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

PMRA Submission Number 2006-4727; ID 1283253 **EPA MRID Number** {.....} 469084-40

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 850.4250

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

Secondary Reviewer: Jack Holland **Date:** 5 June 2007 22/2/08
Australian Government Department of the Environment, Water, Heritage and the Arts

PMRA Reviewer: Émilie Larivière **Date:** 27 June 2007 05/03/08
Environmental Assessment Directorate, PMRA

EPA/EFED/ERBIV: Christopher Salice **Date:** 22 June 2007 4/10/08

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

CITATION:

Eley, R. (2006): Effects of GF-1674 on the Vegetative Vigour of Non-Target Terrestrial Plants (Tier II) – 2005, Dow AgroSciences, unpublished report No. ACE-05-214, 27 June 2006.

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EXECUTIVE SUMMARY:

The effect of pyroxsulam, as an oil dispersion formulation, GF-1674 (29 g ac/L) on the vegetative vigour 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 850.4220, Vegetative Vigour 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, soybean, 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 and corn). 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%). Plants were assessed for visual phytotoxicity 7, 14, and 21 days after application. On day 21 following application, the number of dead plants, shoot height and shoot fresh weight was recorded.

With the exception of soybean and oil seed rape, shoot fresh weight was the most sensitive end-point for both monocots and dicots, based on the reviewer-calculated ER25. Onion and ryegrass were the most sensitive monocotyledon species to post-emergence application of the test substance, with ER25 values of 0.623 and 0.746 g ac/ha, respectively, and ER50 values of 0.883 and 1.33 g ac/ha, respectively (reviewer-calculated). Soybean and carrot were the most sensitive dicotyledon species in terms of shoot weight reduction with ER25 values of 0.243 and 0.465 g ac/ha, respectively, and ER50 values of 0.856 and 0.864 g ac/ha, respectively (reviewer-calculated).

Shoot height was affected the most in onion and ryegrass for monocotyledon species, with ER25 values of 1.33 and 1.45 g ac/ha, respectively, and ER50 values of >15 and >30 g ac/ha, respectively (reviewer-calculated). Oil seed rape, soybean, and carrot were the most sensitive dicotyledon species in terms of shoot height reduction with ER25 values of 0.058, 0.185 and 0.474 g ac/ha, respectively, and ER50 values of 5.77, 9.73 and >15 g ac/ha, respectively (reviewer-calculated). Due to uncertainty associated with the shoot height ER25 value for oil seed rape, as reflected by the large confidence intervals (0.048 to 0.427 g ac/ha), and the less than 25% inhibition observed up to test concentrations of 0.47 g ac/ha, the ER25 for oil seed rape will not be considered by the PMRA. For the PMRA, the most sensitive dicotyledon species in terms of shoot height reduction is considered to be soybean (ER25 of 0.185 g ac/ha).

The main symptom shown by affected plants was stunting. Wilting was also recorded for cabbage, sugar beet and oil seed rape, swollen stems for oil seed rape, necrosis for soybean and sugar beet and also blackened stems for sugar beet. No dead plants were recorded for oat, onion,

oil seed rape, cabbage, carrot, sugar beet or corn, with only an apparent non-treatment related death of a single plant at a lower treatment rate for cucumber. Some mortality was found ryegrass and soybean at the higher end of treatment rates.

Endpoints based on TOXCALC analysis conducted by Reviewer:

Monocot

ER25:	0.623 g ac/ha (95% CI: 0.360-0.694 g ac/ha)
ER50:	0.883 g ac/ha (95% CI: 0.786-0.946 g ac/ha)
NOAEC/ER05:	0.051 g ac/ha (95% CI: 0 – 0.626)
Most Sensitive monocot:	Onion (based on ER25 values)
Most Sensitive Parameter:	Shoot fresh weight

Dicot

ER25:	0.058 g ac/ha (95% CI: 0.048-0.427g ac/ha)
ER50:	5.77 g ac/ha (95% CI: not calculated)
NOAEC/ER05:	0.035 g ac/ha (95% CI: 0.011-0.041 g ac/ha)
Most Sensitive monocot:	Oilseed Rape (based on ER25 values)
Most Sensitive Parameter:	Shoot height

Dicot (PMRA conclusions)

ER25:	0.185 g ac/ha (95% CI: 0.134-0.215 g ac/ha)
ER50:	9.73 g ac/ha (95% CI: not calculated)
NOEC/ER05:	0.046 g ac/ha (95% CI: 0.024-0.172 g ac/ha)
Most Sensitive dicot:	Soybean (based on ER25 values)
Most Sensitive Parameter	Shoot height

This toxicity study is classified as acceptable.

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: OPPTS 850.4250 Vegetative Vigour 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:

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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:

Test Species (Common name/*species*/variety)	Lot number or Date tested	Supplier
Oat/*Avena sativa*/Dala	32949	Herbiseed[1]
Ryegrass/*Lolium perenne*/Herbiseed	Dated 1 July 2004	Herbiseed
Corn/Maize/*Zea mays*/Pioneer	3187/2005/7630	Herbiseed
Onion/*Allium cepa*/White Libson	43820	EW King[2]
Oil seed rape/*Brassica napus*/Wichita	29108	Walnes[3]
Cabbage/*Brassica oleracea*/Golden Acre	43033	EW King
Soybean/*Glycine max*/Hutcheson	Dated 27 July 2005	Herbiseed
Carrot/*Daucus carota*/Early Nantes	35098	EW King
Cucumber *Cucumis sativa*/Gherkin National	46722	EW King
Sugar beet/*Beta vulgaris*/Herbiseed	Dated 13 May 2004	Herbiseed

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;

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

B. <u>STUDY DESIGN</u>:

<u>1. Experimental Conditions</u>

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

b) Definitive Study

Table 1. Experimental Parameters - Vegetative Vigour

Parameters	**Vegetative vigour**	
	Details	**Remarks*Criteria***
Duration of the test	21 days post application	*EPA requires a minimum duration of 14 days following application.* Requirements were met.
Number of seeds/plants/species/ replicate	Six replicate pots were used per treatment and species. The number of plants per replicate varied according to the size of the test species and their growth requirements. All species contained 5 plants per replicate pot (30 plants total per treatment rate) with the exception ryegrass and onion, where 6 plants per replicate pot (36 plants total per treatment level) were tested.	*The number of plants per pot is left to the discretion of the laboratory conducting the test.* Requirements were met.
Number of plants retained after thinning	Not applicable.	
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* Requirements were met.

Parameters	Vegetative vigour	
	Details	**Remarks***Criteria*
Test concentrations (g ac/ha) Nominal:	All species tested with 8 to 10 rates in a 2X progression Nominal application rates: 30, 15, 7.5, 3.75, 1.88, 0.94, 0.47, 0.23, 0.117, 0.059, 0.029, and 0 g ac/ha. Oats, onion, oilseed rape, cabbage, carrot, sugar beet, cucumber and soybean were tested at rates of 15 to 0.029 g ac/ha, while corn and ryegrass were tested at rates of 30 to 0.23 g ac/ha. Agridex, a crop oil concentrate, was added to the spray mixture at 0.8% v/v in the 15 g ac/ha rate, or 1.6% v/v to 30 g ac/ha but diluted along with XDE-742 at lower application rates. Measured rates based on analysis of spray solution and uncorrected for method recovery averaged 81% of nominal and ranged from 77% to 91%. Only spray solutions for the top three application rates were analysed. 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. In separate work testing solutions where analysis was undertaken prior to frozen storage, a mean recovery of 93% is reported (actual data not presented in the test report). Nominal concentrations were used for biological comparisons.	*EPA requires at least 5 test concentrations with a dose range of 2X or 3X progression.* The requirements were met.

Parameters	**Vegetative vigour**	
	Details	**Remarks***Criteria*
Method and interval of analytical verification LOQ: LOD:	HPLC/UV method based on conditions provided in method DAS-AM-05-032. Standard concentrations ranged from 1 mg/L to 24 mg/L, which covered the range of concentrations assayed in spray solutions.	Limit of detection/quantification were not reported.
Solvent (type, percentage, if used)	Not applicable for the spray solutions.	
Test container (pot) Size/Volume Material: (glass/polystyrene)	Not reported. Non-porous plastic pots	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 was met.
Growth facility	Greenhouse	
Method/depth of seeding	Seeds were germinated in Levington F1 Compost and transplanted shortly after emergence (BBCH 10) into pots containing the test soil.	No criteria specified in the guideline.

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Parameters	Vegetative vigour	
	Details	**Remarks***Criteria*
Test material application: Application time including the plant growth stage Number of applications Application interval Method of application	 Post-emergence application; seedlings were in the BBCH 12 to 14, depending on species. Single application event N/A Application performed with a Mardive Cabinet track sprayer fitted with a flat fan Tee Jet nozzle (SS8005E) and spray mixture was applied at 175-185 L/ha using a pressure of 1.9 Kpa. The sprayer was positioned 65 cm above the soil surface and plant heights at the time of application ranged from173 mm (carrot) to 428 mm (oats). The sprayer was calibrated immediately prior to application.	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.
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, 4 L washed quartzite and 100 g nutrient medium (see below for details). 0-1 m. 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 was 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	Osmacot Exact (15% N, 9% K, 3% MgO and trace elements) added to soil.	

Parameters	Vegetative vigour	
	Details	**Remarks***Criteria*
Watering regime and schedules Water source/type: Volume applied: Interval of application: Method of application:	 Tap water from municipal source. Not reported. During the time that plants were being grown to the proper growth stage for treatment, they were watered by sub pot irrigation and overhead watering. All watering following treatment application was by sub-pot irrigation only. Pots were kept moist at all times.	*EPA prefers that bottom watering be utilized, however, top watering under the foliage can also be used to prevent washing pesticide off foliage.* The requirement was met.
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. A nutrient medium (Osmacot Exact) was incorporated into the test soil.	*Pesticide treatments other than the test pesticide should be avoided.* The requirement was met.
Test conditions Temperature: Photoperiod: Light intensity and quality: Relative humidity:	Temperature and humidity were recorded continuously using an electronic data logging system. The range of values measured at midnight, 8:00 am and 4:00 pm were provided as an appendix to the report and those values were used to calculate mean and ranges provided below. Mean 16.0°C (range 14.3-19.2°C) 16 h photoperiod Natural light was supplemented with mercury vapour lighting. Mean 50.6% (range 37.7-73.5%)	*EPA doesn't specify test temperatures but prefers that the cool and warm season plants be tested in two separate groups.* All plants were tested under the same temperature range. It is unclear the impact this has on the test results. However, the control plants appeared normal and healthy throughout. *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 – Vegetative vigour

Parameters	Vegetative vigour	
	Details	**Remarks/*Criteria***
Parameters measured	Shoot height, shoot fresh weight and number of dead plants. Visual phytotoxicity ratings were made.	*Data on plant dry weight, plant shoot height, root dry weight, root length/volume, number of dead plants, height or other growth parameters are recommended test endpoints.* The requirement was met. For this study, symptoms of visual injury such as chlorosis, necrosis, stunting or vigour reduction are reported as being 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.
Observation intervals	Shoot height and shoot weight were measured 21 days after application. Phytotoxicity ratings and number of dead plants were recorded at 7, 14, and 21 days after application.	The test guideline states that shoot heights should be observed on days 7 and 14 (if not more frequently). In this study, they were only recorded on day 21.
Other observations, if any	None	

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:

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

Apart from soybean and oil seed rape, shoot fresh weight appeared the most sensitive end-point for both monocots and dicots. Onion and ryegrass were the most sensitive monocotyledon species to post-emergence application of the test substance, with ER25 values of 0.54 and 0.79 g ac/ha respectively, and ER50 values of 0.99 and 1.26 g ac/ha respectively. Soybean and carrot were the most sensitive dicotyledon species in terms of shoot weight reduction with ER25 values of 0.14 and 0.58 g ac/ha respectively, and ER50 values of 0.86 and 2.49 g ac/ha respectively.

Shoot height was affected the most in onion and ryegrass for monocotyledon species, with ER25 values of 1.78 and 1.61 g ac/ha, respectively, and ER50 values of 99.7 and 135.5 g ac/ha, respectively. Soybean, oil seed rape and carrot were the most sensitive dicotyledon species in terms of shoot height reduction with ER25 values of 0.21, 0.52 and 0.56 g ac/ha, respectively, and ER50 values of 6.31, 6.2 and 59.7 g ac/ha, respectively.

The main symptom of visual injury exhibited by treated plants was stunting. Wilting was also recorded for cabbage, sugar beet and oil seed rape, swollen stems for oil seed rape, necrosis for soybean and sugar beet and also blackened stems for sugar beet.

Overall, plant mortality was very low with no plant deaths observed at the final assessment for oat, onion, oil seed rape, cabbage, carrot, sugar beet and corn. One plant death in one replicate of

cucumber was observed, but this could not be attributed to treatment as it was at a lower application rate. Mortalities in ryegrass and soybean were mainly observed in the higher application rates.

Table 3: Effect of GF-1674 on Vegetative Vigour, according to the study author.

Species	NOEC, ER25 and ER50 (mg ai/kg soil or g ai/ha)								
	Survival	Shoot Height (mm)				Shoot Fresh Weight (mg)			
	No dead plants observed for oat, corn, onion, oil seed rape, cabbage, carrot and sugar beet. Survival effects in cucumber, soybean and ryegrass were observed only among the higher rates tested and since mortality was a less sensitive parameter than height or weight, endpoint values were not calculated.	dry weight*	NOEC	ER25	ER50	height*	NOEC	ER25	ER50
Oat		501 (402-548)	0.117	9.7	636	19.08 (16.97-20.69)	0.47	1.08	2.32
Ryegrass		403 (373-436)	0.23	1.61	135.5	9.93 (7.46-11.8)	0.94	0.79	1.26
Corn		599 (558-644)	0.94	11.40	1380	26.2 (21.9-31.3)	1.88	0.98	26.9
Onion		307 (245-347)	0.47	1.78	99.7	10.61 (7.87-12.76)	0.47	0.54	0.99
Soybean		693 (598-756)	0.059	0.21	6.31	16.88 (15.02-21.33)	0.23	0.14	0.86
Oil seed rape		383 (296-448)	0.23	0.52	6.2	24.67 (21.19-29.71)	3.75	5.3	17.1
Cabbage		158 (152-166)	15	>15	>15	19.99 (18.82-20.98)	7.5	6.03	16.4
Carrot		293 (282-312)	0.059	0.56	59.7	11.85 (9.65-13.26)	0.23	0.58	2.49
Cucumber		301 (279-340)	7.5	83.6	1370	37.07 (31.99-42.96)	7.5	4.3	18.0
Sugar beet		180 (170-200)	1.88	>15	>15	17.66 (15.28-20.73)	1.88	6.9	44.0

* Average of control (range of control values).

Plant Injury Index*											
Control	Solvent Control	Oat	Rye grass	Corn	Onion	Soybean	Oil seed rape	Cabbage	Carrot	Cucumber	Sugar beet
0	N/A	0-53	2-95	0-53	0-68	5-93	7-73	0-63	0-72	0-50	0-50

* Visual rating where: 0 = no injury ; 1-39 = slight injury; 40-69 = moderate injury; 70-99 = severe injury; 100 = all plants dead. Values listed are the range of average visual injury assessment from minimum (not including control) to maximum effect observed across rates when evaluated at 21 days.

Table 5: Effect of GF-1674 on vegetative vigour: most sensitive monocot and dicot plant species, according to the study author.

Statistical Endpoint	**Seedling emergence**
Monocot:	
most sensitive species	Onion (based on ER25)
NOEC for the most sensitive parameter	0.47 (shoot weight) [oat: 0.117 (shoot height)]
ER25 for the most sensitive monocot	0.54 (shoot weight)
ER50 for the most sensitive monocot	0.99 (shoot weight)
Reference chemical	Not applicable
Dicot:	
most sensitive species	Soybean
NOEC for the most sensitive parameter (eg: dry weight)	0.059 (shoot height; also, 0.059 for carrot shoot height)
ER25 for the most sensitive dicot	0.14 (shoot weight)
ER50 for the most sensitive dicot	0.86 (shoot weight)
Reference chemical	Not applicable.

B. REPORTED STATISTICS:

No observable effect concentrations (NOEC) for shoot length and shoot fresh weight were determined using a Dunnett's test in Minitab 12.22. Data were first tested for homogeneity of variance using a Levene's test and when required data were transformed using one of the following transformations; 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 injury ratings, shoot length and fresh weight using ARM 7. ER25 and ER50 values (g ac/ha) were calculated for shoot height and shoot fresh weight data expressed as a percentage (%) untreated control and capped at 100%. Regression models in Minitab 12.22 were used to calculate ER values.

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 ER25 value. The results presented above (see Table 3) for shoot fresh weights show NOECs for ryegrass, corn, soybean, cabbage and cucumber all greater 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. 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 6: Reviewed statistics using TOXCALC for ER05, ER25 and ER50, vegetative vigour test results. 95% CI provided in parentheses.

Species	**ER05, ER25 and ER50 (g as/ha)**					
	Shoot Height (mm)			**Shoot Fresh Weight (mg)**		
	ER05	ER25	ER50	ER05	ER25	ER50
Oat	0.032 (0.005 – 0.059)	>15	>15	0.042 (0.021-0.359)	0.956 (0.587 – 1.37)	2.55 (1.52 – 3.13)
Ryegrass	0.266 (0.047 – 0.429)	1.45 (0.876 – 1.91)	>30	0.307 (0.00 – 0.414)	0.746 (0.380 – 1.19)	1.33 (0.816 – 1.56)
Corn	0.574 (0.081 – 0.942)	20.7 (95% CI not calculated)	>30	0.141* (0.027 – 1.26)	1.36 (0.377 – 5.58)	27.3 (95% CI not calculated)
Onion	0.522 (0.00 – 0.614)	1.33 (0.619 – 2.89)	>15	0.051 (0.00 – 0.626)	0.623 (0.360 – 0.694)	0.883 (0.786 – 0.946)
Soybean	0.076 (0.004 – 0.098)	0.185 (0.134 – 0.215)	9.73 (95% CI not calculated)	0.046 (0.024 – 0.172)	0.243 (0.104 – 0.366)	0.856 (0.320 – 1.31)
Oil seed rape	0.035 (0.011 – 0.041)	0.058 (0.048 – 0.427)	5.77 (95% CI not calculated)	1.039 (0.00 – 2.98)	5.00 (0.88 – 8.17)	>15
Cabbage	12.2	>15	>15	0.99	5.0	>15
Carrot	0.021* (0.011 – 0.100)	0.474 (0.123 – 0.670)	>15	0.025* (0.004 – 0.315)	0.465 (0.140 – 0.656)	0.864 (0.723 - 4.36)
Cucumber	0.346 (0.00 – 0.834)	>15	>15	0.014* (0.006 – 0.058)	9.32 (7.40 – 10.84)	14.62 (95% CI not calculated)
Sugar beet	0.346 (0.028 – 0.863)	>15	>15	0.054 (0.032 – 0.702)	1.53 (0.316 – 2.77)	>15

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

The ER25 for oil seed rape based on shoot height is very low (0.058 g as/ha). However, when looking at the raw data, 25% or less reduction in shoot height was found for test concentrations

0.029 to 0.23 g as/ha with around a 40% reduction at the next test level of 0.47 g as/ha. The percent inhibition in shoot height compared to the control was -15.1 (stimulation), 22.6, 22.5, 15.3, 38.4, 42.8, 40.3, 41.8, 50.6 and 49.6% at the 0.029, 0.059, 0.117, 0.23, 0.47, 0.94, 1.88, 3.75, 7.5 and 15 g as/ha test concentrations, respectively. Therefore, the true ER25 could be argued as being between 0.029 and 0.427 g as/ha, hence the relatively wide confidence intervals associated with this value. With the exception of this result, based on ER25 results, the TOXCALC results support the statistics provided in the test report in that onion is deemed the most sensitive monocot (ER25 0.623 g as/ha, shoot weight) and soybean the most sensitive dicot (ER25 0.185 g as/ha, shoot height).

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

EPA Reviewer: Endpoints are based on the Reviewer's recalculated statistics using TOXCALC.

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 only averaged 81% of nominal and ranged from 77% to 91% 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.
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.
Observation intervals.	The test guideline states that shoot heights should be observed 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.
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.).
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 to recalculate the statistics.

Due to uncertainty associated with the shoot height ER25 value for oil seed rape, as reflected by the large confidence intervals (0.048 to 0.427 g ac/ha), and the less than 25% inhibition observed up to test concentrations of 0.47 g ac/ha, the ER25 for shoot height in oil seed rape will not be considered by the PMRA. For the PMRA, the most sensitive dicotyledon species in terms of shoot height reduction is considered to be soybean (ER25 of 0.185 g ac/ha).

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

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 ER25: Onion, ER25 0.623 g ac/ha (shoot fresh weight, reviewer calculated)
Most sensitive dicot and ER25: Oil Seed Rape ER25 0.058 g ac/ha (reviewer calculated).
PMRA: Most sensitive dicot and ER25: Soybean, ER25 0.185 g ac/ha (shoot height, reviewer-calculated)

III. REFERENCES:

References not provided in the test report. The study was based on:
U.S. Environmental Protection Agency. 1996. Ecological Effects Test Guidelines OPPTS 850.4250. Terrestrial Plant Toxicity Tier II (Vegetative Vigor).

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.