOEC:	0.0011 lb a.i/A	0.000009 lb a.i/A
Most sensitive monocot:	radish	tomato/carrot
Most sensitive parameter	emergence	biomass/ shoot length

Appendix 9.8.4c
PMRA Reviewer: Peter Takacs 10-October-2000

Requirement: Required.

STUDY TYPE: Terrestrial Vascular Plant - Vegetative vigour and seedling emergence: monocotyledonous species: corn, (*Zea mays*), oat (*Avena sativa*), onion (*Allium cepa*) and ryegrass (*Lolium spp.*) and dicotyledonous species: carrot (*Daucus carota*), cucumber (*Cucumis sativus*) radish (*Raphanus satirus*), soybean (*Glycina max*), sunflower (*Helianthus annus*) and tomato (*Lycopersicon esculentum*).

PMRA DATA CODE: 9.8.4 (TGAI) and 9.8.6 (EP);
OECD Data Point IIA 8.12 (TGAI) and IIIA 10.8.1.2 (EP)

TEST MATERIAL: EF-1343 (52.9 g a.i./L)

SYNONYMS:

CITATION: Cordes, R. C. 1998. The Effects of DE-570 on the Emergence and Vegetative Vigor of Non-Target Terrestrial Plants (Tier II Study). Global Environmental Chemistry Laboratory, Indianapolis, Indiana. Testing Lab Name ENV98072. Study ID No. November 20, 1998. Full Date Dow AgroSciences Canada Inc. ENV98072. Volume No. 6. 216 Pages. Calgary, Canada. Unpublished.

SPONSOR: Dow AgroSciences LLC, 9330 Zionsville Road, Indianapolis, Indiana 46268-1054

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In the seedling emergence test, EF-1343 demonstrated no significant soil activity on any of the species tested, except radish. For all other species, the EC25 and EC50 values were all > 10 g ai/ha. Visual phytotoxicity ratings showed a dose-response on radish with an EC25 value of **4.3 g ai/ha**.

In the vegetative vigor test, the greatest phytotoxicity was observed on the dicot species and the least activity on the monocot species. In general visual phytotoxicity ratings provided the lowest EC values. The NOEC values for tomato, carrot, radish, sunflower, cucumber, and soybean, based on visual phytotoxicity, were <0.014, 0.041, 0.041, 0.014, 0.123, and 0.041 g a.i./ha, respectively. The EC25 values for the above species were 0.02, 0.09, 0.07, 0.04, 0.35, and 0.2 g a.i./ha, respectively. Therefore, the most sensitive species for plant vigor is **tomato** with an **EC25 of 0.02 g ai/ha.** These results were presented based on the nominal concentration. Based on this study, EF-1343 is, therefore, considered very highly toxic ($EC_{25} \leq 1.0$ g a.i./ha) to terrestrial dicot vascular plant vegetative vigour at concentrations from 0.014 g a.i./ha.

This acute toxicity study is classified as acceptable, and satisfies satisfy the data requirement for a terrestrial vascular plant vegetative vigour toxicity study (DATA CODE: 9.8.4).

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging Statements were provided.

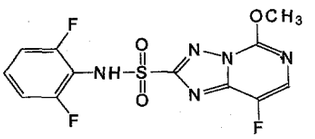
I. MATERIALS AND METHODS:

GUIDELINE FOLLOWED: US EPA Ecological Effects Test Guidelines, OPPTS 850.4225 and OPPTS 850.4250

A. MATERIALS:

1. Test Material: EF-1343

Description: formulated, soluble concentrate
CAS #:145701-23-1
IUPAC name: 2′,6′,8-trifluoro-5-methoxy-s-triazolo[1,5-c]pyrimidine-2-sulphonanilide
Chemical formula: $C_{12}H_8O_3N_5F_3$
Molecular weight: 359.3
Lot/Batch #: TSN 101352, lot no. C0267-44-A
Purity: 52.9 g/L
Water solubility:121 mg/L
pK_a: 4.54
K_{ow}: 0.06
Mode of phytotoxic action: Acetolactate synthase (ALS) inhibitor
Structure:



2. Test organisms:

Monocotyledonous species: corn, (*Zea mays*), oat (*Avena sativa*), onion (*Allium cepa*) and ryegrass (*Lolium spp.*)

Dicotyledonous species: carrot (*Daucus carota*), cucumber (*Cucumis sativus*) radish (*Raphanus satirus*), soybean (*Glycina max*), sunflower (*Helianthus annus*) and tomato (*Lycopersicon esculentum*).

Seed source: not specified

Seed storage: not specified

Seed treatment: none

Pre-exposure conditions:not specified

Pre-exposure period: not specified

B. STUDY DESIGN:

1. Soil:

Table 1: Physicochemical properties of natural soil used in the vegetative vigour studies.

Property	Natural Soil Characteristics	Method
Geographic location	not stated	
Soil type	loam	
Soil depth	not stated	
Soil preparation	not stated	
Texture	32% sand 48% silt 20% clay	
Classification	not stated	
pH	__:__ soil:water	stated to have an overall soil pH of 5.8
Organic carbon (%)	2.7% organic matter	

Moisture at 1/3 atm (%)	30.8	

2. Experimental conditions:

Table 2: Experimental design.

Experimental Design Parameters		Value/Type	Remarks
Storage conditions of soil		not stated	
Average weight of soil per replicate		weight not given,	pots were 10.2 x 12 cm
Controls	Negative	water	
	Solvent control	non-ionic surfactant (Agral 90)	
Number of seeds in each replicate		12	
Test concentrations [g a.i./ha]		0.014, 0.041, 0.123, 0.37, 1.11, 3.33, and 10.0	
Pesticide addition method		sprayed onto soil for seedling emergence and sprayed onto established plants for vegetative vigour. An overhead track sprayer was used, delivering 187L/ha.	
Timing of pesticide application		test pots were sprayed within 24 hours of seed planting or when seedling had emerged for the vigour test.	
Method of analytical verification		HPLC/UV	
Number of replicates	Control	3 replicates with 12 plants/seeds each*	

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	Treatments	3 replicates with 12 plants/seeds each	
Test conditions	Test duration	3 weeks	
	Test container (volume, composition (e.g., glass, polystyrene, etc.))	Each replicate included two pots of size 10.2 x 12 cm	
	Incubation facility	green house	
	Watering regime	dependent on species (0-120 mL/day/pot)	
	Fertilizing regime	Peters 20-20-20 was applied 2-3 times during the study in irrigation water at a rate of 2 tbl/gal of water.	
	Temperature (°C)	18-39	
	Photoperiod	16h light: 8h dark	
	Light fluence rate	1000-1200 μEm^{-2}	
	Light wavelengths	not specified	
	Light source	natural sunlight and high pressure sodium lamps.	

*: Note that several of the treatment groups in the seedling emergence test had fewer than the nominal number of seeds per treatment and some replicates had only half the number of seeds due to human error (see pg. 13 of study).
**Fluence rate = flow rate of light, flux of light, or the amount of light per unit area per unit time. It is sometimes referred to as light intensity, although this is not a desirable term. The photon fluence rate is given in $\mu mol\ m^{-2}\ s^{-1}$.

3. Observations:

Table 3: Observations

Observation Parameters		Details	Remarks/Methods
Test dates	Initiation	24-June-98 and 09-July-98	
	Termination	15-30-July-98	
Observation or sampling intervals		Three evaluations were made for both the emergence and vigour tests one week apart	
Measurement endpoint parameter(s)		number of seedlings emerged, number of live/dead plants at the end of the study, percent visual phytotoxicity, shoot length and shoot fresh weight at the end of the study, composite fresh weight of all live and dead plants.	Visual phytotoxicity was based on a subjective scale ranging from 0-100% effect.

No additional observations were made.

4. Statistical Analysis: All data were analysed using the Minitab statistical software package. All data was initially analysed using ANOVA (all treatments and controls) with Bonferroni means separation to determine whether there were any significant differences between the water and the surfactant controls. All statistics were performed at a 5% level of significance. For either the seedling emergence test or the vegetative vigour test, no significant differences were found between the control and adjuvant blank for any species or any evaluation. Thus, the water control and adjuvant blank treatments were pooled for additional ANOA to determine whether EF-1343 produced any significant effects on emergence, survival, shoot length, and fresh shoot weight or visual phytotoxicity as compared to the pooled controls. For those species which exhibited a statistically significant effect, and /or if a reasonably clear indication of a dose response to EF-1343 was observed, then a four parameter logistics model was fit to the data using least squares non-linear regression. Estimates of EC05, EC25 and EC50 values were calculated using the statistical model. Counts of seed emergence, survival, shoot length and shoot weight had to be transformed to percent effects in order to calculate EC05, EC25 and EC50 values. These calculations were done using Microsoft Excel. The percent effects on the above parameters were determined by comparing replicate means of pooled controls to treated plants. EC05, EC25 and EC50 values were calculated for the most sensitive endpoint only. These values were not extrapolated beyond the maximal range of effects.

II. RESULTS AND DISCUSSION:

Seedling emergence: Except for radish, no significant biological effects on the seed emergence of any species was observed. Except for radish, all EC values were determined to be > 10.0 g a.i./ha. For radish, there was no significant separation of treatment means, however, there was a reasonable indication of a dose response with the rates of EF-1343 at 1.11, 3.33 and 10.0 g a.i./ha causing 4, 10 and 13% reductions in plant emergence at one week after application. The test chemical showed no significant biological effect on the survival of plants after emergence for any of the test species. Visual phytotoxic effects were only significant in radish, having EC05 and EC50 values of 0.87 and 4.3 g a.i./ha, respectively. Carrot, sunflower and tomato showed slight effects at the high concentration of 10 g a.i./ha. There were no significant effects on the shoot length or weight of any of the test species.

Vegetative vigour: EF-1343 showed significant effects on the survival of several test species following foliar application. The small seeded dicot species (carrot, radish and tomato) showed the greatest effects with EC25 values below 0.8 g a.i./ha. Lesser effects were observed in the large seeded dicot species and the monocot species with EC25 values of 8.7 g a.i./ha for cucumber and > 10.0 g a.i./ha for all other species. Shoot length of the dicot species were most effected by a foliar application of EF-1343, especially carrot, sunflower, and tomato which had EC25 values less than 0.15 g ai/ha and EC50 values less than 0.4 g ai/ha. Of the monocot species tested, onion was the most sensitive with EC25 value of 4.7 g ai/ha and EC50 > 10.0 g ai/ha. The shoot weight of the dicot species were most effected by the test chemical, especially carrot, radish, sunflower and tomato which had EC25 values less than 0.2 g ai/ha and EC50 values less than 0.4 g ai/ha. Onion was the most sensitive monocot species with an EC25 value of 0.69 g ai/ha and an EC50 value of 3.5 g ai/ha. Tomato visual phytotoxic effects was the most sensitive dicot species endpoint, having an EC25 value of 0.02 g a.i./ha. Significant effects on visual phytotoxicity in tomato were observed even at the lowest test concentration of 0.014 g a.i./ha.

Generally, visual phytotoxicity was the most sensitive endpoint, while the sensitivity ranking of the test species was (most sensitive to least):
tomato>carrot≥radish≥sunflower>cucumber>soybean>onion>ryegrass>corn>oat.

A. Residue analysis:

Table 4: Concentrations of EF-1343 used in the terrestrial vascular plant seedling emergence/vegetative vigour toxicity test. Note that only the three highest test concentrations were analysed. Actual application rates were estimated based on analysis of tank mix concentrations and sprayer calibration.

Treatment	Nominal Concentration (g a.i./ha)	Actual Application Rate (g a.i./ha) (Validated by chemical analyses)*
		Initial
Negative control	0	0
Solvent control	0	0
Treatment 1	0.014	-
Treatment 2	0.041	-
Treatment 3	0.123	-
Treatment 4	0.37	-
Treatment 5	1.11	1.10/1.13
Treatment 6	3.33	3.31/3.39
Treatment 7	10	9.95/10.21

* Validated by HPLC/UV analysis.

Table 5: Stability of the test compound.

Stability Parameter	Half-life
Shelf life:	No change after 2 weeks at 54°C.
Hydrolysis:	
Photolysis in water:	
Aerobic biotransformation in water:	
Anaerobic biotransformation in water:	

B. Inhibitory effects: Seedling emergence was unaffected in all but one species tested (radish). EF-1343 had the greatest postemergence activity on dicot species, especially carrot, radish, sunflower and tomato, which consistently had EC25 values less than 0.3 g ai/ha. Monocot species were less affected compared to dicot species. In the seedling emergence test visual phytotoxic effects were only significant in radish, having EC05 and EC50 values of 0.87 and 4.3 g a.i./ha, respectively.

Table 6: Effect of EF-1343 on carrot, terrestrial vascular plant vegetative vigour.

Treatment (nominal concentration: g a.i./ha)	**Observation period**	
	Week 3	
	Shoot Length	**% Inhibition**
Negative control	236	0
Solvent control	235	0
0.014	253	8
0.041	235	0
0.123	188	-20
0.37	100	-58
1.11	66	-72
3.33	62	-74
10	47	-80

See study report for other endpoints (pg. 35-59).

Table 7: Statistical endpoint values for carrot in the vegetative vigour tests.

Statistical Endpoint	**Value for Shoot Length**	**Value for visual phytotoxicity**	**Value for shoot weight**	**Value for survival**
NOEC (g a.i./ha)	0.041	0.041	0.041	0.37
LOEC (g a.i./ha)	0.123	0.123	0.123	1.11
EC_{50} (g a.i./ha)	0.39	0.17	0.12	1.1
EC_{25} (g a.i./ha)	0.12	0.09	0.06	0.77

C. <u>Other effects</u>: In the seedling emergence tests, stimulation was observed in several of the dicot and monocot test species at concentrations of up to 10.0 g a.i./ha. In the vegetative vigour tests, monocot species were stimulated at up to 10.0 g a.i./ha.

Table 8: Statistical endpoint values for the most sensitive endpoint, visual phytotoxicity in the vegetative vigor tests.

Species	EC_{25} (g a.i./ha)	EC_{50} (g a.i./ha)
Carrot	0.09	0.17
Corn	>10.0	>10.0
Cucumber	0.35	1
Oat	>10.0	>10.0
Onion	1	1.6
Radish	0.07	0.13
Ryegrass	6.8	>10.0
Soybean	0.2	1.4
Sunflower	0.04	0.11
Tomato	0.02	0.06

IV. Study deficiencies: Several of the replicates in various treatments had unequal number of seeds planted or in some instances only one of two pots were planted per replicate. Soil pH was not monitored and/or reported during the study. These are considered to be minor deficiencies.