Data Evaluation Report on the acute toxicity of GF-1674 (EUP containing pyroxulam) to terrestrial vascular plants [seedling emergence]

PMRA Submission Number 2006-4727; ID 1283252 **EPA MRID Number** {469084-39}

Data Requirement: PMRA DATA CODE: 9.8.6 (EP)
EPA DP Barcode:
OECD Data Point: IIIA 10.8.1.1 (EP)
EPA Guideline: U.S. EPA OPPTS 850.4225

Test material: GF-1674 (XDE-742) **Purity (%):** 29 g ac/L XDE-742 (2.78%)
Common name: Pyroxulam (active constituent)
Chemical name: 3-pyridinesulfonamide, N-(5,7-dimethoxy[1,2,4]triazolo[1,5-α]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)
IUPAC N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide
CAS name N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide
CAS No. 422556-08-9
Synonyms None

Primary Reviewer: Chris Lee-Steere **Date:** 8 May 2007
Australian Government Department of the Environment, Water, Heritage and the Arts (DEWHA)

Secondary Reviewer: Jack Holland **Date:** 14 June 2007
Australian Government Department of the Environment, Water, Heritage and the Arts

PMRA Reviewer: Émilie Larivière **Date:** 26 June 2007
Environmental Assessment Directorate, PMRA

{EPA/EFED/ERBIV} Christopher Salice Date: 25 June 2007

Company Code: DWE
Active Code: JUA
Use Site Category: 13, 14
EPA PC Code:

CITATION:

Eley, R. (2006): Effects of GF-1674 on Seedling Emergence and Seedling Growth of Non-Target Terrestrial Plants (Tier II) – 2005, Dow AgroSciences, unpublished report No. ACE-05-213, 27 June 2006.

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IUPAC	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)pyridine-3-sulfonamide		
CAS name	N-(5,7-dimethoxy[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)-2-methoxy-4-(trifluoromethyl)-3-pyridinesulfonamide		
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Synonyms	None		

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EXECUTIVE SUMMARY:

The effect of pyroxsulam, as an oil dispersion formulation, GF-1674 (29 g ac/L) on the seedling emergence of 4 monocotyledon (oat *Avena sativa*; ryegrass *Lolium perenne*, maize/corn *Zea mays* and onion *Allium cepa*) and 6 dicotyledon (oil seed rape *Brassica napus*, cabbage *Brassica oleracea*, soybean *Glycine max*, carrot *Daucus carota*, cucumber *Cucumis sativa* and sugar beet *Beta vulgaris*) crops was studied under greenhouse conditions. The study was conducted according to EPA Pesticide Assessment Guidelines Subdivision J, Series 123-1, OPPTS 850.4225, Seedling Emergence Tier II. Pyroxsulam was applied in a tank mix with Agridex, an oil crop concentrate, at the following nominal concentrations of GF-1674 (g ac/ha) + Agridex (%v/v): 0.029 + 0.00156, 0.059 + 0.00313, 0.117 + 0.00625, 0.23 + 0.0125, 0.47 + 0.025, 0.94 + 0.05,1.88 + 0.1, 3.75 + 0.2, 7.5 + 0.4 and 15 + 0.8 (oat, onion, oil seed rape, cabbage, carrot, cucumber and sugar beet) or 0.47 + 0.025, 0.94 + 0.05, 1.88 + 0.1, 3.75 + 0.2, 7.5 + 0.4, 15 + 0.8 and 30 + 1.6 (ryegrass, corn and soybean). The growth medium used in the test was natural sandy loam, prepared by mixing 10 L sterile loam soil with 10 L sand and 4 L washed quartzite (pH 7.6, organic carbon 0.8%). On day 21 following 50% emergence in control plants, the surviving plants per pot were recorded and measured for shoot height and shoot fresh weight.

Onion and ryegrass were the most sensitive monocotyledon species to pre-emergence application of pyroxsulam; emergence of the other monocots and all six dicots was unaffected by the test substance. Onion was the most sensitive monocot species with respect to shoot height reduction (ER25 0.32 g ac/ha; ER50 1.04 g ac/ha; reviewer-calculated) and sugar beet was the most sensitive dicot species for this parameter (ER25 0.95 g ac/ha; ER50 2.17 g ac/ha; reviewer-calculated). Shoot fresh weight reduction was generally the more sensitive parameter; most sensitive monocot was onion (ER25 0.25 g ac/ha; ER50 0.42 g ac/ha; reviewer-calculated) and sugar beet was the most sensitive dicot (ER25 0.64 g ac/ha; ER50 1.42 g ac/ha;reviewer-calculated).

The main symptom of visual injury exhibited by treated plants was stunting (reduction in height and/or biomass). In addition carrot exhibited slight chlorosis, cucumber slight chlorosis and distortion, maize leaf necrosis, oilseed rape slight chlorosis and distortion, ryegrass chlorosis, cabbage distortion and discolouration, oat necrosis and sugar beet chlorosis and distortion.

Overall, plant mortality was very low with no plant deaths observed at the final assessment for oats, oil seed rape or cucumber. For all other species, the mean number of dead emerged plants per replicate at the final assessment was <1, generally with no clear dose response.

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Results Synopsis	**Seedling emergence** (Most sensitive species based on EC25)
Monocot	
ER25/IC25:	0.25 g ac/ha (95% CI 0.00-0.49 g ac/ha)
ER50/IC50:	0.42 g ac/ha (onion, 95% CI 0.0.014—1.56 g ac/ha)
NOEC (study determined):	0.47 g ac/ha (ryegrass, shoot height)
ER05:	0.072 g ac/ha (95% CI: 0 – 0.309)
Most sensitive monocot:	Onion (based on ER25 values)
Most sensitive parameter:	Shoot fresh weight.
Dicot	
ER25/IC25:	0.64 g ac/ha (95% CI –0.00-1.27 g ac/ha)
ER50/IC50:	1.42 g ac/ha (sugar beet, 95% CI 0.75-2.27 g ac/ha)
NOEC (study determined):	0.47 g ac/ha (sugar beet, shoot height)
ER05:	0.044 g ac/ha (95% CI: 0-0.322 g ac/ha)
Most sensitive dicot:	Sugar beet (based on ER25 values)
Most sensitive parameter:	Shoot fresh weight.

This toxicity study is classified as acceptable.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OPPTS 850.4225, Seedling Emergence Tier II, no deviations

COMPLIANCE: The study was conducted in accordance with the OECD principles of GLP [EN/MC/CHEM(98) 17]. Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

A. MATERIALS:

1. Test Material

Description: XDE-742
Formulated as GF-1674 OD (Oil dispersion formulation).

Lot No./Batch No. : Lot 190/65/A

Purity: 2.78% ac (29 g ac/L)

Stability of Compound Under Test Conditions: Unclear.

Storage conditions of test chemicals: Ambient room temperature in the dark.

Physicochemical properties of GF-1674 (29 g pyroxsulam/L formulation).

Parameter	Values	Comments
Water solubility at 20°C		No physico-chemical properties were available for the test formulation.
Vapour pressure		
UV absorption		
pKa		
Kow		

2. Test organism:

Monocotyledoneae species tested included four species (including corn) in two families. Dicotyledoneae species tested included six species in five families. Soybean was included as one of the dicots and sugar beet was included as a root crop.

Group/Family	Species	Common Name
MONOCOTYLEDONEAE		
Liliaceae	*Allium cepa*	Onion
Gramineae	*Zea mays*	Corn
Gramineae	*Avena sativa*	Oat
Gramineae	*Lolium perenne*	Ryegrass
DICOTYLEDONEAE		
Cruciferae	*Brassica napus*	Oil seed rape
Cruciferae	*Brassica oleracea*	Cabbage
Leguminosae	*Glycine max*	Soybean
Umbelliferae	*Daucus carota*	Carrot
Cucurbitaceae	*Cucumis sativus*	Cucumber
Chenopodiaceae	*Beta vulgaris*	Sugar beet

Seed source; Germination History:

Test Species (Common name/*species*/variety)	Lot number or Date Tested	Supplier	Germination Rate (%)
Oat/*Avena sativa*/Dala	32949	Herbiseed[1]	>80
Ryegrass/*Lolium perenne*/Herbiseed	Dated 1 July 2004	Herbiseed	>80
Corn/Maize/*Zea mays*/Pioneer	3187/2005/7630	Herbiseed	>80
Onion/*Allium cepa*/White Libson	43820	EW King[2]	75[4]
Oil seed rape/*Brassica napus*/Wichita	29108	Walnes[3]	>80
Cabbage/*Brassica oleracea*/Golden Acre	43033	EW King	>80
Soybean/*Glycine max*/Hutcheson	Dated 20 July 2005	Herbiseed	>80
Carrot/*Daucus carota*/Early Nantes	35098	EW King	>80
Cucumber *Cucumis sativa*/Gherkin National	46722	EW King	>80
Sugar beet/*Beta vulgaris*/Herbiseed	Dated 13 May 2004	Herbiseed	>80

1) Herbiseed Ltd, Twyford, RG10 0NJ, UK;
2) EW King and Co Ltd, Kelvedon, R05 9PG, UK;
3) Walnes Seeds Ltd, Farmlingham, IP13 9EE, UK;
4) Supplied seed of onion was expected to give greater than 80% germination, but in an additional test carried out under conditions similar to test conditions germination was 75%.

Prior seed treatment/sterilization: All species were untreated.
Seed storage, if any: Not specified.

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: No range finding study was performed.

b) Definitive Study

Table 1. Experimental Parameters - Seedling Emergence

Parameters	**Seedling Emergence**	
	Details	**Remarks***Criteria*
Duration of the test	21 days after 50% emergence in the untreated control.	*EPA requires a duration of 14 days.* The requirement was met. *OECD recommends the test should be terminated no sooner than 14 days after 50 percent of the control seedlings have emerged*
Number of seeds/plants/species/ replicate	A minimum of 30 seeds per species per treatment were tested, which is equivalent to the EPA guideline of 10 seeds per replicate with 3 replicates. In this study 5 seeds were planted in each of 6 pots and pots were treated as replicates, so there were 6 replicates each with 5 seeds for a total of 30 seeds per treatment. In the case of onion and ryegrass, there were 6 seeds per replicate, resulting in 36 seeds per treatment.	*EPA requires 10 seeds per replicate.* The requirement was met. *OECD recommends a minimum of five seeds planted in each replicate within 24 hours of incorporation of the test substance. All seeds of each species for each test should be of the same size class. The seed should not be imbibed.*
Number of plants retained after thinning	Not applicable – based on number of seeds planted and does not involve thinning or transplanting after emergence.	
Number of replicates: Control: Solvent control:	6 replicates per treatment. 6 replicates per species. Not applicable. No solvent control used.	*EPA requires at least 3 replicates per dose* The requirement was met. *OECD requires a minimum of four replicates per treatment*

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Parameters	**Seedling Emergence**	
	Details	**Remarks*Criteria***
Test concentrations (g ac/ha) Nominal:	All species tested with 7 to 10 test concentrations in a 2X progression Oats, onion, oilseed rape, cabbage, carrot, sugar beet, and cucumber were tested at nominal rates of 0.029, 0.059, 0.117, 0.23, 0.47, 0.94, 1.88, 3.75, 7.5 and 15 g ac/ha. Corn, soybean and ryegrass were tested at nominal rates of 0.47, 0.94, 1.88, 3.75, 7.5, 15 and 30 g ac/ha. For all crops, an untreated control was also tested using deionised water. Agridex, a crop oil concentrate, was added to the spray mixture at 1.6%v/v in the 30 g ac/ha rate. The surfactant was then diluted with the active constituent to keep the ratio of the test substance to Agridex constant across the dose rates. Measured rates based on analysis of spray solution and uncorrected for method recovery averaged 90% of nominal and ranged from 87% to 95%. Data in report suggests that measured rates may have been reduced due to freezing of spray solution prior to analysis and resulting difficulty in resuspending the active ingredient. Nominal concentrations were used for biological comparisons.	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.* *OECD requires 3 concentrations, preferably with application rates equivalent to 0.0 (control), 1.0, 10.0 and 100 mg substance per kg of oven dried soil.* The requirement was met.
Method and interval of analytical verification LOQ: LOD:	HPLC/UV method based on conditions provided by the sponsor (method DAS-AM-05-032). Calibration solutions with the parent compound ranged from 1 mg/L to 24 mg/L, which covered the range of concentrations assayed in spray solutions.	Limits of detection/quantification were not reported.

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Parameters	Seedling Emergence	
	Details	**Remarks***Criteria*
Solvent (type, percentage, if used)	Not applicable.	
Test container (pot) Size/VolumeMaterial: (glass/polystyrene)	Non-porous plastic pots 7x7x8 cm.	The study plan states that details of pot size and number of plants per pot must be recorded in the raw data and final report. Pot size must be large enough to allow the normal healthy growth of test species. These details were not given in the test report. *EPA recommends that non-porous containers be used.* The requirement is met. The guideline doesn't specify pot sizes. *OECD requires non-porous plastic or glazed pots.*
Growth facility	Greenhouse	
Method/depth of seeding	Hand planted, planting depth of approximately 1-2 cm.	No criteria specified in the guideline.
Test material application: Application time including the plant growth stage Number of applications Application interval Method of application	 Pre-emergence application with seeds planted in soil approximately 24 h prior to application. Single application event N/A Application performed with a Mardive Cabinet track sprayer fitted with a flat fan TeeJet nozzle (SS8005E) and spray mixture was applied at an average of 196 L/ha using a pressure of 1.9 Kpa. The sprayer was calibrated immediately prior to application. Test substance applied to the soil surface with the spray nozzle positioned 65 cm above the soil surface.	While the guideline doesn't specify application equipment for greenhouse experiments, the application equipment in this study simulated conventional farm equipment using the basic components of commercial application equipment. This is considered acceptable.

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Parameters	**Seedling Emergence**	
	Details	**Remarks*Criteria***
Details of soil used Geographic location Depth of soil collection Soil texture % sand % silt % clay pH: % organic carbon CEC Moisture at 1/3 atm (%)	Sandy loam, prepared by mixing 10 L sterile loam soil with 10 L sand and 4 L washed quartzite. Moreton, Essex, UK (Southeast England) Approximately 1 m depth. 73 17 10 7.6 0.8 5.1 meq/100 g Not reported	*EPA prefers soil mixes containing sandy loam, loam, or clay loam soil with no greater than 2% organic matter. Glass beads, rockwool, and 100% acid washed sand are not recommended.* The requirement is met. *OECD prefers the soil to be sieved (0.5 cm) to remove coarse fragments. Carbon content should not exceed 1.5% (3% organic matter). Fine particles (under 20um) should make up between 10 and 20%. The pH should be 5.0 and 7.5.*
Details of nutrient medium, if used	Nothing reported.	
Watering regime and schedules Water source/type: Volume applied: Interval of application: Method of application:	 Tap water. Not reported. All pots were given an initial overhead watering approximately 24 hours after application to promote germination and facilitate movement of the test substance into the soil. Thereafter, watering was via subpot irrigation using individual saucers under each pot. Pots were kept moist at all times.	*Bottom watering of test containers is preferred in order to prevent washing the chemical through the soil during watering. The laboratory should assess the potential for leaching of the pesticide based on solubility and Kd value before attempting the top watering method.* The report does not specify the amount of irrigation initially applied using top watering. There is no discussion of this and whether it would likely lead to any leaching of the test substance through the soil profile in the test report.
Any pest control method/fertilization, if used	Other than the test substance no pesticides were applied to the soil or plants at any time during the study period. No information on fertilization was provided in the test report.	*Pesticide treatments other than the test pesticide should be avoided.* The requirement was met.

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Parameters	**Seedling Emergence**	
	Details	**Remarks*Criteria***
Test conditions Temperature: Photoperiod: Light intensity and quality: Relative humidity:	Mean 15.9°C (range 13.9-21.9°C) 16 h photoperiod Natural light was supplemented with mercury vapour lighting. Mean 66.0% (range 40.4-88.8%)	*EPA doesn't specify test temperatures but prefers that cool vs warm season plants be tested in two separate groups to optimize plant growth.* All plants were tested under the same temperature range. *OECD doesn't specify test conditions but recommends the temperature, humidity and light conditions be suitable for maintaining normal growth of each species for the test period.*
Reference chemical	No reference chemical tested.	
Other parameters, if any	None.	

2. Observations:

Table 2: Observation parameters - Seedling Emergence

Parameters	Seedling Emergence	
	Details	**Remarks*Criteria***
Parameters measured	Seedling emergence. Shoot height and shoot weight Visual phytotoxicity ratings made.	*Parameters measured include percent emergence, number of emerged plants, seedling height, seedling dry weight, root length, and root dry weight (if a root active compound), and observed phytotoxicity.* The requirement is considered met as the guideline doesn't stipulate that all these end-points require measurement. In this test, symptoms of visual injury such as chlorosis, necrosis, stunting or vigour reduction are reported as recorded at each assessment. The raw data for these observations were not provided.
Measurement technique for each parameter	Individual shoot lengths measured from the base of the stem to tip of longest leaf for monocots and dicots with a leaf rosette (i.e. lettuce, oilseed rape, and sugar beet), and from the base of the stem to the apical bud for all other dicots. Fresh replicate weights measured by compositing shoots within a replicate. Plants were fully turgid at the time of harvest.	No specific measurement techniques specified in the guideline.

Parameters	Seedling Emergence	
	Details	**Remarks*Criteria***
Observation intervals	Seedling emergence and phytotoxicity ratings made at 7, 14, and 21 days after 50% emergence in the untreated control. Shoot length and shoot fresh weight recorded after 21 days.	The test guideline states that shoot heights should be recorded on days 7 and 14 (if not more frequently) and compared to control values. In this study, they were only recorded on day 21. While the study report states that assessment was at 7, 14 and 21 days after 50% emergence in the untreated control, the report further discusses other assessments occurring 21 days after application. Further, the raw data provide results in terms of "days after application".
Other observations, if any	Photographs of untreated plants were taken immediately before harvest (not included in test report).	
Were raw data included?	Partially, and in transcribed format.	The raw data provided were in transcribed format only. Observations were not separated. For example, the observations related to phytoxicity were provided as the overall rating. From this, it can not be ascertained what the actual observations were (eg, necrosis, chlorosis, wilting etc.).
Phytotoxicity rating system, if used	0 = no injury 1-39 = slight injury 40-69 = moderate injury 70-99 = severe injury 100 = all plants dead.	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Seed germination: There was no separate seed germination component to this study.

Seedling Emergence:
There was no clear distinction between the monocotyledon and dicotyledon species in terms of their sensitivity to pre-emergence application of the test substance.

Onion and ryegrass were the most sensitive monocotyledon species to pre-emergence application of the test substance, with ER50 values of 6.51 and 15.3 g ac/ha respectively (ER25 1.36 and 4.85 g ac/ha, respectively). The emergence of oats and maize was unaffected by pre-emergence applications. Similarly, the emergence of all six dicotyledon species was unaffected by pre-emergence application of the test substance up to the rates tested. Seedling emergence was the least sensitive end-point.

Onion was the most sensitive monocotyledon in terms of shoot height reduction (ER25 0.35 g ac/ha; ER50 2.18 g ac/ha) while soybean was the most sensitive dicotyledon species based on shoot height reduction (ER25 0.85 g ac/ha; ER50 2.05 g ac/ha).

Ryegrass and onion were the most sensitive monocotyledon species in terms of shoot weight reduction (ER25 0.49 and 0.24 g ac/ha respectively; ER50 0.73 and 1.30 g ac/ha respectively). Sugar beet was the most sensitive dicotyledon species in terms of shoot weight reduction (ER25 0.45 g ac/ha; ER50 1.48 g ac/ha).

The main symptom of visual injury exhibited by treated plants was stunting (reduction in height and/or biomass). In addition carrot exhibited slight chlorosis, cucumber - slight chlorosis and distortion, maize - leaf necrosis, oilseed rape - slight chlorosis and distortion, ryegrass - chlorosis, cabbage - distortion and discolouration, oat - necrosis and sugar beet - chlorosis and distortion.

Overall, plant mortality was very low with no plant deaths observed at the final assessment for oats, oil seed rape or cucumber. For all other species, the mean number of dead emerged plants per replicate at the final assessment was <1, generally with no clear dose response.

Table 3: Effect of GF-1674 on Seedling Emergence

Species	NOEC, ER25 and ER50 (mg ai/kg soil or g ai/ha)											
	Seedling emergence				Shoot Height (mm)				Weight (mg)			
	%*	NOEC	ER25	ER50	Height*	NOEC	ER25	ER50	Weight*	NOEC	ER25	ER50
Oat	87 (60-100)	15	>15	>15	364 (320-437)	0.94	1.23	2.22	6.03 (5.4-7.24)	0.94	0.70	2.68
Ryegrass	89 (83-100)	7.5	4.85	15.3	236 (183-278)	0.47	0.70	2.13	1.11 (0.9-1.33)	1.88	0.49	0.73
Corn	100	30	>30	>30	308 (266-328)	1.88	5.30	9.88	9.24 (7.91-9.95)	3.75	5.79	9.64
Onion	67 (33-100)	3.75	1.36	6.51	79 (40-138)	3.75	0.35	2.18	0.76 (0.13-1.99)	3.75	0.24	1.27
Soybean	100	30	>30	>30	347 (319-382)	0.94	0.85	2.05	12.06 (11.02-13.71)	0.94	1.31	6.18
Oil seed rape	87 (40-100)	15	>15	>15	148 (118-192)	1.88	2.44	3.96	6.45 (5.68-7.53)	1.88	1.91	3.58
Cabbage	87 (60-100)	15	>15	>15	114 (103-125)	0.47	1.34	3.55	5.14 (3.74-6.14)	0.94	1.48	3.73
Carrot	90 (80-100)	15	>15	>15	138 (127-147)	1.88	3.04	6.07	1.38 (0.89-1.63)	1.88	0.39	3.96
Cucumber	90 (80-100)	15	>15	>15	88 (76-102)	3.75	15	>15	6.16 (4.95-7.07)	>15	>15	>15
Sugar beet	87 (40-100)	15	>15	>15	100 (83-115)	0.47	0.94	2.28	3.77 (3.1-4.8)	3.75	0.45	1.48

* Average of control (range of control values) at 21 days.

Plant Injury Index*											
Control	Solvent Control	Oat	Rye grass	Corn	Onion	Soybean	Oil seed rape	Cabbage	Carrot	Cucumber	Sugar beet
0	N/A	0-83	0-80	0-87	3-99	2-75	5-88	8-78	0-76	0-23	0-90

* Visual rating where: 0 = no injury ; 1-39 = slight injury; 40-69 = moderate injury; 70-99 = severe injury; 100 = all plants dead.

Table 4: Effect of GF-1674 on seedling emergence: most sensitive monocot and dicot plant species based on ER25 results.

Statistical Endpoint	Seed germination	Seedling emergence
Monocot:		
most sensitive species	Not applicable	Onion (based on ER25)
NOEC for the most sensitive parameter	Not applicable	0.47 (rye grass, shoot height)
ER25 for the most sensitive monocot	Not applicable	0.24 (shoot weight)
ER50 for the most sensitive monocot	Not applicable	0.73 (rye grass, shoot weight)

Statistical Endpoint	Seed germination	Seedling emergence
Reference chemical, if any: NOEC for most sensitive monocot IR50/ER50	Not applicable	Not applicable
Dicot:		
most sensitive species	Not applicable	Carrot (based on ER25)
NOEC for the most sensitive parameter (eg: dry weight)	Not applicable	0.47 (shoot height; sugar beet and cabbage)
ER25 for the most sensitive dicot	Not applicable	0.39 (shoot weight)
ER50 for the most sensitive dicot	Not applicable	1.48 (sugar beet, shoot weight)
Reference chemical, if any NOEC for most sensitive dicot IR50/ER50	Not applicable	Not applicable

B. REPORTED STATISTICS:

No observable effect concentrations (NOEC) for emergence, shoot length and shoot fresh weight reduction were determined using a Dunnett's test in Minitab 12.22. Data were first tested for variance using a Levene's test and when required data were transformed using one of the following; square root, inverse of y, "1/y to achieve homogeneous variance. In some cases it was not possible to achieve homogeneous variance due to lack of any variance in the data.

The means and standard deviation were calculated for % emergence, injury ratings, shoot length and fresh weight using ARM 7. ER25 and ER50 values (g ac/ha) were calculated for emergence, shoot length and shoot fresh weight data expressed as a percentage of the untreated control and capped at 100%. Regression models in Minitab Statistical package 12.22. were used to calculate ER 25 and ER 50 values. Regression models available in Minitab 12.22 include linear, non-linear, log-probit, log-log, exponential, log-linear, and log-logit. All data for each species were analyzed using each model and the model which best fitted the data (i.e. had the highest r^2 value) was used to calculate the ER 25 and ER 50 values for a given species.

C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

As noted in the test guideline, the lowest test level and the NOEC should not be greater than the EC25 value. The results presented above for shoot fresh weights show NOECs for oat, ryegrass, onion, carrot and sugar beet all greater than the corresponding ER25 while for shoot height, soybean has a reported NOEC higher than the corresponding ER25. The lack of statistically significant differences being detected at lower concentrations is likely a result of the large variability among replicates. Due to the within-treatment variability, only large differences compared to controls would be detected by the test, and the resulting statistical (not biological) NOEC is set at a concentration where greater than 25% inhibition is observed compared to the

controls. However, this reasoning is not provided in the test report and the raw data and summary results tables provided in the test report make no comment relating to statistical significance of results compared to the control. In addition, several EC50 values are also lower than the reported NOECs with no explanation (ryegrass, onion and sugar beet based on shoot fresh weight). Further, the statistics in many cases for ER25 and ER50 values are of concern with very wide confidence intervals and/or quite poor correlation coefficients.

To verify the statistics, the data have been re-analysed using TOXCALC – Toxicity Data Analysis Software v5.0.26. The reviewer estimated the NOEC by the ER05. The following results using linear interpolation were found based on the raw data provided in the study report:

Table 5: Reviewed statistics using TOXCALC for NOEC (ER05), ER25 and ER50, seedling emergence test results. 95% CI provided in parentheses.

Species	**NOEC (ER05), ER25 and ER50 (g as/ha)**					
	Shoot Height (mm)			**Shoot Fresh Weight (mg)**		
	NOEC (ER05)	ER25	ER50	NOEC (ER05)	ER25	ER50
Oat	0.365 (0.00 – 0.646)	1.20 (0.855 – 2.50)	2.40 (1.30 – 3.40)	0.288 (0.00 – 0.661)	1.11 (0.513 – 2.49)	2.51 (1.20 – 3.45)
Ryegrass	0.252* (0.056 – 0.659)	0.751 (0.455 – 2.31)	2.43 (1.39 – 2.82)	0.170* (0.033 – 0.636)	0.612 (0.239 – 0.835)	0.928 (0.761 – 2.46)
Corn	1.95 (0.00 – 2.86)	5.10 (2.95 – 7.79)	10.7 (8.06 – 14.3)	2.55 (0.00 – 4.68)	5.32 (2.86 – 6.72)	9.56 (7.04 – 10.2)
Onion	0.077 (0.063 – 0.319)	0.315 (0.040 – 0.577)	1.042 (0.266 – 3.22)	0.072 (0.00 – 0.309)	0.248 (0.00 – 0.491)	0.418 (0.014 – 1.56)
Soybean	0.426* (0.117 – 1.11)	1.28 (0.789 – 1.69)	2.78 (1.46 – 5.23)	0.330* (0.101 – 1.17)	1.41 (0.784 – 2.06)	4.39 (1.06 – 6.07)
Oil seed rape	1.05 (0.009 – 1.38)	2.08 (1.42 – 2.85)	3.60 (2.87 – 5.19)	0.058 (0.008 – 0.846)	1.69 (0.089 – 4.39)	4.41 (2.44 – 5.98)
Cabbage	0.196 (0.00 – 0.679)	1.32 (0.758 – 2.23)	3.60 (2.70 – 5.34)	0.683 (0.00 – 1.21)	1.56 (0.650 – 2.00)	3.52 (2.50 – 5.74)
Carrot	0.025* (0.012 – 0.922)	3.11 (2.33 – 4.54)	6.34 (5.24 – 7.67)	0.012* (0.004 – 0.438)	1.53 (0.00 – 3.06)	3.91 (2.79 – 5.56)
Cucumber	2.66 (0.00 – 5.30)	>15	>15	2.16 (0.00 – 4.79)	6.95 (95% CI not calculated)	>15
Sugar beet	0.032 (0.001 – 0.434)	0.954 (0.262 – 1.36)	2.17 (1.39 – 3.11)	0.044 (0.00 – 0.322)	0.641 (0.00 – 1.27)	1.42 (0.75 – 2.27)

* - NOEC (ER05) value estimated as less than the lowest test concentration.

The results of this statistical analysis are, based on ER25 data, the most sensitive monocot is identified as onion (ER25 0.248 g as/ha) and the most sensitive dicot is identified as sugar beet (ER25 0.641 g as/ha).

The study author's identified carrot as the most sensitive dicot with an ER25 of 0.39 g as/ha. Results for this species were quite variable. Based on mean responses, the reduction in shoot weight relative to the control was 17, 23, 1, 8, 30, 15, 26, 49, 76 and 80% respectively at test

concentrations of 0.029, 0.059, 0.117, 0.23, 0.47, 0.94, 1.88, 3.75, 7.5 and 15 g as/ha. Such variability at the lower application rates means the ER25 should be treated with some caution.

The PMRA re-calculated the ER25 and ER50 for dry weight of carrot, onion and sugarbeet using linear interpolation and obtained results similar to those of the primary reviewer.

The reviewer-calculated values are recommended and therefore reported in the Executive Summary and Conclusions Sections of this DER.

D. STUDY DEFICIENCIES:

Study component	Deficiency
Application rates.	There is a question over the actual application rates applied. While nominal values were used for reporting results, the measured rates based on analysis of spray solution and uncorrected for method recovery averaged 90% of nominal and ranged from 87% to 95% of nominal values. Data in the report suggests that measured rates may have been reduced due to freezing of spray solution prior to analysis and resulting difficulty in re-suspending the active ingredient. However, while the report states that the test solution was stored under ambient conditions in the dark, there is no mention of the length of time it was stored between making the spray solution and application, nor is there any comment relating to the stability of the spray formulation once prepared.
Watering regime.	All pots were given an initial overhead watering approximately 24 hours after application. The report does not specify the amount of irrigation initially applied using top watering. There is no discussion of this and whether it would likely lead to any leaching of the test substance through the soil profile in the test report.
Test conditions.	All plants were subject to the same temperature and humidity test conditions with no separation of cool vs warm season plants. This is not considered to significantly impact on the test results as control plants, based on available information, were healthy and normal throughout the study (with the exception of poor germination of onions).
Observation intervals.	The test guideline states that shoot heights should be recorded on days 7 and 14 (if not more frequently). In this study, they were only recorded on day 21 with no comment about earlier observations.
Observation reporting inconsistencies.	While the study report states that assessment was at 7, 14 and 21 days after 50% emergence in the untreated control, the report further discusses other assessments occurring 21 days after application. Further, the raw data provide results in terms of "days after application".
Raw data.	Only partial raw data were provided in transcribed format. Observations were not separated. For example, the observations related to phytotoxicity were provided as the overall rating. From this, it could not be ascertained what the actual observations were (such as necrosis, chlorosis, wilting etc.).

Onion results.	The emergence for onion in the control group was relatively low (mean 67% compared to mean emergence >85% for all other species). The NOEC for this species based on emergence was deemed to be 3.75 g ac/ha based on mean emergence of around 28% in the next treatment level of 7.5 g ac/ha. However, at 1.88 g ac/ha, mean emergence was only 30.6%. The guideline does not provide acceptable criteria for levels of emergence in control groups (although it does state a minimum seed germination standard of 70% for onion). The overall onion results should be treated with some caution.
Reported statistics.	While the study report notes the methodology for determining NOECs, no actual workings of NOEC values are provided in the test report. In several instances, NOECs were in excess of corresponding ER25 values, and no reason for this is provided in the test report.

E. REVIEWERS COMMENTS:

The statistical package used for calculation of statistics in the test report (Minitab) was not available for the review. Hence for the statistical review, TOXCALC was used by the primary reviewer to recalculate the statistics.

The PMRA reviewer re-calculated the ER25 and ER50 for dry weight of carrot, onion and sugarbeet using linear interpolation (ICp, Version 2.0; US EPA 1993) and obtained results similar to those of the primary reviewer. The results reported by the primary reviewer are considered acceptable to the PMRA.

F. CONCLUSIONS: The deficiencies noted above are not considered likely to have impacted the study outcomes. The study is considered acceptable.

Most sensitive monocot and EC25: Onion, EC25 0.25 g ac/ha (reviewer-calculated)
Most sensitive monocot and EC25: Sugar beet EC25 0.64 g ac/ha (reviewer-calculated)

III. REFERENCES:

References not detailed in the study report. The test was based on:
U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines OPPTS 850.4225. Terrestrial Plant Toxicity Tier II (Seedling Emergence).

Rosser W, 2006. Study Profile Template for GF-1674 (XDE-742): Effects on Seedling Emergence and Vegetative Vigor of Non-Target Terrestrial Plants (Tier II). Study ID ACE-05-213.SPT. Dow Agrosciences, Indianapolis, Indiana. 26 July 2006.

APPENDIX 1: Output of statistical verification by PMRA reviewer:

Carrot

```
Conc. ID        1    2    3    4    5    6    7    8    9   10   11
------------------------------------------------------------------
Conc. Tested    00.0290.0590.117 0.23 0.47 0.94 1.88 3.75  7.5   15
------------------------------------------------------------------
Response  1  0.89 1.02 0.69 0.97 1.09 1.18 1.21 1.23  0.8 0.35 0.36
Response  2  1.48 1.24 1.36 1.38 1.77 0.96 0.99 0.79 0.73 0.08 0.17
Response  3  1.63 0.82 0.77 1.78 1.13 1.07 1.35 1.34    1 0.22 0.24
Response  4  1.48 0.93    1  0.8 1.21 0.99 0.99 1.06 0.73 0.51 0.11
Response  5  1.31 1.42 1.39 1.49 1.45 0.93 1.31 0.93 0.64 0.53 0.21
Response  6  1.51 1.46 1.19 1.79    1 0.65 1.17 0.77 0.35 0.26 0.54
------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: GF-1674 (29 g pyroxsulam/L formulation)
Test Start Date:    Test Ending Date:
Test Species: Carrot    weight
Test Duration:         21 days
DATA FILE: secarrot.icp
OUTPUT FILE: secarrot.i25
```

Conc. ID	Number Replicates	Concentration g ai/ha	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	1.383	0.262	1.383
2	6	0.029	1.148	0.265	1.215
3	6	0.059	1.067	0.297	1.215
4	6	0.117	1.368	0.411	1.215
5	6	0.230	1.275	0.287	1.215
6	6	0.470	0.963	0.178	1.067
7	6	0.940	1.170	0.154	1.067
8	6	1.880	1.020	0.233	1.020
9	6	3.750	0.708	0.213	0.708
10	6	7.500	0.325	0.174	0.325
11	6	15.000	0.272	0.156	0.272

```
The Linear Interpolation Estimate:     1.5275   Entered P Value: 25
------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   1.2605 Standard Deviation:     0.7859
Original Confidence Limits:   Lower:     0.2443 Upper:     2.6294
Resampling time in Seconds:     0.00  Random_Seed: -278170026


Conc. ID        1    2    3    4    5    6    7    8    9   10   11
------------------------------------------------------------------
Conc. Tested    00.0290.0590.117 0.23 0.47 0.94 1.88 3.75  7.5   15
------------------------------------------------------------------
Response  1  0.89 1.02 0.69 0.97 1.09 1.18 1.21 1.23  0.8 0.35 0.36
```

```
Response  2  1.48 1.24 1.36 1.38 1.77 0.96 0.99 0.79 0.73 0.08 0.17
Response  3  1.63 0.82 0.77 1.78 1.13 1.07 1.35 1.34    1 0.22 0.24
Response  4  1.48 0.93    1  0.8 1.21 0.99 0.99 1.06 0.73 0.51 0.11
Response  5  1.31 1.42 1.39 1.49 1.45 0.93 1.31 0.93 0.64 0.53 0.21
Response  6  1.51 1.46 1.19 1.79    1 0.65 1.17 0.77 0.35 0.26 0.54
-------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: GF-1674 (29 g pyroxsulam/L formulation)
Test Start Date:    Test Ending Date:
Test Species: Carrot    weight
Test Duration:         21 days
DATA FILE: secarrot.icp
OUTPUT FILE: secarrot.i50
```

Conc. ID	Number Replicates	Concentration g ai/ha	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	1.383	0.262	1.383
2	6	0.029	1.148	0.265	1.215
3	6	0.059	1.067	0.297	1.215
4	6	0.117	1.368	0.411	1.215
5	6	0.230	1.275	0.287	1.215
6	6	0.470	0.963	0.178	1.067
7	6	0.940	1.170	0.154	1.067
8	6	1.880	1.020	0.233	1.020
9	6	3.750	0.708	0.213	0.708
10	6	7.500	0.325	0.174	0.325
11	6	15.000	0.272	0.156	0.272

```
-------------------------------------------------------------------
The Linear Interpolation Estimate:     3.9130   Entered P Value: 50
-------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   3.9336 Standard Deviation:     0.6040
Original Confidence Limits:   Lower:     3.0285 Upper:     5.1963
Resampling time in Seconds:     0.00  Random_Seed: 1295525366
```

Onion

```
Conc. ID        1    2    3    4    5    6    7    8    9   10   11
-------------------------------------------------------------------
Conc. Tested    00.0290.0590.117 0.23 0.47 0.94 1.88 3.75  7.5   15
-------------------------------------------------------------------
Response  1  1.99 1.23 1.36 1.25 0.49 0.62 0.19 0.01 0.13 0.15    0
Response  2  0.27 1.19 0.35 0.05 0.31 0.24 0.61 0.05    0    0 0.01
Response  3  1.78 1.79 1.66 1.19 1.32 0.45 1.56 0.05 0.07 0.01    0
Response  4  0.24 0.45 1.61  0.4 1.26 0.05 0.29 0.23 0.35 0.08 0.01
Response  5  0.17 0.71 0.59 0.78 1.07 0.38 0.31 0.11 0.56 0.04 0.02
Response  6  0.13 1.75 0.58 0.15 0.94 0.31 0.03 0.07 0.04    0    0
-------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: GF-1674 (29 g pyroxsulam/L formulation)
Test Start Date:    Test Ending Date:
```

```
Test Species: Onion weight
Test Duration:          21 days
DATA FILE: seonion.icp
OUTPUT FILE: seonion.i25
```

Conc. ID	Number Replicates	Concentration g ai/ha	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	0.763	0.873	0.992
2	6	0.029	1.187	0.539	0.992
3	6	0.059	1.025	0.583	0.992
4	6	0.117	0.637	0.518	0.768
5	6	0.230	0.898	0.413	0.768
6	6	0.470	0.342	0.193	0.420
7	6	0.940	0.498	0.554	0.420
8	6	1.880	0.087	0.077	0.139
9	6	3.750	0.192	0.219	0.139
10	6	7.500	0.047	0.059	0.047
11	6	15.000	0.007	0.008	0.007

```
The Linear Interpolation Estimate:     0.2464   Entered P Value: 25
Number of Resamplings:   80
The Bootstrap Estimates Mean:    0.1835 Standard Deviation:     0.1118
Original Confidence Limits:   Lower:     0.0458 Upper:     0.3748
Resampling time in Seconds:     0.00  Random_Seed: 664974646
```

Conc. ID	1	2	3	4	5	6	7	8	9	10	11
Conc. Tested	0	0.029	0.059	0.117	0.23	0.47	0.94	1.88	3.75	7.5	15
Response 1	1.99	1.23	1.36	1.25	0.49	0.62	0.19	0.01	0.13	0.15	0
Response 2	0.27	1.19	0.35	0.05	0.31	0.24	0.61	0.05	0	0	0.01
Response 3	1.78	1.79	1.66	1.19	1.32	0.45	1.56	0.05	0.07	0.01	0
Response 4	0.24	0.45	1.61	0.4	1.26	0.05	0.29	0.23	0.35	0.08	0.01
Response 5	0.17	0.71	0.59	0.78	1.07	0.38	0.31	0.11	0.56	0.04	0.02
Response 6	0.13	1.75	0.58	0.15	0.94	0.31	0.03	0.07	0.04	0	0

```
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: GF-1674 (29 g pyroxsulam/L formulation)
Test Start Date:    Test Ending Date:
Test Species: Onion weight
Test Duration:          21 days
DATA FILE: seonion.icp
OUTPUT FILE: seonion.i50
```

Conc. ID	Number Replicates	Concentration g ai/ha	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	0.763	0.873	0.992
2	6	0.029	1.187	0.539	0.992
3	6	0.059	1.025	0.583	0.992

4	6	0.117	0.637	0.518	0.768
5	6	0.230	0.898	0.413	0.768
6	6	0.470	0.342	0.193	0.420
7	6	0.940	0.498	0.554	0.420
8	6	1.880	0.087	0.077	0.139
9	6	3.750	0.192	0.219	0.139
10	6	7.500	0.047	0.059	0.047
11	6	15.000	0.007	0.008	0.007

The Linear Interpolation Estimate: 0.4176 Entered P Value: 50

Number of Resamplings: 80
The Bootstrap Estimates Mean: 0.4963 Standard Deviation: 0.3098
Original Confidence Limits: Lower: 0.1127 Upper: 1.2021
Resampling time in Seconds: 0.00 Random_Seed: 328242390

Sugarbeet

Conc. ID	1	2	3	4	5	6	7	8	9	10	11
Conc. Tested	0	0.029	0.059	0.117	0.23	0.47	0.94	1.88	3.75	7.5	15
Response 1	3.1	2.46	2.83	2.52	2.29	3.1	2.66	2.4	0.78	0.73	0.12
Response 2	3.5	3.02	3.37	4.11	2.77	3.09	2.49	3.12	0.54	0.2	0.07
Response 3	3.56	3.54	4.69	3.59	3.98	2.9	3.87	0.72	0.4	0.14	0.12
Response 4	4.8	4.72	4.38	3.63	4.43	2.28	1.3	0.72	0.35	0.66	0.13
Response 5	3.74	4.02	2.17	4.03	2.78	3.94	2.8	0.76	0.35	0.16	0.14
Response 6	3.89	6.03	0.51	6	3.51	3.32	2.11	0.46	0.71	0.14	0.12

*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: GF-1674 (29 g pyroxsulam/L formulation)
Test Start Date: Test Ending Date:
Test Species: Sugarbeet weight
Test Duration: 21 days
DATA FILE: sesgbeet.icp
OUTPUT FILE: sesgbeet.i25

Conc. ID	Number Replicates	Concentration g ai/ha	Response Means	Std. Dev.	Pooled Response Means
1	6	0.000	3.765	0.573	3.865
2	6	0.029	3.965	1.279	3.865
3	6	0.059	2.992	1.537	3.486
4	6	0.117	3.980	1.141	3.486
5	6	0.230	3.293	0.819	3.293
6	6	0.470	3.105	0.542	3.105
7	6	0.940	2.538	0.846	2.538
8	6	1.880	1.363	1.111	1.363
9	6	3.750	0.522	0.188	0.522
10	6	7.500	0.338	0.278	0.338
11	6	15.000	0.117	0.024	0.117

The Linear Interpolation Estimate: 0.6411 Entered P Value: 25

```
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   0.5729 Standard Deviation:     0.2544
Original Confidence Limits:   Lower:     0.0580 Upper:     1.0560
Resampling time in Seconds:     0.00  Random_Seed: 1399317910

Conc. ID        1    2    3    4    5    6    7    8    9   10   11
---------------------------------------------------------------------
Conc. Tested    00.0290.0590.117 0.23 0.47 0.94 1.88 3.75  7.5   15
---------------------------------------------------------------------
Response  1   3.1 2.46 2.83 2.52 2.29  3.1 2.66  2.4 0.78 0.73 0.12
Response  2   3.5 3.02 3.37 4.11 2.77 3.09 2.49 3.12 0.54  0.2 0.07
Response  3  3.56 3.54 4.69 3.59 3.98  2.9 3.87 0.72  0.4 0.14 0.12
Response  4   4.8 4.72 4.38 3.63 4.43 2.28  1.3 0.72 0.35 0.66 0.13
Response  5  3.74 4.02 2.17 4.03 2.78 3.94  2.8 0.76 0.35 0.16 0.14
Response  6  3.89 6.03 0.51    6 3.51 3.32 2.11 0.46 0.71 0.14 0.12
---------------------------------------------------------------------
*** Inhibition Concentration Percentage Estimate ***
Toxicant/Effluent: GF-1674 (29 g pyroxsulam/L formulation)
Test Start Date:    Test Ending Date:
Test Species: Sugarbeet weight
Test Duration:          21 days
DATA FILE: sesgbeet.icp
OUTPUT FILE: sesgbeet.i50
---------------------------------------------------------------------
Conc.      Number      Concentration      Response       Std.       Pooled
 ID     Replicates          g ai/ha         Means        Dev.   Response Means
---------------------------------------------------------------------
  1          6              0.000          3.765       0.573       3.865
  2          6              0.029          3.965       1.279       3.865
  3          6              0.059          2.992       1.537       3.486
  4          6              0.117          3.980       1.141       3.486
  5          6              0.230          3.293       0.819       3.293
  6          6              0.470          3.105       0.542       3.105
  7          6              0.940          2.538       0.846       2.538
  8          6              1.880          1.363       1.111       1.363
  9          6              3.750          0.522       0.188       0.522
 10          6              7.500          0.338       0.278       0.338
 11          6             15.000          0.117       0.024       0.117
---------------------------------------------------------------------
The Linear Interpolation Estimate:     1.4247   Entered P Value: 50
---------------------------------------------------------------------
Number of Resamplings:   80
The Bootstrap Estimates Mean:   1.4453 Standard Deviation:     0.3377
Original Confidence Limits:   Lower:     0.9428 Upper:     2.2557
Resampling time in Seconds:     0.00  Random_Seed: 1295525366
```