FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: A18385B

Product name: SPANDIS

Chemical active substance:

Dicamba, 400 g/kg
Nicosulfuron, 100 g/kg
Prosulfuron, 40 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(new authorization)

Applicant: Syngenta

Submission date: 30/11/2020

MS Finalisation date: 11/07/2022

Version history

When	What
November 2020	dRR submitted by applicant to the Polish Ministry of Agriculture and Rural Development
February 2021	Submission to the evaluation unit
September 2021	Updates following request of Poland (zRMS)
November 2021	Updates based on feedback from zRMS Poland
January 2022	Updates based on feedback from zRMS Poland
April 2022	zRMS evaluation of dRR
May 2022	Comments from applicant on dRR evaluation from zRMS in the annex point 9.5.2 and 9.5.3, including the 5 m SDB and 5 m VFSmod in the aquatic mitigation conclusion
July 2022	Final version prepared by zRMS after Commenting period

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9 Ecotoxicology (KCP 10)

Review Comments:

This document describes the acceptable use conditions required for registration of A18385B, a water dispersible granule formulation (WG) containing 100 g/kg of nicosulfuron, 40 g/kg of prosulfuron and 400 g/kg of dicamba, for use as a herbicide for controls weeds in maize.

This Part B document only reviews data and additional information that has not previously been considered within the EU review process.

Since this document is based on the information provided by the applicant, all review comments, additions and corrections have been made using commenting boxes or highlighted in grey. Any incorrect data or text not evaluated by the zRMS has been crossed out.

All changes in the report made by the applicant at the request of zRMS are marked in pink.

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	15	16	17	18	19	20	21
Use - No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**					PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg A18385B / ha a) max. rate per appl. b) max. total rate per crop/season	g prosulfuron/ ha a) max. rate per appl. b) max. total rate per crop/season	g nicosulfuron/ ha a) max. rate per appl. b) max. total rate per crop/season	g dicamba / ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																						
1	PL	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.4 b) 0.4	16	40	160	200-400		tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)							
1	PL	Maize	F	Annual/ perennial broad leave weeds and grasses	Foliar spray	BBCH 12-18	1 (1 appl. every 3rd year)	N/A	a) 0.5 b) 0.5	20	50	200	200-400		tank-mixed oil-based adjuvant needed (e.g Adigor@ 1.0-1.5L/ha)							

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 "Conclusion"

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

Remarks table:

(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
(6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(8) The maximum number of application possible under practical conditions of use must be provided
(9) Minimum interval (in days) between applications of the same product.
(10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
(13) PHI - minimum pre-harvest interval
(14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

9.1.1.1 Effects on birds (KCP 10.1.1), Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk (including drinking water and secondary poisoning) at either Screening step or Tier 1, indicating that the risk to birds and mammals is acceptable following use of A18385B according to the proposed use pattern.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The PEC/RAC ratios, using worst-case PEC_{SW} values for A18385B, are less than the trigger value of 1, for all aquatic organisms, with the exception of aquatic plants exposed to prosulfuron, nicosulfuron and A18385B. A refined risk assessment is conducted for aquatic plants exposed to prosulfuron, nicosulfuron and A18385B taking into account appropriate mitigation measures.

~~The potential risk to aquatic plants exposed to prosulfuron has been refined by using FOCUS Step 3 7 d TWA values where appropriate.~~ Safe use for prosulfuron for all FOCUS scenarios (except R1 stream for 20 g a.s./ha) is indicated taking into account FOCUS Step 3 values based on field DT_{50} without further mitigation.
In addition, FOCUS Step 4 PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies as an additional refinement option.

The PEC/RAC ratios are <1 for nicosulfuron when based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies as an additional refinement option.

~~The PEC/RAC ratios are <1 for A18385B when consideration is given to a 5 m buffer zone or 75% drift reducing nozzles following application of 1 x 400 g A18385B/ha or 90% drift reducing nozzles or 5 m buffer zone following application of 1 x 500 g A18385B/ha, respectively.~~

Additionally, the mixture toxicity assessment was conducted.

Overall, the risk to aquatic plants is acceptable following the proposed use pattern of 400 or 500 g A18385B/ha implementing drift and run-off mitigation.

To protect aquatic organisms respect an unsprayed, vegetated buffer zone of 20m to surface water bodies.
Based on the additional calculations included in the aquatic section by the applicant and considering the mitigation option of a 5m VFSmod buffer. The overall conclusion on mitigation is **5m VFSmod + 5m drift buffer** for both application rates (or 20m VFS as already concluded from zRMS).

Additional, the mixture toxicity assessment, performed by the Applicant was accepted. Thus, for Poland an unsprayed, vegetated buffer zone of 5 m to surface water bodies is sufficient to conclude safe use of A18385B in maize.

Table 9.1-2: Proposed mitigation measures for application of A18385B to maize according to the proposed use pattern for Poland

Crop group	Use pattern	Scenario		
		D3	D4	R1
Maize	1 x 400 g A18385B/ha	-	-	5-10 m VFS or 5 m VFS_{MOD}
	1 x 500 g A18385B/ha	-	-	10 m VFS or 5 m VFS_{MOD}

VFS = Vegetative filter strip (run-off buffer)

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk of A18385B to honey-bees was assessed from hazard quotients between toxicity endpoints, estimated from acute oral and contact studies with A18385B, prosulfuron, nicosulfuron and dicamba, and the maximum single application rates.

All the hazard quotients are less than 50, indicating that the risk to bees is acceptable following use of A18385B according to the proposed use pattern.

According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): The Applicant should provide chronic test on bees and evaluation of effects on honey bee development with formulated product. The chronic studies were not performed, therefore, for Poland, the deficiencies need to be fulfilled by the entry into force of the revised EFSA bee guideline. Concerned Member States must decide on the consideration of data requirements on national level.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

At Tier I, the in-field HQ values were below the trigger value for the worst case use scenarios (1 x 400 and 1 x 500 g A18385B/ha in maize) indicating the need for further refinement. The off-field HQ values were below the trigger value for all proposed uses indicating that the risk to in-field non-target arthropods is acceptable following the use of A18385B according to the proposed use pattern.

The Tier II, extended laboratory studies showed acceptable foliar in-field and off-field effects from foliar applications of A18385B for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Chrysoperla carnea* and *Aleochara bilineata* for the worst case use scenarios (1 x 400 and 1 x 500 g A18385B/ha in maize). The risk to non-target arthropods is therefore acceptable following use of A18385B according to the proposed use pattern.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

Soil meso- and macrofauna

The acute and long-term risk of A18385B, prosulfuron, nicosulfuron, dicamba and relevant metabolites was evaluated where relevant for earthworms, Collembola and *Hypoaspis*. The risk assessment demonstrated that the risk to non-target soil meso- and macrofauna is acceptable following use of A18385B according to the proposed use pattern.

Soil micro-organisms

All the effect levels for A18385B, prosulfuron, nicosulfuron, dicamba and relevant metabolites exceeded the relevant PEC_{soil} values, indicating that the risk to soil micro-organisms is acceptable following use of A18385B according to the proposed use pattern.

9.1.1.6 Effects on non-target terrestrial plants (KCP 10.6)

The risk of A18385B to non-target terrestrial plants was assessed from toxicity exposure ratios (TERs) using the formulation toxicity data from Tier II studies using a calculated HC5, and the maximum off-field predicted environmental residues (PERs). Higher tier field studies have been used to further refine the risk assessment.

When based on the probabilistic HC_5 approach, the risk to non-target terrestrial plants in off-crop areas is acceptable following use of A18385B according to the proposed use pattern, provided the following mitigation is implemented:

1 x 400 g A18385B/ha:

- No buffer and 90% drift reduction mitigation or
- 5 m buffer with 50% drift reduction or
- 10 m buffer with no drift reduction

1 x 500 g A18385B/ha:

- 5 m buffer with 75% drift reduction or
- 10 m buffer with 50% drift reduction or
- 15 m buffer with no drift reduction.

When based on the most sensitive ER_{50} of the higher tier field studies, the risk to non-target terrestrial plants in off-crop areas is acceptable following use of A18385B according to the proposed use pattern, provided the following mitigation is implemented:

1 x 400 g A18385B/ha:

- 75% drift reduction or
- 5 m buffer

1 x 500 g A18385B/ha:

- 90% drift reduction mitigation or
- 5m buffer

9.1.1.7 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

No further data are required.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

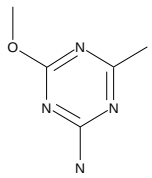
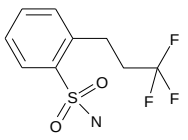
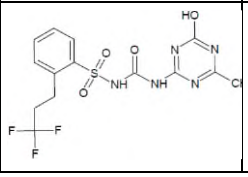
Table 9.1-3: Critical use pattern of A18385B grouped according to criterion

Grouping according to criterion			
Use no.	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
1	Maize	Crop (maize) Growth stage: BBCH 12-18 Application rate: 1 × 400 g A18385B/ha (1 × 16 g prosulfuron/ha, 40 g nicosulfuron/ha and 160 g dicamba/ha) Minimum application rate	Use pattern for all aspects of the ecotox risk assessment to adress the application rate of 1 × 16 g prosulfuron/ha, 40 g nicosulfuron/ha and 160 g dicamba/ha
1	Maize	Crop (maize) Growth stage: BBCH 12-18 Application rate: 1 × 500 g A18385B/ha (1 × 20 g prosulfuron/ha, 50 g nicosulfuron/ha and 200 g dicamba/ha) Maximum application rate	Critical use pattern for all aspects of the ecotox risk assessment to support an application rate 1 × 20 g prosulfuron/ha , 50 g nicosulfuron/ha and 200 g dicamba/ha

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of A18385B is indicated in the table.

Table 9.1-4: Metabolites of prosulfuron

Metabolite	**Molar mass (g/mol)**	**Chemical structure**	**Maximum observed occurrence in compartments (%)**	**Risk assessment required?**
CGA150829 prosulfuron triazine amine	140.1		Soil: > 10 % of a.s. Water: > 10 % of a.s. (aquatic hydrolysis) Water/sediment: > 5 % of a.s.	PEC_s $PEC_{SW/SED}$
CGA159902 prosulfuron phenyl sulfonamide	253.2		Soil: > 10 % of a.s. Water: > 10 % of a.s. (aquatic hydrolysis) Sediment: > 10 % of a.s. Water/sediment: > 10 % of a.s.	PEC_s $PEC_{SW/SED}$
CGA300406 O-desmethyl-prosulfuron	405.4		Soil: > 10 % of a.s. Water: > 10 % of a.s. Sediment: > 10 % of a.s.	PEC_s $PEC_{SW/SED}$

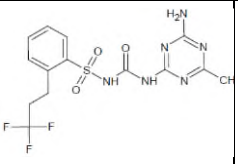
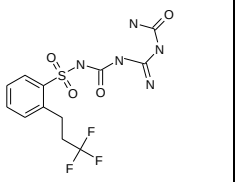
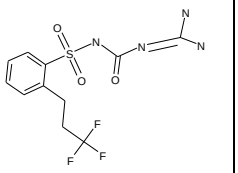
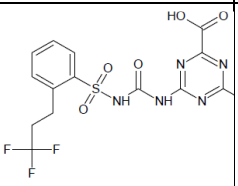
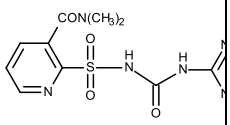
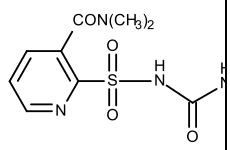
Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence in compartments (%)	Risk assessment required?
CGA325025 demethoxy amino-prosulfosulfuron	404.4		Soil: > 10 % of a.s. Water/sediment: > 5 % of a.s.	PEC_s $PEC_{SW/SED}$
SYN542604	381.3		Soil: > 10 % of a.s. Water/sediment: > 10 % of a.s.	PEC_s $PEC_{SW/SED}$
CGA349707	338.3		Soil: > 10 % of a.s. Water/sediment: > 10 % of a.s.	PEC_s $PEC_{SW/SED}$
SYN547308	449.4		Soil: > 5 % of a.s. and maximum of formation not yet reached at the end of the study Water: < 5 % Sediment: < 5 %	PEC_s

Table 9.1-5: Metabolites of nicosulfuron

Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence in compartments (%)	Risk assessment required?
HMUD 2-{[(4-hydroxy-6-methoxypyrimidin-2-yl)carbamoyl]sulfamoyl}-*N*,*N*-dimethylpyridine-3-carboxamide	396.4		Soil: > 10 % of a.s. Water: > 10 % of a.s. Sediment: > 5 % of a.s. in 2 sequential measurements	PEC_s $PEC_{SW/SED}$
AUSN 2-[(carbamimidoylcarbamoyl)sulfamoyl]-*N*,*N*-dimethylpyridine-3-carboxamide	314.3		Soil: > 10 % of a.s. Water: > 5 % of a.s. and maximum of formation not yet reached at the end of the study Sediment: < 5 % of a.s.	PEC_s $PEC_{SW/SED}$

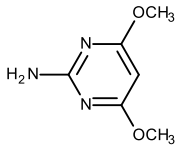
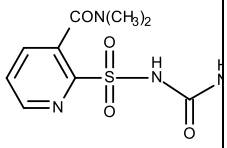
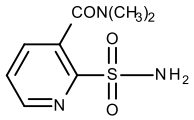
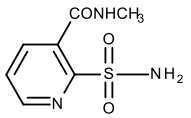
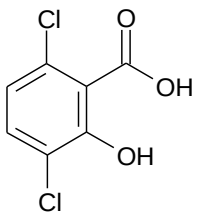
Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence in compartments (%)	Risk assessment required?
ADMP 4,6-dimethoxypyrimidin-2-amine	155.2		Soil: > 5 % of a.s. in 2 sequential measurements Water: < 5 % of a.s. Sediment: < 5 % of a.s.	PEC_s $PEC_{SW/SED}$
UCSN 2-[(carbamoylcarbamoyl)sulfamoyl]-*N*,*N*-dimethylpyridine-3-carboxamide	315.3		Soil: > 10 % of a.s. Water: > 5 % of a.s. and maximum of formation not yet reached at the end of the study Sediment: < 5 % of a.s.	PEC_s $PEC_{SW/SED}$
ASDM *N*,*N*-dimethyl-2-sulfamoylpyridine-3-carboxamide	229.2		Soil: > 10 % of a.s. Water: > 5 % of a.s. and maximum of formation not yet reached at the end of the study Sediment: < 5 % of a.s.	PEC_s $PEC_{SW/SED}$
MU-466 *N*-methyl-2-sulfamoylpyridine-3-carboxamide	215.1		Soil: > 0.1 µg/L in lysimeter leachate Water: < 5 % of a.s. Sediment: < 5 % of a.s.	-

Table 9.1-6: Metabolites of dicamba

Metabolite	Molar mass (g/mol)	Chemical structure	Maximum observed occurrence in compartments (%)	Exposure assessment required due to
DCSA 3,6-dichlorosalicylic acid, 3,6-dichloro-2-hydroxy-benzoic acid	207		Soil: > 10 % of a.s. Water: > 10 % of a.s. Sediment: < 5 % of a.s.	PEC_s $PEC_{SW/SED}$

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with prosulfuron, nicosulfuron and dicamba and prosulfuron relevant metabolites. Full details of these studies are provided in the respective EU RAR or DAR and related

documents.

Effects on birds of formulation A18385B were not evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba. Since mammal studies give no indication of higher toxicity from the formulation and the risk to birds from A18385B can be adequately assessed from risk assessment for the individual active substances and the "virtual formulation" considering additive toxicity, the risk to birds from the proposed uses of A18385B will be assessed using the endpoints for prosulfuron, dicamba and nicosulfuron.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds - prosulfuron

Species	Substance	Exposure System	Results	Reference
Mallard duck	Prosulfuron	Oral 1 d Acute	**LD_{50} = 1300 mg/kg bw**	EFSA Journal 2014;12(9):3815
Mallard duck	Prosulfuron	Oral 1 d Acute	LD_{50} = 2105 mg/kg bw	EFSA Journal 2014;12(9):3815
Bobwhite quail	Prosulfuron	Oral 1 d Acute	LD_{50} > 2150 mg/kg bw	EFSA Journal 2014;12(9):3815
Mallard duck	Prosulfuron	Dietary 8 d Short-term	$LDD_{50} \geq$ 1352 mg/kg bw/d	EFSA Journal 2014;12(9):3815
Bobwhite quail	Prosulfuron	Dietary 8 d Short-term	$LDD_{50} \geq$ 735 mg/kg bw/d	EFSA Journal 2014;12(9):3815
Mallard duck	Prosulfuron	Dietary Reproductive toxicity	**NOEL = ~~2.96~~ 2.95 mg/kg bw/d**	EFSA Journal 2014;12(9):3815

Endpoints in bold were used for the risk assessment

Table 9.2-2: Endpoints and effect values relevant for the risk assessment for birds - nicosulfuron

Species	Substance	Exposure System	Results	Reference
Bobwhite quail, Mallard duck	Nicosulfuron	Oral 1 d Acute	LD_{50} >2000 mg a.s./kg bw	EFSA Scientific Report (2007) 120, 1-91
Bobwhite quail	Nicosulfuron	Acute	**Extrapolated value of 3776 mg/kg [a]**	Please refer to 9.2.1.1
Bobwhite quail	Nicosulfuron	Dietary 8 d Short-term	5 day LC_{50} >1603 mg a.s./kg bw/day	EFSA Scientific Report (2007) 120, 1-91
Mallard duck	Nicosulfuron	Dietary 8 d Short-term	5 day LC_{50} >911 mg a.s./kg bw/day	EFSA Scientific Report (2007) 120, 1-91
Japanese quail	Nicosulfuron	Reproductive toxicity	**NOEL = 171 mg a.s./kg bw/day**	EFSA Scientific Report (2007) 120, 1-

Species	Substance	Exposure System	Results	Reference
				91

[a] The acute toxicity value for the bobwhite can be extrapolated according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)
Endpoints in bold were used for the risk assessment

Table 9.2-3: Endpoints and effect values relevant for the risk assessment for birds - dicamba

Species	Substance	Exposure System	Results	Reference
Anas platyrhynchos (Mallard duck)	Dicamba	Oral 1 d Acute	LD_{50} = 1373 mg/kg bw	EFSA Scientific Report 2011;9(1):1965
Colinus virginianus (Bobwhite quail)	Dicamba	Oral 1 d Acute	LDD_{50} = 216 mg/kg bw	EFSA Scientific Report 2011;9(1):1965
Anas platyrhynchos (Mallard duck), *Colinus virginianus* (Bobwhite quail)	Dicamba	Acute	**Geometric mean of endpoints = 545 mg a.s./kg bw**	Please refer to 9.2.1.1
Anas platyrhynchos (Mallard duck)	Dicamba	Dietary 8 d Short-term	LD_{50} >1567 mg a.s./kg bw/day	EFSA Scientific Report 2011;9(1):1965
Colinus virginianus (Bobwhite quail)	Dicamba	Dietary 8 d Short-term	LD_{50} >995 mg a.s./kg bw/day	EFSA Scientific Report 2011;9(1):1965
Anas platyrhynchos (Mallard duck)	Dicamba	Dietary Reproductive toxicity	NOEL = 89 mg a.s./kg bw/day	EFSA Scientific Report 2011;9(1):1965
Colinus virginianus (Bobwhite quail)	Dicamba	Dietary Reproductive toxicity	NOEL = 170 mg a.s./kg bw/day	EFSA Scientific Report 2011;9(1):1965
Anas platyrhynchos (Mallard duck), *Colinus virginianus* (Bobwhite quail)	Dicamba	Reproductive toxicity	**LD/10 ie 54.5 mg/kg bw [a]**	EFSA Scientific Report 2011;9(1):1965

[a] For the long-term assessment, the geometric mean LD_{50}/10 of 54.5 mg a.s./kg bw is used as an endpoint in the reproductive assessment, since this endpoint is lower than the lowest NOEL from the avian reproduction studies (89 mg a.s./kg bw/d)
Endpoints in bold were used for the risk assessment

Table 9.2-4: Endpoints and effect values relevant for the risk assessment for birds – prosulfuron/nicosulfuron/dicamba mixture

Substance	Exposure system	Proposed endpoint	Reference
Prosulfuron/nicosulfuron/dicamba mixture	Acute	LD_{50} mix = 682.5 mg/kg bw	Refer to section 9.2.1.1

9.2.1.1 Justification for new endpoints

There are no new endpoints for prosulfuron. All endpoints are in line with EFSA Journal 2014;12(9):3815. All endpoints for nicosulfuron, dicamba and the mixture have been already evaluated in Central zone for

for product authorisation of A18385B. For convenience, the endpoints used in the risk assessment for nicosulfuron, dicamba and the mixture are presented below.

Consideration of acute endpoints for nicosulfuron used in the risk assessment

All data have been taken directly from the EU endpoints document; the **EFSA Scientific Report (2007) 120, 1-91**, or the **DAR** for nicosulfuron (Addendum 3 to Annex B for nicosulfuron, section B.9 Ecotoxicology).

In the acute oral toxicity study conducted with the bobwhite (***Cummings, 1991b***, for further details please refer to the **DAR** for nicosulfuron) no mortalities were observed and therefore the LD_{50} was reported as >2000 mg/kg bw/d. According to the **Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438)**[1] the acute toxicity value for the bobwhite can be extrapolated. The extrapolated value is presented in the table below.

Table 9.2-5: Extrapolation of the acute oral toxicity value for nicosulfuron

Test species	Experimental LD_{50} (mg/kg bw/d)	Number of animals tested	Number of mortalities	Extrapolation factor [a]	Corrected LD_{50} (mg/kg bw/d)	Study
Bobwhite quail	>2000	10	0	1.888	3776	Cummings, 1991b (DAR, nicosulfuron)

[a] The extrapolation factor is presented in Table 1 of the guidance document (Point 2.1.2)

The extrapolated LD_{50} value of 3776 mg/kg bw/d will therefore be used in the subsequent risk assessment.

Review Comments:

The proposed extrapolated LD_{50} value of 3776 mg/kg is accepted by zRMS, as it was derived according the EFSA B&M guidance.

Consideration of acute endpoints for dicamba used in the risk assessment

According to EFSA/2009/1438 the geometric mean should be used for the acute assessment, except when the endpoint for the most sensitive species is more than a factor of 10 below the geometric mean of all the tested species. Since this is not the case, the geometric mean was used for the risk assessment. As two acute oral toxicity studies are available with dicamba a geometric mean can be calculated following the approach outlined under Point 2.4.2 of the **Guidance Document**. Before a geometric mean can be calculated we need to ensure that the studies are equivalent in terms of endpoint and in particular the vehicle/solvent used in dosing. Both the studies conducted with the mallard duck by Campbell & Beavers, 1993 and the bobwhite quail by Campbell *et al.*, 1993, were conducted in accordance with Fifra Subdivision E, Section 71-1; dicamba was also dosed in a corn oil solvent by oral gavage in both. The studies were conducted in accordance with the same guidance documents by the same laboratory therefore the studies are equivalent and it is appropriate to calculate a geometric mean. The geometric mean of 1373 mg a.s./kg bw and 216 mg a.s./kg bw/d is 545 mg/kg bw.

[1] European Food Safety Authority (2009): Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA. EFSA Journal 2009; 7(12):1438. [139 pp.]

Review Comments:

The proposed geometric mean LD_{50} value of 545 mg/kg is accepted by zRMS, as it was derived according the EFSA B&M guidance (Points: 2.4.1 and 2.4.2).

Consideration of reproductive endpoints for dicamba used in the risk assessment

According to the EFSA/2009/1438, an estimated reproductive endpoint should be obtained by using the acute oral LD_{50} (from a single species or geometric mean) and divided by 10 to obtain an $LD_{50}/10$. This $LD_{50}/10$ is used as an endpoint in the reproductive assessment to take account of the possibility of reproductive impairment due to sub-lethal effects on pair formation and breeding site selection, incubation, parental care of nestlings, and survival of fledgling birds (in accordance with **Appendix J** of the EFSA Guidance). If the $LD_{50}/10$ is lower than the lowest reproductive endpoint, then this should be used as the Screening Step reproductive endpoint.
For Dicamba the geometric mean $LD_{50}/10$ of 54.5 mg a.s./kg bw is used as an endpoint in the reproductive assessment, since this endpoint is lower than the lowest NOEL from the avian reproduction studies.

Review Comments:

The proposed geometric mean $LD_{50}/10$ value of 54.5 mg/kg bw/d is accepted by zRMS, as it was derived according the EFSA B&M guidance.

Consideration of acute mixture toxicity

According to EFSA/2009/1438 combined action of several toxicants must be specifically considered in the risk assessment when it is obvious that such exposure situations will occur for animals.
For the assessment of acute effects (mortality), a surrogate LD_{50} can be calculated. The EFSA Guidance Document indicates that the following equation should be used for deriving a surrogate LD_{50} for a mixture of active substances with known toxicity assuming dose additivity:

$$\mathrm{LD}_{50}\ (\mathrm{mix}) = \left(\sum_i \frac{\mathrm{X}(a.s._i)}{\mathrm{LD}_{50}(a.s._i)} \right)^{-1}$$

where:
$X\ (a.s._i)$ = fraction of active substance (i) in the formulation mixture
$LD_{50}\ (a.s._i)$ = acute toxicity for the active substance (i)

The LD_{50} of the mix is summarised in the table below.

Table 9.2-6: Acute LD_{50} for the mixture of active substances

Test substance	Concentration of active substance in formulation A18385B (g/kg)	Fraction of active substance in the formulation mixture[a]	Acute toxicity endpoint (mg/kg bw)	Fraction of active substance/LD_{50} for the active substance	LD_{50} mix (mg/kg bw)
Prosulfuron	40	0.074	1300	5.7×10^{-5}	682
Nicosulfuron	100	0.185	3776	4.9×10^{-5}	
Dicamba	400	0.741	545	1.4×10^{-3}	
Total	540	1	-	-	

[a] Concentration of an active substance in the formulation, divided by, the total concentration of all active substances in the formulation.

Review Comments:

The proposed $LD_{50\ MIX}$ value of 682 mg/kg is accepted by zRMS, as it was derived according the EFSA B&M guidance.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA/2009/1438).

9.2.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive screening step risk assessments are summarised in the following tables. A Tier 1 risk assessment is conducted also for uses that are safe at screening step when results can be used for assessing the chronic mixture toxicity.

Prosulfuron

Table 9.2-7: Screening Step assessment of the acute and long-term/reproductive risk for birds due to the use of A18385B in maize – prosulfuron

Intended use		Maize				
Active substance		Prosulfuron				
Acute toxicity (mg/kg bw)		1300				
TER criterion		10				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, BBCH 12 - 18	1 × 16	Small omnivorous bird	158.8	1	2.54	510
	1 × 20	Small omnivorous bird	158.8	1	3.18	410
Reprod. Toxicity (mg/kg bw/d)		2.95				
TER criterion		5				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 16	Small omnivorous bird	64.8	1 × 0.53	0.550	5.4
	1 × 20	Small omnivorous bird	64.8	1 × 0.53	0.687	**4.3**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-8: Tier 1 assessment of the long-term/reproductive risk for birds due to the use of A18385B in maize – prosulfuron

Intended use **Active substance**		Maize Prosulfuron					
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		2.95 5					
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Scenario**	**Generic Focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 16	BBCH 10 - 29	Medium granivorous bird "gamebird"	3.0	1 × 0.53	0.0254	120
		Leaf development BBCH 10 to 19	Small insectivorous/worm feeding species “thrush”	5.7	1 × 0.53	0.0483	61
		BBCH 10 - 29	Small omnivorous bird “lark”	10.9	1 × 0.53	0.0924	32
		BBCH 10 - 29	medium herbivorous/granivorous bird "pigeon"	22.7	1 × 0.53	0.192	15
		BBCH 10 - 19	Small insectivorous bird “wagtail”	11.3	1 × 0.53	0.0958	31
	1 × 20	BBCH 10 - 29	Medium granivorous bird "gamebird"	3.0	1 × 0.53	0.0318	93
		Leaf development BBCH 10 to 19	Small insectivorous/worm feeding species “thrush”	5.7	1 × 0.53	0.0604	49
		BBCH 10 - 29	Small omnivorous bird “lark”	10.9	1 × 0.53	0.116	26
		BBCH 10 - 29	medium herbivorous/granivorous bird "pigeon"	22.7	1 × 0.53	0.241	12
		BBCH 10 - 19	Small insectivorous bird “wagtail”	11.3	1 × 0.53	0.120	25

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Nicosulfuron

Table 9.2-9: Screening Step assessment of the acute and long-term/reproductive risk for birds due to the use of A18385B in maize – nicosulfuron

Intended use		Maize				
Active substance		Nicosulfuron				
Acute toxicity (mg/kg bw)		3776				
TER criterion		10				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, BBCH 12 - 18	1 × 40	Small omnivorous bird	158.8	1	6.35	590
	1 × 50	Small omnivorous bird	158.8	1	7.94	480
Reprod. Toxicity (mg/kg bw/d)		171				
TER criterion		5				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 40	Small omnivorous bird	64.8	1 × 0.53	1.37	120
	1 × 50	Small omnivorous bird	64.8	1 × 0.53	1.72	100

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Dicamba

Table 9.2-10: Screening Step assessment of the acute and long-term/reproductive risk for birds due to the use of A18385B in maize – dicamba

Intended use		Maize				
Active substance		Dicamba				
Acute toxicity (mg/kg bw)		545				
TER criterion		10				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, BBCH 12 - 18	1 × 160	Small omnivorous bird	158.8	1	25.4	21
	1 × 200	Small omnivorous bird	158.8	1	31.8	17
Reprod. Toxicity (mg/kg bw/d)		54.5				
TER criterion		5				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 160	Small omnivorous bird	64.8	1 × 0.53	5.50	9.9
	1 × 200	Small omnivorous bird	64.8	1 × 0.53	6.87	7.9

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 9.2-11: Tier 1 assessment of the long-term/reproductive risk for birds due to the use of A18385B in maize – dicamba

Intended use		Maize					
Active substance		Dicamba					
Reprod. Toxicity (mg/kg bw/d)		54.5					
TER criterion		5					
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Scenario**	**Generic Focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 200	BBCH 10 - 29	Medium granivorous bird "gamebird"	3.0	1 × 0.53	0.3180	170
		Leaf development BBCH 10 to 19	Small insectivorous/worm feeding species “thrush”	5.7	1 × 0.53	0.6042	90
		BBCH 10 - 29	Small omnivorous bird “lark”	10.9	1 × 0.53	1.155	47
		BBCH 10 - 29	medium herbivorous/granivorous bird "pigeon"	22.7	1 × 0.53	2.406	23
		BBCH 10 - 19	Small insectivorous bird “wagtail”	11.3	1 × 0.53	1.198	46

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Prosulfuron/nicosulfuron/dicamba mixture

Acute risk

Table 9.2-12: Screening Step assessment of the acute and long-term/reproductive risk for birds due to the use of A18385B in maize – prosulfuron/nicosulfuron/dicamba mixture

Intended use		Maize				
Active substance		Prosulfuron/nicosulfuron/dicamba mixture				
Acute toxicity (mg/kg bw)		682				
TER criterion		10				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, BBCH 12 - 18	1 × 216	Small omnivorous bird	158.8	1	34.3	20
	1 × 270	Small omnivorous bird	158.8	1	42.9	16

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Chronic risk

For assessment of chronic effects, according to the EFSA guidance, if a given formulation contains several active substances all known to cause similar effects *via* a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effect is actually driving the risk assessment, it is thus recommended to perform an assessment for combined effects on a case by case basis.

For A18385B the active ingredient, prosulfuron and nicosulfuron (sulfonylureas) have a different mode of action in plants than the active ingredient dicamba (benzoic acid), and their toxicity profiles in birds are very different.
Prosulfuron and nicosulfuron cause effects on acetolactate synthase in branched chain amino acid synthesis compared to dicamba which causes effects on synthetic auxins. Consequently an assessment for combined effects is not required. Nevertheless as a worst-case approach an assessment for combined effects will be conducted and is based on a concentration addition approach.

In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration using the following equation:

TERcombi = trigger / ((trigger prosulfuron/TER prosulfuron) + (trigger nicosulfuron/TER nicosulfuron) + (trigger dicamba/TER dicamba))

An acceptable risk is expected when TERcombi > trigger.

In this formula, 'triggers' are the EU triggers.

Table 9.2-13: Assessment of the long-term/reproductive risk for birds due to the use of A18385B in maize: combination risk assessment

Intended use	Maize						
Application rate (g/ha)	1 × 216 and 1 x 270						
$Trigger_{combi}$	5						
TER criterion	5						
Application rate (g a.s./ha)	**$TER_{prosulfuron}$**	**Trigger $5/TER_{prosulfuron}$**	**$TER_{nicosulfuron}$**	**Trigger $5/TER_{nicosulfuron}$**	**$TER_{dicamba}$**	**Trigger $5/TER_{dicamba}$**	**TER_{combi}**
1 x 216	15[a]	0.33	120	0.04	9.9	0.50	5.7
1 x 270	12[a]	0.41	100	0.05	23[a]	0.22	7.4

TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
[a] TERs resulted from Tier 1 assessments

9.2.2.2 Higher-tier risk assessment

Not required.

9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

Since A18385B is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (K_{oc} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{oc} ≥ 500 L/kg).

With a K(f)oc of 11.7 (geomean), 20.7 and 12.4 L/kg, prosulfuron, nicosulfuron and dicamba, respectively, belong to the group of less sorptive substances. The maximum use rates are used to cover the risk to birds from all intended uses (see 9.1.2).

Table 9.2-14: Ratio of effective application rate to toxicity endpoints for birds exposed to prosulfuron

Effective application rate (g/ha)*	=	20		
Acute toxicity (mg/kg bw)	=	1300	quotient =	0.02
Reprod. toxicity (mg/kg bw/d)	=	2.95	quotient =	6.78

* Effective application rate = Maximum application rate x MAF of 1 (one application)

Table 9.2-15: Ratio of effective application rate to toxicity endpoints for birds exposed to nicosulfuron

Effective application rate (g/ha)*	=	50		
Acute toxicity (mg/kg bw)	=	3776	quotient =	0.01
Reprod. toxicity (mg/kg bw/d)	=	171	quotient =	0.29

* Effective application rate = Maximum application rate x MAF of 1 (one application)

Table 9.2-16: Ratio of effective application rate to toxicity endpoints for birds exposed to dicamba

Effective application rate (g/ha)*	=	200		
Acute toxicity (mg/kg bw)	=	545	quotient =	0.37
Reprod. toxicity (mg/kg bw/d)	=	54.5	quotient =	3.67

* Effective application rate = Maximum application rate x MAF of 1 (one application)

The resulting ratios fall below the trigger of 50 indicating that further assessment of the acute and long-term risk to birds from drinking water from puddles is not required for prosulfuron, nicosulfuron and dicamba.

9.2.2.4 Effects of secondary poisoning

Prosulfuron has a log P_{OW} of 1.5 at pH 5, nicosulfuron has a log P_{OW} value of 0.6 and its major aquatic metabolites ASDM, AUSN and HMUD have log P_{OW} values of <1.0. The value for dicamba is 0.55 – 1.9 (at pH 5.0 – 8.9) and for its metabolite DCSA is -0.84 (pH 6.8). Therefore it is not necessary to consider

risk from secondary poisoning for prosulfuron, dicamba or nicosulfuron.

Risk assessment for earthworm-eating birds *via* secondary poisoning

Not required.

Risk assessment for fish-eating birds *via* secondary poisoning

Not required.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.2.4 Overall conclusions

The acute and long-term risks of A18385B to birds were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with prosulfuron, nicosulfuron and dicamba, and maximum residues occurring on food items following applications according to the proposed use pattern. The risk to birds from exposure *via* drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute risk and 5 for long-term risk for prosulfuron, nicosulfuron, dicamba, and the mixture toxicity of the three substances indicating that the risk to birds is acceptable following use of A18385B according to the proposed use pattern. Acceptable risk to birds from exposure *via* drinking water was also shown.

Review Comments:

All TER values exceed the relevant triggers indicating that A18385B does not pose an unacceptable risk to birds following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. Since the log P_{ow} value of prosulfuron, nicosulfuron, dicamba, and their relevant metabolites are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with prosulfuron, nicosulfuron and dicamba. Full details of these studies are provided in the respective EU RAR and DAR and related documents.

Effects on terrestrial vertebrates other than birds of A18385B were not evaluated as part of the EU assessment of the prosulfuron, nicosulfuron and dicamba but were already evaluated in the Central zone for last authorization of A18385B. No new data have been submitted.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat	Prosulfuron	Oral 1 d Acute	**LD_{50} = 986 mg/kg bw**	EFSA Journal 2014;12(9):3815
Rat	Prosulfuron	90d Short-term	**NOAEL = 3 mg/kg bw/d** (screening step) [a]	EFSA Journal 2014;12(9):3815
Rat	Prosulfuron	Long-term	NOAEL = 12 mg/kg bw/d	EFSA Journal 2014;12(9):3815
Rat	Prosulfuron	Developmental toxicity	NOAEL = 200 mg/kg bw (maternal) NOAEL = 50 mg/kg bw (developmental)	EFSA Journal 2014;12(9):3815
Rabbit	Prosulfuron	Developmental toxicity	NOAEL = 10 mg/kg bw (maternal) **NOAEL = 10 mg/kg bw (developmental)** (Tier 1) [a]	EFSA Journal 2014;12(9):3815

Endpoints in bold were used for the risk assessment

[a] During EU evaluation it was discussed and agreed by the experts that the endpoints to be used at the different steps of the long-term risk assessment should be selected as recommended in the EFSA Guidance (2009). Therefore, for the screening step the lowest endpoint from the 90-day rat study was used, while at tier 1 the lowest endpoint from the rabbit developmental study was selected (EFSA Journal 2014;12(9):3815).

Table 9.3-2: Endpoints and effect values relevant for the risk assessment for mammals - nicosulfuron

Species	Substance	Exposure System	Results	Reference
Rat	Nicosulfuron	Oral 1 d Acute	**LD_{50} >5000 mg/kg bw**	EFSA Scientific Report (2007) 120, 1-91
Rat	Nicosulfuron	Dietary Reproductive toxicity Two-generation study	**NOAEL = 3861 mg/kg bw/d** (male) & 4404 mg/kg bw/d (female)	EFSA Scientific Report (2007) 120, 1-91

Endpoints in bold were used for the risk assessment

Table 9.3-3: Endpoints and effect values relevant for the risk assessment for mammals - dicamba

Species	Substance	Exposure System	Results	Reference
Rat, female	Dicamba	Oral 1 d Acute	**LD_{50} = 1581 mg/kg bw**	EFSA Scientific Report 2011;9(1):1965

Species	Substance	Exposure System	Results	Reference
Rat	Dicamba	Dietary Reproductive toxicity Two-generation study	**NOAEL = 150 mg/kg bw/d**	EFSA Scientific Report 2011;9(1):1965

Endpoints in bold were used for the risk assessment

Table 9.3-4: Endpoints and effect values relevant for the risk assessment for mammals – A18385B

Species	Substance	Exposure System	Results	Reference
Rat	A18385B	Oral 1 d Acute	**LD_{50} > 2000 mg/kg bw**	Matting E, 2013 (A18385B_10008; VV-405072) KCP 7.1.1/01

Endpoints in bold were used for the risk assessment

9.3.1.1 Justification for new endpoints

Not relevant.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA/2009/1438).

9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Prosulfuron

Table 9.3-5: Screening Step assessment of the acute and long-term/reproductive risk for mammals due to the use of A18385B in maize – prosulfuron

Intended use		Maize				
Active substance		Prosulfuron				
Acute toxicity (mg/kg bw)		986				
TER criterion		10				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, BBCH 12 - 18	1 × 16	Small herbivorous mammal	136.4	1	2.18	450
	1 × 20	Small herbivorous mammal	136.4	1	2.73	360
Reprod. Toxicity (mg/kg bw/d)		3				
TER criterion		5				
Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 16	Small herbivorous mammal	72.3	1 × 0.53	0.613	**4.9**
	1 × 20	Small herbivorous mammal	72.3	1 × 0.53	0.766	**3.9**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

As explained above, during EU evaluation for prosulfuron it was discussed and agreed by the experts that the endpoints to be used at the different steps of the long-term risk assessment should be selected as recommended in the EFSA Guidance (2009). Therefore, for the screening step the lowest endpoint from the 90-day rat study was used, while at tier 1 the lowest endpoint from the rabbit developmental study was selected (EFSA Journal 2014;12(9):3815).

Table 9.3-6: Tier 1 assessment of the long-term/reproductive risk for mammals due to the use of A18385B in maize – prosulfuron

Intended use	Maize
Active substance	Prosulfuron
Reprod. Toxicity (mg/kg bw/d)	10
TER criterion	5

Crop scenario Growth stage	**Application rate (g a.s./ha)**	**Scenario**	**Generic Focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 16	BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2	1 × 0.53	0.0356	280
		BBCH 10 - 29	Small herbivorous mammal "vole"	72.3	1 × 0.53	0.613	16
		BBCH 10 - 29	Small omnivorous mammal “mouse”	7.8	1 × 0.53	0.066	150
	1 × 20	BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2	1 × 0.53	0.0445	220
		BBCH 10 - 29	Small herbivorous mammal "vole"	72.3	1 × 0.53	0.766	13
		BBCH 10 - 29	Small omnivorous mammal “mouse”	7.8	1 × 0.53	0.0827	120

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Nicosulfuron

Table 9.3-7: Screening Step assessment of the acute and long-term/reproductive risk for mammals due to the use of A18385B in maize – nicosulfuron

Intended use **Active substance**		Maize Nicosulfuron				
Acute toxicity (mg/kg bw) **TER criterion**		>5000 10				
Crop scenario **Growth stage**	**Application rate (g a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, BBCH 12 - 18	1 × 40	Small herbivorous mammal	136.4	1	5.46	>920
	1 × 50	Small herbivorous mammal	136.4	1	6.82	>730
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		3861 5				
Crop scenario **Growth stage**	**Application rate (g a.s./ha)**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 40	Small herbivorous mammal	72.3	1 × 0.53	1.53	2 500
	1 × 50	Small herbivorous mammal	72.3	1 × 0.53	1.92	2 000

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Dicamba

Table 9.3-8: Screening Step assessment of the acute and long-term/reproductive risk for mammals due to the use of A18385B in maize – dicamba

Intended use **Active substance**		Maize Dicamba				
Acute toxicity (mg/kg bw) **TER criterion**		1581 10				
Crop scenario **Growth stage**	**Application rate (g a.s./ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, BBCH 12 - 18	1 × 160	Small herbivorous mammal	136.4	1	21.8	72
	1 × 200	Small herbivorous mammal	136.4	1	27.3	58
Reprod. Toxicity (mg/kg bw/d) **TER criterion**		150 5				
Crop scenario **Growth stage**	**Application rate (g a.s./ha)**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Maize, BBCH 12 - 18	1 × 160	Small herbivorous mammal	72.3	1 × 0.53	6.13	24
	1 × 200	Small herbivorous mammal	72.3	1 × 0.53	7.66	20

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Prosulfuron/nicosulfuron/dicamba mixture

Acute risk

Table 9.3-9: Screening Step assessment of the acute and long-term/reproductive risk for mammals due to the use of A18385B in maize – A18385B

Intended use **Product**		Maize A18385B				
Acute toxicity (mg/kg bw) **TER criterion**		>2000 10				
Crop scenario Growth stage	**Application rate (g A18385B/ha)**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Maize, BBCH 12 - 18	1 × 400	Small herbivorous mammal	136.4	1	54.6	>37
	1 × 500	Small herbivorous mammal	136.4	1	68.2	>29

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.

Chronic risk

For assessment of chronic effects, according to the EFSA guidance, if a given formulation contains several active substances all known to cause similar effects *via* a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effect is actually driving the risk assessment, it is thus recommended to perform an assessment for combined effects on a case by case basis.

For A18385B the active ingredient, prosulfuron and nicosulfuron (sulfonylureas) have a different mode of action in plants than the active ingredient dicamba (benzoic acid), and their toxicity profiles in mammals are very different.

Prosulfuron and nicosulfuron cause effects on acetolactate synthase in branched chain amino acid synthesis compared to dicamba which causes effects on synthetic auxins. Consequently an assessment for combined effects is not required. Nevertheless as a worst-case approach an assessment for combined effects will be conducted and is based on a concentration addition approach.

In case of concentration addition each substance contributes to the total toxicity of a mixture in proportion to its concentration using the following equation:

TERcombi = trigger / ((trigger prosulfuron/TER prosulfuron) + (trigger nicosulfuron/TER nicosulfuron) + (trigger dicamba/TER dicamba))

An acceptable risk is expected when TERcombi > trigger.

In this formula, ‘triggers’ are the EU triggers.

Table 9.3-10: Assessment of the long-term/reproductive risk for mammals due to the use of A18385B in maize: combination risk assessment

Intended use	Maize						
Application rate (g a.s./ha)	1 × 216 and 1 x 270						
Trigger$_{combi}$	5						
TER criterion	5						
Application rate (g a.s./ha)	**TER$_{prosulfuron}$**	**Trigger 5/ TER$_{prosulfuron}$**	**TER$_{nicosulfuron}$**	**Trigger 5/ TER$_{nicosulfuron}$**	**TER$_{dicamba}$**	**Trigger 5/ TER$_{dicamba}$**	**TER$_{combi}$**
1 x 216	16	0.31	2 500	0.002	24	0.20	10
1 x 270	13	0.38	2 000	0.003	20	0.26	7.8

TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.3.2.2 Higher-tier risk assessment

Not required.

9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (K_{oc} < 500 L/kg) or 3000 in the case of more sorptive substances (K_{oc} ≥ 500 L/kg).

With a K(f)oc of 11.7 (geomean), 20.7 and 12.4 L/kg, prosulfuron, nicosulfuron and dicamba, respectively, belong to the group of less sorptive substances. The maximum use rates are used to cover the risk to mammals from all intended uses (see 9.1.2).

Table 9.3-11: Ratio of effective application rate to toxicity endpoints for mammals exposed to prosulfuron

Effective application rate (g/ha)*	=	20		
Acute toxicity (mg/kg bw)	=	986	quotient =	0.02
Reprod. toxicity (mg/kg bw/d)	=	3	quotient =	6.67

* Effective application rate = Maximum application rate x MAF of 1 (one application)

Table 9.3-12: Ratio of effective application rate to toxicity endpoints for mammals exposed to nicosulfuron

Effective application rate (g/ha)*	=	50		
Acute toxicity (mg/kg bw)	=	>5000	quotient =	<0.01
Reprod. toxicity (mg/kg bw/d)	=	3861	quotient =	0.01

* Effective application rate = Maximum application rate x MAF of 1 (one application)

Table 9.3-13: Ratio of effective application rate to toxicity endpoints for mammals exposed to dicamba

Effective application rate (g/ha)*	=	200		
Acute toxicity (mg/kg bw)	=	1581	quotient =	0.13
Reprod. toxicity (mg/kg bw/d)	=	150	quotient =	1.33

* Effective application rate = Maximum application rate x MAF of 1 (one application)

The resulting ratios fall below the trigger of 50 indicating that further assessment of the acute and long-term risk to mammals from drinking water from puddles is not required for prosulfuron, nicosulfuron and dicamba.

9.3.2.4 Effects of secondary poisoning

Prosulfuron has a log P_{OW} of 1.5 at pH 5, nicosulfuron has a log P_{OW} value of 0.6 and its major aquatic metabolites ASDM, AUSN and HMUD have log P_{OW} values of <1.0. The value for dicamba is 0.55 – 1.9 (at pH 5.0 – 8.9) and for its metabolite DCSA is -0.84 (pH 6.8). Therefore it is not necessary to consider risk from secondary poisoning for prosulfuron, dicamba or nicosulfuron.

Risk assessment for earthworm-eating mammals *via* secondary poisoning

Not required.

Risk assessment for fish-eating mammals *via* secondary poisoning

Not required.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.3.4 Overall conclusions

The acute and long-term risks of A18385B to wild mammals were assessed from toxicity exposure ratios between toxicity endpoints, estimated from studies with prosulfuron, nicosulfuron and dicamba and

A18385B, and maximum residues occurring on food items following applications according to the use pattern. The risk to birds from exposure *via* drinking water has also been assessed.

The TER values, calculated for recommended scenarios, all exceed the trigger values of 10 for acute and 5 for long-term risk at Screening step or for chronic exposure to prosulfuron at Tier 1, thus indicating acceptable risk to mammals from the proposed use. Acceptable risk to mammals from exposure *via* drinking water was also shown.

Review Comments:

All TER values exceed the relevant triggers indicating that A18385B does not pose an unacceptable risk to mammals following applications according to recommended use pattern.

Evaluation of exposing to birds through the drinking water demonstrated the acceptable risk. Since the log P_{ow} value of prosulfuron, nicosulfuron, dicamba, and their relevant metabolites are all below the trigger of 3, the evaluation of the risk of secondary poisoning is not triggered.

9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

No relevant data on amphibians and reptiles are available for prosulfuron, nicosulfuron and dicamba. Regarding assessment of potential effects on reptiles and amphibians neither guidance documents nor testing guidelines are available at present. Consequently no further assessment of potential effects on reptiles and amphibians will be presented in this document.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with prosulfuron, nicosulfuron, dicamba and relevant metabolites. Full details of these studies are provided in the respective EU RAR and DAR and related documents.

Effects on aquatic organisms of A18385B were not evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but were evaluated in Central zone for product authorization of A18385B. Product data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process. However, for proposed deviations, justifications are provided below.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – prosulfuron and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Prosulfuron	96 h, s	LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2014;12(9):3815
Oncorhynchus mykiss	Prosulfuron	96 h, f	**LC_{50} > 160 mg a.s./L $_{mm}$**	EFSA Journal 2014;12(9):3815
Lepomis macrochirus	Prosulfuron	96 h, s	LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2014;12(9):3815

Species	Substance	Exposure System	Results	Reference
Lepomis macrochirus	Prosulfuron	96 h, f	LC_{50} > 155 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Cyprinus carpio	Prosulfuron	96 h, s	LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2014;12(9):3815
Cyprinodon variegatus	Prosulfuron	96 h, f	LC_{50} > 155 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Ictalurus punctatus	Prosulfuron	96 h, s	LC_{50} > 100 mg a.s./L $_{nom}$	EFSA Journal 2014;12(9):3815
Oncorhynchus mykiss	CGA159902	96 h, s	**LC_{50} 63 mg a.s./L $_{nom}$**	EFSA Journal 2014;12(9):3815
Oncorhynchus mykiss	CGA300406	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Journal 2014;12(9):3815
Oncorhynchus mykiss	CGA150829	96 h, s	**LC_{50} > 200 mg a.s./L $_{nom}$**	EFSA Journal 2014;12(9):3815
Oncorhynchus mykiss	CGA349707	96 h, f	**LC_{50} > 42 mg a.s./L $_{mm}$**	EFSA Journal 2014;12(9):3815
Pimephales promelas	Prosulfuron	37 d, f	NOEC = 150 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Oncorhynchus mykiss	Prosulfuron	21 d, f	**NOEC = 5.80 mg a.s./L $_{nom}$**	EFSA Journal 2014;12(9):3815
Daphnia magna	Prosulfuron	48 h, f	**EC_{50} > 120 mg a.s./L $_{mm}$**	EFSA Journal 2014;12(9):3815
Mysidopsis bahia	Prosulfuron	96 h, f	EC_{50} > 150 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Crassostrea virginica	Prosulfuron	96 h, f	EC_{50} > 125 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Daphnia magna	CGA159902	48 h, s	**EC_{50} 74 mg/L $_{nom}$**	EFSA Journal 2014;12(9):3815
Daphnia magna	CGA300406	48 h, s	**EC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2014;12(9):3815
Daphnia magna	CGA150829	24 h, s	EC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Daphnia magna	CGA150829	48 h, s	EC_{50} = 16 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Daphnia magna	CGA150829	48 h, s	EC_{50} > 99 mg/L $_{mm}$	EFSA Journal 2014;12(9):3815
Daphnia magna	CGA150829	48 h, s	**EC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2014;12(9):3815 *See justification below*
Daphnia magna	CGA349707	48 h, s	EC_{50} > 100 mg/L $_{nom}$ **> 2.8 mg/L (considering solubility)**	EFSA Journal 2014;12(9):3815
Daphnia magna	Prosulfuron	21 d, ss	**NOEC = 32 mg a.s./L $_{nom}$**	EFSA Journal 2014;12(9):3815

Species	Substance	Exposure System	Results	Reference
Daphnia magna	Prosulfuron	21 d, f	NOEC = 148 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Daphnia magna	CGA150829	21 d, ss	**NOEC ≥ 97 mg/L $_{mm}$**	EFSA Journal 2014;12(9):3815
Pseudokirchneriella subcapitata	Prosulfuron	120 h, s	E_rC_{50} = 0.0106 mg a.s./L $_{mm}$ *(not considered valid during EU review*)	EFSA Journal 2014;12(9):3815
Pseudokirchneriella subcapitata	Prosulfuron	72 h, s	**E_rC_{50} = 0.074 mg a.s./L $_{mm}$ [a]** E_bC_{50} = 0.016 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Anabaena flos-aquae	Prosulfuron	120 h, s	E_rC_{50} > 0.0272 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Navicula pelliculosa	Prosulfuron	120 h, s	E_rC_{50} > 0.0836 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Skeletonema costatum	Prosulfuron	120 h, s	E_rC_{50} > 0.0286 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Anabaena flos-aquae	Prosulfuron	72 h, s	E_rC_{50} = 1.160 mg a.s./L $_{mm}$ E_yC_{50} = 0.530 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Scenedesmus subspicatus	CGA159902	72 h, s	**E_rC_{50} = 238 mg/L $_{nom}$ [a]** E_bC_{50} = 86 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Pseudokirchneriella subcapitata	CGA300406	72 h, s	**E_rC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2014;12(9):3815
Scenedesmus subspicatus	CGA150829	72 h, s	E_bC_{50} > 90 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Pseudokirchneriella subcapitata	CGA150829	72 h, s	**E_rC_{50} > 10 mg/L $_{nom}$**	EFSA Journal 2014;12(9):3815
Pseudokirchneriella subcapitata	CGA150829	72 h, s	E_rC_{50} > 100 mg/L $_{nom}$ [a] E_bC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Pseudokirchneriella subcapitata	CGA349707	72 h, s	**E_rC_{50} > 64.3 mg/L $_{mm}$ [a]** E_bC_{50} > 64.3 mg/L $_{mm}$	EFSA Journal 2014;12(9):3815
Lemna gibba	Prosulfuron	14 d, s	EC_{50} = 0.00126 mg a.s./L $_{mm}$	EFSA Journal 2014;12(9):3815
Lemna gibba	Prosulfuron (tested as A8714C)	7 d, s	**E_rC_{50} = 0.0029 mg f.p./L $_{nom}$ (0.00212 mg a.s./L) [a]** E_yC_{50} = 0.0018 mg f.p./L $_{nom}$ (0.00131 mg a.s./L)	EFSA Journal 2014;12(9):3815
Lemna gibba	CGA150829	7 d, s	**E_rC_{50} > 100 mg/L $_{nom}$ [a]** E_bC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Lemna gibba	CGA150829	7 d, s	**E_rC_{50} > 100 mg/L $_{nom}$ [a]** E_bC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Lemna minor	CGA150829	7 d, ss	E_rC_{50} > 100 mg/L $_{nom}$ [a] E_bC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Lemna gibba	CGA150829	14 d, ss	EC_{50} > 10 mg/L $_{nom}$ [a] E_bC_{50} > 10 mg/L $_{nom}$	EFSA Journal 2014;12(9):3815
Lemna gibba	SYN542604	7 d, s	**E_rC_{50} > 104 mg/L $_{mm}$** E_bC_{50} > 104 mg/L $_{mm}$	EFSA Journal 2014;12(9):3815

Species	Substance	Exposure System	Results	Reference
Lemna gibba	CGA325025	7 d, s	**E_rC_{50} = 1.6 mg/L $_{mm}$** [a] E_yC_{50} = 0.83 mg/L $_{mm}$	EFSA Journal 2014;12(9):3815
Higher-tier studies (micro- or mesocosm studies)				
Not required				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations

[a] According to the provisions of the new Guidance Document on Aquatic Ecotoxicology (2013), E_rC_{50} endpoints were chosen for the risk assessment if available, see justification below

In bold: Endpoints used for risk assessment

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – nicosulfuron and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Nicosulfuron	96 h, s	**LC_{50} = 65.7 mg a.s./L**	EFSA Scientific Report (2007) 120, 1-91
Lepomis macrochirus	ASDM	96 h, s	**LC_{50} > 100 mg/L**	EFSA Scientific Report (2007) 120, 1-91
Brachydanio rerio	AUSN	96 h, s	**LC_{50} > 100 mg/L**	EFSA Scientific Report (2007) 120, 1-91
Oncorhyncus mykiss	MU-466	96 h, s	LC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-91
Oncorhyncus mykiss	HMUD	96 h, s	**LC_{50} > 100 mg/L**	EFSA Scientific Report (2007) 120, 1-91
Oncorhyncus mykiss	ADMP	96 h, s	**LC_{50} > 100 mg/L**	EFSA Scientific Report (2007) 120, 1-91
Oncorhynchus mykiss	Nicosulfuron	28 d, ss	**NOEC = 10 mg a.s./L $_{nom}$**	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	Nicosulfuron	48 h, s	**EC_{50} > 90 mg a.s./L**	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	ASDM	48 h, s	**EC_{50} > 954 mg/L**	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	AUSN	48 h, s	**EC_{50} > 100 mg/L**	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	MU-466	48 h, s	EC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	HMUD	48 h, s	**EC_{50} > 100 mg/L**	EFSA Scientific

Species	Substance	Exposure System	Results	Reference
				Report (2007) 120, 1-91
Daphnia magna	UCSN	48 h, s	**EC_{50} > 100 mg/L**	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	ADMP	48 h, s	**EC_{50} > 100 mg/L**	EFSA Scientific Report (2007) 120, 1-91
Daphnia magna	Nicosulfuron	21 d, ss	**NOEC = 5.2 mg a.s./L**	EFSA Scientific Report (2007) 120, 1-91
Anabaena flos-aque	Nicosulfuron	72 h, s	E_bC_{50} = 7.8 mg a.s./L **E_rC_{50} = 8.4 mg a.s./L**	EFSA Scientific Report (2007) 120, 1-91
Pseudokirchneriella subcapitata	ASDM	72 h, s	**E_rC_{50} > 336 mg/L** E_bC_{50} > 54 mg/L	EFSA Scientific Report (2007) 120, 1-91
Scendesmus subspicatus	AUSN	72 h, s	**E_rC_{50} > 100 mg/L** E_bC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-91
Scendesmus subspicatus	MU-466	72 h, s	E_rC_{50} > 100 mg/L E_bC_{50} > 84.4 mg/L	EFSA Scientific Report (2007) 120, 1-91
Scendesmus subspicatus	HMUD	72 h, s	**E_rC_{50} > 100 mg/L** E_bC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-91
Scendesmus subspicatus	UCSN	72 h, s	**E_rC_{50} > 100 mg/L** E_bC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-91
Scendesmus subspicatus	ADMP	72 h, s	**E_rC_{50} > 100 mg/L** E_bC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-91
Lemna gibba	Nicosulfuron	7 d, s	EC_{50} (frond count) = 0.0017 mg a.s./L **E_rC_{50} (growth rate) = 0.0027 mg a.s./L**	EFSA Scientific Report (2007) 120, 1-91
Myriophyllum aquaticum	Nicosulfuron	7 d, s	EC_{50} = 3.071 mg a.s./L[a]	Wenzel, 2010 Report no. 185 NIS
Lemna gibba	ASDM	7 d, s	**E_rC_{50} > 100 mg/L** E_bC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-91
Lemna gibba	AUSN	7 d, s	**E_rC_{50} > 100 mg/L** E_bC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-91
Lemna gibba	HMUD	7 d, s	**E_rC_{50} > 1 mg/L** E_bC_{50} > 1 mg/L **L**	EFSA Scientific Report (2007) 120, 1-91
Lemna gibba	UCSN	7 d, s	**E_rC_{50} > 100 mg/L** E_bC_{50} > 100 mg/L	EFSA Scientific Report (2007) 120, 1-

Species	Substance	Exposure System	Results	Reference
				91

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations

[a] Additional data generated since the EU review (Wenzel, 2010;unpublished report no. 185 NIS; data owned by Cheminova). Available to Syngenta by Letter of Access from Cheminova and is submitted for the evaluation of product authorisation of A18385B. Not used for risk assessment as it is not most sensitive endpoint.

In bold: Endpoints used for risk assessment

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – dicamba and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Cyprinus carpio	Dicamba	96 h, s	**LC_{50} > 100 mg a.s./L $_{nom}$**	EFSA Journal 2011;9(1):1965
Oncorhynchus mykiss	DCSA	96 h, s	**LC_{50} >100 mg/L $_{mm}$**	EFSA Journal 2011;9(1):1965
Oncorhynchus mykiss	Dicamba	21 d, ss	**NOEC = 180 mg a.s./L $_{nom}$**	EFSA Journal 2011;9(1):1965
Daphnia magna	Dicamba (tested as Banvel 480 SL)	48 h, s	**EC_{50} > 41 mg a.s./L $_{nom}$**	EFSA Journal 2011;9(1):1965
Daphnia magna	DCSA	48 h, s	**EC_{50} = 89 mg/L**	EFSA Journal 2011;9(1):1965
Daphnia magna	Dicamba	21 d, ss	**NOEC = 97 mg a.s./L $_{mm}$**	EFSA Journal 2011;9(1):1965
Pseudokirchneriella subcapitata	Dicamba	72 h, s	**EC_{50} > 3.7 mg a.s./L**	Dicamba DAR 2007, revised September 2010 and October 2010
Skeletonema costatum	Dicamba	72 h, s	E_bC_{50} = 1.8 mg a.s./L E_rC_{50} > 4.1 mg a.s./L	EFSA Journal 2011;9(1):1965
Navicula pelliculosa	Dicamba	72 h, s	E_bC_{50} > 3.8 mg a.s./L **E_rC_{50} > 3.8 mg a.s./L**	EFSA Journal 2011;9(1):1965
Anabaena flos-aque	Dicamba	72 h, s	E_bC_{50} > 32 mg a.s./L E_rC_{50} > 32 mg a.s./L	EFSA Journal 2011;9(1):1965
Pseudokirchneriella subcapitata	DCSA	72 h, s	E_bC_{50} = 118 mg/L $_{mm}$ **E_rC_{50} = 138 mg/L $_{mm}$**	EFSA Journal 2011;9(1):1965
Myriophyllum spicatum	Dicamba	26 d, s	E_bC_{50} > 0.45 mg a.s./L $_{nom}$ **E_rC_{50} > 0.45 mg a.s./L $_{nom}$**	EFSA Journal 2011;9(1):1965
Lemna gibba	Dicamba	7 d, s	**E_bC_{50} > 3.25 mg a.s./L** E_rC_{50} n.a.	EFSA Journal 2011;9(1):1965
Lemna gibba	DCSA	7 d, s	E_bC_{50} = 11.9 mg/L **E_rC_{50} > 73 mg/L**	EFSA Journal 2011;9(1):1965

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations

In bold: Endpoints used for risk assessment

Table 9.5-4: Endpoints and effect values relevant for the risk assessment for aquatic organisms – A18385B

Species	Substance	Exposure System	Results	Reference
Pseudokirchneriella subcapitata	A18385B	72 h, s	**E_rC_{50} = 0.73 mg/L** E_yC_{50} = 0.30 mg/L E_bC_{50} = 0.34 mg/L	Liedtke A, 2013 (A18385B_10020)
Lemna gibba	A18385B	7 d, s	**EC_{50} (growth rate) = 0.010 mg/L** EC_{50} (dry weight) = >0.060 mg/L E_bC_{50} (frond no. yield)= 0.017 mg/L	Liedtke A, 2013a (A18385B_10021)
Higher-tier studies (micro- or mesocosm studies)				
Not required				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
In bold: Endpoints used for risk assessment

Tests conducted with A18385B with fish and *Daphnia* were not considered essential and were therefore not carried out in accordance with the proposals for testing in the Aquatic Guidance Document, since algae and macrophytes are clearly the most sensitive groups for this herbicide.

9.5.1.1 Justification for new endpoints

Acute endpoint for *Daphnia magna* exposed to metabolite CGA150829
Syngenta do not agree with the acute *Daphnia* endpoint for metabolite CGA150829 of 16 mg/L. The study upon which the endpoint is based was not conducted in compliance with the principles of GLP, nor was there any analytical determination of test substance concentrations performed during the test. Another acute *Daphnia* endpoint of >100 mg/L is available (EFSA Scientific Report, 2014; 12(9):3815) and will be used in the risk assessment.

Review Comments:

zRMS does not agree with the change in the acute *Daphnia* endpoint for metabolite CGA150829. Additionally, *Daphnia magna* is not an issue of concern because risk assessment is clearly driven by high toxicity of prosulfuron to aquatic plants. Thus, acute endpoint for invertebrates reported in the LoEP (EC_{50} = 16 mg/L) will be use in the risk assessment.

Relevant endpoint for algae and aquatic macrophytes risk assessment for prosulfuron
According to the recommendations in the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013, 11(7):3290) in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015), focus on growth rate endpoints for algae and aquatic macrophytes are recommended for European risk assessment. The advantages of using growth rate are that growth rate is less dependent on study duration and is relevant to 'recovery potential'. Therefore, E_rC_{50} values will be used as relevant endpoints in the algae and aquatic macrophytes risk assessment.

For algae, the E_rC_{50} derived from the Grade (1996) study with *P. subcapitata* will be used in the risk assessment for prosulfuron. It was agreed during EU review that this study is valid and provides the relevant algae endpoint for use in the risk assessment.

Relevant endpoint for aquatic macrophytes and nicosulfuron

In the final addendum to DAR for nicosulfuron (July 2007), the endpoint for the aquatic macrophyte *Lemna* was further discussed: "*The endpoint for the aquatic macrophyte, Lemna, was queried (Reporting table 5(9). The endpoint for toxicity studies was based on effects on growth not mortality. The RMS acknowledges that the original DAR may have been open to miss-interpretation on this point. The endpoint used in risk assessment was the lowest given in the study which was based on 50% reduction of frond number at day 7. Unlike the E_rC_{50} and E_bC_{50} the calculation of frond number EC_{50} does not involve a logarithmic conversion and is not a standard endpoint in the current OECD 221 test guideline. The standard endpoints, the E_rC_{50} and the E_bC_{50} were 2.7 and 3.4 µg a.s./L respectively. TERs for the lower of these, 2.7 µg a.s./L will be used in the risk assessment as well as the absolute lowest endpoint previously chosen, the EC_{50} based on frond number, 1.7 µg a.s./L which will be retained for comparison*".
Thus, a Tier 1 risk assessment is presented based E_rC_{50} of 0.0027 mg a.s./L as well as on the EC_{50} of 0.0017 mg a.s./L whereas the higher tier risk assessment is based on the relevant growth rate endpoint.

9.5.2 Risk assessment

The evaluation of the risk for aquatic organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013, 11(7):3290) in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

To achieve a concise risk assessment, the risk envelope approach is applied in the Tier 1 risk assessment. Here, for prosulfuron, nicosulfuron, dicamba and relevant metabolites, the relevant endpoint values are compared to the maximum FOCUS PEC_{SW} ensuring that the aquatic risk from all intended uses is covered (see 9.1.2).

For the formulated product A1838B the relevant endpoint values for algae and aquatic macrophytes are compared to the maximum PEC_{SW} from entry through spray-drift immediately after a single application ensuring that the aquatic risk from all intended uses is covered.

Table 9.5-5: Derivation of RAC values used in the Tier 1 risk assessment – prosulfuron and relevant metabolites

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Oncorhynchus mykiss	Prosulfuron	96 h, s	LC_{50} > 160 000	100	1 600
Oncorhynchus mykiss	CGA159902	96 h, s	LC_{50} = 63 000	100	630
Oncorhynchus mykiss	CGA300406	96 h, s	LC_{50} > 100 000	100	1 000
Oncorhynchus mykiss	CGA150829	96 h, s	LC_{50} > 200 000	100	2 000
Oncorhynchus mykiss	CGA349707	96 h, s	LC_{50} > 42 000	100	420

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Oncorhynchus mykiss	Prosulfuron	21 d, f	NOEC = 5 800	10	580
Daphnia magna	Prosulfuron	48 h, f	EC_{50} > 120 000	100	1 200
Daphnia magna	CGA159902	48 h, s	EC_{50} = 74 000	100	740
Daphnia magna	CGA300406	48 h, s	EC_{50} > 100 000	100	1 000
Daphnia magna	CGA150829	48 h, s	EC_{50} = 16 000 ~~>100 000~~	100	160 ~~1 000~~
Daphnia magna	CGA349707	48 h, s	EC_{50} > 2 800	100	28
Daphnia magna	Prosulfuron	21 d, ss	NOEC = 32 000	10	3 200
Daphnia magna	CGA150829	21 d, ss	NOEC ≥ 97 000	10	9 700
Pseudokirchneriella subcapitata	Prosulfuron	72 h, s	E_rC_{50} = 74	10	7.4
Scenedesmus subspicatus	CGA159902	72 h, s	E_rC_{50} = 238 000	10	23 800
Pseudokirchneriella subcapitata	CGA300406	72 h, s	E_rC_{50} > 100 000	10	10 000
Pseudokirchneriella subcapitata	CGA150829	72 h, s	E_rC_{50} > 10 000	10	1 000
Pseudokirchneriella subcapitata	CGA349707	72 h, s	E_rC_{50} > 64 300	10	6 430
Lemna gibba	Prosulfuron (as formulation A8714C)	7 d, s	E_rC_{50} = 2.12	10	0.212
Lemna gibba	CGA150829	7 d, s	E_rC_{50} > 100 000	10	10 000
Lemna gibba	SYN542604	7 d, s	E_rC_{50} > 104 000	10	10 400
Lemna gibba	CGA325025	7 d, s	E_rC_{50} = 1 600	10	160

Table 9.5-6: Derivation of RAC values used in the risk assessment – nicosulfuron and relevant metabolites

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Oncorhynchus mykiss	Nicosulfuron	96 h, s	LC_{50} = 65 700	100	657
Oncorhynchus mykiss	HMUD	96 h, s	LC_{50} > 100 000	100	1 000
Brachydanio rerio	AUSN	96 h, s	LC_{50} > 100 000	100	1 000
Lepomis macrochirus	ASDM	96 h, s	LC_{50} > 100 000	100	1 000
Oncorhynchus mykiss	ADMP	96 h, s	LC_{50} > 100 000	100	1 000
Oncorhynchus mykiss	Nicosulfuron	28 d, ss	NOEC = 10 000	10	1 000

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Daphnia magna	Nicosulfuron	48 h, s	EC_{50} = 90 000	100	900
Daphnia magna	HMUD	48 h, s	EC_{50} > 100 000	100	1 000
Daphnia magna	AUSN	48 h, s	EC_{50} > 100 000	100	1 000
Daphnia magna	ASDM	48 h, s	EC_{50} > 954 000	100	9 540
Daphnia magna	ADMP	48 h, s	EC_{50} > 100 000	100	1 000
Daphnia magna	UCSN	48 h, s	EC_{50} > 100 000	100	1 000
Daphnia magna	Nicosulfuron	21 d, ss	NOEC = 5 200	10	520
Anabaena flos-aquae	Nicosulfuron	72 h, s	E_bC_{50} = 7 800	10	780
Scenedesmus subspicatus	HMUD	72 h, s	EC_{50} > 100 000	10	10 000
Scenedesmus subspicatus	AUSN	72 h, s	EC_{50} > 100 000	10	10 000
Pseudokirchneriella subcapitata	ASDM	72 h, s	E_rC_{50} > 336 000	10	33 600
Pseudokirchneriella subcapitata	UCSN	72 h, s	EC_{50} > 100 000	10	10 000
Scenedesmus subspicatus	ADMP	72 h, s	EC_{50} > 100 000	10	10 000
Lemna gibba	Nicosulfuron	7 d, ss	EC_{50} = 1.70 (frond)	10	0.17
			EC_{50} = 2.70* (growth)	10	0.27
Lemna gibba	HMUD	7 d, ss	EC_{50} > 1 000	10	100
Lemna gibba	AUSN	7 d, ss	EC_{50} > 100 000	10	10 000
Lemna gibba	ASDM	7 d, ss	EC_{50} > 100 000	10	10 000
Lemna gibba	UCSN	7 d, ss	EC_{50} > 100 000	10	10 000
Higher-tier studies (micro- or mesocosm studies)					
Not required.					

Table 9.5-7: Derivation of RAC values used in the risk assessment – dicamba and relevant metabolites

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Cyprinus carpio	Dicamba	96 h, s	LC_{50} > 100 000	100	1 000
Oncorhynchus mykiss	DCSA	96 h, s	LC_{50} > 100 000	100	1 000
Oncorhynchus mykiss	Dicamba	21 d, ss	NOEC = 180 000	10	18 000
Daphnia magna	Dicamba	48 h, s	EC_{50} > 41 000	100	410
Daphnia magna	DCSA	48 h, s	EC_{50} = 89 000	100	890

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Daphnia magna	Dicamba	21 d, ss	NOEC = 97 000	10	9 700
~~*Pseudokirchneriella subcapitata*~~ *Navicula pelliculosa*	Dicamba	72 h, s	EC_{50} > ~~3 700~~ 3 800	10	~~370~~ 380
Skeletonema costatum	Dicamba	72 h, s	E_bC_{50} > 4 100	10	410
Pseudokirchneriella subcapitata	DCSA	72 h, s	E_bC_{50} = 138 000	10	13 800
Myriophyllum spicatum	Dicamba	26 d, ss	E_rC_{50} > 450	10	45
Lemna gibba	Dicamba	7 d, ss	E_bC_{50} > 3 250	10	325
Lemna gibba	DCSA	7 d, ss	E_rC_{50} > 73 000	10	7 300
Higher-tier studies (micro- or mesocosm studies)					
Not required.					

Table 9.5-8: Derivation of RAC values used in the risk assessment – A18385B

Species	Substance	Exposure System	Results (µg/L)	Assessment Safety factor	RAC (µg/L)
Pseudokirchneriella subcapitata	A18385B	72 h, s	E_rC_{50} = 730	10	73
Lemna gibba	A18385B	7 d, s	EC_{50} = 10	10	1.0

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{sed}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario relevant for Poland and each organism group.

Prosulfuron and metabolites

The following risk assessment for prosulfuron was updated based on new PEC_{sw} on request of Poland.
For the initial risk assessment, the TER values for prosulfuron have been calculated using maximum FOCUS Step 1 - 3 PEC_{SW} values for maize at 16 and 20 g a.s./ha. A conservative prosulfuron laboratory degradation rate DT_{50} of 62.1 days was used for Tier 1 together with a PUF = 0 for FOCUS Step 3. For Tier 2 a DT_{50} of 18.7 days was used based on field data together with a PUF =0.15 for FOCUS Step 3. ~~field dissipation studies value of 20.8[2] was considered.~~
The TER values for prosulfuron metabolites have been calculated using FOCUS Step 1 PEC_{SW} values for application to maize at 20 g a.s./ha.

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfuron for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A18385B in maize (16 g a.s./ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophyte**
RAC (µg/L)		1 600	580	1 200	3 200	7.4	0.212
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	5.40	0.003	0.009	0.005	0.002	0.73	**25**
Step 2 (Tier 1, $DT_{50,lab}$ = 62.1 d)							
N-Europe	0.90	<0.001	0.002	<0.001	<0.001	0.12	**4.2**
S-Europe	1.65	<0.001	0.003	<0.001	<0.001	0.22	**7.8**
Step 2 (Tier 2, $DT_{50,field}$ = 18.7 d)							
N-Europe	0.82	<0.001	0.001	<0.001	<0.001	0.11	**3.9**
S-Europe	1.50	<0.001	0.003	<0.001	0.001	0.20	**7.1**

~~[2] The value of 20.8 days was taken from the original issued report by Hardy & Jastrzebski (2015). In the meantime the report was re-issued with a corrected geometric mean value of 21.2 days. Please see Section 8 of the core assessment.~~

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte
Step 3 (Maize, 16 g a.s./ha, Tier 1, $DT_{50,lab}$ = 62.1 d)							
D3 / ditch	0.289	-	-	-	-	-	1.4
D4 / pond	0.376	-	-	-	-	-	1.8
D4 / stream	0.189	-	-	-	-	-	0.89
R1 / pond	0.006	-	-	-	-	-	0.03
R1 / stream	0.172	-	-	-	-	-	0.81
Step 3 (Maize, 16 g a.s./ha, Tier 2, $DT_{50,field}$ = 20.8 d)							
D3 / ditch	0.093	-	-	-	-	-	0.44
D4 / pond	0.024	-	-	-	-	-	0.11
D4 / stream	0.075	-	-	-	-	-	0.35
R1 / pond	0.005	-	-	-	-	-	0.02
R1 / stream	0.168	-	-	-	-	-	0.79
Step 3 (Maize, 16 g a.s./ha, Tier 1, $DT_{50,lab}$ = 62.1 d, PUF = 0)							
D3 / ditch	0.33	-	-	-	-	-	1.6
D4 / pond	0.48	-	-	-	-	-	2.3
D4 / stream	0.24	-	-	-	-	-	1.1
R1 / pond	<0.01	-	-	-	-	-	0.028
R1 / stream	0.18	-	-	-	-	-	0.84
Step 3 (Maize, 16 g a.s./ha, Tier 2, $DT_{50,field}$ = 18.7 d, PUF = 0.15)							
D3 / ditch	0.09	-	-	-	-	-	0.42
D4 / pond	0.02	-	-	-	-	-	0.087
D4 / stream	0.07	-	-	-	-	-	0.35
R1 / pond	<0.01	-	-	-	-	-	0.027

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte
R1 / stream	0.17	-	-	-	-	-	0.81

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prosulfuron for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A18385B in maize (20 g a.s./ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophyte**
RAC (µg/L)		1 600	580	1 200	3 200	7.4	0.212
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	6.75	0.004	0.012	0.006	0.002	0.912	**32**
Step 2 (Tier 1, $DT_{50,lab}$ = 62.1 d)							
N-Europe	1.12	0.001	0.002	0.001	<0.001	0.151	**5.3**
S-Europe	2.06	0.001	0.004	0.002	0.001	0.278	**9.7**
Step 2 (Tier 2, $DT_{50,field}$ = 18.7 d)							
N-Europe	1.03	<0.001	0.002	<0.001	<0.001	0.14	**4.8**
S-Europe	1.88	0.001	0.003	0.002	<0.001	0.25	**8.9**
~~**Step 3 (Maize, 20 g a.s./ha, Tier 1, $DT_{50,lab}$ = 62.1 d)**~~							
~~D3 / ditch~~	~~0.368~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~1.7~~
~~D4 / pond~~	~~0.478~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~2.3~~
~~D4 / stream~~	~~0.240~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~1.1~~
~~R1 / pond~~	~~0.007~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~0.03~~
~~R1 / stream~~	~~0.214~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~1.0~~
~~**Step 3 (Maize, 20 g a.s./ha, Tier 2, $DT_{50,field}$ = 20.8 d)**~~							
~~D3 / ditch~~	~~0.117~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~0.55~~
~~D4 / pond~~	~~0.031~~	~~-~~	~~-~~	~~-~~	~~-~~	~~-~~	~~0.15~~

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Aquatic macrophyte
~~D4 / stream~~	~~0.095~~	-	-	-	-	-	~~0.45~~
~~R1 / pond~~	~~0.007~~	-	-	-	-	-	~~0.03~~
~~R1 / stream~~	~~0.210~~	-	-	-	-	-	~~0.99~~
Step 3 (Maize, 20 g a.s./ha, Tier 1, $DT_{50,lab}$ = 62.1 d, PUF = 0)							
D3 / ditch	0.43	-	-	-	-	-	**2.0**
D4 / pond	0.61	-	-	-	-	-	**2.9**
D4 / stream	0.30	-	-	-	-	-	**1.4**
R1 / pond	<0.01	-	-	-	-	-	0.035
R1 / stream	0.222	-	-	-	-	-	**1.0**
Step 3 (Maize, 20 g a.s./ha, Tier 2, $DT_{50,field}$ = 18.7 d, PUF = 0.15)							
D3 / ditch	0.11	-	-	-	-	-	0.53
D4 / pond	0.02	-	-	-	-	-	0.11
D4 / stream	0.09	-	-	-	-	-	0.44
R1 / pond	<0.01	-	-	-	-	-	0.033
R1 / stream	0.214	-	-	-	-	-	**1.0**

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.

For the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by an E_rC_{50} for *Lemna gibba* of 2.12 µg/L in connection with an assessment factor of 10) in several FOCUS Step 3 scenarios. Therefore, further refinement is required.

Refined risk assessment prosulfuron – 7-day TWA

As recommended in EFSA Journal 2014;12(9):3815 on prosulfuron, at the Pesticides Peer Review expert Meeting 115 (May 2014), the experts agreed on the appropriateness of the use of a 7-day TWA factor in combination with the growth rate endpoint (E_rC_{50}) as an alternative to the yield endpoint. A justification is presented below.

The EFSA guidance (2013) proposes that the TWA approach can be adopted in a chronic risk assessment when certain conditions are met by following the decision scheme in the guidance. A default TWA PEC value of 7 days is proposed. Applying the scheme to prosulfuron has the following outcome:

1. Chronic Assessment. Is $PEC_{sw;max}$ (of highest available tier) > $RAC_{sw;ch}$ (of highest available tier)?

Yes: Go to 2

No: Low chronic risk

For prosulfuron the answer is 'yes' as the $PEC_{sw;max}$ is greater than the RAC of 0.212 µg a.s./L in several of the FOCUS Step 3 scenarios.

2. Is the $RAC_{sw;ch}$ derived from a test with algae, or from a long term (≥7 days) test with another water organism and the following conditions apply: (i) loss of the a.s. from water is more than 20% of nominal at the end of the exposure period and (ii) the toxicity estimate (e.g. EC_{10} or NOEC) is expressed in terms of nominal/initially measured concentration of the a.s.?

Yes: $PEC_{sw;twa}$ not appropriate (low risk not demonstrated)

No: Go to 3

For prosulfuron, the answer is 'no' as prosulfuron concentrations did not fall by more than 20% over the test duration.

3. Is the RACsw;ch based on treatment related responses of the relevant test species early in the chronic test (e.g. during the initial 96 hours observed mortality/immobility in tests with animals, or 50% reduction in growth rate in tests with macrophytes, in the treatment level above the one from which the RACsw;ch is derived) or is the acute to chronic ratio (acute $L(E)C_{50}$/chronic NOEC or acute $L(E)C_{50}$/chronic EC_{10}) based on immobility or mortality <10?

Yes: PECsw;twa not appropriate (low risk not demonstrated)

No: Go to 4

At the workshop on the new EFSA Guidance Document on Aquatic Organisms (November 6-7th Parma, Italy) it was acknowledged that there was an error in the guidance with respect to when TWAs were not appropriate (the erroneous statement in red above).

As the $RAC_{sw;ch}$ for macrophytes is derived from the E_rC_{50}, the concentration above this would be expected to have a >50% effect, assuming a monotonic dose response. This statement, seemingly ruling out using TWAs for macrophytes is an error and should not have been included in the final guidance.

Dr Theo Brock (EFSA expert, PPR panel), acknowledged this error and stated that the evidence shows that TWAs can be appropriate for use in macrophyte risk assessment, subject to all the normal caveats.

EFSA supporting publication 2015:EN-924 (Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology) further clarifies that the sentence refers to 50% effect early in the 7-14 day chronic macrophyte test (e.g. at day 2-4) in the treatment level above the one from which the $RAC_{sw;ch}$ is derived.

In the test from which the most sensitive endpoint is derived for prosulfuron (Liedtke, 2010; A8714C_11409), the treatment level above the one from which the $RAC_{sw;ch}$ is derived is 3.2 µg A8714C/L, equivalent to 2.34 µg a.s./L. A statistical re-evaluation of the acute effects (0 to 3 days after exposure) of

the test item on *Lemna gibba* was performed using the most recent version of ToxRat Professional. The concentration that originated an effect of 50% (E_rC_{50}) on the growth rate (frond number) was calculated using the Logit analysis. The calculated E_rC_{50} and 95% confidence intervals were calculated to be 3.605 and 3.106-4.120 µg product/ respectively. According to the % of active substance inside the formulated product, the calculated E_rC_{50} corresponds to 2.63 µg active substance/L.
Both the dose response curve obtained and the statistical parameters (p(F) <0,001; p(Chi2) = 1,000) suggest that the endpoint present should be considered reliable. It can be concluded that in the first 3 days no effect greater than 50% on the growth rate of *Lemna gibba* when exposed to a concentration of 3.605 µg A8714C/L (2.63 µg a.s./L) is to be expected. Detailed information on the statistical re-evaluation can be made available upon request.

4. Is it demonstrated by the notifier that, for the organisms and the PPP under evaluation and/or PPP with a similar toxic mode of action (read-across information), the following phenomena are not likely: (i) latency of effects due to short-term exposure; (ii) the co-occurrence of exposure and specific sensitive life stages that last a short time only?

Yes: Go to 5

No: $PEC_{sw;twa}$ not appropriate (low risk not demonstrated)

For prosulfuron, the answer is 'yes' as latency is not expected for this mode of action, and there are no expected sensitive life stages.

However, in EFSA supporting publication 2015:EN-924 (Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology) states it was stated that "..until further guidance on reciprocity and latency of effects are available, then the use of TWA approaches are unlikely to be sufficiently robust to be used in regulatory risk assessment."

The applicant does not agree to this general statement. In the case of toxicants whose mode of action is binding to a specific enzyme (thus blocking its activity), it seems obvious that the effect will depend on the number of bound molecules per unit time, and as such, reciprocity can be assumed. Reciprocity relates to Haber's law, assuming that toxicity depends on the product of concentration and time. And linear reciprocity is the basis of the TWA approach, where exposure concentration is integrated over time (area under the curve) and then divided by the duration of the toxicity test.
Sulfonylurea herbicides like prosulfuron are acting by inhibiting the ALS enzyme and as such causing inhibition of growth but not injury or death. As growth is a time dependent rate, large growth inhibitions over short periods are comparable to lower inhibition over longer periods. Therefore, latency is not considered relevant for prosulfuron.
The EFSA Guidance (2013) states "*It is advised to address latency if, through analogy to similar substances or knowledge of mechanisms of action, it is expected to occur. In cases where latency is known not to occur in PPPs with a similar toxic mode of action, it might be disregarded*." This is not the case for sulfonylurea herbicides. There are a number of studies conducted using e.g. the representative sulfonylurea metsulfuron methyl, whose effects on non-target plants have been widely studied showing that there is no latency of effects.
The EFSA Guidance (2013) further states "*Several scientific papers have demonstrated that in laboratory toxicity tests the effects of time-variable exposure concentrations of amino acid biosynthesis-inhibiting herbicides on the growth of Myriophyllum spicatum and Lemna gibba can best be predicted by area under the curve exposure concentrations. […] Under these conditions the Aquatic Guidance Document offers the possibility to use the $PEC_{sw;7d\text{-}twa}$ in the risk assessment.*"

5. Is $PEC_{sw;7d\text{-}twa}$ (of highest available tier) > $RAC_{sw;ch}$ (of highest available tier)?

Yes: Go to 6

No: Low risk demonstrated

Therefore, in line with the recommendation of EFSA Journal 2014;12(9):3815 on prosulfuron and the Pesticides Peer Review expert Meeting 115 (May 2014), the use of a 7-day TWA factor in combination with the growth rate endpoint (E_rC_{50}) is justified.

Refined risk assessments based on 7-day TWA values are presented for those scenarios where PEC/RAC ratios were above the relevant trigger of 1.

Table 9.5-11: Aquatic organisms: higher tier risk assessment for acceptability of risk (PEC/RAC < 1) for prosulfuron based on 7 d TWA approach for one application in maize (16 g a.s./ha)

Group		Aquatic macrophyte		Aquatic macrophyte
RAC (µg/L)		0.212		0.212
FOCUS Scenario	7 d TWA PEC (Tier 1, $DT_{50,lab}$ = 62.1 d) (µg/L)	PEC / RAC	7 d TWA PEC (Tier 2, $DT_{50,field}$ = 20.8 d) (µg/L)	PEC / RAC
Step 3 (Maize, 16 g a.s./ha)				
D3 / ditch	0.220	1.0	n.r.	n.r.
D4 / pond	0.375	**1.8**	n.r.	n.r.

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
n.r. not relevant as scenario is already safe based on max PEC_{sw} (Tier 2), see Table 9.5-9.

Table 9.5-12: Aquatic organisms: higher tier risk assessment for acceptability of risk (PEC/RAC < 1) for prosulfuron based on 7 d TWA approach for one application in maize (20 g a.s./ha)

Group		Aquatic macrophyte		Aquatic macrophyte
RAC (µg/L)		0.212		0.212
FOCUS Scenario	7 d TWA PEC (Tier 1, $DT_{50,lab}$ = 62.1 d) (µg/L)	PEC / RAC	7 d TWA PEC (Tier 2, $DT_{50,field}$ = 20.8 d) (µg/L)	PEC / RAC
Step 3 (Maize, 20 g a.s./ha)				
D3 / ditch	0.282	**1.3**	n.r.	n.r.
D4 / pond	0.477	**2.3**	n.r.	n.r.
D4 / stream	0.230	**1.8**	n.r.	n.r.

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
n.r. not relevant as scenario is already safe based on max PEC_{sw} (Tier 2), see Table 9.5-10.

Refined risk assessment prosulfuron – FOCUS Step 4

Even though safe use can be demonstrated when based on FOCUS Step 3 Tier 2 calculations, for scenarios that are not safe at FOCUS Step 3 Tier 1 further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies as an additional refinement option.

Table 9.5-13: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for prosulfuron incorporating exposure mitigation options for maize (1 × 16 g a.s./ha)

Group			**Aquatic macrophyte**		**Aquatic macrophyte**
RAC (µg/L)			0.212		0.212
Step 4 FOCUS Scenario (Tier 1, $DT_{50,lab}$ = 62.1 d, PUF = 0) [a]					
Vegetated filter strip (m)		~~10~~ **~~12~~**		~~18~~ **~~20~~**	
Spray drift buffer (m)		**10**		**20**	
Drift-reducing nozzles (%)		**-**		**-**	
		PEC_{SW} (µg/L)	**PEC / RAC**	**PEC_{SW} (µg/L)**	**PEC / RAC**
~~D3 / ditch~~		~~0.220~~	~~1.0~~	~~n.r.~~	~~n.r.~~
~~D4 / pond~~		~~0.376~~	~~**1.8**~~	~~0.376~~	~~**1.8**~~
D3 / ditch		0.27	**1.3**	0.27	**1.3**
D4 / pond		0.48	**2.3**	0.48	**2.3**
D4 / steam		0.24	**1.1**	0.24	**1.1**

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

[a] Scenarios are considered for refinement that are not safe based on max FOCUS Step 3 PEC_{SW}, see Table 9.5-9.

Table 9.5-14: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for prosulfuron incorporating exposure mitigation options for maize (1 × 20 g a.s./ha)

Group			**Aquatic macrophyte**		**Aquatic macrophyte**
RAC (µg/L)			0.212		0.212
Step 4 FOCUS Scenario (Tier 1, $DT_{50,lab}$ = 62.1 d, PUF = 0) [a]					
Vegetated filter strip (m)		**10 (only for R1)**		**20 (only for R1)**	
Spray drift buffer (m)		**10**		**20**	
Drift-reducing nozzles (%)		**-**		**-**	
		PEC_{SW} (µg/L)	**PEC / RAC**	**PEC_{SW} (µg/L)**	**PEC / RAC**
~~D3 / ditch~~		~~0.281~~	~~**1.3**~~	~~0.280~~	~~**1.3**~~
~~D4 / pond~~		~~0.478~~	~~**2.3**~~	~~0.478~~	~~**2.3**~~
~~D4 / stream~~		~~0.240~~	~~**1.1**~~	~~0.240~~	~~**1.1**~~
D3 / ditch		0.34	**1.6**	0.34	**1.6**
D4 / pond		0.61	**2.9**	0.61	**2.9**
D4 / stream		0.30	**1.4**	0.30	**1.4**
R1 / stream		0.09	0.43	0.05	0.22
Step 4 FOCUS Scenario (Tier 2, $DT_{50,field}$ = 18.7 d, PUF = 0.15) [a]					
Vegetated filter strip (m)		**10**		**20**	
Spray drift buffer (m)		**10**		**20**	
Drift-reducing nozzles (%)		**-**		**-**	
		PEC_{SW} (µg/L)	**PEC / RAC**	**PEC_{SW} (µg/L)**	**PEC / RAC**
R1 / stream		0.09	0.42	0.04	0.21

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

[a] Scenarios are considered for refinememnt that are not safe based on max FOCUS Step 3 PEC_{SW}, see Table 9.5-10.

Table 9.5-15: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for prosulfuron incorporating exposure mitigation options for maize (1 × 16 g a.s./ha and 1 × 20 g a.s./ha) with the VFSmod module

Group		**Aquatic macrophyte**
RAC (µg/L)		0.212
VFS_{MOD} buffer (m)	**5 m VFS_{MOD}**	**5 m VFS_{MOD}**
	PEC (µg/L)	**PEC / RAC**
(Maize, 16 g a.s./ha, Tier 1, $DT_{50,lab}$ = 62.1 d, PUF = 0)		
R1 / stream	0.06	0.27
(Maize, 20 g a.s./ha, Tier 1, $DT_{50,lab}$ = 62.1 d, PUF = 0)		
R1 / stream	0.07	0.34

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

When based on Tier 1 FOCUS Step 4 values, acceptable risk to aquatic organisms (represented by most sensitive E_rC_{50} for *Lemna gibba* of 2.12 µg/L) is indicated for ~~D3 ditch~~ R1 stream scenario at 16 g a.s./ha and 20 g a.s./ha.

An overview on the safe uses for prosulfuron for all relevant scenarios in maize for Poland is presented in the tables below.

Table 9.5-16: Aquatic organisms: overview refinement options / mitigation requirements for prosulfuron following use in maize (1 x 16 g a.s./ha)

	Tier 1, $DT_{50,lab}$ = 62.1 d, PUF 0			**Tier 2, $DT_{50,field}$ = ~~20.8 d~~ 18.7 d, PUF = 0.15**		
Scenario	**Step 3**	**~~7 d TWA Step 3~~**	**Step 4**	**Step 3**	**~~7 d TWA Step 3~~**	**Step 4**
D3/ditch	R	~~A~~	R	A	~~n.r.~~	n.r.
D4/pond	R	~~R~~	R	A	~~n.r.~~	n.r.
D4/stream	R	~~n.r.~~	R	A	~~n.r.~~	n.r.
R1/pond	A	~~n.r.~~	n.r.	A	~~n.r.~~	n.r.
R1/stream	A	~~n.r.~~	n.r.	A	~~n.r.~~	n.r.

A	Acceptable, Safe use
n.r.	not relevant due to safe use at FOCUS Step 3
R	Further refinement required
VFS	Vegetated filter strip (run-off buffer)

Table 9.5-17: Aquatic organisms: overview refinement options / mitigation requirements for prosulfuron following use in maize (1 x 20 g a.s./ha)

	Tier 1, $DT_{50,lab}$ = 62.1 d, PUF 0			**Tier 2, $DT_{50,field}$ = ~~20.8 d~~ 18.7 d, PUF = 0.15**		
Scenario	**Step 3**	**~~7 d TWA Step 3~~**	**Step 4**	**Step 3**	**~~7 d TWA Step 3~~**	**Step 4**
D3/ditch	R	~~R~~	R	A	~~n.r.~~	n.r.
D4/pond	R	~~R~~	R	A	~~n.r.~~	n.r.
D4/stream	R	~~R~~	R	A	~~n.r.~~	n.r.
R1/pond	A	~~n.r.~~	n.r.	A	~~n.r.~~	n.r.
R1/stream	R	~~n.r.~~	10 m VFS or 5 m VFSmod	R	~~n.r.~~	~~n.r.~~ 10 SD +10 VFS Comment applicant: **Or 5m VFSmod** (as already Tier 1 passes with the same mitigation and run-off is the main entry route)

A	Acceptable, Safe use
n.r.	not relevant due to safe use at FOCUS Step 3
R	Further refinement required
VFS	Vegetated filter strip (run-off buffer)

Metabolites of prosulfuron

Table 9.5-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA159902 for each organism group based on the maximum FOCUS Step 1 (1 x 20 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**
RAC (µg/L)		630	740	23 800
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	2.57	0.0041	0.0035	<0.001
Step 2 & 3				
Not relevant as PEC/RAC ratios < 1 already at Step 1				

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA300406 for each organism group based on the maximum FOCUS Step 1 (1 x 20 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**
RAC (µg/L)		1 000	1 000	10 000
FOCUS Scenario	**PEC gl-max (µg/L)**			
Step 1				
	3.60	0.004	0.004	<0.001
Step 2 & 3				
Not relevant as PEC/RAC ratios < 1 already at Step 1				

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-20: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA150829 for each organism group based on the maximum FOCUS Steps 1 (1 x 20 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophyte**
RAC (µg/L)		2 000	~~1 000~~ 160	9 700	10 000	10 000
FOCUS Scenario	**PEC gl-max (µg/L)**					
Step 1						
	1.02	<0.001	~~0.001~~ 0.006	<0.001	0.001	<0.001
Step 2 & 3						
Not relevant as PEC/RAC ratios < 1 already at Step 1						

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant

trigger of 1 are shown in bold

Table 9.5-21: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA349707 for each organism group based on the maximum FOCUS Steps 1 (1 x 20 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**
RAC (µg/L)		420	28	6 430
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	1.99	0.005	0.071	<0.001
Step 2 & 3				
Not relevant as PEC/RAC ratios < 1 already at Step 1				

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-22: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite SYN542604 for each organism group based on the maximum FOCUS Steps 1 (1 x 20 g a.s./ha)

Group		**Aquatic macrophyte**
RAC (µg/L)		10 400
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	
Step 1		
	2.98	<0.001
Step 2 & 3		
Not relevant as PEC/RAC ratios < 1 already at Step 1		

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-23: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA325025 for each organism group based on the maximum FOCUS Step 1 (1 x 20 g a.s./ha)

Group		**Aquatic macrophyte**
RAC (µg/L)		160
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**	
Step 1		
	1.53	0.010
Step 2 & 3		
Not relevant as PEC/RAC ratios < 1 already at Step 1		

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Nicosulfuron and metabolites

For the initial risk assessment, the TER values for nicosulfuron have been calculated using maximum FOCUS Step 1 - 3 PEC_{SW} values for maize at 40 and 50 g a.s./ha. The TER values for nicosulfuron metabolites have been calculated using FOCUS Step 1 PEC_{SW} values for application to maize at 50 g a.s./ha.

Table 9.5-24: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A18385B in maize (1 x 40 g a.s./ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophyte**	
RAC (µg/L)		657	1 000	900	520	~~780~~ 840	0.17 (Lemna, E_bC_{50})	0.27 (Lemna, E_rC_{50})
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	13.3	0.020	0.013	0.015	0.026	~~0.017~~ 0.016	**78**	**49**
Step 2								
N-Europe	1.98	0.003	0.002	0.002	0.004	~~0.003~~ 0.002	**12**	**7.3**
S-Europe	3.61	0.006	0.004	0.004	0.007	~~0.005~~ 0.004	**21**	**13**
Step 3								
D3 ditch	0.217	<0.001	<0.001	<0.001	<0.001	<0.001	**1.3**	0.804
D4 pond	0.025	<0.001	<0.001	<0.001	<0.001	<0.001	0.149	0.094
D4 stream	0.184	<0.001	<0.001	<0.001	<0.001	<0.001	**1.1**	0.680
R1 pond	0.016	<0.001	<0.001	<0.001	<0.001	<0.001	0.097	0.061
R1 stream	0.449	<0.001	<0.001	<0.001	<0.001	<0.001	**2.7**	**1.7**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-25: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron for each organism group based on maximum FOCUS Steps 1, 2 and 3 calculations for the use of A18385B in maize (1 x 50 g a.s./ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophyte**	
RAC (µg/L)		657	1 000	900	520	780	0.17 (Lemna, E_bC_{50})	0.27 (Lemna, E_rC_{50})
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	16.6	0.025	0.017	0.032	0.018	0.021	**98**	**61**
Step 2								
N-Europe	2.47	0.004	0.002	0.003	0.005	0.003	**15**	**9.2**
S-Europe	4.52	0.007	0.005	0.005	0.009	0.006	**27**	**17**
Step 3								
D3 ditch	0.272	<0.001	<0.001	<0.001	<0.001	<0.001	**1.6**	1.0
D4 pond	0.032	<0.001	<0.001	<0.001	<0.001	<0.001	0.189	0.119
D4 stream	0.230	<0.001	<0.001	<0.001	<0.001	<0.001	**1.4**	0.850
R1 pond	0.020	<0.001	<0.001	<0.001	<0.001	<0.001	0.121	0.076
R1 stream	0.561	<0.001	<0.001	<0.001	0.001	<0.001	**3.3**	**2.1**

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

For the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by the relevant E_rC_{50} for *Lemna gibba* of 2.7 µg/L in connection with an assessment factor of 10) in R1 stream scenario. Therefore, further refinement is required using FOCUS Step 4 estimates implementing drift and run-off mitigation.

Table 9.5-26: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for nicosulfuron incorporating exposure mitigation options for maize (1 × 40 g a.s./ha)

Group			**Aquatic macrophyte**	
RAC (µg/L)			0.27	
Vegetated filter strip (m)	**5**	**10-12**	**5**	**10**
Spray drift buffer (m)	**5**	**10**	**5**	**10**
	PEC (µg/L)	**PEC (µg/L)**	**PEC / RAC**	**PEC / RAC**
R1 / stream	0.274	0.184	**1.0**	0.68

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-27: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for nicosulfuron incorporating exposure mitigation options for maize (1 × 40 g a.s./ha) with the VFSmod module

Group		**Aquatic macrophyte**
RAC (µg/L)		0.27
VFS_{MOD} buffer (m)	**5 m VFS_{MOD}**	**5 m VFS_{MOD}**
	PEC (µg/L)	**PEC / RAC**
R1 / stream	0.143	0.53

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

The calculated PEC/RAC ratios show an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by the relevant E_rC_{50} for *Lemna gibba* of 2.7 µg/L in connection with an assessment factor of 10) in all relevant scenarios when exposed to 40 g/L nicosulfuron.

Table 9.5-28: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for nicosulfuron incorporating exposure mitigation options for maize (1 × 50 g a.s./ha)

Group			**Aquatic macrophyte**	
RAC (µg/L)			0.27	
Vegetated filter strip (m)	**5**	**10-12**	**5**	**10-12**
Spray drift buffer (m)	**5**	**10**	**5**	**10**
	PEC (µg/L)	**PEC (µg/L)**	**PEC / RAC**	**PEC / RAC**
R1 / stream	0.343	0.230	**1.3**	0.85

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-29: Aquatic organisms: higher-tier risk assessment for acceptability of risk (PEC/RAC < 1) for nicosulfuron incorporating exposure mitigation options for maize (1 × 50 g a.s./ha) with the VFSmod module

Group		**Aquatic macrophyte**
RAC (µg/L)		0.27
VFS_{MOD} buffer (m)	**5 m VFS_{MOD}**	**5 m VFS_{MOD}**
	PEC (µg/L)	**PEC / RAC**
R1 / stream	0.178	0.66

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

The calculated PEC/RAC ratios show an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by the relevant E_rC_{50} for *Lemna gibba* of 2.7 µg/L in connection with an assessment factor of 10) in all relevant scenarios when exposed to 50 g/L nicosulfuron.

Metabolites of nicosulfuron

Table 9.5-30: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron metabolite HMUD for each organism group based on FOCUS Step 1 (50 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic macrophyte**
RAC (µg/L)		1 000	1 000	10 000	1 00
FOCUS Scenario	**$PEC_{gl\text{-}max}$ (µg/L)**				
Step 1					
	5.48	0.005	0.005	<0.001	0.055
Step 2					
Not relevant as PEC/RAC ratios < 1 already at Step 1					

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron metabolite AUSN for each organism group based on FOCUS Step 1 (50 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic macrophyte**
RAC (µg/L)		1 000	1 000	10 000	10 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	4.79	0.005	0.005	<0.001	<0.001
Step 2 & 3					
Not relevant as PEC/RAC ratios < 1 already at Step 1					

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron metabolite ASDM for each organism group based on FOCUS Step 1 (50 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic macrophyte**
RAC (µg/L)		1 000	9 540	33 600	10 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	6.78	0.007	<0.001	<0.001	<0.001
Step 2 & 3					
Not relevant as PEC/RAC ratios < 1 already at Step 1					

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-33: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron metabolite ADMP for each organism group based on FOCUS Step 1 (50 g a.s./ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**
RAC (µg/L)		1 000	1 000	10 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	0.425	<0.001	<0.001	<0.001
Step 2 & 3				

Group		Fish acute	Inverteb. acute	Algae
Not relevant as PEC/RAC ratios < 1 already at Step 1				

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-34: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for nicosulfuron metabolite UCSN for each organism group based on FOCUS Step 1 (50 g a.s./ha)

Group		Inverteb. acute	Algae	Aquatic macrophyte
RAC (µg/L)		1 000	10 000	10 000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	2.30	0.002	<0.001	<0.001
Step 2 & 3				
Not relevant as PEC/RAC ratios < 1 already at Step 1				

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Dicamba and metabolites

For the initial risk assessment, the TER values for dicamba have been calculated using maximum FOCUS Step 1 - 2 PEC_{SW} values for very conservative single application of A18385B at 200 g and 160 g dicamba/ha. The TER values for dicamba metabolite has been calculated using maximum FOCUS Step 1 PEC_{SW} values for single application of A18385B at 200 g dicamba/ha.

Table 9.5-35: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for dicamba for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A18385B in maize (1 x 200 g a.s./ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophyte**	
RAC (µg/L)		1 000	18 000	410	9 700	~~370~~ 380	45 (*Myriophyllum*)	325 (*Lemna*)
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	67.4	0.068	0.004	0.17	0.007	0.18	**1.5**	0.21
Step 2								
N-Europe	6.75	0.007	<0.001	0.016	<0.001	0.018	0.15	0.021
S-Europe	11.7	0.012	<0.001	0.029	0.001	0.032	0.26	0.036

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-36: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for dicamba for each organism group based on maximum FOCUS Steps 1 and 2 calculations for the use of A18385B in maize (1 x 160 g a.s./ha)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophyte**	
RAC (µg/L)		1 000	18 000	410	9 700	~~370~~ 380	45 (*Myriophyllum*)	325 (*Lemna*)
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**							
Step 1								
	54.2	0.054	0.003	0.13	0.006	~~0.15~~ 0.14	**1.2**	0.17
Step 2								
N-Europe	5.40	0.005	<0.001	0.013	<0.001	0.015	0.12	0.017
S-Europe	9.35	0.009	<0.001	0.023	<0.001	0.025	0.21	0.029

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-37: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for dicamba metabolite DCSA for each organism group based on maximum FOCUS Step 1

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic macrophyte**
RAC (µg/L)		1 000	890	13 800	7 300
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	26.5	0.027	0.030	0.002	0.004
Step 2 & 3					
Not relevant as PEC/RAC ratios < 1 already at Step 1					

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

For the intended uses, calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic plants as characterised by the relevant EC_{50} for *Myriophyllum* of 450 µg/L in connection with an assessment factor of 10). Therefore, no further refinements are required.

A18385B

Table 9.5-38: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for A18385B for each organism group based on maximum PEC_{SW} for the use of A18385B in maize

Group				**Algae**	**Aquatic macrophyte**
RAC (µg/L)				73	1.0
Use pattern	**Drift[a]**	**Drift reducing nozzle**	**PEC_{sw} (µg/L)**		
1 x 400 g A18385B/ha	1 m (2.77%)	-	3.69	0.051	**3.7**
		50 %	1.85	0.025	**1.8**
		75 %	0.923	0.013	0.92
		90 %	0.369	0.005	0.37
	5 m (0.57%)	-	0.760	0.010	0.76
		50 %	0.380	0.005	0.38
		75 %	0.190	0.003	0.19
		90 %	0.0760	0.001	0.08
1 x 500 g A18385B/ha	1 m (2.77%)	-	4.62	0.063	**4.6**
		50 %	2.31	0.032	**2.3**
		75 %	1.15	0.016	**1.2**
		90 %	0.462	0.006	0.46

Group				Algae	Aquatic macrophyte
	5 m (0.57%)	-	0.950	0.013	0.95
		50 %	0.475	0.007	0.48
		75 %	0.238	0.003	0.24
		90 %	0.0950	0.001	0.10

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

[a] Drift value according to Rautmann *et al.* (2001)[3]

Decision scheme for mixture toxcitiy risk assessment A183855B use in maize.

The applicant appreciates that the zRMS did take care of the mixture toxicity risk assessment. However, as it seems that the mitigation refinements with 5m VFSmod buffer are not considered yet, additional calculations are included during this commenting below, to demonstrate that also less stringent mitigation measures than proposed from the zRMS are possible. Furthermore additional mitigation options are included for the D scenarios which result in less stringent mitigation measures as well.

STEP 1. Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?

Only for the a.s. (ECxa.s.): Go to 7
For both formulation (ECxPPP) and a.s. (ECxa.s.): Go to 2

Answer: Measured toxicity data for the formulation and the a.s. are available for algae and macrophytes. As these are the most sensitive aquatic organisms, it is justified to conduct the mixture toxicity risk assessment only for these two organism groups. Go to 2

STEP 2. Check the plausibility of the measured formulation toxicity (EC_xPPP) against the calculated mixture toxicity EC_{xmix}-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (EC_xPPP) by means of the model deviation ratio (MDR = EC_{xmix}-CA/EC_xPPP).

If MDR = 0.2–5 (CA approximately holds for the mixture)
If MDR > 5 (mixture more toxic than CA)
If MDR < 0.2 (mixture less toxic than CA)

Equation 13:

$$EC_{X_{mix-CA}} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{X_i}} \right)^{-1}$$

Equation 15:

$$MDR = \frac{EC_{X_{mix-CA}}\ (calculated\ mixture\ toxicity)}{EC_{X_{PPP}}\ (measured\ mixture\ toxicity)}$$

Calculation of the acute mixture toxicity of the formulation

[3] D. Rautmann, M. Streloke, M. Winkler (2001): New basic drift values in the authorisation procedure for plant protection products. In: R. Forster, M. Streloke: Workshop on Risk Assessment and Risk Mitigation Measures in the Context of the Authorization of Plant Protection Products (WORMM). Mitt. Biol. Bundesanst. Land-Forstwirtsch, Berlin-Dahlem, Heft 381

Composition

Name/code of the product	**A183855B**
Name of the active substance A	PROSULFURON
Name of the active substance B	NICOSULFURON
Name of the active substance C	DICAMBA
Density [g product/cm^3]	1

	Nominal [g a.s./kg product]	Fraction considering density [%]	$p_{i\ mix}$ = Fraction of active substance i in the mixture with $\sum p_{i\ mix} = 100$ [%]
Concentrations of the active substance A in the product	40	4.0%	7.4%
Concentrations of the active substance B in the product	100	10.0%	18.5%
Concentrations of the active substance C in the product	400	40.0%	74.1%

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) ($EC_{x\ PPP}$) [mg a.s./L]	Toxicity of the a.s. A ($EC_{x\ A}$) [mg a.s./L]	Toxicity of the a.s. B ($EC_{x\ B}$) [mg a.s./L]	Toxicity of the a.s. C ($EC_{x\ C}$) [mg a.s./L]	Triggers (from EFSA Journal 2013;11(7):3290)
E_rC_{50} algae	0.73	0.394	0.074	8.4	3.8	0.1
E_rC_{50} lemna	0.01	0.005	0.00212	0.0027	3.25	0.1

Substance A	Exposure tier (FOCUS step)	Step 4 R1 10+10	Step 4 R1 20+20	Step 3 D3	Step 3 D4	Step 4 D3 10+10	Step 4 D4 10+10
	PECsw [mg a.s./L]	0.000090	0.000044	0.000113	0.000093	0.000026	0.000024
	Relative proportions of the individual mixture components in the environment ($p_{i\ PEC}$)	0.013	0.006	0.016	0.013	0.004	0.004
Substance B	Exposure tier (FOCUS step)	Step 4 10+10	Step 4 R1 20+20	Step 3 D3	Step 3 D4	Step 4 D3 10+10	Step 4 D4 10+10
	PECsw [mg a.s./L]	0.000230	0.000116	0.000272	0.000230	0.000055	0.000055
	Relative proportions of the individual mixture components in the environment ($p_{i\ PEC}$)	0.033	0.017	0.038	0.033	0.008	0.008
Substance C	Exposure tier (FOCUS step)	STEP 2	Step 2	Step 2	Step 2	Step 2	Step 2
	PECsw [mg a.s./L]	0.006750	0.006750	0.006750	0.006750	0.006750	0.006750
	Relative proportions of the individual mixture components in the environment ($p_{i\ PEC}$)	0.955	0.977	0.946	0.954	0.988	0.988
	Total exposure concentration of the mixture (a.s. based) (PEC_{mix}) [mg/L]	0.007070	0.006910	0.007135	0.007073	0.006831	0.006829

Endpoint/Test species	Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\text{-}CA} = 1/\sum (p_{i\ PEC}/EC_{x\ i})$) [mg a.s./L]					
E_rC_{50} algae	2.341	2.898	2.139	2.311	3.201	3.241
E_rC_{50} lemna	0.055	0.105	0.046	0.054	0.197	0.202

Endpoint/Test species	Toxicity per fraction of the a.s. A ($1/TU_A$) [mg a.s./L]	Toxicity per fraction of the a.s. B ($1/TU_B$) [mg a.s./L]	Toxicity per fraction of the a.s. C ($1/TU_C$) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA} = 1/\sum (TU_i)$) [mg a.s./L]	Model deviation ratio (MDR = $EC_{x\ mix\text{-}CA}/EC_{x\ PPP}$)	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) (at lower exposure tier)
E_rC_{50} algae	0.999	45.36	5.13	0.821	2.083	0.351
E_rC_{50} lemna	0.02862	0.01458	4.3875	0.010	1.785	0.177

Answer: With an MDR in the range of 0.2 to 5 the predicted endpoint for CA is interpreted as to be in line with the measured toxicity. Go to Step 3

STEP 3: Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPPP with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation.

If ECxmix-CA (a.s. in PPP)/ECxmix-CA (a.s. in PECmix) = 0.8–1.2 (mixture similar): Go to 4

If not (mixture not similar): Go to 5

Answer: Calculated factors fall outside 0.8-1.2, thus go to 5

STEP 5: Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (ECxPPP), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation (≥ 90 %) comes from a single a.s. (TUi)?

Yes (single 'driver' of mixture toxicity identified): Go to 6

No: Go to 8

		Active substance A		Active substance B		Active substance C		Triggers	
Endpoint/Test species	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA}$) [mg a.s./L]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x (1/$EC_{x\ mix\text{-}CA}$-TU_i) [%]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x (1/$EC_{x\ mix\text{-}CA}$-TU_i) [%]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x (1/$EC_{x\ mix\text{-}CA}$-TU_i) [%]	>=90 % for one a.s.	>=90% for no a.s.
ErC50 algae	0.821	0.999	82.2%	45.360	1.8%	5.130	16.0%		Go to 8
ErC50 lemna	0.010	0.029	33.7%	0.015	66.1%	4.388	0.2%		Go to 8

STEP 8: Conduct a mixture RA based on calculated mixture toxicity according to:

$$ETR_{mix} = \frac{PEC_{mix}}{EC_{X_{mix-CA}}}$$

If ETRmix-CA < trigger: Low risk

If ETRmix-CA > trigger: Low risk not demonstrated, check single-substance refinement options.

MAIZE 1 x 0.5 kg formulation/ha

Exposure							
Exposure tier (FOCUS step)		Step 4 R1 10+10	Step 4 R1 20+20	Step 3 D3	Step 3 D4	Step 4 D3 10 ns	Step 4 D4 10 ns
PECsw [mg a.s./L]		0.000090	0.000044	0.000113	0.000093	0.000026	0.000024
Exposure tier (FOCUS step)		Step 4 10+10	Step 4 R1 20+20	Step 3 D3	Step 3 D4	Step 4 D3 10+10	Step 4 D4 10+10
PECsw [mg a.s./L]		0.000230	0.000116	0.000272	0.000230	0.000055	0.000055
Exposure tier (FOCUS step)		Step 2	Step 2	Step 2	Step 2	Step 2	Step 2
PECsw [mg a.s./L]		0.006750	0.006750	0.006750	0.006750	0.006750	0.006750
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]		0.007070	0.006910	0.007135	0.007073	0.006831	0.006829

Endpoint/Test species		Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\text{-}CA} = \sum (p_{i\ PEC}/EC_{x\ i})$) [mg a.s./L]					
ErC50 algae		2.339	2.896	2.137	2.310	3.200	3.240
ErC50 lemna		0.055	0.105	0.046	0.054	0.197	0.202

Endpoint/Test species		$ETR_{mix} = PEC_{mix}/EC_{x\ PPP}$						Triggers
ErC50 algae		0.003	0.002	0.003	0.003	0.002	0.002	0.10
ErC50 lemna		0.130	0.066	0.156	0.131	0.035	0.034	0.10

In addition for the rate of 1 x 0.5 kg formulation/ha calculations with 5m VFSmod buffer for prosulfuron and nicosulfuron are included which result in a less stringent mitigation for the R1 scenario. It should be noted, that for the R1 scenario, no additional drift buffer zone is required if the risk assessment is passed with run-off buffer (VFSmod). This can be proofed for prosulfuron PEC_{sw} in Step 3 (Table 8.9- 6 and 8 in Part B8) and Step 4 Table A 53 and 55 which all show that the R1 scenario is run-off dominated even if run-off is mitigated with 10 or 20 m VFS. For nicosulfuron this can be proofed comparing Step 3 (Table 8.9- 18) and Step 4 with 5 m drift buffer (Table A 71) in Part B8. Both values are identical and are indicated as being run-off dominated.
To archive a less stringent mitigation for the D scenarios, additional mixture toxicity calculations are included based on 5 m drift buffer values for nicosulfuron and

dicamba FOCUS Step 3 values (please see updated Part B8). Updated mitigation measures are highlighted in **bold** below. Furthermore as Lemna is clearly driving the risk assessment, only Lemna risk assessment is presented (covering algae).

Exposure					
Exposure tier (FOCUS step)		Step 4 R1 **5m VFS**	Step 4 R1 **5m VFS**	Step 3 D3	Step 3 D4
PECsw [mg a.s./L]		0.000071	0.000071	0.000113	0.000093
Exposure tier (FOCUS step)		Step 4 R1 **5m VFS**	Step 4 R1 **5m VFS**	Step **4** D3 **5m ns**	Step **4** D4 **5m ns**
PECsw [mg a.s./L]		0.000178	0.000178	0.000095	0.000100
Exposure tier (FOCUS step)		Step 2	**Step 3 R1**	**Step 3**	**Step 3**
PECsw [mg a.s./L]		0.006750	0.001830	0.00105	0.000899
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]		0.006999	0.006999	0.001258	0.001092

Endpoint/Test species		Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\text{-}CA} = \sum (p_{i\ PEC}/EC_{x\ i})$) [mg a.s./L]					
ErC50 lemna		0.069	0.021	0.014	0.013		

Endpoint/Test species		$ETR_{mix} = PEC_{mix}/EC_{x\ PPP}$						Triggers
ErC50 lemna		0.1015	0.09998	0.088	0.081			0.10

Based on the 5m VFSmod buffer for prosulfuron and nicosulfuron the combined risk assessment is still very slightly above the trigger. As for dicamba only PEC_{sw} up to FOCUS Step 2 were calculated so far, additional PEC_{sw} with FOCUS Step 3 are calculated (please refer to commentet version of Part B8, Chapter 8.9). The maximum dicamba Step 3 PEC_{sw} value is 0.00183 µg/L for the R1 stream scenario. This value is included in the mixtox calculation (see above). In addition PEC_{sw} with 5 m drift buffer (ns) for nicosulfuron (D scenarios) are included. Based on these additional assessments the overall mitigation of **5m VFSmod + 5m SDB** is also applicable.

Maize 1 x 0.4 kg /ha

Endpoint/Test species	Toxicity of the product [mg product/L]	Toxicity of the product (a.s. based) ($EC_{x\ PPP}$) [mg a.s./L]	Toxicity of the a.s. A ($EC_{x\ A}$) [mg a.s./L]	Toxicity of the a.s. B ($EC_{x\ B}$) [mg a.s./L]	Toxicity of the a.s. C ($EC_{x\ C}$) [mg a.s./L]	Triggers (from EFSA Journal 2013;11(7):3290)
E_rC_{50} algae	0.73	0.394	0.074	8.4	3.8	0.1
E_rC_{50} lemna	0.01	0.005	0.00212	0.0027	3.25	0.1

Substance A	Exposure tier (FOCUS step)	Step 4 R1 10+10	Step 4 R1 20+20	Step 3 D3	Step 3 D4	Step 4 D3 10+10	Step 4 D4 10+10
	PECsw [mg a.s./L]	0.000071	0.000036	0.000113	0.0000	0.000021	0.000019
	Relative proportions of the individual mixture components in the environment ($p_{i\ PEC}$)	0.013	0.006	0.016	0.013	0.004	0.004
Substance B	Exposure tier (FOCUS step)	Step 4 10+10	Step 4 R1 20+20	Step 3 D3	Step 3 D4	Step 4 D3 10+10	Step 4 D4 10+10
	PECsw [mg a.s./L]	0.000230	0.000116	0.000272	0.000230	0.000055	0.000055
	Relative proportions of the individual mixture components in the environment ($p_{i\ PEC}$)	0.033	0.017	0.038	0.033	0.008	0.008
Substance C	Exposure tier (FOCUS step)	STEP 2	Step 2	Step 2	Step 2	Step 2	Step 2
	PECsw [mg a.s./L]	0.006750	0.006750	0.006750	0.006750	0.006750	0.006750
	Relative proportions of the individual mixture components in the environment ($p_{i\ PEC}$)	0.955	0.977	0.946	0.954	0.988	0.988
	Total exposure concentration of the mixture (a.s. based) (PEC_{mix}) [mg/L]	0.007070	0.006910	0.007135	0.007073	0.006831	0.006829

Endpoint/Test species	Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\text{-}CA} = 1/\sum (p_{i\ PEC}/EC_{x\ i})$) [mg a.s./L]					
E_rC_{50} algae	2.354	2.882	2.143	2.316	3.196	3.246
E_rC_{50} lemna	0.055	0.104	0.046	0.054	0.196	0.203

Endpoint/Test species	Toxicity per fraction of the a.s. A ($1/TU_A$) [mg a.s./L]	Toxicity per fraction of the a.s. B ($1/TU_B$) [mg a.s./L]	Toxicity per fraction of the a.s. C ($1/TU_C$) [mg a.s./L]	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA} = 1/\sum (TU_i)$) [mg a.s./L]	Model deviation ratio ($MDR = EC_{x\ mix\text{-}CA}/EC_{x\ PPP}$)	$EC_{x\ mix\text{-}CA}$ (a.s. in product)/$EC_{x\ mix\text{-}CA}$ (a.s. in PEC_{mix}) (at lower exposure tier)
E_rC_{50} algae	0.999	45.36	5.13	0.821	2.083	0.349
E_rC_{50} lemna	0.02862	0.01458	4.3875	0.010	1.785	0.176

Answer: With an MDR in the range of 0.2 to 5 the predicted endpoint for CA is interpreted as to be in line with the measured toxicity. Go to Step 3

STEP 3: Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPPP) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxPPP with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation.

If ECxmix-CA (a.s. in PPP)/ECxmix-CA (a.s. in PECmix) = 0.8–1.2 (mixture similar): Go to 4

If not (mixture not similar): Go to 5

Answer: Calculated factors fall outside 0.8-1.2, thus go to 5

STEP 5: Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (ECxPPP), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation (≥ 90 %) comes from a single a.s. (TUi)?

Yes (single 'driver' of mixture toxicity identified): Go to 6

No: Go to 8

		Active substance A		Active substance B		Active substance C		Triggers	
Endpoint/Test species	Calculated mixture toxicity (a.s. in product) ($EC_{x\ mix\text{-}CA}$) [mg a.s./L]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x (1/$EC_{x\ mix\text{-}CA}$-TU_i) [%]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x (1/$EC_{x\ mix\text{-}CA}$-TU_i) [%]	Toxicity per fraction ($1/TU_i$) [mg a.s./L]	Deviation from mixture toxicity = 1-$EC_{x\ mix\text{-}CA}$ x (1/$EC_{x\ mix\text{-}CA}$-TU_i) [%]	>=90 % for one a.s.	>=90% for no a.s.
ErC50 algae	0.821	0.999	82.2%	45.360	1.8%	5.130	16.0%		Go to 8
ErC50 lemna	0.010	0.029	33.7%	0.015	66.1%	4.388	0.2%		Go to 8

STEP 8: Conduct a mixture RA based on calculated mixture toxicity according to:

$$ETR_{mix} = \frac{PEC_{mix}}{EC_{X_{mix-CA}}}$$

If ETRmix-CA < trigger: Low risk

If ETRmix-CA > trigger: Low risk not demonstrated, check single-substance refinement options.

MAIZE ~~1 x 0.5 kg formulation/ha~~ 1 x 0.**4** kg formulation/ha

Exposure							
Exposure tier (FOCUS step)		Step 4 R1 10+10	Step 4 R1 20+20	Step 3 D3	Step 3 D4	Step 4 D3 10+10	Step 4 D4 10+10
PECsw [mg a.s./L]		0.000071	0.000036	0.000090	0.000074	0.000021	0.000019
Exposure tier (FOCUS step)		Step 4 10+10	Step 4 R1 20+20	Step 3 D3	Step 3 D4	Step 4 D3 10+10	Step 4 D4 10+10
PECsw [mg a.s./L]		0.000184	0.000093	0.000217	0.000184	0.000044	0.000044
Exposure tier (FOCUS step)		Step 2	Step 2	Step 2	Step 2	Step 2	Step 2
PECsw [mg a.s./L]		0.0054	0.0054	0.0054	0.0054	0.0054	0.0054
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]		0.005655	0.005529	0.005707	0.005658	0.005465	0.005463

Endpoint/Test species		Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\text{-}CA} = \sum (p_{i\ PEC}/EC_{x\ i})$) [mg a.s./L]					
ErC50 algae		2.354	2.882	2.143	2.316	3.196	3.246
ErC50 lemna		0.055	0.104	0.046	0.054	0.196	0.203

Endpoint/Test species		$ETR_{mix} = PEC_{mix}/EC_{x\ PPP}$						Triggers
ErC50 algae		0.002	0.002	0.003	0.002	0.002	0.002	0.10
ErC50 lemna		0.103	0.053	0.124	0.105	0.028	0.027	0.10

In addition for the rate of 1 x 0.4 kg formulation/ha calculations with 5m VFSmod buffer for prosulfuron and nicosulfuron are included which result in a less stringent mitigation for the R1 scenario. It should be noted, that for the R1 scenario, no additional drift buffer zone is required if the risk assessment is passed with run-off buffer (VFSmod). This can be proofed for prosulfuron PEC_{sw} in Step 3 (Table 8.9- 5 and 7 in Part B8) and Step 4 Table A 53 and 55 which all show that the R1 scenario is run-off dominated even if run-off is mitigated with 10 or 20 m VFS. For nicosulfuron this can be proofed comparing Step 3 (Table 8.9- 17) and Step 4 with 5 m drift buffer (Table A 71) in Part B8. Both values are identical and are indicated as being run-off dominated.

To archive a less stringend mitigation for the D scenarios, additional mixture toxicity calculations are included based on 5 m drift buffer values for nicosulfuron and dicamba FOCUS Step 3 values (please see updated Part B8). Updated mitigation measures are highlighted in **bold**.
Furthermore as Lemna is clearly driving the risk assessment, only Lemna risk assessment is presented (covering algae).

Exposure				
Exposure tier (FOCUS step)		Step 4 R1 **5m VFS**	Step 3	Step 3
PECsw [mg a.s./L]		0.000057	0.00009	0.000074
Exposure tier (FOCUS step)		Step 4 R1 **5m VFS**	Step **4** D3 **5m ns**	Step **4** D4 **5m ns**
PECsw [mg a.s./L]		0.000143	0.000076	0.000080
Exposure tier (FOCUS step)		Step 2	**Step 3**	**Step 3**
PECsw [mg a.s./L]		0.0054	0.00084	0.000719
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]		0.006999	0.001006	0.000873

Endpoint/Test species		Calculated mixture toxicity (a.s. in PEC_{mix}) ($EC_{x\ mix\text{-}CA} = \sum (p_{i\ PEC}/EC_{x\ i})$) [mg a.s./L]					
ErC50 lemna		0.069	0.014	0.013			

Endpoint/Test species		$ETR_{mix} = PEC_{mix}/EC_{x\ PPP}$						Triggers
ErC50 lemna		0.082	0.071	0.065				0.10

Based on the 5m VFSmod buffer for prosulfuron and nicosulfuron the combined risk assessment is passed. In addition PEC_{sw} with 5 m drift buffer (ns) for nicosulfuron (D scenarios) and Step 3 values for dicamba are included. Based on these additional assessments the overall mitigation of **5m VFSmod + 5m SDB** is also applicable.

9.5.3 Overall conclusions

The PEC/RAC ratios, using worst-case PEC_{SW} values for A18385B, are less than the trigger value of 1, for all aquatic organisms, with the exception of aquatic plants exposed to prosulfuron, nicosulfuron and A18385B. A refined risk assessment is conducted for aquatic plants exposed to prosulfuron, nicosulfuron and A18385B taking into account appropriate mitigation measures.

~~The potential risk to aquatic plants exposed to prosulfuron has been refined by using FOCUS Step 3 7 d TWA values where appropriate.~~ Safe use for prosulfuron for all FOCUS scenarios (except R1 stream for 20 g a.s./ha) is indicated taking into account FOCUS Step 3 values based on field DT_{50} without further mitigation.
In addition, FOCUS Step 4 PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies as an additional refinement option.

The PEC/RAC ratios are <1 for nicosulfuron when based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies as an additional refinement option.

The PEC/RAC ratios are <1 for A18385B when consideration is given to a 5 m buffer zone or 75% drift reducing nozzles following application of 1 x 400 g A18385B/ha or 90% drift reducing nozzles or 5 m buffer zone following application of 1 x 500 g A18385B/ha, respectively.

Overall, the risk to aquatic plants is acceptable following the proposed use pattern of 400 or 500 g A18385B/ha implementing drift and run-off mitigation.

Table 9.5-39: Proposed mitigation measures for application of A18385B to maize according to the proposed use pattern for Poland

~~Crop group~~	~~Use pattern~~	~~Scenario~~		
		~~D3~~	~~D4~~	~~R1~~
~~Maize~~	~~1 x 400 g A18385B/ha~~	~~-~~	~~-~~	~~5 10 m VFS or 5 m VFS_{MOD}~~
	~~1 x 500 g A18385B/ha~~	~~-~~	~~-~~	~~10 m VFS or 5 m VFS_{MOD}~~
Crop group	**Use pattern**	**Scenario**		
Maize	1 x 400 g A18385B/ha	5 SDB (formulation) + 5 VFS (nicosulfuron VFS_{MOD})		
	1 x 500 g A18385B/ha	(10 SDB + 10 VFS) (nicosulfuron and prosulfuron Tier 2) Or 5 SDB (formulation) + 5 VFSmod (nicosulfuron and prosulfuron)		
Mixture toxicity assessment				
Maize	1 x 400 g A18385B/ha and 1 x 500 g A18385B/ha	20 SDB + 20 VFS Or **5 SDB + 5 VFSmod**		

SDB= Spray drift buffer
VFS = Vegetative filter strip (run-off buffer)

Review Comments:

The relevant predicted environmental concentrations in water (PEC_{sw}) for risk assessments covering the proposed use pattern are taken from Part B Section 8 (Environmental Fate). The risk assessment was based on the worst case PEC values and the results of laboratory toxicity testing.

A18385B pose no unacceptable risk to aquatic organisms according to the label with appropriate buffer

zone.

Additional the mixture toxicity assessment, performed by the Applicant was accepted. Thus, for Poland an unsprayed, vegetated buffer zone of 5 m to surface water bodies is sufficient to conclude safe use of A18385B in maize.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with prosulfuron, nicosulfuron and dicamba. Full details of these studies are provided in the respective EU RAR and DAR and related documents.

Effects on bees of A18385B were not evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but were evaluated in the Central zone for product authorization of A18385B. Product data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees - prosulfuron

Species	Substance	Exposure System	Results	Reference
Honeybee	Prosulfuron	Oral	LD_{50} > 100 µg/bee	EFSA Journal 2014;12(9):3815
Honeybee	Prosulfuron	Contact	LD_{50} > 100 µg/bee	EFSA Journal 2014;12(9):3815
Higher-tier studies (tunnel test, field studies)				
Not required.				

Table 9.6-2: Endpoints and effect values relevant for the risk assessment for bees - nicosulfuron

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Nicosulfuron	Oral	LD_{50} > 5.24 µg a.s./bee [a]	EFSA Scientific Report (2007) 120, 1-91
Apis mellifera	Nicosulfuron	Contact	LD_{50} = 76 µg a.s./bee	EFSA Scientific Report (2007) 120, 1-91
Higher-tier studies (tunnel test, field studies)				
Not relevant.				

[a] Derived from formulation study (SL-950 4% SC)

Table 9.6-3: Endpoints and effect values relevant for the risk assessment for bees - dicamba

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Dicamba	Oral	LD_{50} > 100 µg a.s./bee	EFSA Journal 2011;9(1):1965
Apis mellifera	Dicamba	Contact	LD_{50} > 100 µg a.s./bee	EFSA Journal 2011;9(1):1965
Higher-tier studies (tunnel test, field studies)				
Not relevant.				

Table 9.6-4: Endpoints and effect values relevant for the risk assessment for bees – A18385B

Species	Substance	Exposure System	Results	Reference
Apis mellifera	A18385B	Oral	LD_{50} = 140 µg/bee	Kling, A., 2013 (A18385B_10014)
		Contact	LD_{50} > 256 µg/bee	
Higher-tier studies (tunnel test, field studies)				
Not relevant.				

9.6.1.1 Justification for new endpoints

Not relevant.

9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The assessment of the risk for bees is based on the maximum application rates (see 9.1.2).

9.6.2.1 Hazard quotients for bees

Table 9.6-5: First-tier assessment of the risk for bees due to the use of A18385B in maize - prosulfuron

Intended use **Active substance**	Maize Prosulfuron		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 100	16	< 0.16
		20	< 0.20
Contact toxicity	> 100	16	< 0.16
		20	< 0.20

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Table 9.6-6: First-tier assessment of the risk for bees due to the use of A18385B in maize - nicosulfuron

Intended use **Active substance**	Maize Nicosulfuron		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 5.24	40	< 7.6
		50	< 9.5
Contact toxicity	76	40	0.53
		50	0.66

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Table 9.6-7: First-tier assessment of the risk for bees due to the use of A18385B in maize - dicamba

Intended use **Active substance**	Maize Dicamba		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 100	160	< 1.6
		200	< 2.0
Contact toxicity	> 100	160	< 1.6
		200	< 2.0

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Table 9.6-8: First-tier assessment of the risk for bees due to the use of A18385B in maize– A18385B

Intended use **Product**	Maize A18385B		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	140	400	2.9
		500	3.6
Contact toxicity	> 256	400	< 1.6
		500	< 2.0

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

9.6.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Not relevant.

9.6.3 Effects on bumble bees

No data or information is currently available for bumble bees.

9.6.4 Effects on solitary bees

No data or information is currently available for solitary bees.

9.6.5 Overall conclusions

The risk of A18385B to honey-bees was assessed from hazard quotients between toxicity endpoints, estimated from acute oral and contact studies with A18385B, prosulfuron, nicosulfuron and dicamba, and the maximum single application rates.

All the hazard quotients are less than 50, indicating that the risk to bees is acceptable following use of A18385B according to the proposed use pattern.

Review Comments:

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The submitted risk assessment, based on laboratory studies, has been accepted. It can therefore be concluded that there will be negligible risk associated with the exposure of bees to A18385B.

According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees): The Applicant should provide chronic test on bees and evaluation of effects on honey bee development with formulated product. The chronic studies were not performed, therefore, for Poland, the deficiencies need to be fulfilled by the entry into force of the revised EFSA bee guideline. Concerned Member States must decide on the consideration of data requirements on national level.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with the representative solo formulations of prosulfuron, nicosulfuron and dicamba. Full details of these studies are provided in the respective EU RAR and DAR and related documents.

Effects on non-target arthropods of A18385B were not evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but were evaluated in the Central zone for last authorization of A18385B. Product data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	A18385B	Laboratory test glass plates (2D)	LR_{50} > 165.6 g A18385B /ha	Fallowfied L., 2013 (A18385B_10017)
Aphidius rhopalosiphi (adults)	A18385B	Laboratory test glass plates (2D)	LR_{50} < 62.5 g A18385B /ha	Stevens J., 2013 (A18385B_10013)
Typhlodromus pyri (protonymphs)	A18385B	Extended laboratory test bean leaves (2D)	ER_{50} = 1266.3 g A18385B /ha	Fallowfield L., 2014 (A18385B_10070)
Aphidius rhopalosiphi (adults)	A18385B	Extended laboratory test barley seedling (3D)	ER_{50} > 1000 g A18385B /ha	Stevens J., 2013a (A18385B_10034)
Chrysoperla carnea (larvae)	A18385B	Extended laboratory test bean leaves (2D)	ER_{50} > 1000 g A18385B /ha	Vaughan R., 2014 (A18385B_10081)
Aleochara bilineata (adults)	A18385B	Extended laboratory test soil (2D)	ER_{50} > 1000 g A18385B /ha	Tew G., 2014 (A18385B_10072)
Field or semi-field tests				
Not required				

9.7.1.1 Justification for new endpoints

Studies with non-target arthropods are always conducted with a formulated product and no testing is carried out with unformulated technical material. Therefore it may not be appropriate to rely on the data from the individual solo formulation(s,) submitted as representative formulations for the EU review, for the risk assessment for non-target arthropods.

The toxicity of A18385B to non-target arthropods has been investigated by carrying out Tier I and Tier II on a range of species including the representative non-target arthropods *Aphidius rhopalosiphi* and *Typhlodromus pyri* in accordance with ESCORT 2.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

The assessment of the risk for non-target arthropods is based on the maximum application rates (see 9.1.2).

The $PER_{in\text{-}field}$ value according to ESCORT 2 was calculated as: Application rate × MAF.

Table 9.7-2: First tier assessment of the in-field risk for non-target arthropods due to the use of A18385B

Intended use	Maize		
Product	A18385B		
Application rate (g/ha)	1 × 400, 1 x 500		
MAF	1		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 165.6	400	**< 2.6**
		500	**< 3.2**
Aphidius rhopalosiphi	< 62.5	400	**> 6.4**
		500	**> 8.0**
Test species Higher-tier	**Rate with ≤ 50 % effect* (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	1266.3	400	yes
		500	yes
Aphidius rhopalosiphi	> 1 000	400	yes
		500	yes
Chrysoperla carnea	> 1 000	400	yes
		500	yes
Aleochara bilineata	> 1 000	400	yes
		500	yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the maximum application rate covers the risk for non-target arthropods from all intended uses (see 9.1.2).

The $PER_{off\text{-}field}$ value according to ESCORT 2 was calculated as:

Application rate × MAF × (drift factor/vegetation distribution factor)

The corrected $PER_{off\text{-}field}$ values according to ESCORT 2 was calculated as:

corr. $PER_{off\text{-}field}$ = $PER_{off\text{-}field}$ × correction factor

Table 9.7-3: First tier assessment of the off-field risk for non-target arthropods due to the use of A18385B

Intended use	Maize				
Product	A18385B				
Application rate (g/ha)	1 × 400, 1 x 500				
MAF	1				
Drift rate(%)	2.77				
vdf	10 (Tier 1, 2-D test) / 10 (Tier 2 – 2-D test) / 1 (Tier 2 – 3-D test)				
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift factor**	**$PER_{off-field}$ (g/ha)**	**CF**	**$HQ_{off-field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 165.6	0.0277	1.108	10	< 0.067
			1.385		< 0.084
Aphidius rhopalosiphi	< 62.5		1.108		> 0.18
			1.385		> 0.22
Test species Tier I	**Rate with ≤ 50 % effect* (g/ha)**	**Drift factor**	**$PER_{off-field}$ (g/ha)**	**CF**	**corr. $PER_{off-field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	1266.3	0.0277	1.108	5	yes
			1.385		yes
Aphidius rhopalosiphi	> 1 000		11.08		yes
			13.85		yes
Chrysoperla carnea	> 1 000		1.108		yes
			1.385		yes
Aleochara bilineata	> 1 000		1.108		yes
			1.385		yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.3 Additional higher-tier risk assessment

Not required.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

At Tier I, the in-field HQ values were ~~below~~ above the trigger value for the worst case use scenarios (1 x 400 and 1 x 500 g A18385B/ha in maize) indicating the need for further refinement. The off-field HQ values were below the trigger value for all proposed uses indicating that the risk to in-field non-target arthropods is acceptable following the use of A18385B according to the proposed use pattern.

The Tier II, extended laboratory studies showed acceptable foliar in-field and off-field effects from foliar applications of A18385B for *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Chrysoperla carnea* and *Aleochara bilineata* for the worst case use scenarios (1 x 400 and 1 x 500 g A18385B/ha in maize). The risk to non-target arthropods is therefore acceptable following use of A18385B according to the proposed use pattern.

Review Comments:

Based on the results of the conducted risk assessment it can be concluded that low risk for non-target arthropods is expected from the use of A18385B according to the proposed use pattern. No unacceptable effects on non-target arthropods are expected in in-field and off-field habitats.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with prosulfuron, nicosulfuron, dicamba and relevant metabolites. Full details of these studies are provided in the respective EU RAR and DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of A18385B were not evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but were evaluated in the Central zone for product authorization of A18385B. Product data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – prosulfuron and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Acute				
Eisenia fetida	Prosulfuron	14 d, acute 10 % peat content	**LC_{50} > 1000 mg a.s./kg dw**	EFSA Journal 2014;12(9):3815
Eisenia fetida	CGA150829	14 d, acute 10 % peat content	**LC_{50} > 1000 mg a.s./kg dw**	EFSA Journal 2014;12(9):3815
Eisenia fetida	CGA349707	14 d, acute 10 % peat content	**LC_{50} > 1000 mg a.s./kg dw**	EFSA Journal 2014;12(9):3815
Eisenia fetida	CGA159902	14 d, acute 10 % peat content	**LC_{50} = 420 mg a.s./kg dw**	EFSA Journal 2014;12(9):3815
Eisenia fetida	SYN542604	Not applicable	**LC_{50} > 100 mg a.s./kg dw**	EFSA Journal 2014;12(9):3815 Assumed to be 10 times more toxic than the parent prosulfuron
Eisenia fetida	CGA325025	Not applicable	**LC_{50} > 100 mg a.s./kg dw**	
Eisenia fetida	CGA300406	Not applicable	**LC_{50} > 1000 mg a.s./kg dw**	

Species	**Substance**	**Exposure System**	**Results**	**Reference**
Eisenia fetida	SYN547308	Not applicable	**LC_{50} > 1000 mg a.s./kg dw**	Assumed to be 10 times more toxic than the parent prosulfuron *See justification below*
Chronic				
Eisenia fetida	Prosulfuron (tested as A8714C)	Mixed into substrate 56 d, chronic 10 % peat content	**NOEC** = 1 mg product/kg d.w.soil (equivalent to **0.73 mg a.s./kg d.w.soil**)	EFSA Journal 2014;12(9):3815
Eisenia fetida	CGA150829	Mixed into substrate 56 d, chronic 5% peat content	**NOEC = 30 mg/kg dw**	EFSA Journal 2014;12(9):3815
		Mixed into substrate 56 d, chronic 10% peat content	NOEC = 8 mg/kg dw	EFSA Journal 2014;12(9):3815
Eisenia fetida	CGA349707	Mixed into substrate 56 d, chronic 5% peat content	**NOEC = 100 mg/kg dw**	Friedrich, 2012a (12 10 48 068 S)
Eisenia fetida	CGA159902	Mixed into substrate 56 d, chronic 5% peat content	**NOEC = 17.1 mg/kg dw**	Friedrich, 2012b (12 10 48 066 S)
Eisenia fetida	SYN542604	Mixed into substrate 56 d, chronic 5% peat content	**NOEC = 100 mg/kg dw**	Friedrich, 2012c (12 10 48 070 S)
Eisenia fetida	CGA325025	Mixed into substrate 56 d, chronic 5% peat content	**NOEC = 100 mg/kg dw**	Friedrich, 2012d (12 10 48 064 S)
Eisenia fetida	CGA300406	Mixed into substrate 56 d, chronic 5% peat content	**NOEC = 95 mg/kg dw**	Friedrich, 2015 (15 10 48 138 S)
Eisenia fetida	SYN547308	Not applicable	**NOEC = 0.073 mg kg d.w.soil**	Assumed to be 10 times more toxic than the parent prosulfuron *See justification below*
Folsomia candida	CGA150829	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 0.225 mg/kg dw	EFSA Journal 2014;12(9):3815 Lührs, U. (2004) *See justification below*
		Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 100 mg/kg dw**	RAR 2014; B.9.7.2 Frommholz U. (2011) *See justification below*
Folsomia candida	CGA349707	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 100 mg/kg dw**	Friedrich, 2012e (12 10 48 067 S)

Species	Substance	Exposure System	Results	Reference
Folsomia candida	CGA159902	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 30.9 mg/kg dw**	Friedrich, 2012f (12 10 48 065 S)
Folsomia candida	SYN542604	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 100 mg/kg dw**	Friedrich, 2012g (12 10 48 069 S)
Folsomia candida	CGA325025	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 100 mg/kg dw**	Friedrich, 2012h (12 10 48 063 S)
Folsomia candida	CGA300406	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 1000 mg/kg dw**	Friedrich, 2015 (15 10 48 139 S)
Folsomia candida	SYN547308	Not applicable	**NOEC = 10 mg kg d.w.soil**	Assumed to be 10 times more toxic than the parent prosulfuron *See justification below*
Hypoaspis aculeifer	CGA150829	Mixed into substrate 14 d, chronic 5 % peat content	**NOEC = 100 mg/kg dw**	EFSA Journal 2014;12(9):3815
Field studies				
Not relevant				
Litter bag test				
Not relevant				

Endpoints in bold were used for the risk assessment

Table 9.8-2: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – nicosulfuron and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Acute				
Eisenia fetida	Nicosulfuron	Mixed into substrate 14 d, acute 10 % peat content	**LC_{50} > 1000 mg a.s./kg dw**	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	ASDM	Mixed into substrate 14 d, acute 10 % peat content	**LC_{50} > 1000 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	HMUD	7 d, acute	**LC_{50} > 1250 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	AUSN	14 d, acute	**LC_{50} > 1250 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	ADMP	04 d, acute	**LC_{50} > 1250 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	MU-466	14 d, acute	LC_{50} > 1250 mg/kg dw	EFSA Scientific Report (2007) 120, 1-91

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	UCSN	14 d, acute	**LC_{50} > 1250 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Chronic				
Eisenia fetida	AUSN	56 d, chronic	**NOEC = 0.100 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	UCSN	56 d, chronic	**NOEC = 0.050 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Eisenia fetida	ASDM	56 d, chronic	**NOEC = 0.350 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Folsomia candida	Nicosulfuron	DT_{90} = 30 - 210 days; only one application/crop, concentration at which sublethal effects seen in acute study gave no cause for concern, so study on long-term effects not required.		EFSA Scientific Report (2007) 120, 1-91
Folsomia candida	AUSN	28 d, chronic	**NOEC = 0.100 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Folsomia candida	UCSN	28 d, chronic	**NOEC = 0.050 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Folsomia candida	ASDM[a]	28 d, chronic	**NOEC = 0.350 mg/kg dw**	EFSA Scientific Report (2007) 120, 1-91
Field studies				
Not required.				
Litter bag test				
Not required.				

[a] EFSA Scientific Report (2007) 120, 1-91 does not state this endpoint but twice the same endpoint for AUSN. Syngenta believes this is an error in the review documents as the addendum to the DAR presents a NOEC of 0.35 mg/kg soil for metabolite ASDM.

Endpoints in bold were used for the risk assessment

Table 9.8-3: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – dicamba and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Acute				
Eisenia fetida	Dicamba	Mixed into substrate 14 d, acute 10 % peat content	LC_{50} > 1000 mg a.s./kg dw	EFSA Journal 2011;9(1):1965
Eisenia fetida	Dicamba (tested as Banvel 480 SL)	Mixed into substrate 14 d, acute 10 % peat content	**LC_{50} > 480 mg a.s./kg dw**	EFSA Journal 2011;9(1):1965
Eisenia fetida	DCSA	Mixed into substrate 14 d, acute 10 % peat content	**LC_{50} > 1000 mg/kg dw**	EFSA Journal 2011;9(1):1965
Chronic				
Not required.				

Species	Substance	Exposure System	Results	Reference
Field studies				
Not required.				
Litter bag test				
Not required.				

Endpoints in bold were used for the risk assessment

Table 9.8-4: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) – A18385B

Species	Substance	Exposure System	Results	Reference
Chronic				
Eisenia fetida	A18385B	Mixed into substrate 56 d, chronic 10 % peat content	**NOEC = 50 mg/kg dw**	Friedrich, 2012 (A18385B_10000)
Folsomia candida	A18385B	Mixed into substrate 28 d, chronic 5 % peat content	**NOEC = 29 mg/kg dw**	Friedrich, 2013 (A18385B_10011)
Hypoaspis aculeifer	A18385B	Mixed into substrate 14 d, chronic 5 % peat content	**NOEC = 95 mg/kg dw**	Schult, 2013 (A18385B_10012)
Field studies				
Not required.				
Litter bag test				
Not required.				

Endpoints in bold were used for the risk assessment

9.8.1.1 Justification for new endpoints

Prosulfuron metabolites

Since the renewal of approval of prosulfuron new studies for prosulfuron metabolites CGA349707, CGA159902, SYN542604, CGA325025 and CGA300406 have been performed on earthworm and Collembola to adequately address these metabolites in the risk assessment and as a result there are new endpoints for use in the risk assessment. Apart from studies with metabolite CGA300406, the studies were evaluated at zonal level for authorization of A18385B. The endpoints are presented in Table 9.8-1 above.

For metabolite CGA150829 an additional chronic earthworm NOEC is reported in the EFSA Journal 2014;12(9):3815. During peer review of prosulfuron (November 2013) Germany mentioned this study (Lührs, 2007) and a summary was included by the Co-RMS in the Final addendum to RAR (June 2014). The study was carried out based on application rates of 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg. The resulting endpoint was determined as NOEC 8 mg CGA150829/kg the highest concentration tested. As stated in the Final addendum to RAR (June 2014), this study brings only supportive information. Furthermore, Syngenta does not have access to this study as it was not part of the AMT agreement, and the source of this study is unknown to Syngenta.
In the study submitted by Syngenta for EU renewal (Leicher, 2011), CGA150829 was tested at higher rates ranging from 9.5 mg/kg up to 95 mg/kg. The NOEC reproduction was determined to be 30 mg/kg. For the purposes of risk assessment it was considered appropriate to use the higher NOEC of 30 mg/kg.

For metabolite CGA150829, there were two Collembola reproduction studies evaluated during EU review (Final addendum to RAR, 2014). In the study by Lührs, 2004, the NOEC was determined to be 0.225 mg CGA150829/kg, the highest concentration tested. In the study by Frommholz, 2011, CGA150829 was tested at a single rate of 100 mg/kg. The NOEC was determined to be 100 mg/kg. For the purposes of risk assessment it was considered appropriate to use the higher NOEC of 100 mg/kg.

For soil metabolite SYN547308 no study is available. Therefore, 10fold toxicity of the parent prosulfuron is assumed as worst case approach.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate, Chapter 8.7.2). According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for prosulfuron, nicosulfuron, dicamba and dicamba metabolite DCSA but for prosulfuron metabolites CGA150829, CGA159902, SYN542604, CGA349707 and SYN547308 and nicosulfuron metabolites AUSN, UCSN and ASDM.

Here, for A18385B, prosulfuron, nicosulfuron, dicamba and relevant metabolites, the relevant endpoints are compared to the maximum PEC_{soil} ensuring that the risk for earthworms and other non-target soil organisms from all intended uses is covered (see 9.1.2).

Table 9.8-5: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of A18385B in maize

Intended use	Maize (1 x 400 g A18385B/ha)		
~~**Acute effects on earthworms**~~			
~~**Test substance**~~	~~**LC_{50} (mg/kg dw)**~~	~~**PEC_{soil} (mg/kg dw)**~~	~~**TER_a (criterion TER ≥ 10)**~~
~~Prosulfuron~~	~~> 1 000~~	~~0.016~~	~~> 63 000~~
~~CGA150829~~	~~> 1 000~~	~~0.004~~	~~> 270 000~~
~~CGA159902~~	~~420~~	~~0.009~~	~~47 000~~
~~CGA300406~~	~~> 1 000~~	~~0.004~~	~~> 250 000~~
~~SYN542604~~	~~> 100~~	~~0.004~~	~~> 25 000~~
~~CGA349707~~	~~> 1 000~~	~~0.005~~	~~> 200 000~~
~~CGA325025~~	~~> 100~~	~~0.003~~	~~> 33 000~~
~~SYN547308~~	~~> 100 [a]~~	~~0.002~~	~~> 50 000~~
~~Nicosulfuron~~	~~> 1 000~~	~~0.040~~	~~> 25 000~~
~~HMUD~~	~~> 1 250~~	~~0.006~~	~~> 210 000~~

~~AUSN~~	~~> 1 250~~	~~0.009~~	~~> 140 000~~
~~ADMP~~	~~> 1 250~~	~~0.001~~	~~> 1 300 000~~
~~UCSN~~	~~> 1 250~~	~~0.004~~	~~> 310 000~~
~~ASMD~~	~~> 1 000~~	~~0.016~~	~~> 63 000~~
~~Dicamba (tested as Banvel 480 SL)~~	~~> 480~~	~~0.160~~	~~> 3 000~~
~~DCSA~~	~~> 1 000~~	~~0.112~~	~~> 8 900~~
Chronic effects on earthworms			
Test substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
A18385B	50	0.400	130
Prosulfuron (tested as A8714C)	0.73	0.016	46
CGA150829	30	0.004	8 200
CGA159902	17.1	0.009	1 900
CGA300406	95 0.073	0.004	24 000 18.25
SYN542604	100	0.004	25 000
CGA349707	100	0.005	20 000
CGA325025	100	0.003	33 000
SYN547308	0.073 [a]	0.002	37
AUSN	0.1	0.009	11
UCSN	0.05	0.004	13
ASDM	0.35	0.016	22
Chronic effects on other soil macro- and mesofauna			
Test substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
A18385B (Collembola)	29	0.400	73
A18385B (Spring mite)	95	0.400	240
CGA150829 (Collembola)	100	0.016	6 300
CGA150829 (Spring mite)	100	0.004	27 000
CGA159902	30.9	0.009	3 400
CGA300406	1 000	0.004	250 000
SYN542604	100	0.004	25 000
CGA349707	100	0.005	20 000
CGA325025	100	0.003	33 000
SYN547308	10 [a]	0.002	5 000
AUSN	0.1	0.009	11
UCSN	0.05	0.004	13
ASDM	0.35	0.016	22

[a] No study available for metabolite SYN547308. As worst case approach, 10 fold toxicity of the parent is assumed.

Table 9.8-6: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of A18385B in maize

Intended use	Maize (1 x 500 g A18385B/ha)		
~~**Acute effects on earthworms**~~			
~~**Test substance**~~	~~**LC_{50} (mg/kg dw)**~~	~~**PEC_{soil} (mg/kg dw)**~~	~~**TER_a (criterion TER ≥ 10)**~~
~~Prosulfuron~~	~~> 1 000~~	~~0.020~~	~~> 50 000~~
~~CGA150829~~	~~> 1 000~~	~~0.005~~	~~> 200 000~~
~~CGA159902~~	~~420~~	~~0.011~~	~~38 000~~
~~CGA300406~~	~~> 1 000~~	~~0.005~~	~~> 200 000~~
~~SYN542604~~	~~> 100~~	~~0.006~~	~~> 17 000~~
~~CGA349707~~	~~> 1 000~~	~~0.006~~	~~> 170 000~~
~~CGA325025~~	~~> 100~~	~~0.003~~	~~> 33 000~~
~~SYN547308~~	~~> 100 [a]~~	~~0.002~~	~~> 50 000~~
~~Nicosulfuron~~	~~> 1 000~~	~~0.050~~	~~> 20 000~~
~~HMUD~~	~~> 1 250~~	~~0.007~~	~~> 180 000~~
~~AUSN~~	~~> 1 250~~	~~0.011~~	~~>110 000~~
~~ADMP~~	~~> 1 250~~	~~0.001~~	~~> 1 300 000~~
~~UCSN~~	~~> 1 250~~	~~0.005~~	~~> 250 000~~
~~ASMD~~	~~> 1 000~~	~~0.021~~	~~> 48 000~~
~~Dicamba (tested as Banvel 480 SL)~~	~~> 480~~	~~0.200~~	~~> 2 400~~
~~DCSA~~	~~> 1 000~~	~~0.140~~	~~> 7 100~~
Chronic effects on earthworms			
Test substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
A18385B	50	0.500	100
Prosulfuron (tested as A8714C)	0.73	0.020	37
CGA150829	30	0.005	6 000
CGA159902	17.1	0.011	1 600
CGA300406	95	0.005	19 000
SYN542604	100	0.006	17 000
CGA349707	100	0.006	17 000
CGA325025	100	0.003	33 000
SYN547308	0.073 [a]	0.002	37
AUSN	0.1	0.011	9.1
UCSN	0.05	0.005	10
ASDM	0.35	0.021	17
Chronic effects on other soil macro- and mesofauna			

Test substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
A18385B (Collembola)	29	0.500	58
A18385B (Spring mite)	95	0.500	190
CGA150829 (Collembola)	100	0.005	20 000
CGA150829 (Spring mite)	100	0.005	20 000
CGA159902	30.9	0.011	2 800
CGA300406	1 000	0.005	200 000
SYN542604	100	0.006	17 000
CGA349707	100	0.006	17 000
CGA325025	100	0.003	33 000
SYN547308	10 [a]	0.002	5 000
AUSN	0.1	0.011	9.1
UCSN	0.05	0.005	10
ASDM	0.35	0.021	17

[a] No study available for metabolite SYN547308. As worst case approach, 10 fold toxicity of the parent is assumed.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

The acute and long-term risk of A18385B to earthworms was assessed from acute and long-term toxicity exposure ratios (TERs) between the selected toxicity endpoints for A18385B, prosulfuron, nicosulfuron, dicamba and relevant metabolites, and the maximum PEC_{soil} values. All acute and chronic TER values are greater than the Regulation (EU) 546/2011 triggers of 10 and 5, respectively, indicating that the risk to earthworms is acceptable following use of A18385B according to the proposed use pattern.

The risk of A18385B to other non-target soil macro-organisms, as represented by Collembola and Hypoaspis, was assessed from long-term toxicity exposure ratios (TERs) between the selected no-effect concentrations, derived from laboratory tests on relevant metabolites, and the maximum PEC_{soil}. The TER_{LT} values are all greater than the recommended trigger value of 5, indicating that the risk to soil macro-organisms, as represented by Collembola and Hypoaspis, is acceptable following use of A18385B according to the proposed use pattern.

Review Comments:

All TER values for A18385B, the active substances and relevant metabolites for chronic exposure of earthworms and other non-target soil organisms (meso- and macrofauna) are considerably higher than the Commission Regulation (EU) 546/2011 trigger value of 5. This indicates that A18385B poses no unacceptable risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use pattern.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with prosulfuron, nicosulfuron, dicamba and relevant metabolites. Full details of these studies are provided in the respective EU RAR and DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on soil microorganisms of A18385B were not evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but were evaluated in the Central zone for product authorization of A18385B. Product data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms – prosulfuron and relevant metabolites

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Prosulfuron	-	NOEC = 0.2 mg a.s./kg dry soil	EFSA Journal 2014;12(9):3815
C-mineralisation				
N-mineralisation	Prosulfuron (tested as A8714C)	56 d, aerobic loamy sand	**NOEC =** 0.18 mg A8714C/ kg dw soil (equivalent to **0.131 mg a.s./kg soil**)	EFSA Journal 2014;12(9):3815
C-mineralisation		28 d, aerobic loamy sand	**NOEC =** 0.18 mg A8714C/ kg dw soil (equivalent to **0.131 mg a.s./kg soil**)	EFSA Journal 2014;12(9):3815
N-mineralisation	CGA150829	42 d, aerobic loamy sand	**NOEC = 0.204 mg/kg dw soil**	EFSA Journal 2014;12(9):3815
		28 d, aerobic	NOEC = 0.0397 mg/kg dw soil	EFSA Journal 2014;12(9):3815 *See justification below*
C-mineralisation		28 d, aerobic loamy sand	**NOEC = 0.204 mg/kg dw soil**	EFSA Journal 2014;12(9):3815
N-mineralisation	CGA159902	28 d, aerobic loamy sand	**NOEC = 0.135 mg/kg dw soil**	EFSA Journal 2014;12(9):3815
C-mineralisation				
N-mineralisation	CGA300406	28 d, aerobic silty loamy sand	**NOEC = 0.135 mg/kg dw soil**	EFSA Journal 2014;12(9):3815
C-mineralisation				
N-mineralisation	CGA349707	28 d, aerobic silty loam	**NOEC = 0.135 mg/kg dw soil**	Hutcheson, 2015 CEMR-6587
C-mineralisation				
N-mineralisation	SYN542604	Not applicable	**NOEC = 0.0131 mg/kg dw soil**	EFSA Journal 2014;12(9):3815 Assumed to be 10 times more toxic than the parent prosulfuron
C-mineralisation				

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	CGA325025	Not applicable	**NOEC = 0.0131 mg/kg dw soil**	EFSA Journal 2014;12(9):3815 Assumed to be 10 times more toxic than the parent prosulfuron
C-mineralisation				
N-mineralisation	SYN547308	Not applicable	**NOEC = 0.0131 mg/kg dw soil**	Assumed to be 10 times more toxic than the parent prosulfuron *See justification below*
C-mineralisation				

Table 9.9-2: Endpoints and effect values relevant for the risk assessment for soil microorganisms – nicosulfuron and relevant metabolites

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Nicosulfuron	28 d, aerobic sand and sandy silt	< 25 % effect up to 0.8 mg a.s./kg soil dw	EFSA Scientific Report (2007) 120, 1-91
C-mineralisation				
N-mineralisation	AUSN	28 d, aerobic sandy loam	< 25 % effect up to 0.082 mg/kg soil dw	EFSA Scientific Report (2007) 120, 1-91
C-mineralisation				
N-mineralisation	UCSN	28 d, aerobic sandy loam	< 25 % effect up to 0.034 mg/kg soil dw	EFSA Scientific Report (2007) 120, 1-91
C-mineralisation				
N-mineralisation	ASDM	28 d, aerobic sandy loam	< 25 % effect up to 0.191 mg/kg soil dw	EFSA Scientific Report (2007) 120, 1-91
C-mineralisation				

Table 9.9-3: Endpoints and effect values relevant for the risk assessment for soil microorganisms - dicamba

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Dicamba	28 d, aerobic sandy loam	< 25 % effect up to 6.4 mg a.s./kg soil dw	EFSA Journal 2011;9(1):1965
C-mineralisation				

Table 9.9-4: Endpoints and effect values relevant for the risk assessment for soil microorganisms – A18385B

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	A18385B	28 d, aerobic soil type	< 25 % effect up to 3.3 mg/kg soil dw	Schulz, 2013a (A18385B_10010)
C-mineralisation				

9.9.1.1 Justification for new endpoints

Prosulfuron metabolites

Since renewal of approval of prosulfuron a new study with metabolite CGA349707 on N- and C – mineralisation has been performed to adequately address this metabolite and as a result there are new endpoints for use in the risk assessment. Endpoints are presented in Table 9.9-1, a full study summary can be found in Appendix 2.

For metabolite CGA150829 an additional NOEC of 0.0397 mg/kg dw soil after 28 days is reported in the EFSA Journal 2014;12(9):3815. During peer review of prosulfuron (November 2013) Germany mentioned this study (Reis, 2006) and a summary was included by the Co-RMS in the Final addendum to RAR (June 2014) for reasons of completeness. According to this summary, no adverse effects on N-mineralisation > 25% were found at test end after 28 days at the highest test rate of 0.0397 mg/kg dw. This endpoint will not be used in the risk assessment as Syngenta does not have access to the study as it was not part of the AMT agreement, and the source of this study is unknown to Syngenta.

In the study submitted by Syngenta for EU renewal (Reis, 2003), higher rates were tested resulting in a NOEC of 0.204 mg/kg dw soil for metabolite CGA150829. This endpoint was also considered reliable during EU evaluation and will therefore be used in the risk assessment.

For soil metabolite SYN547308 no study is available. Therefore, 10fold toxicity of the parent prosulfuron is assumed as worst case approach.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) above.

Here, for A18385B, prosulfuron, nicosulfuron, dicamba and relevant metabolites, the relevant endpoints are compared to the maximum PEC_{soil} values ensuring that the risk for soil micro-organism organisms from all intended uses is covered (see 9.1.2).

Table 9.9-5: Assessment of the risk for effects on soil micro-organisms due to the use of A18385B in maize (1 x 400 g A18385B/ha)

Intended use	Maize (1 x 400 g A18385B/ha)		
N-mineralisation / C-mineralisation			
Test substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
A18385B	3.3	0.400	yes
Prosulfuron (tested as A8714C)	0.131	0.016	yes
CGA150829	0.204	0.004	yes
CGA159902	0.135	0.009	yes
CGA300406	0.135	0.004	yes
SYN542604	0.0131 [a]	0.004	yes
CGA349707	0.135	0.005	yes

CGA325025	0.0131 [a]	0.003	yes
SYN547308	0.0131 [a]	0.002	yes
Nicosulfuron	0.8	0.040	yes
AUSN	0.082	0.009	yes
UCSN	0.034	0.004	yes
ASDM	0.191	0.016	yes
Dicamba	6.4	0.160	yes

[a] No study available for the metabolite. As worst case approach, 10 fold toxicity of the parent is assumed.

Table 9.9-6: Assessment of the risk for effects on soil micro-organisms due to the use of A18385B in maize (1 x 500 g A18385B/ha)

Intended use	Maize (1 x 500 g A18385B/ha)		
N-mineralisation / C-mineralisation			
Test substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
A18385B	3.3	0.500	yes
Prosulfuron (tested as A8714C)	0.131	0.020	yes
CGA150829	0.204	0.005	yes
CGA159902	0.135	0.011	yes
CGA300406	0.135	0.005	yes
SYN542604	0.0131 [a]	0.006	yes
CGA349707	0.135	0.006	yes
CGA325025	0.0131 [a]	0.003	yes
SYN547308	0.0131 [a]	0.002	yes
Nicosulfuron	0.8	0.050	yes
AUSN	0.082	0.011	yes
UCSN	0.034	0.005	yes
ASDM	0.191	0.021	yes
Dicamba	6.4	0.200	yes

[a] No study available for the metabolite. As worst case approach, 10 fold toxicity of the parent is assumed.

9.9.3 Overall conclusions

The risk of A18385B, prosulfuron, nicosulfuron, dicamba and relevant metabolites to soil micro-organisms was evaluated by comparison of the maximum concentrations with effects ≤25% derived from laboratory tests, with maximum PEC_{soil}.

All the effect levels exceeded the relevant PEC_{soil} values, indicating that the risk to soil micro-organisms is acceptable following the use of A18385B according to the proposed use pattern.

Review Comments:

For the formulation A18385B, the active substances as well as for the relevant metabolites, the maximum concentration with effects < 25% (SANCO/10329/2002 trigger) are all above the maximum PEC_{soil} values. Therefore, it is concluded that the use of A18385B will not pose an unacceptable risk to non-target soil micro-organisms, if applied according to good agricultural practice.

9.10 Effects on non-target terrestrial plants (KCP 10.6)

9.10.1 Toxicity data

Studies with non-target terrestrial plants are always conducted with a formulated product and no testing is carried out with unformulated technical materials, therefore it is not appropriate to present the data for the individual solo formulations submitted as representative formulations for the EU review to address the risk to non-target plants for A18385B.

Effects on non-target terrestrial plants for A18385B have not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but were evaluated in the Central zone for last authorization of A18385B. Product data submitted with this application are listed in Appendix 1 and summarized in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Most sensitive species	Substance	Exposure System	Results[a]	Reference
Beta vulgaris (sugar beet) $_d$ [1)] *Brassica napus* (oilseed rape) $_d$ [2)] *Cucumis sativus* (cucumber) $_d$ [3)] *Daucus carota* (carrot) $_d$ [4)] *Lactuca sativa* (lettuce) $_d$ [5)] *Lycopersicon esculentum* (tomato) $_d$ [6)] *Raphanus sativus* (radish) $_m$ [7)] *Avena sativa* (oat) $_m$ [8)] *Lolium perenne* (ryegrass) $_m$ [9)] *Oryza sativa* (rice) $_m$ [10)]	A18385B	21 d Seedling emergence	[1)] ER_{50} = 10.6 g A18385B/ha [2)] ER_{50} = ´2.49 g A18385B/ha [3)] ER_{50} = 23.8g A18385B/ha [4)] ER_{50} = 13.1 g A18385B/ha [5)] ER_{50} = 12.4 g A18385B/ha [6)] ER_{50} = 5.18 g A18385B/ha [7)] ER_{50} = 2.44 g A18385B/ha [8)] ER_{50} >500 g A18385B/ha [9)] ER_{50} = 32.0 g A18385B/ha [10)] ER_{50} = 51.1 g A18385B/ha	Bramby-Gunary J, 2013a (A18385B_10003)
9 NTPS species	A18385B	21 d Seedling emergence	HC_5 = 1.74 g A18385B /ha	Refer to Section 9.10.1.1
Brassica napus (oilseed rape) $_d$ [1)] *Raphanus sativus* (radish) $_m$ [2)] *Lycopersicon esculentum* (tomato) $_d$ [3)]	A18385B	39 d, field study, seedling emergence	[1)] ER_{50} > 81 g A18385B/ha [2)] ER_{50} > 81 g A18385B/ha [3)] ER_{50} = 18 g A18385B/ha	Dickinson R.A., 2015a (A18385B_10377)
Beta vulgaris (sugar beet) $_d$ [1)] *Brassica napus* (oilseed rape)	A18385B	21 d Vegetative	[1)] ER_{50} = 20.2 g A18385B/ha [2)] ER_{50} = ´11.8 g A18385B/ha	Bramby-Gunary J, 2013

Most sensitive species	Substance	Exposure System	Results[a]	Reference
$_{d}$ [2)] *Cucumis sativus* (cucumber) $_{d}$ [3)] *Daucus carota* (carrot) $_{d}$ [4)] *Lactuca sativa* (lettuce) $_{d}$ [5)] *Lycopersicon esculentum* (tomato) $_{d}$ [6)] *Raphanus sativus* (radish) $_{m}$ [7)] *Avena sativa* (oat) $_{m}$ [8)] *Lolium perenne* (ryegrass) $_{m}$ [9)] *Oryza sativa* (rice) $_{m}$ [10)]		vigour	[3)] ER_{50} = 80.9 g A18385B/ha [4)] ER_{50} = 6.14 g A18385B/ha [5)] ER_{50} = 3.27 g A18385B/ha [6)] ER_{50} = 2.39 g A18385B/ha [7)] ER_{50} = 3.88 g A18385B/ha [8)] ER_{50} = 119 g A18385B/ha [9)] ER_{50} = 16.6 g A18385B/ha [10)] ER_{50} > 500 g A18385B/ha	(A18385B_10004)
9 NTPS species	A18385B	21 d Vegetative vigour	**HC_5 = 1.17 g A18385B /ha**	Refer to Section 9.10.1.1
Brassica napus (oilseed rape) $_{d}$ [1)] *Daucus carota* (carrot) $_{d}$ [2)] *Lactuca sativa* (lettuce) $_{d}$ [3)] *Lycopersicon esculentum* (tomato) $_{d}$ [4)] *Raphanus sativus* (radish) $_{m}$ [5)]	A18385B	Up to 39 d, field study, Vegetative vigour	[1)] ER_{50} = 25 g A18385B/ha [2)] ER_{50} = 63.1 g A18385B/ha [3)] ER_{50} = 36.8 g A18385B/ha [4)] **ER_{50} = 14.7 g A18385B/ha** [5)] ER_{50} (estimated) > 9 < 27 g A18385B/ha	Dickinson R.A., 2015 (A18385B_10378)

m: monocotyledonous; d: dicotyledonous
[a] Most sensitive endpoints all based on biomass
Values in bold are used in the risk assessment

9.10.1.1 Justification for new endpoints

Studies with non-target terrestrial plants are always conducted with a formulated product and no testing is carried out with unformulated technical material. Therefore it may not be appropriate to rely on the data from the individual solo formulations, submitted as representative formulations for the EU review, for the risk assessment for non-target terrestrial plants
The data are summarised in Table 9.10-1.

A Tier II vegetative vigour and seedling emergence study with formulated product A18385B has been conducted.

For support the risk assessment in Member States not accepting e.g. buffer zones, the potential effects of A18385B on vegetative vigour and seedling emergence of non-target terrestrial plants have been further investigated in higher tier field studies on up to 5 of the most sensitive species from the Tier II studies, all dicotyledons i.e. carrot, lettuce, oil seed rape, radish and tomato. These studies have not been evaluated before at either EU or zonal level for product authorisation of A18385B.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.10.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

The risk to non-target terrestrial plants for all intended uses is presented using worst-case HC_5 for vegetative vigour (see 9.10.2.3 below for details).

The $PER_{off\ field}$ for each crop use was calculated as Application rate × drift factor

Table 9.10-2: Assessment of the risk for non-target plants due to the use of A18385B in maize

Intended use	Maize			
Product	A18385B			
Application rate (g/ha)	1 × 400, 1 x 500			
Drift rate (%)	2.77% at 1 m			
MAF	Not applicable			
Test species	**HC_5 (g/ha)**	**Drift factor**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 1**
All species	1.17	0.0277	11.1	**0.11**
			13.9	**0.084**

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The TER values, based on PERs estimated without mitigation measures, are below the trigger of 1, indicating that A18385B pose a potential risk to non-target plant. Therefore a refined risk assessment using mitigation measures is presented below (see 9.10.2.4).

9.10.2.3 Higher-tier risk assessment

Species sensitivity distribution (SSD)

The risk of A18385B to non-target plants has been further refined using the probabilistic HC_{05} approach (***Aldenberg & Jaworska 2000***[4]) to investigate the distribution of sensitivities of all the tested plant species. This approach considers the whole sensitivity distribution of species in an ecosystem, represented by the tested species, to derive a hazard concentration protective of 95% of the species (HC_{05}) instead of just using the lowest ER_{50} value. Because of the large data set (9 definite ER_{50} values of species of varying classes and morphologies) the uncertainty in extrapolation of the data to the natural environment is reduced, and accordingly the assessment factor can also be reduced.

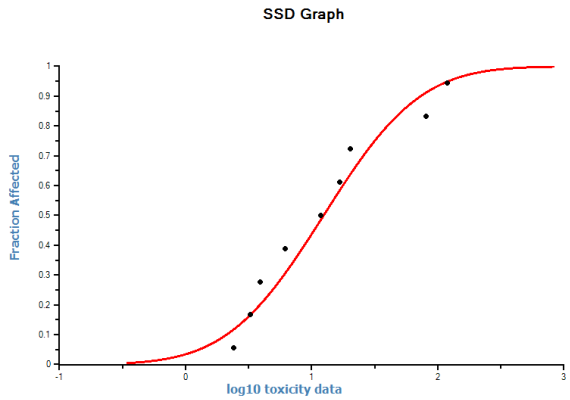
The vegetative vigour and seedling emergence data for the 9 species of plants, as summarised in Table Table 9.10-1, are used with the species sensitivity distribution (SSD) method for evaluating the risk. The statistical analysis was conducted to investigate the distribution of sensitivities of the tested species, and estimate the proportion of species affected at a range of concentrations and derive the HC_{05} value from laboratory to be protective of ecosystems in the field. This method has been proposed and accepted by leading authorities and ecotoxicologists and is a clearly defined probabilistic risk assessment method, with

[4] Aldenberg T & Jaworska JS (2000): Estimation of the hazardous concentration and fraction affected from normally distributed species sensitivity distributions. Ecotoxicology and Environmental Safety 46, 1-18.

supporting software (RIVM program *ETX* 2.0[5]).

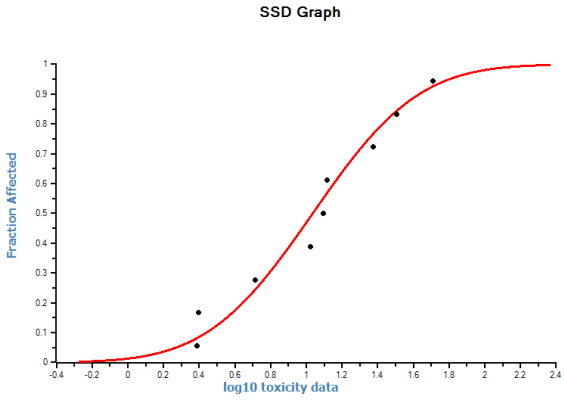
The results of the species sensitivity distribution for A18385B for vegetative vigour and seedling emergence are presented below.

Figure 9.10-1: Species Sensitivity Distribution for Plants in the Vegetative Vigour Study (9 species)



The calculated HC_{05} value was:
Median HC_{05} for plants = 1.17 g A18385B/ha
The lower and upper 90% confidence limits were: 0.188 g/ha and 3.16 g/ha.
The mean (log 10) for the plant data was 1.09 with a sample deviation of 0.600.
Goodness of fit: Acceptable - p = 0.1 (Anderson-Darling test for normality)

Figure 9.10-2: Species Sensitivity Distribution for Plants in the Seedling Emergence Study (9 species)



The calculated HC_{05} value was:
Median HC_{05} for plants = 1.74 g A18385B/ha
The lower and upper 90% confidence limits were 0.422 g/ha and 3.76 g/ha.
The mean (log 10) for the plant data was 1.04 with a sample deviation of 0.465.
Goodness of fit: Acceptable - p = 0.1 (Anderson-Darling test for normality)

The toxicity data (ER_{50}) were subjected to three different goodness of fit tests (Anderson-Darling, Kolmogorov-Smirnov and the Cramer von Mises), where normality at the 0.01 significance level was checked.

Goodness of fit tests - results for plants (veg.vigour)

Sign. level	Tests for normality n=9					
	Anderson-Darling		Kolmogorov-Smirnov		Cramer von Mises	
0.1	0,631	a	0,819	a	0,104	a
0.05	0,752	a	0,895	a	0,126	a
0.025	0,873	a	0,995	a	0,148	a
0.01	1,035	a	1,035	a	0,179	a
Statistic	0,326451		0,459957		0,033075	

a- acceptable

[5] Van Vlaardingen PLA, Traas TP, Wintersen AM, Aldenberg T (2004): *ETX 2.0.* A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 601501028/2004, 68pp.

Goodness of fit tests - results for plants (Seedling Emergence)

Sign. level	Tests for normality n=9					
	Anderson-Darling		Kolmogorov-Smirnov		Cramer von Mises	
0.1	0,631	a	0,104	a	0,104	a
0.05	0,752	a	0,126	a	0,126	a
0.025	0,873	a	0,148	a	0,148	a
0.01	1,035	a	0,179	a	0,179	a
Statistic	0,272198		0,515522		0,027028	

a- acceptable

The analysis of the EC_{50} values show normal distribution of the data.

In addition, the potential effects of A18385B on vegetative vigour and seedling emergence of non-target terrestrial plants has been investigated in higher tier field studies on up to 5 of the most sensitive species from the Tier II studies, all dicotyledons i.e. carrot, lettuce, oil seed rape, radish and tomato.

Endpoints from the studies are presented in the tables below. Full study summaries can be found in Appendix 2.

Table 9.10-3: Effect rates of A18385B on the vegetative vigour of terrestrial non-target plants in a field study

Test species (Common name)	**Day No.**	**Fresh weight ER_{50} (g A18385B/ha)**	**Fresh weight NOER (g A18385B/ha)**
Oilseed rape	23	*	*
	36	25.0	9
Carrot	24	65.0	27
	37	63.1	27
Lettuce	26	36.8	9
	35	>81	27
Tomato	25	14.7	9
	39	33.6	9
Radish*	15	ND	9
	29	ND	9

*The number of oilseed rape plants was not counted in error at this sampling occasion. Therefore it was not possible to calculate ER_{50} and NOER values for this sampling point; this deviation was not considered significant or to have affected the integrity of the study.

ND = Not determined as there was total inhibition of growth at 27 and 81 g A18385B/ha. Therefore the projected ER_{50} was between 9 and 27 g A18385/ha.

Table 9.10-4: Effect Rates of A18385B on the fresh weight of seedlings of terrestrial non-target plants in a field study

Test species (Common name)	**Day No.**	**Fresh weight ER_{50} (g A18385B/ha)**	**Fresh weight NOER (g A18385B/ha)**
Oilseed rape	26	>81	27
	39	>81	27
Radish	26	>81	27
	39	>81	27
Tomato	23	18.0	9
	37	19.3	9

Based on the available data, the lowest ER_{50} value for tomato (Day 25; vegetative vigour) of 14.7 g a.s./ha

will be used in the refined risk assessment.

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5, 10 m and 15 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Table 9.10-5: Risk assessment for non-target terrestrial plants due to the use of A18385B in maize using HC_5 endpoint and considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use **Product** **Application rate (g/ha)** **MAF**		Maize A18385B 1 × 400 Not applicable			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g/ha)**
1	2.77	11.1	5.54	2.77	1.11
5	0.57	2.28	1.14	0.570	0.228
10	0.29	1.16	0.58	0.290	0.116
Toxicity value HC_5 = 1.17 g/ha		**TER** **criterion: TER ≥ 1**			
1	2.77	**0.11**	**0.21**	**0.42**	1.1
5	0.57	**0.51**	1.0	2.1	5.1
10	0.29	1.0	2.0	4.0	10
Application rate (g/ha) **MAF**		1 × 500 Not applicable			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (g/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (g/ha)**
1	2.77	13.9	6.93	3.46	1.39
5	0.57	2.85	1.43	0.713	0.285
10	0.29	1.45	0.725	0.363	0.145
15	0.20	1.00	0.500	0.250	0.100
Toxicity value HC_5 = 1.17 g/ha		**TER** **criterion: TER ≥ 1**			
1	2.77	**0.084**	**0.17**	**0.34**	**0.84**
5	0.57	**0.41**	**0.82**	1.6	4.1
10	0.29	**0.81**	1.6	3.2	8.1
15	0.20	1.2	2.3	4.7	12

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. TER values shown in bold breach the relevant trigger.

Table 9.10-6: Risk assessment for non-target terrestrial plants due to the use of A18385B in maize using higher tier field endpoint and risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Maize			
Product		A18385B			
Application rate (g/ha)		1 × 400			
MAF		Not applicable			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	2.77	11.1	5.54	2.77	1.11
5	0.57	2.28	1.14	0.570	0.228
Toxicity value		**TER**			
ER_{50} = 14.7 g/ha		**criterion: TER ≥ 5**			
1	2.77	**1.3**	**2.7**	5.3	13
5	0.57	6.4	13	26	64
Application rate (g/ha)		1 × 500			
MAF		Not applicable			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (g/ha)**	**$PER_{off-field}$ 50 % drift red. (g/ha)**	**$PER_{off-field}$ 75 % drift red. (g/ha)**	**$PER_{off-field}$ 90 % drift red. (g/ha)**
1	2.77	13.9	6.93	3.46	1.39
5	0.57	2.85	1.43	0.713	0.285
Toxicity value		**TER**			
ER_{50} = 14.7 g/ha		**criterion: TER ≥ 5**			
1	2.77	**1.1**	**2.1**	**4.2**	11
5	0.57	5.2	10	21	52

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. TER values shown in bold breach the relevant trigger.

9.10.3 Overall conclusions

The risk of A18385B to non-target terrestrial plants was assessed from toxicity exposure ratios (TERs) using the formulation toxicity data from Tier II studies using a calculated HC5, and the maximum off-field predicted environmental residues (PERs). Higher tier field studies have been used to further refine the risk assessment.

When based on the probabilistic HC_5 approach, the risk to non-target terrestrial plants in off-crop areas is acceptable following use of A18385B according to the proposed use pattern, provided the following mitigation is implemented:

1 x 400 g A18385B/ha:

- No buffer and 90% drift reduction mitigation or
- 5 m buffer with 50% drift reduction or
- 10 m buffer with no drift reduction

1 x 500 g A18385B/ha:

- 5 m buffer with 75% drift reduction or
- 10 m buffer with 50% drift reduction or
- 15 m buffer with no drift reduction.

When based on the most sensitive ER_{50} of the higher tier field studies, the risk to non-target terrestrial plants in off-crop areas is acceptable following use of A18385B according to the proposed use pattern, provided the following mitigation is implemented:

1 x 400 g A18385B/ha:

- 75% drift reduction or
- 5 m buffer

1 x 500 g A18385B/ha:

- 90% drift reduction mitigation or
- 5m buffer
-

Review Comments:

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002).

Based on the probabilistic and higher tier risk assessment it can be concluded that the proposed use of A18385B poses acceptable risk to non-target plants, if applied according to the recommended use pattern. Particular precautions to reduce the environmental concentrations resulting from A18385B applications are required.

For Poland, zRMS agrees to apply mitigation measures derived from risk assessment performed with most sensitive ER_{50} from higher tier field studies.

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9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

There are no other relevant data for the active substances or product on organisms in the environment generated from monitoring schemes.

9.13 Classification and Labelling

Based upon all the available aquatic endpoints for A8714C, the proposed classification and labelling of A8714C, driven by effects on aquatic plants is:

Acute Category 1 / Chronic Category 1
H410: Very toxic to aquatic life with long lasting effects.
P391: Collect spillage
P501: Dispose of contents/ container in accordance with national law

Appendix 1 Lists of data considered in support of the evaluation

Product studies relied on and submitted with this application are listed in the table below. These studies were not evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba. List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1 / 01	Liedtke A.	2013	Prosulfuron/dicamba/nicosulfuron WG (A18385B) plus adigor (A12127R) - Toxicity to Pseudokirchneriella subcapitata in a 96-hour algal growth inhibition test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D75032 GLP not published Syngenta File No A18385B_10020; VV-405587	N	Syngenta
KCP 10.2.1 / 02	Liedtke A.	2013a	Prosulfuron/dicamba/nicosulfuron WG (A18385B) plus adigor (A12127R) - Toxicity to the aquatic higher plant Lemna gibba in a 7-day growth inhibition test Syngenta Harlan Laboratories Ltd., Itingen, Switzerland, D75010 GLP not published Syngenta File No A18385B_10021; VV-405419	N	Syngenta
KCP 10.3.1.1 / 01	Kling A.	2013	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Acute Oral and Contact Toxicity to the Honeybee, Apis mellifera L. under Laboratory Conditions Syngenta Eurofins Agroscience Services EcoChem GmbH, N-Osch., Germany, S13-00901 GLP not published Syngenta File No A18385B_10014; VV-404782	N	Syngenta
KCP 10.3.2.1 / 01	Fallowfield L.	2013	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - A rate-response laboratory bioassay of the effects of fresh residues on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) Syngenta	N	Syngenta

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	Vertebrate study Y/N	Owner
			Mambo-Tox Ltd., Southampton, United Kingdom, SYN-13-19 GLP not published Syngenta File No A18385B_10017; VV-404789		
KCP 10.3.2.1 / 02	Stevens J.	2013	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - A rate-response laboratory bioassay of the effects of fresh residues on the parasitic wasp Aphidius rhopalosiphi (Hymenoptera, Braconidae) Syngenta Mambo-Tox Ltd., Southampton, United Kingdom, SYN-13-18 GLP not published Syngenta File No A18385B_10013; VV-405152	N	Syngenta
KCP 10.3.2.2 / 01	Fallowfield L.	2014	Prosulfuron/Dicamba/Nicosulfuron WG (A1835B) plus Adigor (A12127R) - A rate-response extended laboratory bioassay of the effects of fresh residues on the predatory mite Typhlodromus pyri Syngenta Mambo-Tox Ltd., Southampton, United Kingdom, SYN-13-53 GLP not published Syngenta File No A18385B_10070; VV-406997	N	Syngenta
KCP 10.3.2.2 / 02	Stevens J.	2013a	Prosulfuron/Dicamba/Nicosulfuron WG (A1835B) plus Adigor (A12127R) - A rate-response extended laboratory bioassay of the effects of fresh residues on the parasitic wasp Aphidius rhopalosiphi (Hymenoptera, Braconidae) Syngenta Mambo-Tox Ltd., Southampton, United Kingdom, SYN-13-52 GLP not published Syngenta File No A18385B_10034; VV-406310	N	Syngenta
KCP 10.3.2.2 / 03	Tew G.	2014	Prosulfuron/Dicamba/Nicosulfuron WG (A1835B) plus Adigor (A12127R) - A rate-response extended laboratory bioassay of the effects of fresh residues on the rove beetle Aleochara bilineata Syngenta	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Mambo-Tox Ltd., Southampton, United Kingdom, SYN-13-54 GLP not published Syngenta File No A18385B_10072; VV-407006		
KCP 10.3.2.2 / 04	Vaughan R.	2014	Prosulfuron / dicamba / nicosulfuron WG (A18385B) plus Adigor (A12127R) - a rate-response extended laboratory test to evaluate the effects of fresh residues on the green lacewing Chrysoperla carnea (Neuroptera, Chrysopidae) Syngenta Mambo-Tox Ltd., Southampton, United Kingdom, SYN-13-55 GLP not published Syngenta File No A18385B_10081; VV-407544	N	Syngenta
KCP 10.4.1.1 / 01	Friedrich S.	2015	CGA300406 - Sublethal Toxicity to the Earthworm Eisenia fetida in Artificial Soil with 5 % peat Syngenta BioChem Agrar, Gerichshain, Germany, 15 10 48 138 S GLP not published Syngenta File No CGA300406_10018; VV-414538	N	Syngenta
KCP 10.4.1.1 / 02	Friedrich S.	2012	CGA349707 - Sublethal toxicity to the earthworm Eisenia fetida in artificial soil with 5 % peat Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 068 S GLP not published Syngenta File No CGA349707_10001; VV-402755	N	Syngenta
KCP 10.4.1.1 / 03	Friedrich S.	2012a	CGA159902 - Sublethal toxicity to the earthworm Eisenia fetida in artificial soil with 5 % peat Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 066 S GLP not published Syngenta File No CGA159902_10003; VV-402932	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.1.1 / 04	Friedrich S.	2012b	SYN542604 - Sublethal toxicity to the earthworm Eisenia fetida in artificial soil with 5 % peat Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 070 S GLP not published Syngenta File No SYN542604_10007; VV-402929	N	Syngenta
KCP 10.4.1.1 / 05	Friedrich S.	2012c	CGA325025 - Sublethal toxicity to the earthworm Eisenia fetida in artificial soil with 5 % peat Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 064 S GLP not published Syngenta File No CGA325025_10003; VV-402933	N	Syngenta
KCP 10.4.1.1 / 06	Friedrich S.	2012d	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Sublethal Toxicity to the Earthworm Eisenia fetida in Artificial Soil Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 115 S GLP not published Syngenta File No A18385B_10000; VV-403301	N	Syngenta
KCP 10.4.2 / 01	Friedrich S.	2012e	CGA349707 - Effects on the reproduction of the collembolans Folsomia candida Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 067 S GLP not published Syngenta File No CGA349707_10002; VV-402756	N	Syngenta
KCP 10.4.2 / 02	Friedrich S.	2012f	CGA159902 - Effects on the reproduction of the collembolans Folsomia candida Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 065 S GLP not published	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Syngenta File No CGA159902_10002; VV-402759		
KCP 10.4.2 / 03	Friedrich S.	2012g	SYN542604 - Effects on the reproduction of the collembolans Folsomia candida Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 069 S GLP not published Syngenta File No SYN542604_10006; VV-402926	N	Syngenta
KCP 10.4.2 / 04	Friedrich S.	2012h	CGA325025 - Effects on the reproduction of the collembolans Folsomia candida Syngenta BioChem Agrar, Gerichshain, Germany, 12 10 48 063 S GLP not published Syngenta File No CGA325025_10002; VV-402757	N	Syngenta
KCP 10.4.2 / 05	Friedrich S.	2015a	CGA300406 - Effects on the Reproduction of the Collembolan Folsomia candida Syngenta BioChem Agrar, Gerichshain, Germany, 15 10 48 139 S GLP not published Syngenta File No CGA300406_10017; VV-414529	N	Syngenta
KCP 10.4.2 / 06	Friedrich S.	2013	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Effects on the Reproduction of the Collembolan Folsomia candida Syngenta BioChem Agrar, Gerichshain, Germany, 13 10 48 084 S GLP not published Syngenta File No A18385B_10011; VV-404932	N	Syngenta
KCP 10.4.2 / 07	Schulz L.	2013	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Effects on the Reproduction of the Predatory Mite Hypoaspis aculeifer Syngenta BioChem Agrar, Gerichshain, Germany, 13 10 48 085 S	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			GLP not published Syngenta File No A18385B_10012; VV-404934		
KCP 10.5 / 01	Hutcheson K.	2015	CGA349707 - Effect on soil microbial activity, carbon and nitrogen transformations Syngenta CEM Analytical Services Ltd (CEMAS) - Berkshire, UK, CEMR-6587 GLP not published Syngenta File No CGA349707_10012; VV-411802	N	Syngenta
KCP 10.5 / 02	Schulz L.	2013a	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests) Syngenta BioChem Agrar, Gerichshain, Germany, 13 10 48 061 C/N GLP not published Syngenta File No A18385B_10010; VV-405078	N	Syngenta
KCP 10.6.2 / 01	Bramby-Gunary J.	2013	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Vegetative Vigour Test Syngenta AgroChemex Ltd, Manningtree, United Kingdom, ACE-12-183 GLP not published Syngenta File No A18385B_10004; VV-403949	N	Syngenta
KCP 10.6.4 / 01	Dickinson R.	2015	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Vegetative Vigour Test in a higher tier field study Syngenta Agrochemex International Ltd., Aldhams Farm Research Station, Lawford, United Kingdom, ACE-14-062 GLP not published	N	Syngenta

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Syngenta File No A18385B_10378; VV-413377		
KCP 10.6.2 / 02	Bramby-Gunary J.	2013a	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Seedling Emergence and Seedling Growth Test Syngenta AgroChemex Ltd, Manningtree, United Kingdom, ACE-12-182 GLP not published Syngenta File No A18385B_10003; VV-403948	N	Syngenta
KCP 10.6.4 / 02	Dickinson R.	2015a	Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Seedling Emergence and Seedling Growth in a higher tier field study Syngenta Agrochemex International Ltd., Aldhams Farm Research Station, Lawford, United Kingdom, ACE-14-061 GLP not published Syngenta File No A18385B_10377; VV-413376	N	Syngenta

Appendix 2 Detailed evaluation of the new studies

Review Comment:

Most of the studies have been evaluated in fRR of A18385B (zRMS – SK) dated 16/08/2016. Only four studies have been assessted in this report (KCP 10.4.1.1/01, 10.4.2/05, 10.6.4/01 and 10.6.4/02).

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.2.1.1 Algae

The following study on algae has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zone for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.2.1/01
Report	Liedtke A, 2013, Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Toxicity to *Pseudokirchneriella subcapitata* in a 96-hour algal growth inhibition test, Report Number D75032, Harlan Laboratories Ltd., Zelgiwelg 1, 4452 Itingen, Switzerland, (Syngenta File

	No. A18385B_10020)
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006) Commission Regulation (EC) No. 761/2009 C.3: Algal Inhibition Test, 2009 US EPA Ecological Effects Test Guidelines, OPPTS 850.5400: Algal Toxicity, Tiers I and II, (1996)
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

The toxicity of Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus adjuvant Adigor (A12127R) to the green alga *Pseudokirchneriella subcapitata* was determined. The nominal test item concentrations tested were 0.046, 0.10, 0.22, 0.46, 1.0 and 2.2 mg A18385B plus A12127R/L. Additionally, a control group was tested in parallel.

Based on nominal concentrations of the formulation A18385B plus A12127R the 72-hour E_rC_{50} was 0.73 mg/L, the E_yC_{50} was 0.30 mg/L and the E_bC_{50} was 0.34 mg/L. The 96-hour E_rC_{50} was 0.86 mg/L, the E_yC_{50} was 0.38 mg/L and the E_bC_{50} was 0.36 mg/L.

Materials

Test Material	A18385B Prosulfuron/Dicamba/Nicosulfuron WG	
Lot/Batch #:	SMU2BP004	
Actual content of active ingredients:	Prosulfuron:	4.32% w/w
	Dicamba:	41.0% w/w
	Nicosulfuron:	10.5% w/w
Description:	Brown granules	
Stability of test compound:	Stable when stored at room temperature at about 20°C, in the dark.	
Reanalysis/expiry date:	30 September 2014	
Density:	Not applicable	
Test material	A12127R Adigor	
Lot/Batch #:	UNI2IB0912	
Content of Sum of Fatty Acid Methyl Esters (sum of main components):	42.9% (w/w) corresponding to 398 g/L	
Description:	Light yellow liquid	
Stability of test compound:	Stable when stored at room temperature at about 20°C, in the dark.	
Reanalysis/expiry date:	31 October 2016	
Density:	927 kg/m^3	
Treatments		
Test concentrations:	Culture medium control and nominal formulation concentrations of 0.046, 0.10, 0.22, 0.46, 1.0 and 2.2 mg A18385B/L	
Solvent:	None	
Positive control:	Potassium dichromate is used at twice a year	
Analysis of test concentrations:	Yes, 0 and 96 hours (based on measurements of prosulfuron by HPLC-MS/MS)	
Test organism		
Species:	*Pseudokirchneriella subcapitata*, Strain No. 61.81 SAG	
Source:	Collection of Algal Cultures (SAG, Institute for Plant Physiology, University of Göttingen, 37073 Göttingen/ Germany).	
Test design		

Test vessels:	50 mL Erlenmeyer flasks covered with glass dish containing 15 mL of media	
Test medium:	Reconstituted water (AAP algal medium)	
Replication:	Six vessels for the control and three vessels for each test concentration	
Starting cell density:	0.5×10^4 cells/mL	
Exposure regime:	Static	
Aeration:	No	
Duration:	96 hours	
Environmental conditions		
Test temperature:	22°C	
pH:	test start:	7.4 to 7.5
	test end:	8.7 to 9.1
Lighting:	Continuous illumination at 4800 to 5900 Lux	

Study Design and Methods

Experimental dates: 23rd May 2013 to 17th July 2013.

Since the test item comprises two components (A18385B and A12127R) the preparation of the stock solution was performed by mixing these two components at a ratio of 1:3 (w/v) in test water. For this preparation, 278.7 mg of A12127R were carefully mixed into approximately 200 mL test water. Thereafter, 100.4 mg of A18385B were carefully mixed into the first preparation and subsequently step by step and under stirring, filled up to 1000 mL. The solution was stirred for 15 minutes at room temperature. This intensively mixed stock solution was used in a series of dilutions steps to prepare the test media of all test item concentrations. The test media were prepared just before the start of the test.

The test was started using a nominal algal cell density of 5000 cells/mL. Test solutions were continuously stirred with magnetic stirrers and were held in a temperature controlled water bath at a temperature of 22°C and illuminated by fluorescent tubes.

A small volume of the algal suspension was taken from each test flask daily for the measurement of the biomass. At the end of the test, a sample was taken from the control and from the test concentration of nominal 0.22 mg A18385B/L. The shape and size of the algal cells were examined microscopically in these samples.

The pH was measured at the start and at the end of the test. The water temperature was measured and recorded daily in a flask incubated under the same conditions as the test flasks. The appearance of the test media was also recorded daily.

The test concentrations were verified by chemical analysis of prosulfuron at 0 and 96 hours, using HPLC-MS/MS.

Results and Discussion

Since the test item comprises two components (A18385B and A12127R) the preparation of the stock solution was performed by mixing these two components at a ratio of 1:3 (w/v) in test water.
The measured concentrations of the active ingredient prosulfuron of the formulation A18385B plus A12127R in the test media of the concentrations of 0.046 to 2.2 mg/L were between 95 and 107% of the nominal values at the start of the test and between 91 and 107% at the end of the test. The reported biological results were based on the nominal concentrations of the test item since the correct dosage of the formulation and the stability of the active ingredient was confirmed.

Table A 1 Analytical results

Nominal concentrations of A18385B plus A12127R (mg/L)	Nominal concentrations of prosulfuron (µ/L)	% of nominal measured at 0 hours	% of nominal measured at 96 hours
Control	Control	n.a.	n.a.
0.046	1.99	102	91
0.10	4.32	101	102
0.22	9.50	104	103
0.46	19.9	107	107
1.0	43.2	95	97
2.2	95.0	102	103

The algal biomass was measured at 24, 48, 72 and 96 hours and the biomass integral, growth rate and yield were calculated. The 72-hour and 96-hour E_bC_{50}, E_yC_{50} and E_rC_{50} values (defined as the concentration resulting in 50% reduction of each parameter) were calculated using Probit Analysis using linear maximum likelihood regression. A Williams t-test or a Welch t-test, as appropriate, was used to identify significant differences in the calculated mean biomass, growth rate and yield of test item treatments compared to the control.

There were no abnormalities, observed microscopically, in the control or 0.22 mg A18385B/L test culture at 96 hours.

Growth rates

The growth rate 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table A 2 Mean values at each concentration of A18385B plus A12127R for the growth rate at 72 and 96 hours for Pseudokirchneriella subcapitata and relevant endpoints

Nominal concentrations of A18385B plus A12127R (mg/L)	Mean growth rate (1/day) 0 – 72 hrs	Percentage inhibition	Mean growth rate (1/day) 0 – 96 hrs	Percentage inhibition
Control	1.50	0.0	1.36	0.0
0.046	1.47	1.6	1.35	0.5
0.10	1.43#	4.3	1.35	0.5
0.22	1.34#	10.4	1.29#	5.3
0.46	1.20#	19.7	1.16#	14.5
1.0	0.45#	70.2	0.52#	62.1
2.2	-0.04#	102.9	0.16#	88.3
E_rC_{50} mg A18385B plus A12127R/L	0.73		0.86	
(95% confidence limits)	0.68-0.79		0.80-0.92	
NOEC	0.046		0.10	
LOEC	0.10		0.22	

#: mean value significantly lower than in the control (according to Welch t-test, one-sided smaller, $\alpha = 0.05$)

Yield

The yield 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table A 3: Mean values at each concentration of A18385B plus A12127R for the yield at 72 and 96 hours for Pseudokirchneriella subcapitata and relevant endpoints

Nominal concentrations of A18385B plus A12127R (mg/L)	Mean yield (x 10^3 cells/mL) 0 – 72 hrs	Percentage inhibition	Mean yield (x 10^3cells/mL) 0 – 96 hrs	Percentage inhibition
Control	80.4	0.0	207.5	0.0
0.046	74.5*	7.3	202.3	2.5
0.10	66.0*	17.9	201.3	3.0
0.22	50.0*	37.8	155.2*	25.2
0.46	32.8*	59.3	93.9*	54.7
1.0	2.6*	96.8	6.3*	97.0
2.2	-0.1*	100.1	0.9*	99.6
E_yC_{50} mg A18385B plus A12127R/L	0.30		0.38	
(95% confidence limits)	0.26-0.34		0.35-0.42	
NOEC	n.d.		0.10	
LOEC	0.046		0.22	

*: mean value significantly lower than in control (according to Williams t-test, one-sided smaller, α = 0.05)

Biomass (area under the growth curve)

The areas under the growth curve for 0 to 72 hours and 0 to 96 hours were calculated for each replicate culture and the means are shown below, alongside the estimated EC_{50} values.

Table A 4: Mean values at each concentration of A18385B plus A12127R for the biomass integral (area under the growth curve) at 72 and 96 hours for Pseudokirchneriella subcapitata and relevant endpoints

Nominal concentrations of A18385B plus A12127R (mg/L)	Mean biomass integral (103 * day) 0 – 72 hrs	Percentage inhibition	Mean biomass integral (103 * day) 0 – 96 hrs	Percentage inhibition
Control	65.6	0.0	209.5	0.0
0.046	61.8*	5.7	200.2	4.4
0.10	55.8*	14.8	189.5*	9.6
0.22	43.6*	33.5	146.2*	30.2
0.46	30.2*	54.0	93.5*	55.4
1.0	3.7*	94.4	8.1*	96.1
2.2	-0.2*	100.3	0.2*	99.9
E_bC_{50} mg A18385B plus A12127R/L	0.34		0.36	
(95% confidence limits)	0.30-0.38		0.32-0.39	
NOEC	n.d.		0.046	
LOEC	0.046		0.10	

*: mean value significantly lower than in control (according to Williams t-test, one-sided smaller, α = 0.05)

Validity criteria

- In the control the biomass increased by a factor of 89.1 over 72 hours (should be at least 16).
- The mean coefficient of variation of the daily growth rates in the control during 72 and 96 hours was 14.5 and 24%, respectively (must not be higher than 35%).
- The coefficient of variation of the average specific growth rates in the replicates of the control after 72 and 96 hours was 1.6 and 1.1%, respectively (must not be higher than 7%).
- The coefficient of variation of the fluorescence values in replicate control cultures during 72 and 96 hours was 7.2 and 6.1%, respectively (must not exceed 20%).
- The pH rose in the control from 7.5 at test start to 9.1 at test end (should not increase by more than 1.5 units). The increase in pH during the test was caused by the uptake of CO_2 by the algae due to

their rapid growth, despite the test media being stirred continuously during the test. However, since all validity criteria concerning the growth are fulfilled the test is considered to be valid.

Conclusions

Based on nominal concentrations of the formulation A18385B plus A12127R the 72-hour E_rC_{50} was 0.73 mg/L, the E_yC_{50} was 0.30 mg/L and the E_bC_{50} was 0.34 mg/L. The 96-hour E_rC_{50} was 0.86 mg/L, the E_yC_{50} was 0.38 mg/L and the E_bC_{50} was 0.36 mg/L.

Based on nominal concentrations of the formulation A18385B plus A12127R the LOECs at 72 hours, based on growth rate, yield and biomass integral, were 0.10, 0.046 and 0.046 mg/L respectively, and at 96 hours based on growth rate, yield and biomass integral were 0.22, 0.22 and 0.10 mg/L, respectively.
The NOEC at 72 hours, based on growth rate was 0.046 mg/L. The NOECs at 96 hours, based on growth rate, yield and biomass integral, were 0.10, 0.10 and 0.046 mg/L, respectively.

(Liedtke A, 2013)

A 2.2.1.2 Aquatic macrophyte

The following study on Lemna has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zone for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.2.1/02
Report	Liedtke A, 2013a, Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – Toxicity to the Aquatic Higher Plant *Lemna gibba* in a 7-Day Growth Inhibition Test. Report Number D75010. Harlan Laboratories Ltd., Zelgliweg 1, 4452 Itingen, Switzerland. Syngenta file no A18385B_10021
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 221: *Lemna* sp. Growth Inhibition Test (2006) Commission Regulation (EC) No. 761/2009 laying down test methods pursuant to Regulation (EC) No. 1907/2006 of the European Parliament and of the Council on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), 2009, C.26: Lemna sp. Growth Inhibition Test. US EPA Ecological Effects Test Guidelines, OPPTS 850.4400: Aquatic Plant Toxicity using *Lemna* spp., Tiers I and II, (1996).
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

The toxicity of A18385B plus A12127R to the aquatic plant *Lemna gibba* was determined in a 7-day semi-static test with medium renewal every 48 or 72 hours. The *Lemna* were exposed to nominal concentrations of 2.8, 6.0, 13, 28 and 60 µg A18385B/L alongside a dilution water control. Since the test item comprises two components (A18385B and A12127R) the preparation of the stock solution was performed by mixing these two components at a ratio of 1:3 (w/v) in test water.

For frond number, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) for A18385B plus A12127R to *Lemna gibba*, were 10 and 17 µg A18385B/L, respectively, based on nominal concentrations. For dry weight, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) were 21 and >60mg A18385B/L, respectively, based on nominal concentrations.

Materials

Test Material	A18385B Prosulfuron/Dicamba/Nicosulfuron
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	30 September 2014
Test Material	A12127R Adigor
Lot/Batch #:	UNI2IB0912
Actual content of active ingredients:	Content of sum of fatty acid methyl esters : 42.9% corresponding to 398 g/L Some of the main components: Methyl oleate 26.9% corresponding to 249 g/L Methyl linoleate 8.92% corresponding to 82.7 g/L Methyl linolenate 4.29% corresponding to 39.8 g/L Methyl palminate 2.08% corresponding to 19.3 g/L Methyl stearate 0.72% corresponding to 6.7 g/L
Description:	Light yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/expiry date:	31 October 2016
Density:	927 kg/m^3
Treatments	
Test concentrations:	Dilution water control; nominal concentration of 2.8, 6.0, 13, 28 and 60 µg A18385B/L
Solvent:	None
Vehicle and/or positive control:	3,5-dichlorophenol is used as a positive control twice a year. (Latest positive control test performed in April 2013, study #: D74266)
Analysis of test concentrations:	Yes, analysis of the active ingredient prosulfuron in freshly prepared and aged test media on days 0, 3, 5 and 7 using HPLC-MS/MS analysis. Duplicate samples taken.
Test organisms	
Species:	*Lemna gibba* G3 (family Lemnaceae, Macrophyta)
Source:	The original culture was supplied by Bayer CropScience AG, 40789 Monheim, Germany in 2007. The plants were axenically cultivated at Harlan Laboratories Ltd., for more than four weeks prior to the test. The pre-culture was maintained under the conditions of the test (nutrient medium, light conditions and temperature) for at least seven days prior to the start of the test. The test was started with plants from an exponentially growing culture. Only young, rapidly growing colonies without visible lesions were used.
Test design	
Test vessels:	250 mL glass dishes (diameter of approx. 9.5 cm) filled with 150 mL of test medium with glass dish covers
Test medium:	20X AAP growth medium according to OECD guideline
Replication:	Three vessels for the control and each test concentration
Initial frond number:	4 fronds per plant, total 12 fronds per replicate
Exposure regime:	Semi-static; test medium renewal every 48 or 72 hours
Duration:	7 days
Environmental conditions	
Temperature:	24°C
pH:	7.5 – 8.3 new solutions; 8.5 – 8.9 aged solutions
Lighting:	Continuous illumination at 7770 - 8700 Lux

Study Design and Methods

Experimental dates: 3rd June 2013 to 17th July 2013

Since the test item comprises two components (A18385B and A12127R) the preparation of the stock solution was performed by mixing the two components at a ratio of 1:3 (w/v) in test water. A stock solution was prepared by mixing 167.21mg (Day 0), 167.1 mg (Day 3) or 167.17 mg (Day 5) mg of A12127R in approximately 200 mL test water. Thereafter, 60.04 mg (Day 0), 60.0 mg (Day 3) or 60.23 mg (Day 5) of A18385B was carefully mixed into the first preparation and subsequently made up to 1000mL with stirring and finally stirred for 15 minutes at room temperature on days 0, 3 and 5. The stock solution was used in a series of dilutions with test water to prepare the test media of the lower test concentrations. The control consisted of culture medium only.

150 mL of the test solutions were transferred into 250 mL glass dishes and inoculated with *Lemna* plants. Cultures were then transferred to a temperature-controlled room where they were maintained under the conditions indicated above.

Assessments of frond number were made on days 0, 3, 5 and 7. Fronds were harvested for measurement of dry weight after 7 days, and the initial dry weight was determined using a sample of 12 fronds at the start of the test.

At test initiation, light intensity was measured at nine locations distributed over the test area, level with the surface of the test media. The pH was measured and recorded in each treatment at the start and end of each test medium renewal period. The water temperature was measured in a vessel filled with water (incubated under the same conditions as the test vessels) on each working day. The appearance of the test media was recorded on the counting days of the plants. The water temperature in the temperature-controlled water bath was also measured continuously.

The test concentrations were verified by chemical analysis of the active ingredient prosulfuron in samples from the freshly prepared and aged test media of all test concentrations, and from the control, on days 0, 3, 5 and 7, using HPLC-MS/MS analysis. For sampling of the aged test media, the test media of three replicates per test concentration were pooled.

Results and Discussion

The analytically determined concentrations of A18385B plus A12127R (based on the measurement of the active ingredient prosulfuron) were between 79 to 94% of the nominal values in fresh solutions and 77 to 96% in aged solutions (see table below). The limit of quantification in this study was 0.0489 µg prosulfuron/L. Nominal concentrations were used for the calculation and reporting of results.

Table A 5 Analytical results

Nominal concentrations µg A18385B/L	A18385B plus A12127R					
	% of nominal measured at 0 days, 0 hours	% of nominal measured at 3 days, 72 hours	% of nominal measured at 3 days, 0 hours	% of nominal measured at 5 days, 48 hours	% of nominal measured at 5 days, 0 hours	% of nominal measured at 7 days, 48 hours
Control	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2.8	81	79	89	87	89	82
6.0	81	77	94	91	90	86
13	80	80	85	91	88	85
28	80	78	91	81	84	83
60	79	83	90	96	82	78

The tabulated values represent rounded results obtained by calculation using the exact raw data
n.a. = not applicable

Data for frond number and dry weight was used to calculate growth rates and yield for the control and each exposure concentration. Probit analysis using linear maximum likelihood regression was then used to calculate the 7-day ErC_{50} and EyC_{50}, based on percent inhibition relative to the control. For the No Observed Effect Concentration and Lowest Observed Effect Concentration, a multiple Williams t-test or Welch t-test was used to determine values significantly different to the control.

Mean frond numbers are presented below along with the growth rate, yield and respective inhibition values, alongside estimated EC_{50} values:

Table A 6 Effect of A18385B plus A12127R on growth rate and yield (frond number) of *Lemna gibba*

Nominal concentration (µg A18385B/L)	Mean No. fronds/replicate (day 7)	Based on Frond Number (0-7 days)			
		Growth Rate (day^{-1})	Inhibition of Growth Rate (%)	Yield	Inhibition of Yield (%)
Control	183.0	0.389	0.0	171.0	0.0
2.8	187.7	0.393	-0.9	175.7	-2.7
6.0	178.0	0.385	1.0	166.0	2.9
13	49.3	0.201#	48.3	37.3*	78.2
28	27.7	0.114#*	70.7	14.7*	91.4
60	20.7	0.078#	80.1	8.7*	94.9
EC_{50} (µg A18385B/L)		17		10	
95% confidence limits		14 – 21		9.1 - 11	
NOEC (µg A18385B/L)		60		6.0	
LOEC (µg A18385B/L)		13		13	

(-) = increase in growth relative to that of control
= Mean value statistically significantly lower than in the control (according to Welch t-test, one-sided smaller, $\alpha = 0.05$)
* = Mean value statistically significantly lower than in the control (according to Williams t-test, one-sided smaller, $\alpha = 0.05$)

Mean dry weights are presented below along with the growth rate, yield and respective inhibition values, alongside estimated EC_{50} values:

Table A 7 Effect of A18385B plus A12127R on growth rate and yield (dry weight) of *Lemna gibba*

Nominal	Mean Dry	Based on Dry Weight (0-7 days)

concentration (µg A18385B/L)	Weight (mg per test vessel) (day 7)	Growth Rate (day^{-1})	Inhibition of Growth Rate (%)	Yield (mg)	Inhibition of Yield (%)
Control	22.7	0.446	0.00	21.7	0.00
2.8	24.5	0.456	-2.24	23.5	-8.29
6.0	21.7	0.439	1.57	20.7	4.61
13	12.6	0.361*	19.06	11.6#	46.54
28	8.9	0.312*	30.04	7.9#	63.59
60	7.4	0.285*	36.10	6.4#*	70.51
EC_{50} (µg A18385B/L)		>60		21	
95% confidence limits		n.d.		17 - 27	
NOEC (µg A18385B/L)		6.0		6.0	
LOEC (µg A18385B/L)		13		13	

Inoculum = 1.01 mg dry weight per vessel; the dry weight at the start of the test was determined from a sample of the inoculum culture representative of what was used to begin the test. This value was used for calculation of growth rate and yield.
- = increase in growth relative to that of control
* = Mean value statistically significantly lower than in the control (according to Williams t-test, one-sided smaller, α = 0.05)
# = Mean value statistically significantly lower than in the control (according to Welch t-test, one-sided smaller, α = 0.05)
n.d. = not determined

No abnormalities in appearance of the test plants were recorded in the control and the test concentrations of 2.8 and 6.0µg A18385B/L. Based on visual assessment, at the test concentrations of 13 to 60 µg A18385B/L, chlorosis was observed on day 3 and 5 and the roots of the plants were also shorter on Day 5. Additionally, at the end of the test, the fronds were smaller at test concentration 13 µg A18385B/L and brown coloured at the two highest concentrations. No mortality of fronds was observed during the test.

Validity criteria

The validity criterion for the study was fulfilled:

- the doubling time (T_d) of frond number in the control must be <2.5 days (observed: 1.8 days)

Conclusions

For frond number, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) for A18385B plus A12127R to *Lemna gibba*, were 10 and 17 µg A18385B/L, respectively, based on nominal concentrations. For dry weight, the 7-day EC_{50} for yield (E_yC_{50}) and growth rate (E_rC_{50}) were 21 and >60mg A18385B/L, respectively, based on nominal concentrations.

For frond number and dry weight, the 7-day NOEC, based on growth rate and yield, was 6.0 µg A18385B/L, and the 7-day LOEC, based on growth rate and yield, was 13 µg A18385B/L

(Liedtke A, 2013a)

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.2.1 Aquatic invertebrate

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

The following acute study on bees has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zone for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.3.1.1/01
Report	Kling A, (2013), Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Acute Oral and Contact Toxicity to the Honeybee, *Apis mellifera* L. under Laboratory Conditions, Report Number S13-00901. Eurofins Agroscience Services EcoChem GmbH, Eutinger Str. 24, 75223 Niefern-Öschelbronn, Germany. (Syngenta file No. A18385B_10014).
Guideline(s):	OECD Guidelines for Testing of Chemicals, Section 2 - Effects on Biotic Systems, Method 211: *Daphnia magna* Reproduction test (1998) US EPA Ecological Effects Test Guidelines, OPPTS 850.1300: *Daphnia* Chronic Toxicity Test (1996) 92/69/EEC, C.20 (2001)
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

The 48-hour oral LD_{50} value for A18385B was >256 µg product/bee and the 48-hour contact LD_{50} was 140 µg product/bee.

Materials

Test Material	A18385B Prosulfuron/Dicamba/Nicosulfuron
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 September 2014
Density:	n/a
Treatments	
Test rates:	Oral: Nominal – 15.6, 31.3, 62.5, 125 and 250 µg A18385B/bee

	Measured – 17.3, 35.5, 71.5, 139 and 256 µg A18385B/bee Contact: Nominal – 15.6, 31.3, 62.5, 125 and 250 µg A18385B/bee
Control:	Oral: 50% w/v aqueous sucrose solution Contact: Tap water
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). Application concentrations equivalent to 3:1 relative to the test item
Toxic standard:	Perfekthion/BAS 152 11 I (nominally 400 g dimethoate/L; measured 411.7 g dimethoate/L) Oral: Nominal – 0.06, 0.08, 0.11 and 0.15 µg dimethoate/bee Contact: Nominal – 0.10, 0.14, 0.19 and 0.25 µg dimethoate/bee
Administration:	Contact: cuticular absorption following the application of droplets dorsally to the thorax of each bee Oral: ingestion in aqueous sucrose solution
Test organisms	
Species:	*Apis mellifera* L. (Hymenoptera, Apidae)
Source:	Young adult worker bees from a healthy colony descended from a breeding line of a beekeeper in Mayen, Germany (responsible beekeeper: Gerald Wolters, Im Bannen 38 – 54, G-56727 Mayen, Germany), collected the day before test start and kept under test conditions.
Food:	50% w/v aqueous sucrose solution
Test design	
Test cage description:	Stainless steel chambers (approx. 8.0 x 4.0 x 6.0 cm) with a transparent window in the front and a perforated steel bottom. The test cages were lined with filter paper.
Replication:	4
No. of bees/arena :	10
Duration of test:	48 hours
Environmental test conditions	
Temperature:	24.3 – 25.7ºC
Humidity:	59.4 – 67.3% RH
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 7th May 2013 to 9th May 2013

Honeybees (*Apis mellifera* L.) were exposed to A18385B *via* two routes of administration: (1) contact, i.e. cuticular absorption following the application of a droplet to the dorsal body surface of a solution in tap water; after each application the applicator needle was cleaned with a mixture of water and water-wetting agent; and (2) oral ingestion in aqueous sucrose solution. To immobilise the bees during the course of treatment, they were anaesthetised using CO_2.

Contact test procedures: Bees were treated with a 2 µL droplet of A18385B, control, or toxic standard, applied to the dorsal surface of the thorax using a micro applicator. A 2 µL droplet was chosen in deviation to the guideline recommendation of 1 µL, since a higher volume was considered to ensure a more reliable dispersion of the test item. No adverse effects on the outcome of the study were expected. The bees were returned to the test unit, allowed to recover with a continuous supply of 50% w/v aqueous sucrose solution.

Oral test procedures: Bees were starved for 2 hours until treatment. Each group of bees was offered 250 µL (equivalent to 25 µL/bee) of A18385B or toxic standard dispersed in aqueous sucrose solution. Treatments were calculated so that the target dose was contained in 20 µL, however 25 µL was actually

provided per bee. This was to ensure sufficient consumption of the test material so that the target dose was achieved. The doses were measured into eppendorf cups and the weights of these were recorded before the doses were made available to the bees. The bees were allowed to consume the test solutions up to a maximum of six hours after which the eppendorf cups were replaced and 50% w/v aqueous sucrose solution provided *ad libitum*. All cups with test solutions were weighed after feeding in order to calculate actual mean consumption per bee for each treatment.

In both the contact and oral tests there were four replicates per treatment. Mortality and sublethal effects were assessed at 4, 24 and 48 hours.

The mortality [%] per treatment was calculated from the number of dead bees and the total number of introduced bees per treatment group. The calculation of corrected mortality in the test item and reference item treatments according to Schneider-Orelli (1947) was not necessary.

The LD_{50} values with 95% confidence limits of the reference and test item treatments were calculated by means of a probit analysis. The oral LD_{50} values for the test and reference item treatment were calculated with the single consumption values per replicate.

Results and Discussion

Mortality data for the test material and toxic standard are summarised in the table below.

Table A 8 Summary of acute toxicity of A18385B to the honeybee

Treatment	Exposure		LD_{50} values	95% confidence interval
	Route	Duration (hours)		
Test material (µg A18385B/bee)	Contact	24	160	137-191
		48	140	116-176
	Oral	24	>256	n.d.
		48	>256	n.d.
Toxic standard (µg dimethoate/bee)	Contact	24	0.17	0.15-0.19
	Oral	24	0.15	0.14-0.16

n.d. = not determined

In the contact toxicity test at the 4 and 24 hours assessment, sublethal effects like affected, apathetic or moribund bees occurred over all tested dose levels, especially in the two highest levels (125 and 250 µg product/bee). No sublethal effects were noticed at the 48 hours assessment.

In the oral toxicity test, sublethal effects like affected, apathetic or moribund bees were observed in the dose levels of ≥125 µg product/bee at the 4 and 24 hours assessments. At the final assessment, 48 hours after test start, affected bees were noticed in the highest tested dose level (256 µg product/bee).

Validity Criteria

The study is considered to be valid because:

- the mean mortality of the control in the oral and contact toxicity test was ≤10% (observed 0% after 48 hours)
- the 24 h LD_{50} of the reference item in the oral toxicity test was within the range of 0.10 to 0.35 µg a.s./bee (measured 0.15 µg dimethoate/bee)
- the 24 h LD_{50} of the reference item in the contact toxicity test was within the range of 0.10 to 0.30 µg a.s./bee (measured 0.17 µg dimethoate/bee)

Conclusions

The 48-hour oral LD_{50} value for A18385B was >256 µg product/bee and the 48-hour contact LD_{50} was 140 µg product/bee.

(Kling A, 2013)

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Please see A 2.3.1.1.1.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

A 2.3.2.1.1 *Typhlodromus pyri*

The following study has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zone for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.3.2.1/01
Report	Fallowfield L, (2013), Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – A rate-response laboratory bioassay of the effects of fresh residues on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae). Report Number SYN-13-19 Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK. (Syngenta file No. A18385B_10017).
Guideline(s):	Blümel *et al.* (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory

	testing of plant protection products. IOBC Publication. ISBN 92-9067-129-7.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

In a worst-case laboratory test to determine the effects of A18385B, plus the adjuvant A12127R (Adigor), on the predatory mite *Typhlodromus pyri*, the 7-day median lethal rate (LR_{50}) was calculated to be 165.6 g product/ha, with 95% confidence limits of 60.8 and 276.1 g A18385B/ha.

For reproduction, the median effect rate (ER_{50}) was estimated as being >250 g A18385B/ha.

Based on statistical comparisons with the control, the no-observed-effect rate (NOER) for mortality was considered to lie below 62.5 g A18385B/ha, the lowest rate tested, and the NOER for reproduction was 250 g A18385B/ha.

Materials

Test Material	A18385B prosulfuron/dicamba/nicosulfuron WG (4/40/10)
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	September 2014
Density:	n/a
Treatments	
Test rates:	1000, 500, 250, 125 and 62.5 g formulation/ha
Control:	Purified water
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters). Application concentrations equivalent to 3:1 relative to the test item
Toxic standard:	BAS 152 11I Perfekthion (nominally 400 g dimethoate/L, analysed 411.7 g dimethoate/L) applied at a rate of 15 mL product per 200 L water/ha (6 g a.s./ha)
Spray volume rate:	200 L spray solution/ha
Application method:	Laboratory track sprayer (3 bar pressure, 80° flat fan nozzle)
Test organisms	
Species:	*Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Age:	Less than 24 h old protonymphs
Source:	Culture maintained at Test Facility, originally obtained (April 1995) from P.K. Nützlingszuchten, Welzheim, Germany, supplemented with mites from same source in 1996 and 1997.
Feeding:	1:1 v/v mixture of almond (*Prunus* sp. var Butte) and apple (*Malus* sp. var. Red Delicious) pollen
Test design	
Arenas:	Glass plates formed from two microscope slide cover slips with a narrow channel between them were mounted on damp tissue paper with an oblong ring of a sticky non-drying gel drawn onto the plates to create an arena in which the mites were confined. The arena was approximately 3 cm x 4 cm, enclosing an area of ca. 12 cm^2.
Replication:	3

No. of mites/arena :	20
Duration of test:	Mortality assessment: 0-7 days Fecundity assessment: 7-14 days
Environmental test conditions	
Temperature:	25 – 26°C
Humidity:	67– 84% RH
Photoperiod:	16 h photoperiod (600 – 1250 Lux)

Study Design and Methods

Experimental dates: 11th June 2013 to 25th June 2013

Treatments were applied to the glass plates and the bioassay initiated approximately 1 h later, once residues had dried. The glass plates were placed onto damp tissue paper and an oblong ring of a sticky non-drying gel drawn on the plates to create arenas in which mites were confined. The survival of the mites was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined, and where necessary males were moved between replicates to ensure a male to female ratio of 1:5 in each treatment, they were then left *in situ* so that their reproduction could be assessed over a further 7 days. Any eggs produced prior to 7 DAT were removed and discarded. For 7 days, the total egg production (numbers of eggs plus live and dead juvenile stages) was recorded for each unit. Assessments of oviposition activities were carried out at 10, 13 and 14 DAT. Any eggs and nymphs present were recorded and then removed. In addition, the condition of the adult female and male mites in each arena was recorded on each date.

The numbers of any stuck, drowned or missing mites were added to the number of dead mites found in each treatment to derive the overall "mortality". The mean percentage mortality after 7 days was calculated for the individual treatments and then corrected for any losses in the control treatment using Abbott's formula (Abbott, 1925). A Probit analysis (Finney, 1952; SPSS, 2012) was performed on the 7-day mortality data from the test, in order to derive the median lethal rate (LR_{50}). In order to determine the NOER, the percentage mortality in each treatment was compared to the control using Fisher's Exact Test ($\alpha = 0.05$).

In order to determine the NOER for assessments of reproduction, the results were compared by one-way ANOVA ($\alpha = 0.05$). The median effect rate (ER_{50}) for reproduction was estimated without recourse to statistical analysis. The effect of treatments on mite fecundity relative to the control was calculated using the formula:

% change = [1-(Rt/Rc)] * 100
where Rt and Rc are the absolute values observed in the treatment and control groups respectively.

Results and Discussion

Mortality and fecundity are summarised in the table below. All values were calculated using the original raw data and were not based on rounded values.

Table A 9 Effects of A18385B on mortality and fecundity of Typhlodromus pyri, when exposed under extended laboratory test conditions

Treatment (g A18385B/ha)	Mean % mortality at 7 DAT [a)]	Mean corrected % mortality at 7 DAT [b)]	Mean eggs/female from 7 to 14 DAT [c)]	% Effect on reproduction compared to control [d)]
Control	3	-	8.3	-
1000	77*	76	-	-

Treatment (g A18385B/ha)	**Mean % mortality at 7 DAT [a)]**	**Mean corrected % mortality at 7 DAT [b)]**	**Mean eggs/female from 7 to 14 DAT [c)]**	**% Effect on reproduction compared to control [d)]**
500	75*	74	-	-
250	48*	47	6.1	26.4
125	55*	53	6.8	18.1
62.5	32*	29	7.5	9.8
Toxic reference	80*	79	-	-

[a)] Results for mortality in individual treatments at 7 DAT were compared to that in the control by Fisher's Exact Test ($\alpha = 0.05$). Treatment means that differed significantly from the control are indicated with an asterisk (*).
[b)] Calculated using Abbott's formula
[c)] Results for reproduction over the assessment period were compared by one-way ANOVA ($\alpha = 0.05$). No treatment means differed significantly from the control.
[d)] Egg production, relative to the control. A positive value indicates a decrease.

Validity Criteria

The validity criteria for the test were met:

- mortality in the control treatment over the initial 7 days should not exceed 20% (3% observed)
- mortality in the toxic reference treatment should be 50-100% (80% observed)
- the mean cumulative number of eggs produced from 7 to 14 days should be ≥4.0 per female in the control treatment (8.3 observed)

Conclusions

In a worst-case laboratory test to determine the effects of A18385B, plus the adjuvant A12127R (Adigor), on the predatory mite *Typhlodromus pyri*, the 7-day median lethal rate (LR_{50}) was calculated to be 165.6 g product/ha, with 95% confidence limits of 60.8 and 276.1 g A18385B/ha.

For reproduction, the median effect rate (ER_{50}) was estimated as being >250 g A18385B/ha.

Based on statistical comparisons with the control, the no-observed-effect rate (NOER) for mortality was considered to lie below 62.5 g A18385B/ha, the lowest rate tested, and the NOER for reproduction was 250 g A18385B/ha.

(Fallowfield L, 2013)

A 2.3.2.1.2 Aphidius rhopalosiphi

The following study has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zone for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.3.2.1/02
Report	Stevens J, (2013), Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – A rate-response laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). Report Number SYN-13-18. Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK. (Syngenta file No. A18385B_10013).

Guideline(s):	Mead-Briggs *et al.* (2000). A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (DeStephani-Perez) (Hymenoptera, Braconidae). IOBC Publication. ISBN 92-9067-129-7.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

In a laboratory test to determine the effects of A18385B, applied in conjunction with the adjuvant A12127R, on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h LR_{50} (median lethal rate) was <62.5 g A18385B/ha, the minimum rate tested. In terms of effects on wasp survival, the lowest-observed-effect rate (LOER) was 62.5 g product/ha and the no-observed-effect rate (NOER) was not determined.

Materials

Test Material	A18385B prosulfuron/dicamba/nicosulfuron WG (4/40/10)
Lot/Batch #:	SMU2BP004
Content of active ingredients (analysed):	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	September 2014
Density:	not applicable
Treatments	
Test rates:	1000, 500, 250, 125 and 62.5 g A18385B/ha
Control:	Purified water
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). Application concentrations equivalent to 3:1 relative to the test item
Toxic standard:	BAS 152 11 I Perfekthion (nominally 400 g dimethoate/L, analysed 411.7 g dimethoate/L) in purified water, applied at a rate of 0.10 mL product/ha in 200 L water/ha (0.04g a.s./ha).
Spray volume rate:	200 L spray solution/ha
Application method:	Schachtner track sprayer (3 bar pressure, 80° flat fan nozzle)
Test organisms	
Species:	*Aphidius rhopalosiphi* (Hymenoptera: Braconidae).
Age:	Adults within 48 hours of their emergence
Source:	Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*), originally obtained from Katz Biotech AG, Baruth, Germany,
Feeding:	1:3v/v solution of honey in water
Test design - Mortality phase	

Arenas:	Treated glass plates fitted to a square frame (10 cm x 10 cm external dimensions) made from metal casing (1.8 cm x 0.5 cm in cross-section). Three holes (10 mm in diameter) drilled through each of the side walls of the frame covered with fine-gauge, stainless steel mesh. One hole was left uncovered for the introduction of the parasitoids and was sealed with a cotton wool bung. The complete units were held together with elastic bands. Air was forced through the units to prevent a build-up of pesticide vapours and to maintain environmental conditions.
Replication:	4
No. of wasps/arena :	10 (minimum of 5 females)
Test design - Fecundity phase	
Arenas:	N/A
Replication:	N/A
No. of wasps/arena :	N/A
Duration of test:	Mortality assessment: 48 hours Fecundity assessment: not assessed
Environmental test conditions	
Temperature:	Mortality assessment phase: 20 - 21°C.
Humidity:	Mortality assessment phase: 71 - 77% RH.
Photoperiod:	Mortality assessment phase: 16 h photoperiod (810 lux).

Study Design and Methods

Experimental dates: 4th June 2013 to 6th June 2013

Treatments were applied to glass plates which, once dry were used to construct the arenas. The wasps were introduced to these arenas and their behaviour and mortality was assessed 2, 24 and 48 h later.
It was not possible to assess sub-lethal effects on reproduction due to high levels of mortality. The duration of the bioassay was 2 days.

Mortality was defined as the numbers of moribund and dead insects combined. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott's formula (Abbott, 1925).

The mortality in each treatment at 48 h was compared to that in the control using Fisher's Exact Test (Sokal & Rohlf, 1981; SPSS, 2012). It was the intention that the results of this analysis would be used to determine values for the 'lowest-observed-effect rate' (LOER) and the 'no-observed-effect rate' (NOER).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 10 Effects of fresh residues of A18385B + A12127R on mortality of Aphidius rhopalosiphi, when exposed under laboratory test conditions

Treatment* A18385B g/ha	**Mean % mortality at 48 h[a]**	**Mean % corrected mortality at 48 h [b]**
Control	12.5	-
62.5	95.0*	94.3
125	100*	100
250	100*	100

Treatment* A18385B g/ha	**Mean % mortality at 48 h[a]**	**Mean % corrected mortality at 48 h [b]**
500	100*	100
1000	100*	100
Toxic reference	100*	100

[a)] The results for the test-item and toxic-reference were compared to the control using Fisher's Exact Test ($\alpha = 0.05$). Significant differences are indicated by an asterisk (*).
[b)] Derived using Abbott's formula.

Validity criteria

The validity criteria for the control groups were met:
- Mean mortality in control ≤13% (observed: 12.5%)
- Mortality in toxic reference ≥50% at 48 hours (observed: 100%)

Conclusions

In a laboratory test to determine the effects of A18385B, applied in conjunction with the adjuvant A12127R, on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h LR_{50} (median lethal rate) was <62.5 g A18385B/ha, the minimum rate tested. In terms of effects on wasp survival, the lowest-observed-effect rate (LOER) was 62.5 g product/ha and the no-observed-effect rate (NOER) was not determined.

(Stevens J, 2013)

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

A 2.3.2.2.1 Typhlodromus pyri

The following study has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zonefor authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.3.2.2/01
Report	Fallowfield L, (2014), Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – A rate-response extended laboratory bioassay of the effects of fresh residues on the predatory mite Typhlodromus pyri (Acari: Phytoseiidae). Report Number SYN-13-53 Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, UK. (Syngenta file No. A18385B_10070).
Guideline(s):	Blümel *et al.* (2000). Laboratory residual contact test with the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. IOBC Publication. ISBN 92-9067-129-7.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

The 7-day LR_{50} for effects of A18385B (applied in conjunction with A12127R) on mortality of *Typhlodromus pyri* under extended laboratory test conditions was calculated to be 1412.5 g product/ha, with confidence limits of 796.2 and 3810.7 g product/ha.

For reproduction the median effect rate (ER_{50}) was estimated as being 1266.3 g product/ha.

The no observed effect rate (NOER), defined as the highest rate tested that did not produce a statistically significant adverse effect relative to the control, based on survival, was 500 g product/ha and the NOER for reproduction was 1000 g product/ha.

Materials

Test Material	Product: A18385B - Prosulfuron/dicamba/nicosulfuron WG (4/40/10) Adjuvant: A12127R - Adigor
Lot/Batch #:	Product: SMU2BP004 Adjuvant: UN121B0912
Actual content of product active ingredients:	prosulfuron: nominal: 4 % w/w; analysed: 4.32 % w/w dicamba: nominal: 40 % w/w; analysed: 41.0 % w/w nicosulfuron: nominal: 10 % w/w; analysed: 10.5 % w/w
Description:	Product: Brown granules Adjuvant: Clear yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	Product: End of September 2014 Adjuvant: End of October 2016
Density:	Adjuvant: 927 kg/m^3
Treatments	
Test rates:	Product: 2000, 1000, 500, 250, 125 and 62.5 g product/ha Adjuvant: 6000, 3000, 1500, 750, 375, 187.5 mL adjuvant/ha (included in spray mixture respectively)
Control:	Purified water
Toxic standard:	BAS 152 11I Perfekthion (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L) applied at a rate of 30 mL product per 200 L water/ ha (12 g a.i./ha)
Spray volume rate:	200 L spray solution/ha
Application method:	Laboratory track sprayer (3 bar pressure, 80° flat fan nozzle)
Test organisms	
Species:	*Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)
Age:	Less than 24 h old protonymphs
Source:	Culture maintained at Test Facility, originally obtained (April 1995) from P.K. Nützlingszuchten, Welzheim, Germany, supplemented with mites from same source in 1996 and 1997
Feeding:	1:1 v/v mixture of almond (*Prunus* sp. var Butte) and apple (*Malus* sp. var. Red Delicious) pollen
Test design	
Arenas:	Glass plates formed from two microscope slide cover slips with a narrow channel between them were mounted on damp tissue paper with an oblong ring of a sticky non-drying gel drawn onto the plates to create an arena in which the mites were confined. The arena was approximately 3 cm x 4 cm, enclosing an area of ca. 12 cm^2.
Replication:	3
No. of mites/arena :	20
Duration of test:	Mortality assessment: 0-7 days Fecundity assessment: 7-14 days

Environmental conditions test	
Temperature:	25 – 26 °C
Humidity:	72– 87 % RH
Photoperiod:	16 h photoperiod (950 – 1850 Lux)

Study Design and Methods

Experimental dates: 3 December 2013 to 10 February 2014

The test item in this study, prosulfuron/dicamba/nicosulfuron WG (4/40/10), hereafter referred to as A18385B, is a water-dispersible granule formulation nominally containing 4% w/w prosulfuron, 40% w/w dicamba and 10% w/w nicosulfuron. For this study, this test item was applied in conjunction with the adjuvant A12127R (Adigor), with the two being used at a fixed mixture ratio of 1:3 respectively, where results are described in terms of the test item this will always include the adjuvant at this ratio.

A definitive rate-response bioassay was then carried out to evaluate six application rates of the test item plus adjuvant, a water-treated control and a toxic reference treatment. The endpoints were an assessment of mite mortality at 7 DAT and an assessment of the reproduction of the surviving mites between 7 and 14 DAT. Treatments were applied to the glass plates and the bioassay initiated approximately 1 h later, once residues had dried. The glass plates were placed onto damp tissue paper and an oblong ring of a sticky non-drying gel drawn on the plates to create arenas in which mites were confined. The survival of the mites was assessed over a 7-day period, by which time they were adult. The sex of the adult mites was determined, and where necessary males were moved between replicates to ensure a male to female ratio of 1:5 in each treatment, they were then left in situ so that their reproduction could be assessed over a further 7 days. Any eggs produced prior to 7 DAT were removed and discarded. For 7 days, the total egg production (numbers of eggs plus live and dead juvenile stages) was recorded for each unit. Assessments of oviposition activities were carried out at 9, 11 and 14 DAT. Any eggs and nymphs present were recorded and then removed. In addition, the condition of the adult female and male mites in each arena was recorded on each date.

The numbers of any stuck, drowned or missing mites were added to the number of dead mites found in each treatment to derive the overall "mortality". The percentage mortality at each treatment rate was corrected for mortality in the control treatment using Abbott's formula (Abbott, 1925). A Probit regression analysis (Finney, 1952) of the results was performed. The 95% confidence intervals for the LR_{50} value were calculated and a Chi-square goodness of fit test ($\alpha = 0.05$) performed on the Probit line. In order to determine the 'no-observed effect rate' (NOER) for mortality, the percentage mortality in each treatment was compared to the control using Fisher's Exact Test ($\alpha = 0.05$) (Sokal & Rohlf, 1981; SPSS, 2012).

The data for mite reproduction was analysed by one-way ANOVA and Dunnett's test ($\alpha = 0.05$). The effect of treatments on mite fecundity relative to the control was calculated using the formula:

$$\% \text{ change} = [1-(R_t/R_c)] * 100$$

where R_t and R_c are the absolute values observed in the treatment and control groups respectively.

Results and Discussion

Mortality and fecundity are summarised in the table below. All values were calculated using the original raw data and were not based on rounded values.

Table A 11 Effects of A18385B on mortality and fecundity of *Typhlodromus pyri*, when exposed under extended laboratory test conditions

Treatment (g A18385B/ha)	Mean % mortality at 7 DAT[a)]	Mean corrected % mortality at 7 DAT[b)]	Mean eggs/female from 7 to 14 DAT [c)]	% Effect on reproduction compared to control[d)]
Control	13	-	6.7	-
2000	58 *	52	2.6 *	61.6
1000	60 *	54	5.1	23.6
500	23	12	5.9	12.3
250	15	2	5.5	18.0
125	13	0	5.6	17.2
62.5	12	0	4.6	31.3
Toxic reference	77 *	73	-	-

a) Results for mortality in individual treatments at 7 DAT were compared to that in the control by Fisher's Exact Test ($\alpha = 0.05$). Treatment means that differed significantly from the control are indicated with an asterisk (*).
b) Calculated using Abbott's formula
c) Results for reproduction over the assessment period were compared by one-way ANOVA and Dunnett's test ($\alpha = 0.05$). Treatment means that differed significantly from the control are indicated with an asterisk (*).
d) Egg production, relative to the control. A positive value indicates a decrease.
n.d. = not determined

Validity Criteria

The validity criteria for the test were met:

- mortality in the control treatment over the initial 7 days should not exceed 20%
- mortality in the toxic reference treatment should be 50-100%
- the mean cumulative number of eggs produced from 7 to 14 days should be ≥ 4.0 per female in the control treatment

Conclusions

In an extended laboratory test in which the predatory mite *Typhlodromus pyri* was exposed to fresh dry residues of A18385B (applied in conjunction with the adjuvant A12127R), the 7-day LR_{50} was 1412.5 g A18385B/ha, with confidence limits of 796.2 and 3810.7 g A18385B/ha. Based on statistical comparisons with the control, the NOER for mortality was 500 g A18385B/ha.

The median effect rate (ER_{50}) was calculated to be 1266.3 g A18385B/ha, with 95% confidence limits of 601.5 and 14759.8 g A18385B/ha. Based on statistical comparisons with the control, the NOER for reproduction was 1000 g A18385B/ha.

(Fallowfield L, 2014)

A 2.3.2.2.2 Aphidius rhopalosiphi

The following study has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zonefor authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.3.2.2/02
Report	Stevens J, (2013a), Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus

	Adigor (A12127R) – A rate-response extended laboratory bioassay of the effects of fresh residues on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera, Braconidae), Report Number SYN-13-52. Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. A18385B_10034).
Guideline(s):	Mead-Briggs *et al.* (2009). An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae). BioControl (DOI 10.2007/S10526-009-9260-7). Published online 5 December 2009. Springer
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

In an extended laboratory test to determine the effects of A18385B, applied in conjunction with the adjuvant A12127R, on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h median lethal rate (LR_{50}) was >1000 g product/ha, the highest rate tested. Based on statistical comparison with the control, the NOER (no-observed-effects rate) for mortality was 1000 g product/ha.

In terms of effects on the reproductive performance of surviving wasps, the median effect rate (ER_{50}) for A18385B, applied in conjunction with the adjuvant A12127R, was >1000 g product/ha, the highest rate tested. Based on statistical comparison with the control, the NOER for reproduction was 1000 g product/ha.

Materials

Test Material	A18385B prosulfuron/dicamba/nicosulfuron WG (4/40/10)
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	September 2014
Density:	NA
Treatments	
Test rates:	1000, 500, 250, 125, 62.5 and 31.25 g A18385B/ha. A12127R was therefore included in the spray mixture and applied at rates equivalent to 3000, 1500, 750, 375, 187.5 and 93.75 mL adjuvant/ha.
Control:	Purified water
Toxic standard:	Perfekthion BAS 152 11 I (nominally 400 g dimethoate/L, analysed 400.9 g dimethoate/L), applied at a rate of 10 mL product/ha in 400 L water/ha
Spray volume rate:	400 L spray solution/ha
Application method:	Schachtner track sprayer (3 bar pressure, 80° flat fan nozzle)
Test organisms	
Species:	*Aphidius rhopalosiphi* De Stefani-Perez. (Hymenoptera: Braconidae)
Age:	<48 hours
Source:	Culture maintained at Test Facility on cereal aphids (*Metopolophium dirhodum* and *Rhopalosiphum padi*). Originally obtained from Katz Biotech AG, Baruth, Germany.
Feeding:	1:3 v/v solution of honey and water
Test design – Mortality phase	

Arenas:	Clear acrylic cylinders (8 cm diameter, 20 cm high, tops covered with nylon netting) were placed over pots containing approximately 10 sprayed barley seedlings (*Hordeum vulgare* Westminster)
Replication:	6
No. of wasps/arena :	5
Test design - Fecundity phase	
Arenas:	Clear acrylic cylinders (9 cm diameter, 20 cm high, tops covered with nylon netting) were placed over pots containing 15 barley seedlings (*Hordeum vulgare* Westminster). The untreated barley had been infested eight days previously with host aphids (>100 adults and nymphs of *Metopolopium dirhodum* and *Rhopalosiphum padi*).
Replication:	15 female wasps/treatment
No. of wasps/arena :	1
Duration of test:	Mortality assessment: 48 hours Fecundity assessment: 24 hours Observation of mummies developing: 10 days after adult removal
Environmental test conditions	
Temperature:	Mortality assessment phase: 21°C Fecundity assessment phase: 20°C – 21°C
Humidity:	Mortality assessment phase: 69% - 75% RH
Photoperiod:	Mortality assessment phase: 16 h photoperiod (1884 lux) Fecundity assessment phase: 16 h photoperiod (4811 lux)

Study Design and Methods

Experimental dates: 29th October 2013 to 25th November 2013

Treatments were applied to test plants (seedlings of barley - *Hordeum vulgare* var. Westminster) which, once dry, were used to construct arenas. The wasps were introduced to these arenas and their behaviour and mortality were assessed 2, 24 and 48 h later.

To assess any sub-lethal effects, reproduction assessments were then carried out using surviving females from the control and from the three highest treatment rates of the test item that had resulted in ≤60% corrected mortality (1000, 500 and 250 g A18385B/ha). Wasps were confined individually over untreated aphid-infested barley plants for 24 hours, before being removed. The plants were left for a further 10 days before the number of aphid mummies that had developed on plants where wasps had been found alive after the 24-h oviposition period was recorded.

The percentage mortality, defined as the number of moribund and dead insects combined, was calculated over 48 hours. The corrected percentage mortality (taking into account any control treatment losses) was derived using Abbott's (1925) formula. Probit regression analysis proved to be unsuitable. Where there was treatment mortality at 48 hours, this was compared to the control using Fisher's Exact Test ($\alpha = 0.05$).

The numbers of mummies produced per female found alive after the 24-h parasitism period were analysed by one-way ANOVA ($\alpha = 0.05$) of the square root-transformed data. The percentage change in numbers of mummies produced in individual test item treatments, relative to the control, was also calculated using the equation:

$$(1-R_t/R_c)*100\%$$

where R_t and R_c are the absolute values for reproduction observed in the treatment and control groups, respectively.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 12 Effects of fresh residues of A18385B on mortality and fecundity of *Aphidius rhopalosiphi*, when exposed under extended laboratory test conditions

Treatment (g A18385B/ha)	Mean % mortality at 48 h[a]	Mean % corrected mortality at 48 h (M-value)[b]	Number females successfully assessed for reproductive capacity	Mean number mummies per surviving female[c]	% Effect on reproduction compared to control (R-value)[d]
Control	0.0	-	13	13.9	-
1000	6.7	6.7	11	12.0	13.8
500	3.3	3.3	13	13.2	5.5
250	3.3	3.3	14	12.2	12.3
125	0.0	0.0	n.d.	n.d.	n.d.
62.5	0.0	0.0	n.d.	n.d.	n.d.
31.25	0.0	0.0	n.d.	n.d.	n.d.
Toxic reference	83.3*	83.3	n.d.	n.d.	n.d.

[a] The results for the individual treatments were compared to the control using Fisher's Exact Test (α=0.05). Significant differences are indicated by an asterisk (*).
[b] Derived using Abbott's formula.
[c] The results for the test item treatments were compared to the control by one-way ANOVA (α=0.05), but the results did not differ significantly.
[d] Percentage effect on reproduction, relative to the control. A positive value indicates a decrease relative to the control.
n.d. Not determined

Validity criteria

The validity criteria for the control groups were met:

- Mean mortality in control ≤17% (observed 0.0%)
- Mortality in toxic reference ≤25% at 2 hours (observed: 0%), ≥50% at 48 hours (observed 83.3%)
- Mean number of mummies per female in the control ≥5.0 with no more than two zero values (observed 13.9, no zero values)

Conclusions

In an extended laboratory test to determine the effects of A18385B, applied in conjunction with the adjuvant A12127R, on the parasitic wasp *Aphidius rhopalosiphi*, the 48-h median lethal rate (LR_{50}) was >1000 g product/ha, the highest rate tested. Based on statistical comparison with the control, the NOER (no-observed-effects rate) for mortality was 1000 g product/ha.

In terms of effects on the reproductive performance of surviving wasps, the median effect rate (ER_{50}) for A18385B, applied in conjunction with the adjuvant A12127R, was >1000 g product/ha, the highest rate tested. Based on statistical comparison with the control, the NOER for reproduction was 1000 g product/ha.

(Stevens J, 2013)

A 2.3.2.2.3 Aleochara bilineata

The following study has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated in the Central zone for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.3.2.2/03
Report	Tew, G. 2014, Prosulfuron/dicamba/nicosulfuron WG (A18385B) plus Adigor (A12127R) – A rate-response extended laboratory bioassay of the effects of fresh residues on the rove beetle *Aleochara bilineata* (Coleoptera; Staphylinidae). Report Number SYN-13-54, Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. A18385B_10072).
Guideline(s):	Grimm *et al.* (2000). A test for evaluating the chronic effects of plant protection products on the rove beetle *Aleochara bilineata* Gyll. (Coleoptera: Staphylinidae) under laboratory and extended laboratory test conditions.
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

In an extended laboratory test where adults of the rove beetle *Aleochara bilineata* were exposed to fresh residues of A18385B (with adjuvant A12127R) on a natural sandy loam soil, there were no significant effects on the parasitism success of the beetles at application rates up to and including 1000 g product/ha, the maximum tested. In terms of the reproductive capacity of the beetles, the median effect rate (ER_{50}) was determined as > 1000 g product/ha and, the no-observed-effect rate (NOER) was 1000 g product/ha.

Materials

Test Material	Product: A18385B - Prosulfuron/dicamba/nicosulfuron WG (4/40/10) Adjuvant: A12127R - Adigor
Lot/Batch #:	Product: SMU2BP004 Adjuvant: UN121B0912
Actual content of active ingredients:	Prosulfuron: nominal: 4% w/w analysed: 4.32 % w/w dicamba: nominal: 40 % w/w analysed: 41.0 % w/w nicosulfuron: nominal: 10% w/w analysed: 10.5 % w/w
Description:	Product: Brown granules Adjuvant: Clear yellow liquid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	Product: end of September 2014 Adjuvant: end of September 2016
Density:	Adjuvant: 927 kg/m^3
Treatments	
Test rates:	Product: 1000, 500, 250, 125, 62.5 g A18385B/ha Adjuvant: 3000, 1500, 750, 375, 187.5 mL A12127R/ha (included in spray mixture respectively)
Control:	Purified water
Toxic standard:	Cyren (Headland Agrochemicals Ltd.) (chlorpyrifos, nominally 480 g/L) applied at a rate equivalent to 192 g a.i./ha
Application method:	Schachtner track sprayer (3 bar pressure, 80° flat fan nozzle)
Test organisms	
Species:	*Aleochara bilineata*
Age:	5 to 6 day old adults
Source:	Parasitised pupae of the onion fly, *Delia antiqua* Meig. (Diptera: Anthomyiidae) were obtained from a commercial supplier, De Groene

	Vlieg, Nieuwe Tonge, The Netherlands.
Food:	Raw minced beef, fed approximately one hour after test start and then every 2 to 3 days throughout test
Host pupae for larvae to parasitize:	500 onion fly *Delia antiqua* (MEIG.) pupae obtained from De Groene Vlieg, Nieuwe Tonge, The Netherlands were incorporated to the soil on days 7, 14 and 21
Test design - Mortality phase	
Arenas:	Polystyrene boxes (17.1 cm x 11.3 cm x 6 cm high) with tightly fitting lids with 4 holes covered with nylon netting (0.5 x 0.5 mm mesh). Each box was filled with approximately 910 g of sandy soil to a depth of at least 4 cm.
Substrate:	Sandy soil type LUFA 2.1 Organic carbon content: 0.65 ± 0.10% pH: 5.1 ± 0.3 WHC: maintained at 35 ± 5 %
Replication:	4
No. of beetles/arena :	20 (10 male + 10 female)
Test design – Fecundity phase	
Arenas:	The soil from the mortality phase test vessels was transferred to two sizes of plastic pot placed one inside the other, measuring 9 cm diameter x 5 cm deep, and 9 cm diameter x 9 cm deep. Fine mesh (0.5 x 0.5 mm) nylon netting covered a hole in the lids of the smaller pots, and a coarser mesh (*ca*. 2.0 x 2.5 mm) covered a large hole in the base, allowing the emerging adults to fall through into the larger pot beneath.
Replication:	4
Duration of test:	73 days. Mortality phase: 0 – 28 days after treatment (DAT). Fecundity phase: 35 - 73 DAT.
Environmental test conditions	
Temperature:	Mortality phase: 18.7 – 21.1 °C Fecundity phase: 19.6 – 20.8 °C
Humidity:	Mortality phase: 66 - 72 % RH Fecundity phase: 56 – 74 % RH
Photoperiod:	16 h photoperiod, 800 - 900 lux

Study Design and Methods

Experimental dates: 31 October 2013 to 27 January 2014

Treatments were applied to the test arenas and the adult beetles were introduced. At days 7, 14 and 21 during exposure, approximately 500 *Delia antiqua* pupae were incorporated beneath the soil. After 28 days all surviving adult beetles were removed from the substrate. The substrate containing the parasitized onion fly pupae was left to dry for one week. Thirty-five days after application the pupae were separated from the soil using a coarse sieve (*ca*. 1.5 mm mesh) and the pupae of each replicate were transferred to separate emergence pots and stored in a controlled environment room. Emerging beetles were counted and removed from the emergence containers every 2 – 3 days; emergence of the F_1 generation was monitored until the control treatment fell below a rate of two beetles per replicate per day (73 DAT).

Percentage mortalities were calculated, both before and after correction for control treatment losses using Abbott's formula.

The mean number of offspring produced per beetle and a measure of standard deviation was calculated for each treatment. The percentage effect on reproductive performance in the treated groups, compared to the

control group, was calculated using the following equation:

% effect = (1 – (Rt/Rc)) * 100

Where Rt and Rc are the numbers of offspring observed in the treatment and control groups, respectively.

The numbers of progeny per replicate in the test item and control treatments were analysed by one-way analysis of variance (ANOVA). Since none of the treatments resulted in > 50% effect on reproduction, the data was not deemed suitable for Probit analysis (Finney, 1952).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 13 Effects of A18385B on survival and reproduction of *Aleochara bilineata*

Treatment Rate (g A18385B/ha)	**% Mortality at 28 days[1]**	**Corrected % mortality at 28 days[2]**	**Mean number of F_1 progeny (per replicate)[3]**	**% Effect on reproduction (R value)[4]**
Control	20.0	-	1041.3	-
1000	25.0	6.0	996.8	4.3
500	15.0	0.0	1074.3	-3.2
250	15.0	0.0	1125.0	-8.0
125	23.8	5.0	1092.8	-4.9
62.5	13.8	0.0	1038.8	0.2
Toxic reference	100.0	100.0 *	0.0	100.0

1 The mortality in individual treatments was compared to that in the control using Fisher's Exact Test (α = 0.05). An asterisk (*) indicates a significant increase in mortality relative to the control.
2 Corrected percentage mortality calculated using Abbott's formula (Abbott, 1925).
3 The numbers of progeny per replicate in the test-item and control treatments were analysed by one-way ANOVA (α = 0.05). There were no significant differences. The toxic reference treatment was not included in this analysis, due to a lack of variance within the data.
4 The percentage change in numbers of F1 progeny, relative to the control was calculated using the formula: R = (1-(Rt/Rc)) x 100, where Rt and Rc are the numbers of offspring observed in the treatment and control groups, respectively. A positive value indicates a decrease relative to the control, negative values an increase.

Validity Criteria

The mean number of beetles emerging from fly pupae in the control should be > 400 per replicate (nominally 27% of those provided). The mean number of beetles emerging in the toxic reference treatment should be reduced by >50%, relative to the control. Both these criteria were met.

Conclusions

In an extended laboratory test where adults of the rove beetle *Aleochara bilineata* were exposed to fresh residues of A18385B (applied in conjunction with A12127R) on a natural sandy loam soil, there were no significant effects on the parasitisation success of the beetles at application rates up to and including 1000 g product/ha, the maximum tested. In terms of the reproductive capacity of the beetles, the median effect rate (ER_{50}) was determined as > 1000 g product/ha and, the no-observed-effect rate (NOER) was 1000 g product/ha.

(Tew G, 2014)

A 2.3.2.2.4 Chrysoperla carnea

The following study has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but is submitted at zonal level for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.3.2.2/04
Report	Vaughan, G (2014),Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – A rate-response extended laboratory test to evaluate the effects of fresh residues on the green lacewing *Chrysoperla carnea* (Neuroptera, Chrysopidae). Report Number SYN-13-55, Mambo-Tox Ltd., 2 Venture Road, Chilworth Science Park, Southampton SO16 7NP, United Kingdom. (Syngenta file No. A18385B_10081).
Guideline(s):	Vogt *et al.* (2000). Laboratory method to test effects of plant protection products on larvae of *Chrysoperla carnea* (Neuroptera: Chrysopidae).
Deviations:	No.
GLP:	Yes.
Acceptability:	Yes/No/Supplementary

Executive Summary

The effects of A18385B, applied in conjunction with the adjuvant A12127R (Adigor) at a ratio of 1:3, on the green lacewing, *Chrysoperla carnea*, were evaluated under extended laboratory test conditions. No effects >50% on either mortality or reproduction were observed after exposure of larvae to fresh dry residues at application rates up to and including 1000 g A18385B/ha. It was therefore concluded that the median lethal rate (LR_{50}) and the ER_{50} based on effects on reproduction were higher than 1000 g A18385B/ha. The no-observed-effects rate (NOER) was 125 g A18385B/ha.

Materials

Test material	Product: A18385B - Prosulfuron/dicamba/nicosulfuron WG (4/40/10) Adjuvant: A12127R - Adigor
Lot/Batch #:	Product: SMU2BP004 Adjuvant: UN121B0912
Actual content of active ingredients:	Prosulfuron: nominal: 4% w/w analysed: 4.32 % w/w dicamba: nominal: 40 % w/w analysed: 41.0 % w/w nicosulfuron: nominal: 10% w/w analysed: 10.5 % w/w
Description:	Product: Brown granules Adjuvant: Clear yellow liquid
Stability of test compound:	Stable under test conditions.
Reanalysis/expiry date:	Product: end of September 2014 Adjuvant: end of September 2016
Density:	Adjuvant: 927 kg/m^3
Ratio of formulation/surfactant:	1:3 (w:v)
Treatments	
Test concentrations:	1000, 500, 250, 125 and 62.5 g A18385B/ha
Control:	Purified water
Spray volume rate:	200 L spray solution/ha
Toxic standard:	Perfekthion EC (nominally 400 g/L dimethoate) in purified water, applied at a rate of 80 mL product/ha.
Test organisms	

Species:	*Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae).
Source:	Culture maintained at Test Facility
Food:	Larvae: UV-killed eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver) (Lepidoptera, Gelechiidae) provided 2 – 3 times per week. Adults: Artificial diet, water and honey:water (1:3)
Age:	2-3 days old
Test design – Mortality phase	
Test vessels:	Larvae confined over treated excised leaves of dwarf French bean (*Phaseolus vulgaris* var. Montana) using Fluon-coated cylindrical collars.
Replication:	40
No. of organisms per arena:	1
Test design – Fecundity phase	
Test vessels:	Expanded-polystyrene boxes, the ventilated lids of which were lined with a removable fibrous sheet on which the adult lacewings laid their eggs
Replication:	Insects from the control were grouped in two boxes while those from each treatment rate were grouped in a single box
Environmental conditions	
Temperature:	24.2 – 26.6ºC
Humidity:	65-76% RH
Photoperiod:	16 h photoperiod (2300-4400 lux).
Duration of test:	30 days

Study Design and Methods

Experimental dates: 19th February 2014 – 26th March 2014

Larvae of the lacewing *Chrysoperla carnea* (2-3 days old) were exposed to fresh product residues of A18385B (plus adjuvant 'Adigor') on treated foliage.

A18385B was evaluated at five application rates, equivalent to1000, 500, 250, 125 and 62.5 g A18385B/ha. The adjuvant was applied with the test item at a constant ratio of 1:3. These were compared to a water-treated control and a toxic reference treatment of BASF Perfekthion (nominally 400 g/L dimethoate), applied at a rate of 80 mL/ha (nominally 32 g a.i./ha).

Treatments were applied to excised French bean leaves (*Phaseolus vulgaris* L.) and, once residues had dried, the leaves were used to line the floor of test arenas (n = 40 per treatment) into which individual larvae of *C. carnea* (2-3 days old) were introduced. The larvae were fed with untreated eggs of the Angoumois grain moth, *Sitotroga cerealella* (Oliver), and any pre-imaginal mortality of the lacewings was recorded. A check was then made for sub-lethal effects on the reproductive performance of the adult lacewings in the highest three treatment rates of the test item and the control. For this, the egg-laying activity of grouped females was monitored for two 24-h periods and the subsequent viability of the eggs was determined.

The bioassay lasted a total of 36 days, with pre-imaginal mortality assessed up to 23 days after treatment and reproduction/egg viability being assessed from 28-36 days after treatment.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 14 Effects of fresh residues of A18385B on mortality and fecundity of *Chrysoperla carnea*, when exposed under extended laboratory test conditions.

Treatment Rate (g A18385B/ha)	% pre-imaginal mortality[1]	Corrected % mortality [2]	Mean number eggs/female/ day[3]	Mean percentage egg viability[4]	Mean viable eggs/female/ day	% Effects on reproduction [5]
Control	7.5	-	42.7	92.3	39.4	-
1000	35.0*	29.7	31.7	92.5	29.3	25.6
500	30.0*	24.3	25.8	93.7	24.2	38.6
250	32.5*	27.0	31.8	93.4	29.7	24.6
125	22.5	16.2	-	-	-	-
62.5	22.5	16.2	-	-	-	-
Toxic reference	100.0*	100.0	-	-	-	-

[1] Pre-imaginal mortality in individual treatments was compared to that in the control using Fisher's Exact Test ($\alpha = 0.05$). An asterisk indicates where differences were significant (*).
[2] Corrected percentage mortality calculated using Abbott's formula (Abbott, 1925).
[3] Based on two 24-h long assessments made for the oviposition box in each treatment
[4] Based on all eggs laid on the fibrous tissue sheet lining the lid of the oviposition box
[5] Percentage change in mean number of viable eggs per female, relative to control. A positive value indicates a decrease relative to the control, whilst a negative value indicates an increase.

For the test to be deemed valid, the protocol indicated that pre-imaginal mortality should have been ≤ 20% in the control treatment. Also, mean egg production in the control should have been ≥ 15 eggs per female per day and mean viability of the eggs should have been ≥ 70%. In addition, mortality should have been ≥ 50% in the toxic reference treatment. These criteria were met.

Conclusions

In an extended laboratory test in which the foliar-active predator *Chrysoperla carnea* was exposed to freshly-dried foliar residues of A18385B, applied in conjunction with the adjuvant A12127R, the LR_{50} (median lethal rate) was found to be > 1000 g product/ha, the maximum tested. Based on statistical comparison with the control, the NOER for mortality was 125 g product/ha. The reproductive performance of the surviving lacewings was not significantly affected by treatment rates up to and including 1000 g product/ha and this rate was considered to be the NOER for reproduction. The ER_{50} (median effect rate) for A18385B was considered to be > 1000 g product/ha, the maximum tested.

(Vaughan, 2014)

A 2.3.2.3 KCP 10.3.2.3 Semi-field studies with non-target arthropods

A 2.3.2.4 KCP 10.3.2.4 Field studies with non-target arthropods

A 2.3.2.5 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

The following sub-lethal earthworm studies with prosulfuron metabolites CGA349707, CGA159902, SYN542604, CGA325025 and CGA300406 are new, and have not been previously evaluated at EU peer review for prosulfuron.

Comments of zRMS:	The study was conducted to OECD guideline 222 and according to the principles of GLP. In the definitive test all the validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4.1.1/01
Report	Friedrich S. (2015): CGA300406 - Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil with 5 % Peat Report No 15 10 48 138 S BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No CGA300406_10018
Guideline(s):	OECD Guideline No 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*) (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Executive Summary

In a chronic toxicity test in which earthworms (Eisenia fetida) were exposed to CGA349707 the NOEC (based on mortality, reproduction and biomass) was determined to be 100 mg CGA349707/kg soil dry weight.

The LC_{50}, and the EC_{50} (based on reproduction), could not be calculated, but it can be concluded that they are greater than 100 mg CGA349707/kg soil dry weight, the highest concentration tested.

Materials

Test Material	CGA349707 CSAA433641
Parent:	Prosulfuron (CGA152005)
Lot/Batch #:	KGL 4933/6 R 3
Purity:	99 ± 2% w/w
Description:	White solid
Stability of test compound:	Stable when stored <10°C
Reanalysis/Expiry date:	30 June 2015
Density:	Not applicable
Treatments	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg CGA349707/kg soil dry weight (not corrected for purity)
Control:	Untreated control (prepared with quartz sand only)
Toxic standard:	Nutdazim 50 FLOW (Carbendazim, SC 500) was tested at concentrations of 5 and 10 mg product/kg soil dry weight (separate study - No.: R 11 10 48 005 S, dated 02 September 2011)
Test organisms	
Species:	Eisenia fetida (Savigny, 1826) [subspecies Eisenia andrei (Bouché, 1972)]

Age and weight range at test start:	Adult worms, approximately 3 months old with clitellum; 252 – 450 mg/worm
Source:	Reared in the test facility (original breeding animals purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Feeding:	Air-dried and finely ground horse manure
Test design	
Vessels:	Plastic (Bellaplast) vessels (inside dimensions: 16.5 × 12 × 6 cm) with a lid pervious to air and light
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.7% industrial quartz sand (>50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate. 750 g wet weight soil, corresponding to 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	8 for control, 4 for treatment
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	19.2 to 21.5°C
pH of soil*:	Test start: 5.95 to 6.07 Test end: 5.70 to 5.86
Water content of soil*:	Test start: 24.9 to 25.1% (equivalent to 56.6 to 57.0% of water holding capacity) Test end: 24.2 to 24.9% (equivalent to 55.0 to 56.6% of water holding capacity)
Photoperiod:	16 hours light:8 hours dark, 570 lux

*pooled replicates per treatment group

Study Design and Methods

Experimental dates: 12th July 2012 to 6th September 2012

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60% of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. On the day of the test start, the test item was mixed with a small quantity of finely ground quartz sand (10 g per vessel), such that the required test concentrations were achieved once mixed with the artificial soil. The acclimatised test animals were weighed and randomly placed onto the test substrate (10 animals per test vessel). After approximately 30 minutes the test vessels were covered with perforated transparent lids.

One day after application, 5 g dried and ground horse manure was scattered on the soil surface of each test vessel. This was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 15 Effect of CGA349707 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA349707/kg soil dry weight)								
	Control	**1.63**	**2.94**	**5.29**	**9.53**	**17.1**	**30.9**	**55.6**	**100**
Mean adult mortality at 28 days (%)	1.3	0.0	5.0	5.0	0.0	2.5	0.0	0.0	2.5
Mean % biomass change of adults from 0-28 days	38.3	33.2	37.3	35.8	39.1	37.0	34.3	36.7	39.3
Mean number of juveniles after 8 weeks	81.9	79.8	84.8	80.3	86.3	81.0	76.5	79.0	77.5
Coefficient of variation for reproduction (cv %)	20.4	17.1	21.9	37.0	23.8	32.3	13.1	24.4	22.8
% difference in reproduction relative to the control	-	2.6	-3.5	2.0	-5.3	1.1	6.6	3.5	5.3
LC_{50}	>100 mg CGA349707/kg soil dw								
EC_{50} (reproduction)	>100 mg CGA349707/kg soil dw								
NOEC (mortality, reproduction and biomass)	100 mg CGA349707/kg soil dw								

No statistically significant differences between the control and test item were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction, p >0.05, one-sided greater), biomass and reproduction (Williams-t-test, p >0.05, one-sided smaller).
dw: dry weight (of artificial soil)
Negative values = increase, relative to control

Validity criteria

Validity criteria for the control groups were met:

- Adult mortality after 4 weeks: ≤10% (being 1.3)
- Number of juveniles per replicate: ≥30 (being ≥ 54)
- Coefficient of variation for reproduction: ≤30% (being 20.4%)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA349707 the NOEC (based on mortality, reproduction and biomass) was determined to be 100 mg CGA349707/kg soil dry weight.

The LC_{50}, and the EC_{50} (based on reproduction), could not be calculated, but it can be concluded that they are greater than 100 mg CGA349707/kg soil dry weight, the highest concentration tested.

(Friedrich S, 2012a)

Reference:	KCP 10.4.1.1/02
Report	Friedrich S, (2012): CGA349707 – Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil with 5% Peat Report No 12 10 48 068 S

	BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No CGA349707_10001
Guideline(s):	OECD Guideline for testing of chemicals No 222 (adopted 13 April 2004): Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*).
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

In a chronic toxicity test in which earthworms (Eisenia fetida) were exposed to CGA159902 the NOEC based on reproduction was determined to be 17.1 mg CGA159902/kg soil dry weight. The EC_{50} based on reproduction was determined to be 71.2 mg CGA159902/kg soil dry weight.

Materials

Test Material	CGA159902 CA1118
Lot/Batch #:	AMS 597/101
Purity:	99.8%
Description:	White crystals
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg CGA159902/kg soil dw
Control:	Untreated substrate, amended with quartz sand only and irrigated with deionised water
Toxic standard:	Nutdazim 50 FLOW (Carbendazim SC 500) was tested at concentrations of 5 and 10 mg product/kg soil dry weight (separate study - No.: R11 10 48 005 S dated 02 September 2011).
Test organisms	
Species:	Eisenia fetida (Savigny, 1826) [subspecies Eisenia andrei (Bouché, 1972)]
Age and weight range at test start:	Adult worms, approximately 3 months old with clitellum; 283 – 448 mg/worm
Source:	Reared in the test facility (original breeding animals purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Feeding:	Air-dried and finely ground horse manure
Test design	
Vessels:	Plastic vessels (16.5 × 12 × 6 cm) with a lid pervious to air and light.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.7% industrial quartz sand (>50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate. 750 g wet weight soil, corresponding to 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	8 for control, 4 for treatment
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18.0 – 20.8°C
pH of soil*:	Test start: 6.06 – 6.10

	Test end: 5.68 – 5.79
Water content of soil*:	Test start: 24.9 to 25.1% (equivalent to 56.6% to 57.0% of water holding capacity) Test end: 24.2 to 24.8% (equivalent to 55.0 to 56.4% of water holding capacity)
Photoperiod:	16 hours light:8 hours dark, 630 Lux

Study Design and Methods

Experimental dates: 24th July 2012 to 18th September 2012

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60% of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate (mixed with horse manure) for approximately 24 hours before test start. The test concentrations were prepared by mixing the test item with a small quantity of finely ground quartz sand (10 g per vessel), such that the required test concentrations were achieved once mixed with the artificial soil. The treated soil was then added to the test vessels and the acclimatised test animals were weighed and randomly placed onto the test substrate (10 animals per test vessel). After approximately 30 minutes the test vessels were covered with perforated transparent lids.

One day after application, 5 g dried and ground horse manure was scattered on the soil surface of each test vessel. This was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Behavioural and pathological symptoms were observed weekly.

The EC_{50} (number of juveniles) were calculated by Probit analysis using the maximum likelihood method, and the corresponding 95% confidence limits were computed by normal approximation. Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 16 Effect of CGA159902 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	**Treatment groups (mg CGA159902/kg soil dry weight)**								
	Control	**1.63**	**2.94**	**5.29**	**9.53**	**17.1**	**30.9**	**55.6**	**100**

Mean adult mortality at 28 days (%)	1.3	0.0	2.5	5.0	0.0	0.0	5.0	2.5	10.0
Mean % biomass change of adults from 0-28 days	35.4	33.7	40.5	33.6	35.0	32.5	27.7	20.1*	15.1*
Mean number of juveniles after 8 weeks	80.4	83.5	76.5	84.8	76.0	72.3	60.0*	48.0*	30.5*
Coefficient of variation for reproduction (cv %)	20.4	8.1	33.6	29.3	28.8	18.2	29.6	18.5	38.1
% difference in reproduction relative to the control	-	-3.9	4.8	-5.4	5.4	10.1	25.3	40.3	62.1
EC_{50} (reproduction)	71.2 mg CGA159902/kg soil dw (95% confidence limits 64 – 79 mg CGA159902/kg soil dw)								
NOEC(mortality)	100 mg CGA159902/kg soil dw								
NOEC (biomass)	30.9 mg CGA159902/kg soil dw								
NOEC (reproduction)	17.1 mg CGA159902/kg soil dw								

Not statistically significant differences between the control and test item were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction, p ≤0.05, one-sided greater)
* statistically significantly different compared to control (Williams-t-test, p ≤0.05, one-sided smaller)
dw: dry weight (of artificial soil)
Negative values = increase, relative to control

Validity criteria

Validity criteria for the control groups were met:

- Adult mortality after 4 weeks: ≤10% (being 1.3%)
- Number of juveniles per replicate: ≥30 (being ≥ 61)
- Coefficient of variation for reproduction: ≤30% (being 20.4%)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA159902 the NOEC based on reproduction was determined to be 17.1 mg CGA159902/kg soil dry weight. The EC_{50} based on reproduction was determined to be 71.2 mg CGA159902/kg soil dry weight.

(Friedrich S, 2012b)

Reference:	KCP 10.4.1.1/03
Report	Friedrich, S. (2012a): CGA159902 - Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil with 5% Peat Report No 12 10 48 066 S BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No CGA159902_10003

Guideline(s):	OECD Guideline for testing of chemicals No 222 (adopted 13 April 2004): Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*).
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

In a chronic toxicity test in which earthworms (Eisenia fetida) were exposed to CGA325025 the NOEC (based on mortality, reproduction and biomass) was determined to be 100 mg CGA325025/kg soil dry weight. The LC_{50}, and the EC_{50} (based on reproduction), could not be calculated, but it can be concluded that they are greater than 100 mg CGA325025/kg soil dry weight, the highest concentration tested.

Materials

Test Material	CGA325025 CSAA406448
Parent:	Prosulfuron (CGA152005)
Lot/Batch #:	MES 240/1
Purity:	95 ± 2% w/w
Description:	Beige solid
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 April 2013
Density:	Not applicable
Treatments	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6, 100 mg CGA325025/kg soil dry weight (not corrected for purity)
Control:	Untreated control (prepared with quartz sand only)
Toxic standard:	Nutdazim 50 FLOW (Carbendazim, SC 500) was tested at concentrations of 5 and 10 mg product/kg soil dry weight (separate study - No.: R 11 10 48 005 S, dated 02 September 2011)
Test organisms	
Species:	Eisenia fetida (Savigny, 1826) [subspecies Eisenia andrei (Bouché, 1972)]
Age and weight range at test start:	Adult worms, approximately 4 months old with clitellum; 352 – 534 mg/worm
Source:	Reared in the test facility (original breeding animals purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Feeding:	Air-dried and finely ground horse manure
Test design	
Vessels:	Plastic (Bellaplast) vessels (inside dimensions: 16.5 × 12 × 6 cm) with a lid pervious to air and light
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.7% industrial quartz sand (>50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate. 750 g wet weight soil, corresponding to 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	8 for control, 4 for treatment
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	19.2 to 21.4°C
pH of soil*:	Test start: 5.91 to 5.98 Test end: 5.58 to 5.68

Water content of soil*: Test start: 24.9 to 25.0% (equivalent to 56.6 to 56.8% of water holding capacity)
Test end: 24.3 to 25.0% (equivalent to 55.2 to 56.8% of water holding capacity)

Photoperiod: 16 hours light:8 hours dark, 580 lux

**pooled replicates per treatment group*

Study Design and Methods

Experimental dates: 18th July 2012 to 12th September 2012

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60% of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. On the day of the test start, the test item was mixed with a small quantity of finely ground quartz sand (10 g per vessel), such that the required test concentrations were achieved once mixed with the artificial soil. The acclimatised test animals were weighed and randomly placed onto the test substrate (10 animals per test vessel). After approximately 30 minutes the test vessels were covered with perforated transparent lids.

One day after application, 5 g dried and ground horse manure was scattered on the soil surface of each test vessel. This was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test.

After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.

Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 17 Effect of CGA325025 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg CGA325025/kg soil dry weight)								
	Control	1.63	2.94	5.29	9.53	17.1	30.9	55.6	100

Mean adult mortality at 28 days (%)	2.5	0.0	7.5	0.0	2.5	2.5	2.5	5.0	0.0
Mean % biomass change of adults from 0-28 days	28.9	28.8	33.1	29.9	28.1	33.2	26.8	25.3	28.5
Mean number of juveniles after 8 weeks	101.6	100.0	97.3	112.8	105.8	89.0	84.3	93.3	92.8
Coefficient of variation for reproduction (cv %)	19.3	30.5	29.6	21.5	21.7	26.7	21.2	20.5	27.4
% difference in reproduction relative to the control	-	1.6	4.3	-10.9	-4.1	12.4	17.1	8.2	8.7
LC_{50}	>100 mg CGA325025/kg soil dw								
EC_{50} (reproduction)	>100 mg CGA325025/kg soil dw								
NOEC (mortality, reproduction and biomass)	100 mg CGA325025/kg soil dw								

No statistically significant differences between the control and test item were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction, p >0.05, one-sided greater), biomass and reproduction (Williams-t-test, p >0.05, one-sided smaller).
dw: dry weight (of artificial soil)
Negative values = increase, relative to control

Validity criteria

Validity criteria for the control groups were met:

- Adult mortality after 4 weeks: ≤10% (being 2.5%)
- Number of juveniles per replicate: ≥30 (being ≥ 74)
- Coefficient of variation for reproduction: ≤30% (being 19.3%)

Conclusions

In a chronic toxicity test in which earthworms (Eisenia fetida) were exposed to CGA325025 the NOEC (based on mortality, reproduction and biomass) was determined to be 100 mg CGA325025/kg soil dry weight. The LC_{50}, and the EC_{50} (based on reproduction), could not be calculated, but it can be concluded that they are greater than 100 mg CGA325025/kg soil dry weight, the highest concentration tested.

(Friedrich S, 2012a)

Reference:	KCP 10.4.1.1/04
Report	Friedrich S. (2012b): SYN542604 - Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil with 5% Peat Report No 12 10 48 070 S BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No SYN542604_10007

Guideline(s):	OECD Guideline No 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*) (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to SYN542604 the NOEC was determined to be 100 mg SYN542604/kg soil dry weight. Since no concentration response was observed, the EC_{50} could not be calculated, but it can be concluded that the EC_{50} > 100 mg SYN542604/kg soil dry weight, this being the highest concentration tested.

Materials

Test Material	SYN542604 CSAC271531
Parent:	CGA152005
Lot/Batch #:	MES 148/1
Purity:	93 % w/w ± 2 %
Description:	Beige solid
Stability of test compound:	Stable when stored < 10°C
Reanalysis/Expiry date:	31 August 2014
Treatments	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg SYN542604/kg soil d.w.
Control:	Untreated substrate, amended with quartz sand only
Toxic standard:	Nutdazim 50 FLOW (carbendazim, SC 500) was tested at concentrations of 5 and 10 mg product/kg soil dry weight (separate study - No.: R11 10 48 005 S, dated 02 September 2011).
Test organisms	
Species:	Eisenia fetida (Savigny, 1826) [subspecies Eisenia andrei (Bouché, 1972)]
Age and weight range at test start:	Adult worms, approximately 3 months old with clitellum; 306 – 495 mg/worm
Source:	Reared in the test facility (original breeding animals were purchased from W. Neudorff GmbH KG. An der Mühle 3, 31860 Emmerthal, Germany
Feeding:	Air-dried and finely ground horse manure
Test design	
Vessels:	Plastic vessel of a Bellaplast (internal dimensions ~16.5 × 12 × 6 cm) with a lid pervious to air and light.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolin clay, 74.7 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate. 750 g wet weight soil, corresponding to 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	4 per treatment group, 8 per control
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18.0 – 21.1 ºC

pH of soil*:	Test start; 6.04 – 6.16
	Test end; 5.81 – 5.91
Water content of soil*:	Test start; 24.9 – 25.0 % (equivalent to 56.6 – 56.8 % of WHC)
	Test end; 24.4 – 25.0 % (equivalent to 55.5 – 56.8 % of WHC)
Photoperiod:	16 hours light : 8 hours dark
	620 Lux

Study Design and Methods

Experimental dates: 03 August 2012 to 28 September 2012
Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60 % of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. The test concentrations were prepared by mixing an amount of the test material with a small quantity of quartz sand (10 g per vessel) such that the required concentrations were achieved once mixed with the artificial soil. The control substrate contained 10 g of quartz sand only. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate (10 animals per test vessel). Worms which had not dug themselves in after 30 minutes were replaced.
One day after application, 5 g dried and ground horse manure was scattered on the soil surface of each test vessel. This was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test.
After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined, behaviour and pathological symptoms were also recorded. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded, and the water content and the pH of the artificial soil determined. Observations of behavioural and pathological symptoms were observed weekly.
The arithmetic mean and the standard deviation per treatment and per control for reproduction and biomass were calculated. Fisher's Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality, growth and fecundity are summarised in the table below.

Table A 18: Effect of SYN542604 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	**Treatment groups (mg SYN542604/kg soil dry weight)**								
	Control	**1.63**	**2.94**	**5.29**	**9.53**	**17.1**	**30.9**	**55.6**	**100**
Mean adult mortality at 28 days (%)	3.8	0.0	2.5	0.0	0.0	2.5	7.5	2.5	7.5
Mean % biomass change of adults from 0-28 days	38.7	37.0	40.8	36.1	32.5	41.3	37.9	39.8	35.4
Mean number of juveniles after 8 weeks	95.4	91.3	92.3	87.3	94.3	104.0	83.0	88.8	88.0
Coefficient of variation for reproduction (cv %)	20.9	23.3	20.9	14.7	17.5	22.1	27.4	27.2	19.0

% difference in reproduction relative to the control	-	4.3	3.3	8.5	1.2	-9.0	13.0	6.9	7.7
LC_{50}	> 100 mg SYN542604/kg								
NOEC (mortality)	100 mg SYN542604/kg								
NOEC (biomass)	100 mg SYN542604/kg								
NOEC (reproduction)	100 mg SYN542604/kg								
EC_{50}	> 100 mg SYN542604/kg[a]								

No statistically significant differences between the control and test item were calculated for mortality (Fisher's Exact Binomial Test with Bonferroni Correction, $p > 0.05$, one-sided greater), biomass and reproduction (Williams-t-test, $p > 0.05$, one-sided smaller)
Negative (-) values = increase, relative to the control
[a]Based on reproduction

Validity Criteria

Validity criteria for the control group were met:

- Adult mortality after 4 weeks: ≤ 10% (being 3.8% after 4 weeks)
- Number of juveniles per replicate: ≥ 30 (being ≥ 65)
- Coefficient of variation for reproduction: ≤ 30% (being 20.9%)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to SYN542604 the NOEC based on reproduction was determined to be 100 mg SYN542604/kg soil dry weight. Since no concentration response was observed, the EC_{50} based on reproduction could not be calculated, but it can be concluded that the EC_{50} > 100 mg SYN542604/kg soil dry weight, this being the highest concentration tested.

(Friedrich S, 2012b)

Reference:	KCP 10.4.1.1/05
Report	Friedrich, S. (2012c): CGA325025 – Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil with 5% Peat Report No 12 10 48 064 S BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No CGA325025_10003
Guideline(s):	OECD Guideline for testing of chemicals No 222 (adopted 13 April 2004): Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*).
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA300406 the NOEC (based on reproduction) was determined to be 95 mg/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be 102, 160 and 383 mg/kg soil d.w., respectively.

Materials

Test Material	CGA300406
Lot/Batch #:	MES 235/3
Purity:	90 % w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of January 2017
Density:	n/a
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556, 1000 mg/kg soil dry weight
Control:	Untreated
Toxic standard:	Nutdazim 50 FLOW (carbendazim, SC 500)
Test organisms	
Species:	*Eisenia fetida* (subspecies *Eisenia fetida andrei*)
Age and weight range at test start:	adult worms (approximately 3 months old with clitellum) 296 – 496 mg/worm
Source:	Maintained at test facility, originally: W. Neudorff GmbH KG An der Mühle 3, 31860 Emmerthal, Germany
Feeding:	air-dried and finely ground horse manure
Test design	
Vessels:	Plastic trays (inside dimensions: about 16.5 cm x 12 cm x 6 cm) with a lid pervious to air and light.
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate. 750 g wet weight soil, corresponding to about 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	Control: 8 Treated: 4
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	20.2 – 22.0 °C
pH of soil:	test start: 6.07 – 6.12 test end: 5.71 – 5.96
Water content of soil:	test start: 24.9 – 25.0 (equivalent to 59.7 – 60.0 % of WHC) test end: 24.1 – 24.6 (equivalent to 57.8 – 59.0 % of WHC)
Photoperiod:	16 hour light (570 lux)

Study Design and Methods

Experimental dates: 29 July to 23 September 2015

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60 % of its maximum water holding capacity (WHC). The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. The test concentrations were prepared by dispersing an exactly weighed amount of the test material in deionised water to make a stock solution. This stock solution was diluted with deionised water for each concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60 % of WHC. The acclimatised test animals were washed, gently dried on a paper towel, weighed and randomly placed onto the test substrate

(10 animals per test vessel).
After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were observed weekly.
The endpoints were mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (the number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated.

The statistical analysis was performed with the software ToxRat Professional 3.1.0 (2015). The EC_{10}, EC_{20} and EC_{50} values (number of juveniles) were estimated by Probit analysis using linear max. likelihood regression. Confidence limits (95 %) of the ECx values were computed by normal approximation. For identifying the NOEC values the Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and reproduction are summarised in the table below.

Table A 19 Effect of CGA300406 on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	Treatment groups (mg/kg soil dry weight)								
	Control	**16**	**29**	**53**	**95**	**171**	**309**	**556**	**1000**
Mean adult mortality at 28 days (%)	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5
Mean % biomass change of adults from 0-28 days	24.9	25.5	27.4	25.8	23.5	28.1	23.9	25.3	19.9
Mean number of juveniles after 8 weeks	133.4	142.5	140.3	127.8	138.8	100.0*	75.0*	44.5*	30.3*
Coefficient of variation for reproduction (cv %)	18.0	14.8	6.2	11.9	9.8	17.2	23.0	40.1	37.7
% difference in reproduction relative to the control	-	-6.8	-5.2	4.2	-4.0	25.0	43.8	66.6	77.3
NOEC (mortality)	1000								
NOEC (biomass)	1000								
NOEC (reproduction)	95								
LC_{50}	>1000								
EC_{10}	102 (95 % confidence limits 71 to 146)								
EC_{20}	160 (95 % confidence limits 123 to 209)								
EC_{50}	383 (95 % confidence limits 323 to 453)								

* statistically significant compared to control (Williams-t-test, $p \leq 0.05$, one-sided smaller)
Negative % values for change of reproduction = increase, relative to control

Validity criteria

The test is considered valid as:

- Adult mortality was 1.3 % in the control (< 10% required)
- The mean number of juveniles per control replicate was 133.4 (> 30 required)
- The coefficient of variation for reproduction was 18.0 % (< 30% required)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to CGA300406 the NOEC (based on reproduction) was determined to be 95 mg/kg soil d.w. The EC_{10}, EC_{20} and EC_{50} values for reproduction were estimated to be 102, 160 and 383 mg/kg soil d.w., respectively.

(Friedrich S, 2012c)

The following chronic study on earthworms with A18385B has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated at zonal level for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.4.1.1/06
Report	Friedrich S, (2012d), Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – Sublethal Toxicity to the Earthworm *Eisenia fetida* in Artificial Soil, Report Number 12 10 48 115 S. BioChem agrar Labor für biologische und chemische Analytik GmbH, Kupferstraße 6 04827 Gerichshain, Germany. (Syngenta file No. A18385B_10000).
Guideline(s):	OECD Guideline for testing of chemicals No. 222 (adopted 13 April 2004): Earthworm Reproduction Test (*Eisenia fetida*/*Eisenia andrei*).
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to A18385B plus adjuvant Adigor A12127R, the NOEC was determined to be 100 mg A18385B/kg soil dry weight for mortality and biomass, and 50 mg A18385B/kg soil dry weight for reproduction. The EC_{50} (reproduction) could not be calculated, but was concluded to be >100 mg A18385B/kg soil dry weight, the highest concentration tested.

Materials

Test Material	A18385B Prosulfuron/Dicamba/Nicosulfuron WG (4/40/10)
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions

Reanalysis/Expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test rates:	0.78, 1.56, 3.15, 6.25, 12.5, 25, 50 and 100 mg A18385B/kg soil dw
Control:	Untreated substrate irrigated with deionised water
Toxic standard:	Nutdazim 50 FLOW (Carbendazim SC 500) was tested at concentrations of 5 and 10 mg product/kg soil dry weight (separate study - No.: R12 10 48 004 S dated 29 October 2012)
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methyl ester). A18385B and A12127R were mixed at a ratio of 1:3 (A18385B:A12127R). Results are expressed in terms of this ratio.
Test organisms	
Species:	*Eisenia fetida* (Savigny, 1826) [subspecies *Eisenia andrei* (Bouché, 1972)]
Age and weight range at test start:	Adult worms (approximately 3 months old with clitellum); 279 – 420 mg/worm
Source:	Reared in the test facility (original breeding animals purchased from W. Neudorff GmbH KG, An der Mühle 3, 31860 Emmerthal, Germany)
Feeding:	Air-dried, finely ground horse manure
Test design	
Vessels:	Plastic (Bellaplast) vessel (inner dimensions: 16.5 × 12 × 6 cm) with a lid pervious to air and light
Substrate:	Artificial soil comprising 10% sphagnum peat, 20% kaolin clay, 69.5% industrial quartz sand (>50% of the particles between 0.05 mm and 0.2 mm) and 0.5% calcium carbonate. 810 g wet weight soil, corresponding to 600 g dry weight, of artificial soil was added to each test vessel.
Replication:	8 for control, 4 for treatment group
No. of worms/arena :	10
Duration of test:	8 weeks
Environmental test conditions	
Temperature:	18.0 – 21.2°C
pH of soil*:	Test start: 6.17 – 6.24 Test end: 5.73 – 6.02
Water content of soil*:	Test start: 34.9 – 35.0% (equivalent to 55.4 – 55.6% of WHC) Test end: 34.2 – 34.8% (equivalent to 54.3 – 55.2% of WHC)
Photoperiod:	16 hours light:8 hours dark, 510 Lux

* pooled replicates per treatment groups.

Study Design and Methods

Experimental dates: 20th September 2012 to 15th November 2012

Approximately 24 hours prior to test start, the artificial soil was prepared and deionised water was added to the dry components to obtain approximately 50% of the final water content. The worms were acclimatised in a separate batch of the untreated artificial substrate for approximately 24 hours before test start. On the day of test start, the test item (A18385B + A12127R) was introduced by dispersing the quantity of the test item required to obtain the desired test concentration in the volume of water required to hydrate the soil to 40-60% of its WHC. The treated substrate was thoroughly mixed using a laboratory mixer immediately after application. The acclimatised test animals were weighed and randomly placed onto the test substrate (10 animals per test vessel). After approximately 30 minutes the test vessels were covered with perforated transparent lids.
One day after application, 5 g dried and ground horse manure was scattered on the soil surface of each test vessel. This was sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test.
After four weeks, the adult worms were removed from the test vessels, and mortality and the body weight of the surviving worms were determined. After all of the adult worms had been removed, the soil in each vessel was mixed with 5 g horse manure. Four weeks later, the number of surviving juveniles and any morphological alterations were recorded. Observations of behavioural and pathological symptoms were

observed weekly.

The arithmetic mean and the standard deviation per treatment and per control for mortality, biomass and reproduction were calculated. Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 20 Effect of A18385B + A12127R on mortality, growth and reproduction of *Eisenia fetida*

Endpoints	**Treatment groups (mg A18385B/kg soil dry weight)**								
	Control	**0.78**	**1.56**	**3.15**	**6.25**	**12.5**	**25**	**50**	**100**
Mean adult mortality at 28 days (%)	1.3	5.0	0.0	5.0	0.0	0.0	2.5	0.0	7.5
Mean % biomass change of adults from 0-28 days	36.1	33.3	39.1	38.8	35.8	38.3	34.1	35.5	33.7
Mean number of juveniles after 8 weeks	114.5	117.8	110.0	109.8	115.0	118.8	108.3	102.5	81.8*
Coefficient of variation for reproduction (cv %)	18.7	24.9	15.9	14.8	16.8	11.1	17.7	13.9	19.3
% difference in reproduction relative to the control	-	-2.8	3.9	4.1	-0.4	-3.7	5.5	10.5	28.6
LC_{50}	>100 mg A18385B/kg soil dw								
EC_{50} (reproduction)	>100 mg A18385B/kg soil dw								
NOEC (mortality and biomass)	100 mg A18385B/kg soil dw								
NOEC (reproduction)	50 mg A18385B/kg soil dw								

* Statistically significant compared to control (Williams-t-test, p ≤0.05, one-sided smaller)
Negative values = increase, relative to control
No statistically significant differences between control and test item were calculated for mortality or change in biomass (Fisher's Exact Binomial Test with Bonferroni Correction, p >0.05, one-sided greater, and Williams-t-test, p >0.05, one-sided smaller, respectively)
dw: dry weight (of artificial soil)

Validity criteria

Validity criteria for the control groups were met:

- Adult mortality after 4 weeks: ≤10% (being 1.3)
- Number of juveniles per replicate: ≥30 (being ≥ 114.5)
- Coefficient of variation for reproduction: ≤30% (being 18.7%)

Conclusions

In a chronic toxicity test in which earthworms (*Eisenia fetida*) were exposed to A18385B plus adjuvant

Adigor A12127R, the NOEC was determined to be 100 mg A18385B/kg soil dry weight for mortality and biomass, and 50 mg A18385B/kg soil dry weight for reproduction. The EC_{50} (reproduction) could not be calculated, but was concluded to be >100 mg A18385B/kg soil dry weight, the highest concentration tested.

(Friedrich S, 2012d)

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

The following studies on *Folsomia candida* with prosulfuron metabolites CGA349707, CGA159902, SYN542604, CGA325025 and CGA300406 are new, and have not been previously evaluated at EU peer review for prosulfuron.

Reference:	KCP 10.4.2/01
Report	Friedrich S. (2012e): CGA349707 – Effects on the Reproduction of the Collembolans *Folsomia candida.* Report No 12 10 48 067 S BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No CGA349707_10002
Guideline(s):	OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 232 (adopted 7 September 2009): Collembolan reproduction test in soil. ISO 11267 (1999): Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Standard, First edition 1999-04-01.
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

The toxicity of CGA349707 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for both parental mortality and reproduction was determined to be 100 mg CGA349707/kg soils dry weight. Neither the LC_{50} nor the EC_{50} (based on reproduction) could be calculated, but it can be concluded that they are >100 mg CGA349707/kg soil dw, the highest concentration tested.

Materials

Test Material	CGA349707 CSAA433641

Lot/Batch #:	KGL 4933/6 R 3
Parent:	CGA152005
Purity:	99% w/w (estimated error: ± 2%)
Description:	White solid
Stability of test compound:	Stable when stored at <10°C
Reanalysis/Expiry date:	30 June 2015
Density:	Not applicable
Treatments	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6, and 100 mg CGA349707/kg soil dw (factor: 1.8, not corrected for purity)
Control:	Untreated substrate, amended with quartz sand only
Toxic standard:	Boric acid at rates of 44, 67, 100, 150 and 225 mg/kg soil dry weight (Separate study – BioChem project No: R 12 10 48 003 S, dated 24 May 2012).
Application method:	Mixing solution with finely ground quartz sand before introduction of collembolans.
Test organisms	
Species:	*Folsomia candida* (Willem)
Age:	Juvenile collembolans (9 – 12 days)
Source:	Culture maintained at Test Facility. Originally purchased from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem in May 2000
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	Glass container (approximately 150 mL) covered with a glass lid
Replication:	Treated groups 4 (+ 2 replicates not loaded with collembolans for measurement purposes) Control group 8 (+ 2 replicates not loaded with collembolans for measurement purposes)
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.2 – 21.4°C
pH:	Test initiation: 5.99 – 6.04 Test completion: 5.73 – 5.76
Photoperiod:	Light : dark 16 h : 8 h (light intensity 670 lux)

Study Design and Methods

Experimental dates: 6th August 2012 to 3rd September 2012

Two days before test start, the dry artificial soil was moistened by adding deionised water to adjust the water content to 40-60% of WHC. On the day of the test start, the test item was mixed with a small quantity of finely ground quartz sand (10 g treated sand per treatment group), such that the required test concentration was achieved once mixed with the artificial soil. The control substrate contained the corresponding amount of untreated quartz sand only.

Ten juvenile collembolans, were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight replicates (+ two replicates not loaded with collembolans for measurement purposes) were used for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The glass lids covering the test vessels were briefly opened twice a week for aeration. The water content was checked weekly by reweighing the two additional test vessels. Water loss was compensated in all vessels if exceeding 2% of the initial water content. The temperature was 19.2 – 21.4°C, the pH was 5.73 – 6.04, the water content of the artificial soil was 55.5 – 56.8% of WHC and there was a 16 hour light : 8

hour dark photoperiod (670 lux).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 21 Effects of residues of CGA349707 on mortality and reproduction of *Folsomia candida*

Endpoints	Treatment groups (mg CGA349707/kg soil dw)								
	Control	**1.63**	**2.94**	**5.29**	**9.53**	**17.1**	**30.9**	**55.6**	**100**
% Mortality of parental collembolans after 4 weeks	6.3	7.5	7.5	2.5	5.0	5.0	0.0	2.5	0.0
% corrected mortality (Abbott)	-	1	1	-4	-1	-1	-7	-4	-7
Mean number of juveniles after 4 weeks	1147	1138	1091	1105	1187	1093	1074	1120	1179
SD	115.7	100.9	167.0	70.7	226.3	173.8	113.3	131.0	222.5
CV %	10.1	8.9	15.3	6.4	19.1	15.9	10.5	11.7	18.9
% reduction compared to control	-	1	5	4	-4	5	6	2	-3
NOEC (mortality)	**100 mg CGA349707/kg soil dw**								
NOEC (reproduction)	**100 mg CGA349707/kg soil dw**								
EC_{50}/LC_{50}	**>100 mg CGA349707/kg soil dw**								

Not statistically significant compared to the control regarding mortality (Fisher's Exact Binomial Test with Bonferroni Correction, p >0.05, one-sided greater) and reproduction (Williams-t-test, p >0.05, one-sided smaller)
Abbott's formula for corrected mortality (Abbott, 1925): M (%) = ((A-B)/A) * 100%, where
A = mean number of surviving parental collembolans in the control group, and
B = mean number of surviving parental collembolans in the treated groups
Percent reduction: (1-Rt/Rc) * 100%, where Rt = mean number of juveniles observed in the treated groups, and Rc = mean number of juveniles observed in the control group
dw = dry weight
Negative values = increase, relative to control

Validity criteria

The validity criteria for the control group were met:

- Mean adult mortality: ≤20% (observed: 6.3%)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 1147/vessel)
- Coefficient of variation for the mean number of juveniles: ≤30% (observed: 10.1%)

The requirement of the ISO guideline concerning the precision of the counting method (average error <10%) was fulfilled, the determined overall error of counting amounted to 4.4%.

Conclusions

The NOEC for both parental mortality and reproduction was determined to be 100 mg CGA349707/kg soils dry weight. Neither the LC_{50} nor the EC_{50} (based on reproduction) could be calculated, but it can be

concluded that they are >100 mg CGA349707/kg soil dw, the highest concentration tested.

Friedrich S. (2012e)

Reference:	KCP 10.4.2/02
Report	Friedrich S. (2012f): CGA159902 – Effects on the Reproduction of the Collembolans *Folsomia candida* Report No 12 10 48 065 S BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No CGA159902_10002
Guideline(s):	OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 232 (adopted 7 September 2009): Collembolan reproduction test in soil. ISO 11267 (1999): Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Standard, First edition 1999-04-01.
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

The toxicity of CGA159902 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for parental mortality was determined to be 55.6 mg CGA159902/kg soil dw and for reproduction was determined to be 30.9 mg CGA159902/kg soil dw. The LC_{50} could not be calculated but it can be concluded that it is greater than 100 mg CGA159902/kg soil dw, the highest concentration tested. The EC_{50} (based on reproduction) was determined to be 69.0 mg CGA159902/kg soil dw, with corresponding 95% confidence limits of 60.8 – 78.2 mg CGA159902/kg soil dw.

Materials

Test Material	CGA159902 CA1118
Lot/Batch #:	AMS 597/101
Purity:	99.8% w/w
Description:	White crystals
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg CGA159902/kg soil dw
Control:	Untreated quartz sand only
Toxic standard:	Boric acid at rates of 44, 67, 100, 150 and 225 mg/kg soil dry weight (separate study – BioChem project No: R 12 10 48 003 S, dated 24 May 2012)
Application method:	Mixtures of CGA159902 with quartz sand were added to pre-moistened artificial soil prior to introduction of collembolans

Test organisms	
Species:	Collembolans *Folsomia candida* (Willem)
Age:	Juvenile collembolans (9-12 days)
Source:	Culture maintained at Test Facility. Originally purchased from Biologische Bundesanstalt (BBA), Berlin-Dahlem in May 2000
Feeding:	2 mg granulated dry yeast per replicate at the start of the test and after 14 days
Test design	
Arenas:	Glass container (approximately 150 mL) covered with a glass lid
Replication:	Treated groups 4 (+ 2 replicates not loaded with collembolans for measurement purposes) Control group 8 (+ 2 replicates not loaded with collembolans for measurement purposes)
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.3 – 21.5°C
pH:	Test start: 5.92 – 5.96 Test end: 5.58 – 5.68
Photoperiod:	Light : dark 16 h : 8 h (light intensity 670 Lux)

Study Design and Methods

Experimental dates: 12th July 2012 to 9th August 2012

Two days prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60% of its maximum water holding capacity (WHC). The test concentrations were prepared immediately prior to application by mixing an exactly weighed amount of the test material in quartz sand (10g treated sand per treatment group) such that the required test concentrations were achieved once mixed with the artificial soil. The control consisted of untreated quartz sand only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight replicates (+ two replicates not loaded with collembolans for measurement purposes) for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The glass lids covering the test vessels were briefly opened twice a week for aeration. The water content was checked weekly by reweighing the two additional test vessels. Water loss was compensated in all vessels if exceeding 2% of the initial water content. The temperature was 19.3 – 21.5°C, the pH was 5.58 – 5.96, the water content of the artificial soil was 55.5 – 57.0% of WHC and there was a 16 hour light : 8 hour dark photoperiod (670 Lux).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 22 Effects of residues of CGA159902 on mortality and reproduction of *Folsomia candida*

Endpoints	Treatment groups (mg CGA159902/kg soil dw)								
	Control	**1.63**	**2.94**	**5.29**	**9.53**	**17.1**	**30.9**	**55.6**	**100**

% Mortality of parental collembolans after 4 weeks	10.0	5.0	12.5	5.0	12.5	10.0	10.0	20.0	42.5*[1]
% corrected mortality (Abbott)	-	-6	3	-6	3	0	0	11	36
Mean number of juveniles after 4 weeks	516	486	509	527	497	518	539	310*[2]	144*[2]
SD	73.7	31.6	48.8	57.1	40.5	59.9	74.8	53.9	11.6
CV %	14.3	6.5	9.6	10.8	8.2	11.6	13.9	17.4	8.1
% reduction compared to control	-	6	1	-2	4	-1	-5	40	72
NOEC (mortality)	**55.6 mg CGA159902/kg soil dw**								
NOEC (reproduction)	**30.9 mg CGA159902/kg soil dw**								
LC_{50}	**>100 mg CGA159902/kg soil dw**								
EC_{50} (reproduction)	**69.0 mg CGA159902/kg soil dw** (95% confidence limits 60.8 to 78.2 mg CGA159902/kg soil dw)								

* Statistically significantly different compared to the control ([1]Fisher's Exact Binomial Test with Bonferroni Correction ($p \leq 0.05$, one-sided greater), [2]Williams-t-test ($p \leq 0.05$, one-sided smaller))
Abbott's formula for corrected mortality (Abbott, 1925): M (%) = ((A-B)/A) * 100%, where
A = mean number of surviving parental collembolans in the control group, and
B = mean number of surviving parental collembolans in the treated groups
dw = dry weight
Percent reduction: (1-Rt/Rc) * 100%, where
Rt = mean number of juveniles observed in the treated groups, and
Rc = mean number of juveniles observed in the control group
Negative values = increase, relative to control

Validity criteria

The validity criteria for the control group were met:

- Mean adult mortality: ≤20% (observed: 10.0%)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 516/vessel)
- Coefficient of variation for the mean number of juveniles: ≤30% (observed: 14.3%)

The requirement of the ISO guideline concerning the precision of the counting method (average error <10%) was fulfilled, the determined overall error of counting amounted to 4.4%.

Conclusions

The toxicity of CGA159902 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for parental mortality was determined to be 55.6 mg CGA159902/kg soil dw and for reproduction was determined to be 30.9 mg CGA159902/kg soil dw. The LC_{50} could not be calculated but it can be concluded that it is greater than 100 mg CGA159902/kg soil dw, the highest concentration tested. The EC_{50} (based on reproduction) was determined to be 69.0 mg CGA159902/kg soil dw, with corresponding 95% confidence limits of 60.8 – 78.2 mg CGA159902/kg soil dw.

Friedrich S. (2012f)

Reference:	KCP 10.4.2/03
Report	Friedrich S. (2012g): SYN542604 – Effects on the Reproduction of the Collembolans *Folsomia candida* Report No 12 10 48 069 S BioChem agrar Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No. SYN542604_10006
Guideline(s):	OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 232 (adopted 7 September 2009): Collembolan reproduction test in soil. ISO 11267 (1999): Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Standard, First edition 1999-04-01.
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

The toxicity of SYN542604 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for both parental mortality and reproduction was determined to be 100 mg SYN542604/kg soil dw. The LC_{50}, and the EC_{50} (based on reproduction), could not be calculated but it can be concluded that they are greater than 100 mg SYN542604/kg soil dw, the highest concentration tested.

Materials

Test Material	SYN542604 CSAC271531
Parent:	CGA152005
Lot/Batch #:	MES 148/1
Purity:	93% w/w (estimated error ± 2%)
Description:	Beige solid
Stability of test compound:	Stable when stored at <10°C
Reanalysis/Expiry date:	31 August 2014
Density:	Not applicable
Treatments	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg SYN542604/kg soil dw
Control:	Untreated substrate, amended with quartz sand only
Toxic standard:	Boric acid at rates of 44, 67, 100, 150 and 225 mg/kg soil dry weight (separate study – BioChem project No: R 12 10 48 003 S, dated 24 May 2012)
Application method:	Weighed amounts of SYN542604 mixed with quartz sand and then mixed into pre-moistened artificial soil prior to introduction of collembolans
Test organisms	
Species:	Collembolans *Folsomia candida* (Willem)
Age:	Juvenile collembolans (9-12 days)
Source:	Culture maintained at Test Facility. Originally purchased from Biologische Bundesanstalt (BBA), Berlin-Dahlem in May 2000
Feeding:	2 mg granulated dry yeast per replicate at the start of the test and after 14 days

Test design	
Arenas:	Glass container (approximately 150 mL) covered with a glass lid
Replication:	Treated groups 4 (+ 2 replicates not loaded with collembolans for measurement purposes) Control group 8 (+ 2 replicates not loaded with collembolans for measurement purposes)
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	19.2 – 21.3°C
pH:	Test initiation: 6.17 – 6.21 Test completion: 5.81 – 5.92
Photoperiod:	Light : dark 16 h : 8 h (light intensity 710 Lux)

Study Design and Methods

Experimental dates: 9th August 2012 to 6th September 2012

Two days prior to test start, the artificial soil was prepared and deionised water was added to the dry components to adjust the water content to approximately 40-60% of its maximum water holding capacity (WHC). The test concentrations were prepared immediately prior to application by mixing an exactly weighed amount of the test material in quartz sand (10 g treated sand per treatment group) such that the required test concentrations were achieved once mixed with the artificial soil. The control consisted of untreated quartz sand only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight replicates (+ two replicates not loaded with collembolans for measurement purposes) for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The glass lids covering the test vessels were briefly opened twice a week for aeration. The water content was checked weekly by reweighing the two additional test vessels. Water loss was compensated in all vessels if exceeding 2% of the initial water content. The temperature was 19.2 – 21.3°C, the pH was 5.81 – 6.21, the water content of the artificial soil was 55.2 – 57.0% of WHC and there was a 16 hour light : 8 hour dark photoperiod (710 Lux).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 23 Effects of residues of SYN542604 on mortality and reproduction of *Folsomia candida*

Endpoints	Treatment groups (mg SYN542604/kg soil dw)								
	Control	**1.63**	**2.94**	**5.29**	**9.53**	**17.1**	**30.9**	**55.6**	**100**
% Mortality of parental collembolans after 4 weeks	2.5	0	2.5	5.0	0	2.5	2.5	0	0

% corrected mortality (Abbott)	-	-3	0	3	-3	0	0	-3	-3
Mean number of juveniles after 4 weeks	775	762	764	812	801	798	744	762	767
SD	83.3	96.6	131.6	128.1	62.9	94.1	70.4	182.6	122.4
CV %	10.7	12.7	17.2	15.8	7.9	11.8	9.5	24.0	16.0
% reduction compared to control	-	2	1	-5	-3	-3	4	2	1
NOEC (mortality and reproduction)	**>100 mg SYN542604/kg soil dw**								
LC_{50}	**>100 mg SYN542604/kg soil dw**								
EC_{50} (reproduction)	**>100 mg SYN542604/kg soil dw**								

Not statistically significant compared to the control ([1]Fisher's Exact Binomial Test with Bonferroni Correction, (p >0.05, one-sided greater); [2]Williams-t-test, (p >0.05, one-sided smaller))
Abbott's formula for corrected mortality (Abbott, 1925): M (%) = ((A-B)/A) * 100%, where
A = mean number of surviving parental collembolans in the control group, and
B = mean number of surviving parental collembolans in the treated groups
dw = dry weight
Percent reduction: (1-Rt/Rc) * 100%, where
Rt = mean number of juveniles observed in the treated groups, and
Rc = mean number of juveniles observed in the control group
Negative values = increase, relative to control

Validity criteria

The validity criteria for the control group were met:

- Mean adult mortality: ≤20% (observed: 2.5%)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 775/vessel)
- Coefficient of variation for the mean number of juveniles: ≤30% (observed: 10.7%)

The requirement of the ISO guideline concerning the precision of the counting method (average error <10%) was fulfilled, the determined overall error of counting amounted to 2.5%.

Conclusions

The NOEC for both parental mortality and reproduction was determined to be 100 mg SYN542604/kg soil dw The LC_{50}, and the EC_{50} (based on reproduction), could not be calculated but it can be concluded that they are greater than 100 mg SYN542604/kg soil dw, the highest concentration tested.

Friedrich S. (2012g)

Reference:	KCP 10.4.2/04
Report	Friedrich S. (2012h): CGA325025 – Effects on the Reproduction of the Collembolans *Folsomia candida* Report No 12 10 48 063 S BioChem agrar, Labor für biologische und chemische Analytik GmbH Kupferstraße 6, 04827 Gerichshain, Germany

	Syngenta file No. CGA325025_10002
Guideline(s):	OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 232 (adopted 7 September 2009): Collembolan reproduction test in soil. ISO 11267 (1999): Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants. International Standard, First edition 1999-04-01.
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

The toxicity of CGA325025 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC for both parental mortality and reproduction was determined to be 100 mg CGA325025/kg soil dry weight. Neither the LC_{50} nor the EC_{50} (based on reproduction) could be calculated, but it can be concluded that they are >100 mg CGA325025/kg soil dw, the highest concentration tested.

Materials

Test Material	CGA325025 CSAA406448
Lot/Batch #:	MES 240/1
Parent:	Prosulfuron (CGA152005)
Purity:	95% (estimated error: ± 2%)
Description:	Beige solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	30 April 2013
Density:	Not applicable
Treatments	
Test rates:	1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg CGA325025/kg soil dw (factor 1.8, not corrected for purity)
Control:	Untreated substrate, amended with quartz sand only
Toxic standard:	Boric acid at rates of 44, 67, 100, 150 and 225 mg/kg soil dry weight (Separate study – BioChem project No: R 12 10 48 003 S, dated 24 May 2012).
Application method:	Mixing solution with finely ground quartz sand before introduction of collembolans.
Test organisms	
Species:	*Folsomia candida* (Willem)
Age:	Juvenile collembolans (9 – 12 days)
Source:	Culture maintained at Test Facility. Originally purchased from "Biologische Bundesanstalt (BBA)", Berlin-Dahlem in May 2000
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	Glass container (approximately 150 mL) covered with a glass lid
Replication:	Treated groups 4 (+ 2 replicates not loaded with collembolans for measurement purposes) Control group 8 (+ 2 replicates not loaded with collembolans for measurement purposes)
No./arena :	10
Duration of test:	28 days

Environmental conditions	**test**
Temperature:	19.2 – 21.4°C
pH:	Test initiation: 6.02 – 6.05 Test completion: 5.72 – 5.75
Photoperiod:	Light : dark 16 h : 8 h (light intensity 610 lux)

Study Design and Methods

Experimental dates: 2nd August 2012 to 30th August 2012

Two days before test start, the dry artificial soil was moistened by adding deionised water to adjust the water content to 40-60% of WHC. On the day of the test start, the test item was mixed with a small quantity of finely ground quartz sand (10 g treated sand per treatment group), such that the required test concentration was achieved once mixed with the artificial soil. The control substrate contained the corresponding amount of untreated quartz sand only.

Ten juvenile collembolans, were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight replicates (+ two replicates not loaded with collembolans for measurement purposes) were used for the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The glass lids covering the test vessels were briefly opened twice a week for aeration. The water content was checked weekly by reweighing the two additional test vessels. Water loss was compensated in all vessels if exceeding 2% of the initial water content. The temperature was 19.2 – 21.4°C, the pH was 5.72 – 6.05, the water content of the artificial soil was 55.2 – 57.0% of WHC and there was a 16 hour light : 8 hour dark photoperiod (610 lux).

Calculation and Statistics

Fisher`s Exact Binomial Test with Bonferroni Correction and Williams-t-test were used to compare the control with the independent test item groups. Mortality of adult collembolans was corrected using the formula by Abbott (1925).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 24 Effects of residues of CGA325025 on mortality and reproduction of *Folsomia candida*

Endpoints	Treatment groups (mg CGA325025/kg soil dw)								
	Control	**1.63**	**2.94**	**5.29**	**9.53**	**17.1**	**30.9**	**55.6**	**100**
% Mortality of parental collembolans after 4 weeks	2.5	2.5	0.0	2.5	0.0	2.5	0.0	0.0	2.5
% corrected mortality (Abbott)	-	0	-3	0	-3	0	-3	-3	0

Mean number of juveniles after 4 weeks	1059	1023	1025	1052	1012	1087	1066	1075	1040
SD	144.5	162.2	149.3	168.1	198.0	150.1	53.7	143.7	113.2
CV %	13.6	15.9	14.6	16.0	19.6	13.8	5.0	13.4	10.9
% reduction compared to control	-	3	3	1	4	-3	-1	-2	2
NOEC (mortality)	**100 mg SYN542604/kg soil dw**								
NOEC (reproduction)	**100 mg SYN542604/kg soil dw**								
EC_{50}/LC_{50}	**>100 mg SYN542604/kg soil dw**								

Not statistically significant compared to the control regarding mortality ([1]Fisher's Exact Binomial Test with Bonferroni Correction, p >0.05, one-sided greater) and reproduction ([2]Williams-t-test, p >0.05, one-sided smaller)
Abbott's formula for corrected mortality (Abbott, 1925): M (%) = ((A-B)/A) * 100%, where
A = mean number of surviving parental collembolans in the control group, and
B = mean number of surviving parental collembolans in the treated groups
Percent reduction: (1-Rt/Rc) * 100%, where Rt = mean number of juveniles observed in the treated groups, and Rc = mean number of juveniles observed in the control group
dw = dry weight
Negative values = increase, relative to control

Validity criteria

The validity criteria for the control group were met:

- Mean adult mortality: ≤20% (observed: 2.5%)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 1059/vessel)
- Coefficient of variation for the mean number of juveniles: ≤30% (observed: 13.6%)

The requirement of the ISO guideline concerning the precision of the counting method (average error <10%) was fulfilled, the determined overall error of counting amounted to 3.6%.

Conclusions

The NOEC for both parental mortality and reproduction was determined to be 100 mg CGA325025/kg soil dry weight. Neither the LC_{50} nor the EC_{50} (based on reproduction) could be calculated, but it can be concluded that they are >100 mg CGA325025/kg soil dw, the highest concentration tested.

Friedrich S. (2012h)

Comments of zRMS:	The study was conducted to OECD guideline 232 and according to the principles of GLP. In the definitive test all the validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.4.2/05
Report	Friedrich, S. (2015a): CGA300406 - Effects on the Reproduction of the Collembolan *Folsomia candida* Report No 15 10 48 139 S BioChem agrar Labor für biologische und chemische Analytik GmbH

	Kupferstraße 6, 04827 Gerichshain, Germany Syngenta file No CGA300406_10017
Guideline(s):	OECD Guidelines No 232. Collembolan Reproduction test in soil (2009) ISO 11267: Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants (1999)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Executive Summary

The toxicity of CGA300406 to the reproduction and the parental mortality of collembola species *Folsomia candida* were determined. The NOEC was determined to be 1000 mg/kg soil dry weight. The EC_{50} for number of juvenile collembolans was calculated to be >1000 mg/kg soil d.w.

Materials

Test Material	CGA300406
Lot/Batch #:	MES 235/3
Purity:	90 % w/w
Description:	White solid
Stability of test compound:	Stable under standard conditions.
Reanalysis/Expiry date:	End of January 2017
Density:	n/a
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556, 1000 mg/kg soil dry weight
Control:	Untreated
Toxic standard:	Boric acid
Application method:	Mixed with artificial sediment
Test organisms	
Species:	*Folsomia candida*
Age:	9-12 days (juvenile collembolans)
Source:	Culture maintained at Test Facility
Feeding:	2 mg granulated dry yeast at the start of the test and after 14 days
Test design	
Arenas:	glass container (approximately 150 mL) covered with a lid
Substrate:	Artificial soil comprising 5% sphagnum peat, 20 % kaolinite clay, 74.7 % industrial quartz sand (> 50% of the particles between 0.05 mm and 0.2 mm) and 0.3% calcium carbonate. 30 g wet weight soil added to each vessel
Replication:	Control: 8 (+ 2 replicates not loaded with collembolans for measurement purposes) Treated: 4 (+ 2 replicates not loaded with collembolans for measurement purposes)
No./arena :	10
Duration of test:	4 weeks
Environmental test conditions	
Temperature:	20.1 – 21.6 ºC
pH of soil:	test start: 6.00 – 6.11

	test end: 5.74 – 5.80
Water content of soil:	test start: 24.8 – 25.0 (equivalent to 59.5 – 60.0 % of WHC)
	test end: 24.4 – 24.8 (equivalent to 58.5 – 59.5 % of WHC)
Photoperiod:	16 hour light (540 lux)

Study Design and Methods

Experimental dates: 31 August to 28 September 2015

An exact weighed amount of the test item was mixed with finely ground quartz sand, immediately prior to application. This stock mixture was diluted with quartz sand in a way that 10 g of the mixture contained the amount of test item required for one treatment group to adjust the selected concentration. The treated quartz sand (10 g per treatment group) was added to the prepared amount of artificial soil (302.5 g wet weight) yielding 312.5 g wet artificial soil (corresponding to 250 g dry weight). The test item mixture was then mixed thoroughly with the artificial soil by intensive stirring in a laboratory mixer. The control was left untreated.
Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. Four replicates (+ two replicates not loaded with collembolans for measurement purposes) were used per test concentration and eight were used in the control. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.
All values presented throughout this report were calculated using the original raw data and were not based on rounded values.

The statistical analysis was performed with the software ToxRat Professional 3.1.0 (2015). Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm and Williams-t-test were used to compare the control with the independent test item groups. Mortality of adult collembolans was corrected using the formula by Abbott (1925).

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 25 Effects of residues of CGA300406 on mortality and reproduction of *Collembola candida*

Endpoints	Treatment groups (mg/kg soil dry weight)								
	Control	**16**	**29**	**53**	**95**	**171**	**309**	**556**	**1000**
% Mortality of parental collembolans after 4 weeks	3.8	2.5	0.0	2.5	2.5	2,5	5.0	0.0	2.5
% corrected mortality (Abbott)	-	-1	-4	-1	-1	-1	1	-4	-1
Mean number of juveniles after 4 weeks	757	777	765	742	769	735	765	769	767
SD	85.2	62.0	93.2	153.5	51.0	61.4	159.9	85.2	180.3
CV %	11.3	8.0	12.2	20.7	6.6	8.3	20.9	11.1	23.5
% reduction compared to control	-	-3	-1	2	-2	3	-1	-2	-1
NOEC (mortality)	1000								
NOEC (reproduction)	1000								
LC_{50}	>1000								
EC_{10}	>1000								
EC_{20}	>1000								
EC_{50}	>1000								

Mortality not statistically significant compared to control regarding mortality (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, $p > 0.05$, one-sided greater) and reproduction (Williams-t-test, $p > 0.05$, one-sided smaller)
Negative values = increase, relative to control

Validity criteria

The test is considered valid as:

- Control treatment mortality was 3.8% (must be < 20%)
- The mean number of juvenile recorded in the control treatment was 757 (must be > 100 per replicate)
- The coefficient of variation of reproduction in the control was 11.3% (must not be > 30%)

Conclusions

In a chronic toxicity test in which collembolans were exposed to CGA300406 the NOEC for mortality of the parental collembolans was determined to be 1000 mg/kg soil dry weight. The LC_{50} could not be calculated, but it can be concluded that the LC_{50} is higher than 1000 mg/kg soil d.w., the highest concentration tested. The NOEC for reproduction was determined to be 1000 mg/kg soil dry weight. The EC_{10}, EC_{20} and EC_{50} values for reproduction could not be calculated, but it can be concluded that these values are higher than 1000 mg/kg soil d.w., the highest concentration tested.

Friedrich S. (2015a)

The following chronic study on Folsomia with A18385B has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated at zonal level for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.4.2/06
Report	Friedrich S. (2013) Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Effects on the Reproduction of the Collembolan *Folsomia candida*, Report Number 13 10 48 084 S. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta file No. A18385B_10011).
Guideline(s):	OECD Guideline for Testing of Chemicals, Section 2 – Effects on Biotic Systems, Method 232: Collembolan reproduction test in soil (2009). ISO 11267: Soil quality – inhibition of reproduction of Collembola (*Folsomia candida*) by soil pollutants (1999).
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

In a chronic toxicity test in which the Collembolan Folsomia candida were exposed to nominal concentrations of 16, 29, 53, 95, 171, 309, 556 and 1000 mg test item/kg soil dry weight, the NOEC for mortality of the parental collembolans was determined to be 53 mg A18385B/kg soil dry weight. The LC_{50} was calculated to be 118 mg A18385B/kg soil dw. The NOEC for reproduction was determined to be 29 mg A18385B/kg soil dry weight. The EC_{50} (based on reproduction) was calculated to be 98 mg A18385B/kg soil dw.

Materials

Test Material	A18385B Prosulfuron/Dicamba/Nicosulfuron WG
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	September 2014
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556 and 1000 mg test item/kg soil dry weight
Control:	Deionised water
Adjuvant	A12127R (Adigor) a mixture of emulsified fatty acid esters on basis of oleic acid methyl ester. The ratio of A18385B to A12127R was 1: 3 (A18385B : A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio.
Toxic standard:	Boric acid at rates of 44, 67, 100, 150 and 225 mg/kg soil dry weight (separate study – BioChem project No: R 13 10 48 004 S, dated 16 July 2013)
Test organisms	
Species:	Collembolans *Folsomia candida* (Willem)
Age:	Juveniles (9-12 days)
Source:	Culture maintained at Test Facility. Originally purchased from Biologische

	Bundesanstalt (BBA), Berlin-Dahlem in May 2000
Feeding:	2 mg granulated dry yeast per replicate at the start of the test and after 14 days
Test design	
Arenas:	Glass container (approximately 150 mL) covered with a glass lid
Substrate:	Artificial soil comprising 5% sphagnum peat, 20% kaolinite clay, 74.7% industrial quartz sand and 0.3% calcium carbonate. 30 g wet weight soil was added to each test vessel
Replication:	4 (treatments) 8 (controls)
No./arena :	10
Duration of test:	28 days
Environmental test conditions	
Temperature:	18.8 – 21.6°C
pH of soil:	Test start: 5.95 – 6.03 Test end: 5.79 – 5.89
Water content of soil:	Test start: 24.9 to 25.1% (equivalent to 58.0% to 58.5% of water holding capacity) Test end: 24.3 to 24.8% (equivalent to 56.6 to 57.8% of water holding capacity)
Photoperiod:	Light : dark 16 h : 8 h (light intensity 550 lux)

Study Design and Methods

Experimental dates: 28th May to 25th June 2013

Two days before the start of the test, the dry artificial soil was pre-moistened by adding deionised water to obtain approximately half of the final water content. The test concentrations were prepared by dispersing weighed amounts of the test item in deionised water to make a stock solution. This stock solution was diluted with deionised water for each test concentration and was thoroughly mixed with the artificial soil using a laboratory mixer, achieving a final nominal water content of 40-60% of WHC. The control was treated with deionised water only.

Ten juvenile collembolans were transferred after the application to the substrate surface of each test vessel using an exhauster. The test organisms were fed twice during the experiment (at the start of the test and after 14 days) with approximately 2 mg of granulated dry yeast per test vessel. Four weeks after introducing the test organisms, the surviving parental collembolans and offspring (juveniles) were counted.

The glass lids covering the test vessels were briefly opened twice a week for aeration. The water content was checked weekly by reweighing the two additional test vessels. Water loss was compensated in all vessels if exceeding 2% of the initial water content.

Results and Discussion

The statistical analysis was performed with the software ToxRat Professional 2.10.06 (RATTE 2010). Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. The $LC_{50}$ was calculated using linear weighted regression. Confidence limits (95%) of the $LC_{50}$ value were computed according to Fieller`s theorem. The EC_{10}, EC_{20} and EC_{50} were calculated by linear max. likelihood regression (Finney 1971). Confidence limits (95%) of the EC_x values were computed by normal approximation. Mortality of adult collembolans was corrected using the formula by Abbott (1925).

Mortality and fecundity are summarised in the table below.

Table A 26 Effect of A18385B on mortality and reproduction of *Folsomia candida*

Endpoints	Treatment groups (mg A18385B/kg soil dw)								
	Control	**16**	**29**	**53**	**95**	**171**	**309**	**556**	**1000**
% Mortality of parental collembolans after 4 weeks	2.5	2.5	0.0	5.0	32.5*	75.0*	100.0*	100.0*	100.0*
% Corrected mortality (Abbott)	-	0	-3	3	31	74	100	100	100
Mean number of juveniles after 4 weeks	948	989	955	798*	554*	54*	7*	0*	0*
% Reduction compared to control	-	-4	-1	16	42	94	99	100	100
NOEC (mortality)	53 mg A18385B/kg soil dw								
NOEC (reproduction)	29 mg A18385B/kg soil dw								
LC_{50}	118 mg A18385B/kg soil dw (95% confidence limits 98 to 146 mg A18385B/kg soil dw)								
EC_{10} (reproduction)	55 mg A18385B/kg soil dw (95% confidence limits 44 to 68 mg A18385B/kg soil dw)								
EC_{20} (reproduction)	67 mg A18385B/kg soil dw (95% confidence limits 57 to 78 mg A18385B/kg soil dw)								
EC_{50} (reproduction)	98 mg A18385B/kg soil dw (95% confidence limits 89 to 109 mg A18385B/kg soil dw)								

* statistically significant compared to control regarding mortality (Fisher's Exact Binomial Test with Bonferroni Correction, p ≤0.05, one-sided greater) and reproduction (Williams-t-test, p ≤0.05, one-sided smaller)

Validity criteria

The validity criteria for the control group were met as mean adult mortality was ≤20% (being 2.5%), mean number of juveniles per test vessel was ≥100 (being 948/vessel) and the coefficient of variation for the mean number of juveniles was ≤30% (being 14.9%). The requirement of the ISO guideline concerning the precision of the counting method (average error <10%) was fulfilled, the determined overall error of counting amounted to 2.5%.

Conclusions

In a chronic toxicity test in which the Collembolan *Folsomia candida* were exposed to concentrations of A18385B, the NOEC for mortality of the parental collembolans was determined to be 53 mg A18385B/kg soil dry weight. The LC_{50} was calculated to be 118 mg A18385B/kg soil dw. The NOEC for reproduction was determined to be 29 mg A18385B/kg soil dry weight. The EC_{50} (based on reproduction) was calculated to be 98 mg A18385B/kg soil dw.

(Friedrich, S., 2013)

The following chronic study on Hypoaspis with A18385B has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba but was evaluated at zonal level for authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.4.2/07
Report	Schulz L. (2013) Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Effects on the Reproduction of the Predatory Mite *Hypoaspis aculeifer*, Report Number 13 10 48 085 S, BioChem agrar Labor für biologische und chemische, Analytik GmbH, Kupferstraße 6, 04827 Gerichshain, Germany. (Syngenta file No. A18385B_ 10012).
Guideline(s):	OECD (2008). OECD Guidelines for testing of chemicals, No. 226. Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil.
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

The purpose of this study was to determine potential effects of the test item on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) as a representative of soil micro arthropods during a test period of 14 days.

The LC_{50} for mortality and the EC_{50} for reproduction was calculated to be 527.7 mg A18385B/kg soil dry weight and 83.2 mg A18385B/kg soil dry weight, respectively. The NOEC for mortality and the NOEC for reproduction were determined to be 309 mg and 95 mg A18385B/kg soil dry weight, respectively.

Materials

Test Material	A18385B Prosulfuron/Dicamba/Nicosulfuron WG (A18385B)
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	September 2014
Treatments	
Test rates:	16, 29, 53, 95, 171, 309, 556, 1000 mg A18385B/kg soil dw + 49, 88, 159, 286, 514, 926, 1667, 3000 mg A12127R/kg soil dw
Control:	Soil prepared with deionised water only
Toxic standard:	Dimethoate EC 400 at 4.10, 5.12, 6.40, 8.00 and 10.00 mg a.i./kg soil dw
Adjuvant	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18385B to A12127R was 1: 3 (A18385B: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio
Test system:	Exposure of mites to different concentrations of the test item mixed into artificial soil substrate (5% peat)
Test organisms	
Species	*Hypoaspis aculeifer* (CANESTRINI)

Source:	Cultured at Test Facility. Original source; Katz Biotech AG, Germany.
Food:	Two to three times a week with *Tyrophagus putrescentiae* (SCHRANK)
Age at test start:	adults from a synchronised culture with an age difference of 2 days
Test design	
Arenas:	100 mL SCHOTT-bottles with screw cap (4 cm diameter, 11 cm high)
Replication:	8 replicate arenas for the control and 4 for each of the test item treatments.
No. of mites/arena :	10 adult females
Environmental test conditions	
Temperature:	19.5 – 21.4°C
Photoperiod:	16 h photoperiod
Duration of test:	14 days

Study Design and Methods

Experimental dates: 5th March to 4th April 2013

Adult females of the soil mite *Hypoaspis aculeifer* were exposed to concentrations of A18385B + A12127R incorporated into the test soil. For each concentration the desired amount of the test item was diluted in deionised water and mixed thoroughly with the artificial soil by means of a hand stirrer. Adult females from a synchronised culture were transferred to the test vessels which contained untreated (control) or test item treated artificial soil. Ten adult females were introduced to each test vessel together with the food mite *Tyrophagus putrescentiae*. The test was carried out under controlled light-dark cycle. The water content was maintained and food was added at regular intervals throughout the duration of the test. Fourteen days after introducing the test organisms, the surviving mites and the juveniles of *Hypoaspis aculeifer* were extracted by heat/light extraction. Any adult mites not found after extraction were recorded as dead. From these data the mortality of the adult females and the reproductive output were calculated.

The corrected mortality in the treatment groups was calculated according to ABBOTT (1925). The statistical analysis was performed with the software ToxRat Professional 2.10.05 (RATTE 2010). Fisher's Exact Binomial Test with Bonferroni Correction and Welch t-test were used to compare reproductive output from the control with the independent test item groups. Weibull and Probit analyses were performed to calculate LC_{50} and EC_{50} values.

Results and Discussion

Mortality and fecundity are summarised in the table below.

Table A 27 Effect of A18385B on mortality and reproduction of *Hypoaspis aculeifer*

Endpoints	Treatment groups (mg A18385B/kg soil dw)								
	Control	**16**	**29**	**53**	**95**	**171**	**309**	**556**	**1000**
	Mortality of adult mites after 14 days								
% mortality	0.0	0.0	0.0	2.5	0.0	2.5	12.5	92.5*[1]	100.0*[1]
% corrected mortality (Abbott)	-	0.0	0.0	2.5	0.0	2.5	0.0	92.5	100.0
	Number of juveniles after 14 days								
mean	247.3	233.3	266.0	190.3	105.5	19.0*[2]	0.0	0.3*[2]	0.0
standard deviation	35.6	22.7	63.8	62.7	97.8	20.4	0.0	0.5	0.0
coefficient of variation %	14.4	9.7	24.0	32.9	92.7	107.6	-	200.0	-
% change compared to control	-	-5.7	+7.6	-23.1	-57.3	-92.3	-100.0	-99.9	-100.0
NOEC (mortality)	**309 mg A18385B/kg soil dry weight**								
LC_{50} (mortality)	**527.5 mg A18385B/kg soil dry weight (95% confidence limits 296.0 to 2206.9 mg A18385B/kg soil dry weight)**								
NOEC (reproduction)	**95 mg A18385B/kg soil dry weight**								
EC_{50} (reproduction)	**83.2 mg A18385B/kg soil dry weight (95% confidence limits 76.9 to 89.9 mg A18385B/kg soil dry weight)**								

*1 statistically significant compared to control (Fisher's Exact Binomial with Bonferroni Correction for mortality, $p \leq 0.05$, one-sided greater)
*2 statistically significant compared to control (Welch-t-test for reproduction, $p \leq 0.05$, one-sided smaller)

Validity criteria

The validity criteria were fulfilled. Control treatment mortality did not exceed 20% (0%), the mean number of juveniles recorded in the control treatment was at least 50 per replicate at the end of the test (247.3) and the coefficient of variation of reproduction in the control did not exceed 30% (14.4%).

Conclusions

The LC_{50} for mortality and the EC_{50} for reproduction was calculated to be 527.7 mg A18385B/kg soil dry weight and 83.2 mg A18385B/kg soil dry weight, respectively. The NOEC for mortality and the NOEC for reproduction were determined to be 309 mg and 95 mg A18385B/kg soil dry weight, respectively.

Schulz L. (2013)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

The following study on N- and C–mineralisation with prosulfuron metabolite CGA349707 is new, and has not been previously evaluated at EU peer review for prosulfuron.

Reference:	KCP 10.5/01
Report	Hutcheson, K. (2015): CGA349707 - Effect on Soil Microbial Activity, Carbon and Nitrogen Transformations Report No CEMR-6587 CEM Analytical Services Limited Imperial House, Oaklands Business Centre, Oaklands Park, Wokingham, Berkshire, RG41 2FD, UK Syngenta file No CGA349707_10012
Guideline(s):	OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000) OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

CGA349707 was applied to the soil at concentrations of 0.027 and 0.135 mg CGA349707/kg dry soil. The test item CGA349707 caused no adverse effects on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as CO_2-production) at the end of the 28-day incubation period.

Materials

Test Material	CGA349707
Lot/Batch #:	KGL 4933/6 R 3
Purity:	99 % w/w
Description:	White solid
Stability of test compound:	Stable under test conditions
Reanalysis/Expiry date:	30 June 2015
Density:	n/a
Treatments	
Test rates:	0.027 and 0.135 mg CGA349707/kg dry soil
Control:	Deionised water
Toxic standard:	None
Test design	
Soil:	LUFA standard soil type 2.3 (batch number F2.32614, supplied by LUFA-Speyer, Obere Langgasse 40, 67346 Speyer, Germany)
Soil type:	Sand content: 59.9 %, pH: 5.6 (measured in water), organic carbon: 0.67 %, and microbial biomass: 4.15 %. MWHC was determined as 26.2 %.
Test units:	Nitrogen transformation test: 500 g soil dry weight in a 2-L plastic container

	(16.7 x 16.7 x 9 cm) with a perforated lid Carbon transformation test: 1000 g soil dry weight in a 2-L plastic container (16.7 x 16.7 x 9 cm) with a perforated lid
Replication:	3 per treatment rate and control
Sampling intervals :	0, 7, 14 and 28 days after application
Duration of test:	28 days
Environmental test conditions	
Temperature:	20 ± 2 °C
pH of soil:	5.5 (soil pH (measured in water) taken from the carbon test replicate 1 vessel)
Soil moisture content:	42 % of MWHC (moisture content obtained from measurements taken from the carbon test replicate 1 vessel from each treatment)
Photoperiod:	Constant darkness

Study Design and Methods

Experimental dates: 22 August – 26 September 2014

Soil samples were treated with CGA349707 at two doses, 0.027 and 0.135 mg CGA349707/kg dry soil. The test item was mixed with acetone and added to a fine quartz sand carrier. The dosed sand was left for 18 hours to allow evaporation of the acetone and then added with a volume of deionised water to the test soil to achieve a water content of approximately 40 % of WHC. The treated soil was then homogenised in a laboratory mixer. The water content of the soil in each test vessel was determined at test start (after application) and adjusted once a week to the required range of 40 ± 5 % of WHC.

Three replicate soil samples were prepared for each treatment rate and the controls (acetone and deionised water) for the nitrogen transformation test and carbon transformation test.

Mean nitrogen content (mg NO_3-N/kg soil d.w.), standard deviation and coefficient of variation as well as the mean nitrogen content/day (mg NO_3-N/kg soil d.w./day) were calculated for each treatment group and sampling date. For the evaluation of the results the relative deviations (%) of the test item treatment groups from the control were calculated (based on the mean nitrogen content/day) for each sampling date.

CO_2 concentrations were measured on each sampling date and the mean CO_2 production rate and relative deviation (%) of the test item treatment groups from the control treatment were calculated.

Results and Discussion

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table A 28 Effects on Nitrogen Transformation in Soil after Treatment with CGA349707

Days after application	Solvent Control			0.027 mg CGA349707/kg soil d.w.				0.135 mg CGA349707/kg soil d.w.			
	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	CV [%]	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	CV [%]	Deviation from control [%][1)]	NO_3-N [mg/kg soil d.w.]	NO_3-N [mg/kg soil d.w./day]	CV [%]	Deviation from solvent control [%]
0	8.59	-	0.34	8.57	-	0.69	-	8.60	-	1.03	-
7	4.76	-0.55	0.00	4.76	-0.54	2.15	-0.45	5.04	-0.51	2.56	-6.7
14	29.7	1.51	0.75	29.3	1.48	2.42	-1.6	29.7	1.51	0.34	-0.1

28	55.2	1.67	0.19	54.6	1.65	1.68	-1.21	54.8	1.65	0.86	-0.9

CV [%] = Coefficient of Variation

Table A 29 Effects on Carbon Transformation in Soil after Treatment with CGA349707

Days after application	Solvent Control	0.027 mg CGA349707/kg soil d.w.		0.135 mg CGA349707/kg soil d.w.	
	CO_2-production [mg/kg soil d.w./h]	CO_2-production [mg/kg soil d.w./h]	Deviation from control [%][1)]	CO_2-production [mg/kg soil d.w./h]	Deviation from control [%][1]
0	21.39	21.65	+1.21[2]	21.97*	+2.72[2]
7	21.47	21.99	+2.43[2]	22.99*	+7.10[2]
14	13.84	14.02	+1.32[2]	15.60*	+12.73[2]
28	13.74	13.80	-1.48[3]	13.89	-0.86[3]

Some calculations were performed with non-rounded values
CV [%] = Coefficient of Variation
[1]: based on CO_2 production; - = inhibition; + = stimulation
[2]: Deviation from solvent control
[3]: Deviation from pooled control (used when solvent and water control means were not significantly different
*: Statistically different to control (Dunnett's Test, two tail, $p \leq 0.05$)

Validity criteria

The test is considered valid as the variation between replicate control samples was no greater than ± 15 % at day 28.

Conclusions

CGA349707 was applied to the soil at concentrations of 0.027 and 0.135 mg CGA349707/kg dry soil. The test item CGA349707 caused no adverse effects on soil nitrogen transformation (measured as NO_3-N-production) and on soil carbon transformation (measured as CO_2-production) at the end of the 28-day incubation period.

(Hutcheson, K., 2015)

Reference:	KCP 10.5/02
Report	Schulz L, (2013a), Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – Effects on the Activity of Soil Microflora (Nitrogen and Carbon Transformation Tests), Report Number 13 10 48 061 C/N. BioChem agrar, Labor für biologische und chemische Analytik GmbH, Kupferstraβe 6, 04827 Gerichshain, Germany (Syngenta file No. A18385B_10010).
Guideline(s):	OECD guidelines 216, Soil Microorganisms: Nitrogen Transformation Test (2000) OECD guidelines 217, Soil Microorganisms: Carbon Transformation Test (2000)
Deviations:	No

GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

A18385B plus adjuvant A12127R was applied to the soil at concentrations of 0.67 mg A18385B/kg dry soil weight and 3.33 mg A18385B/kg dry soil weight. No adverse effects are to be expected on either short-term microbial respiration or on the nitrification process and hence on soil fertility.

Materials

Test Material	A18385B Prosulfuron/Dicamba/Nicosulfuron WG (4/40/10)
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions
Reanalysis/Expiry date:	30 September 2014
Density:	Not applicable
Treatments	
Test rates:	0.67 mg A18385B/kg soil dw (equivalent to 0.5 kg A18385B/ha) and 3.3 mg A18385B soil dw (equivalent to 2.5 kg A18385B/ha)
Control:	Deionised water only
Toxic standard:	Dinoterb (purity 98.0 ± 0.5%) at concentrations of 6.8, 16.0 and 27.0 mg Dinoterb/kg (Separate study – BioChem project No: R 13 10 48 001 C/N, date 04.01 to 01.02.2013)
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methyl ester). The ratio of A18385B to A12127R was 1: 3. Results are expressed in terms of the test item containing the adjuvant in this ratio.
Test design	
Soil:	Agricultural sandy loam soil, supplied by BioChem agrar GmbH
Soil type:	Sandy loam: 10.3% clay (<0.002 mm), 35.4% silt (0.002 - 0.050 mm) and 54.3% sand (0.050 – 2.0 mm) (USDA classification)
Test units:	Nitrogen transformation test: 200 g soil dry weight in 500 mL wide-mouthed glass flasks Carbon transformation test: 1000 g soil dry weight in 4 L steel test vessels
Replication:	Nitrogen transformation test: 3 replicates per treatment rate and control Carbon transformation test: 3 replicates per treatment rate and control
Sampling intervals :	Nitrogen transformation test: 3 hours, 7 days, 14 days and 28 days after application Carbon transformation test: 3 hours, 7 days, 14 days and 28 days after application
Duration of test:	28 days
Environmental test conditions	
Temperature:	20.0 – 21.0°C
pH of soil:	Nitrogen transformation test: 6.3 – 6.4 at test start, 6.2 at test end Carbon transformation test: 6.3 – 6.5 at test start, 6.3 – 6.4 at test end
Soil moisture content:	Nitrogen transformation test: 14.72 – 16.04 g/100 g soil dw (equivalent to 40.21 – 43.80% of WHC) Carbon transformation test: 15.84 – 16.38 g/100 g soil dw (equivalent to 43.26 – 44.73% of WHC)
Photoperiod:	Darkness

Study Design and Methods

Experimental dates: 15th April 2013 to 13th May 2013

Soil samples were treated with A18385B, together with the adjuvant A12127R, at two doses – 0.67 mg A18385B/kg + 1.85 mg A12127R/kg dry soil weight (low dose) and 3.33 mg A18385B/kg + 9.25 mg A12127R/kg dry soil (high dose) relating to a soil depth of 5 cm and a soil density of 1.5 g/cm^3. The test item was mixed with deionised water, which was added to the soil samples and mixed thoroughly. The soil moisture content of all samples was adjusted to 45% of the WHC by adding deionised water and the samples incubated in the dark at a temperature of 20.0 – 21.0°C. The soil moisture content was checked weekly, and adjusted with purified water to maintain 40 – 50% of the soil WHC.

Respiration and nitrification were determined for all treatments at 3 hours, 7, 14 and 28 days after treatment. In order to measure the short-term respiration of soil microbes, 100 g soil dw were taken from each treatment at each sampling occasion. The samples were amended with glucose and the evolved CO_2 measured over a period of 12 hours. To determine the nitrification, the soil samples were amended with Lucerne meal after application and 10 g soil dw per replicate were taken at each sampling point. The samples were extracted with KCl, and analysed for nitrite-nitrogen, nitrate-nitrogen and ammonium-nitrogen.

Data of nitrate formation and O_2 consumption were used to calculate the percentage deviation from the control on each sampling date which was then analysed statistically (2-sided Student-t-test or Welch-t-test at 5% significance level).

Results and Discussion

Results from the Nitrogen transformation test and the Carbon transformation test are summarised in the tables below.

Table A 30 Effects on Nitrogen Transformation in Soil after Treatment with A18385B + A12127R

Days after application	Control		0.67 mg A18385B/kg + 1.85 mg A12127R/kg soil dw (equivalent to 0.5 kg A18385B/ha + 1.5 L A12127R/ha)			3.3 mg A18385B + 9.25 mg A12127R/kg soil dw (equivalent to 2.5 kg A18385B/ha + 7.5 L A12127R/ha)		
	NO_3-N [mg/kg soil dw]	CV [%]	NO_3-N [mg/kg soil dw]	CV [%]	Deviation from control [%][1)]	NO_3-N [mg/kg soil d w.]	CV [%]	Deviation from control [%][1)]
0	12.4	1.6	12.3	6.2	-1.1	12.2	3.3	-1.9
7	39.5	2.6	42.5*^{s}	2.1	+7.6	45.6*^{s}	5.8	+15.4
14	47.9	4.0	53.2*^{s}	4.8	+11.1	54.3*^{s}	4.0	+13.5
28	54.5	5.8	63.0*^{s}	1.5	+15.5	64.8*^{s}	3.8	+19.0

The calculations were performed with non-rounded values
CV [%] = Coefficient of Variation
1) based on NO_3-nitrogen production; - = inhibition; + = stimulation
*^{s} statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p ≤0.05)

Table A 31 Effects on Carbon Transformation in Soil after Treatment with A18385B + A12127R

Days after application	Control		0.67 mg A18385B/kg + 1.85 mg A12127R/kg soil dw (equivalent to 0.5 kg A18385B/ha + 1.5 L A12127R/ha)			3.3 mg A18385B + 9.25 mg A12127R/kg soil dw (equivalent to 2.5 kg A18385B/ha + 7.5 L A12127R/ha)		
	O_2 consumption [mg/kg soil dw/h]	CV [%]	O_2 consumption [mg/kg soil dw/h]	CV [%]	Deviation from control [%][1)]	O_2 consumption [mg/kg soil dw/h]	CV [%]	Deviation from control [%][1)]
0	13.20	0.3	12.27*^{s}	1.0	-7.0	11.47*^{w}	3.4	-13.1
7	12.41	1.5	11.68*^{s}	2.5	-5.9	10.87*^{s}	2.2	-12.5
14	12.27	1.3	11.30*^{s}	0.9	-7.9	10.45*^{s}	3.4	-14.8
28	12.18	0.4	11.07*^{s}	0.7	-9.1	10.12*^{s}	2.1	-16.9

The calculations were performed with non-rounded values
CV [%] = Coefficient of Variation
1) based on O_2 consumption; - = inhibition; + = stimulation
*^{s} statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)
*^{w} statistically significantly different to control (Welch-t-test for inhomogeneous variances, 2-sided, $p \leq 0.05$)

Validity criteria

The validity criteria were fulfilled. The coefficients of variation in the control group in both the nitrogen and carbon transformation tests were ≤15% (maximum 5.8 and 1.5%, respectively).

The results with the reference substance for both the nitrogen and carbon transformation tests demonstrated the sensitivity of the test system.

Conclusions

A18385B plus adjuvant A12127R was applied to the soil at concentrations of 0.67 mg A18385B/kg dry soil weight and 3.33 mg A18385B/kg dry soil weight. No adverse effects are to be expected on either short-term microbial respiration or on the nitrification process and hence on soil fertility.

(Schulz L, 2013a)

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Vegetative vigour

The following vegetative vigour study with A18385B has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba. It was evaluated at zonal level for last authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.6.2/01
Report	Bramby-Gunary J., 2013, Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Vegetative Vigour Test, Report No: ACE-12-183, AgroChemex Ltd, Manningtree, United Kingdom. (Syngenta File No. A18385B_10004)
Guideline(s):	OECD Guideline for the Testing of Chemicals. Guideline 227: Terrestrial Plant Test: Vegetative Vigour Test (2006).
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

A foliar application of A18385B plus adjuvant A12127R, at rates up to 500 g A18385B per hectare resulted in ER_{50} values ranging from 2.39 to >500 g A18385B/ha. *Lycopersicon esculentum* (tomato) was the most sensitive species, with an ER_{50} of 2.39 g A18385B/ha based on foliar dry weight with 95% confidence limits of 2.03 to 2.78 g A18385B/ha.

Materials

Test material	A18385B Prosulfuron/Dicamba/Nicosulfuron WG (4/40/10)
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under test conditions.
Reanalysis/expiry date:	30 September 2014
Treatments	
Test concentrations:	0.2, 0.6, 1.8, 5.4, 16.2, 48.6, 145.8 and 500 g A18385B/ha
Control:	Water only
Spray volume:	200L/ha ± 10%
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18385B to A12127R was 1: 3 (A18385B: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio
Application method:	Mardrive cabinet track sprayer with 8004E TeeJet flat fan nozzle
Test organisms	
Species:	*Avena sativa* (oat) *Lolium perenne* (ryegrass) *Oryza sativa* (rice) *Beta vulgaris* (sugar beet) *Brassica napus* (oilseed rape) *Cucumis sativa* (cucumber) *Daucus carota* (carrot) *Lactuca sativa* (lettuce) *Lycopersicon esculentum* (tomato) *Raphanus sativus* (radish)
Test soil:	Sandy loam mixed as follows: 20 litres of sterile loam + 10 litres of sand. The soil was determined to consist of 75% w/w sand (2.00 – 0.063mm), 8% silt

	w/w (0.063 – 0.002 mm), 17% w/w clay (<0.002 mm). The organic carbon content was 1.3% w/w. 100 g slow release fertiliser (Osmocote®Extract®) was incorporated into 30 L of soil mix.
Test design	
Test vessels:	Non-porous plastic pots (8 x 8 x 8 cm) placed in saucers filled with enough water to ensure that the pots were kept moist at all times
Sampling interval:	Plants were assessed at 7, 14 and 21 days after application for mortality and visual phytotoxicity. Biomass and height were assessed at test termination
Replication:	Five pots per treatments, four plants per pot
Duration:	21 days
Environmental conditions	
Test temperature:	8.1 – 23.0°C (Mean; 18.2 ºC)*
Humidity:	57.2 – 100.0% (Mean; 81.4%)*
Soil pH:	7.4
Lighting:	Ambient lighting was supplemented by sodium vapour lamps giving at least a 16 hour day. The mean ambient light intensity for the study period was 5.0 kilo lux (Kl), and the maximum intensity was 30.1 Kl

*The temperature fell below the range specified in the study plan on a few occasions and the humidity rose above and fell below the range specified in the study plan on a few occasions; however the plants were healthy and grew well. This minor deviation had no impact on the study.

Study Design and Methods

Experimental dates: 17th October 2012 to 20th November 2012
Young plants of three monocot species (*Avena sativa*, *Lolium perenne* and *Oryza sativa*) and seven dicot species (*Beta vulgaris, Brassica napus, Cucumis sativus, Daucus carota, Lactuca sativa, Lycopersicon esculentum* and *Raphanus sativus*) were sprayed with a series eight test concentrations of A18385B. Nominal test concentrations used in the definitive test for all test species ranged from 0.2 to 500 g A18385B per hectare.
Plants used for the vegetative growth tests were germinated in seed trays and transplanted shortly after emergence at BBCH growth stage 10. The spray application to the plants was performed at the 2 – 4 true leaf growth stage. The pots were then transferred to a greenhouse.
Observations were made 7, 14 and 21 days after application (DAA) to document mortality and visual phytotoxicity, expressed as a percentage of healthy untreated control plants. Each plant was then assigned a numerical score that described the plant condition. This was a scale from 0 to 100% - a subjective or qualitative assessment that determines whether damage is absent (0%), slight (1 – 39%), moderate (40 – 69%), severe (70 – 99%) or all plants dead (100%). A score of 10 does not mean that 10% of the plant is showing the effect (e.g. chlorosis), merely that the severity of the effect (e.g. chlorosis) is very slight.
The growth of test plants was evaluated at the end of the test (21 DAA) by assessing height and biomass. Plant biomass was estimated by measuring the total dry weight of the shoots within each replicate. Plant height was measured with a ruler to the nearest whole centimetre from the surface of the soil to the tip of the tallest leaf. Dead or non-emerged seedlings were assigned a height of 0 cm. Plants were then clipped at soil level, the shoots of all living plants within a replicate were placed in a labelled paper container, dried in an oven, and weighed as a group. Mean height and total replicate biomass were determined for each treatment group.

Results and Discussion

Statistical analyses were used to evaluate effects of test substance application on plant emergence, height, biomass, and survival. Least square difference (LSD) was used for calculating analysis of variances of means. Effect rates (i.e. ER_{50}) and their confidence limits were determined using the maximum likelihood estimation method. The No Observed Effect Rate (NOER) is the highest concentration at which no statistically significant adverse effect was observed ($p \leq 0.05$) when compared to the control.
The NOER and ER_{50} for each of the ten test species are presented in tables below:

Table A 32 Effect Rates of A18385B + A12127R on 21-Day Final Foliar dry weight and Final Height

Species	Biomass (g A18385B/ha)			Final Height (g A18385B/ha)		
	ER_{50}	**95% confidence limits**	**NOER**	**ER_{50}**	**95% confidence limits**	**NOER**
Monocots						
***Avena sativa* (oat)**	119	91.4, 160	16.2	>500	N/A*	5.4
***Lolium perenne* (ryegrass)**	16.6	14.6, 19.0	1.8	236	186, 308	1.8
***Oryza sativa* (rice)**	>500	N/A*	5.4	>500	N/A*	5.4
Dicots						
***Beta vulgaris* (sugar beet)**	20.2	17.5, 23.4	0.6	>500	N/A*	1.8
***Brassica napus* (oilseed rape)**	11.8	10.3, 13.5	1.8	54.0	45.3, 65.0	1.8
***Cucumis sativus* (cucumber)**	80.9	66.4, 100	1.8	92.1	73.3, 118	0.6
***Daucus carota* (carrot)**	6.14	5.10, 7.36	0.2	45.2	36.8, 56.4	1.8
***Lactuca sativa* (lettuce)**	3.27	2.81, 3.79	0.6	138	101, 198	0.6
***Lycopersicon esculentum* (tomato)**	2.39	2.03, 2.78	N.D.**	14.8	12.9, 16.9	0.2
***Raphanus sativus* (radish)**	3.88	3.24, 4.60	0.6	15.3	13.2, 17.8	0.6

*N/A = not applicable
**N.D. = not determined

Validity criteria

The validity criteria for the test were met:

- The control plants did not exhibit any phytotoxic effects
- The was more than 90% survival in the control plants
- The environmental conditions were identical for all the tested species

Conclusions

A foliar application of A18385B plus adjuvant A12127R, at rates up to 500 g A18385B per hectare resulted in ER_{50} values ranging from 2.39 to >500 g A18385B/ha. *Lycopersicon esculentum* (tomato) was the most sensitive species, with an ER_{50} of 2.39 g A18385B/ha based on foliar dry weight with 95% confidence limits of 2.03 to 2.78 g A18385B/ha.

(Bramby-Gunary J., 2013)

A 2.6.2.2 Seedling emergence

The following seedling emergence study with A18385B has not been evaluated as part of the EU assessment of prosulfuron and dicamba. It was evaluated at zonal level for last authorization of A18385B and considered acceptable. For convenience, a full study summary is presented below.

Reference:	KCP 10.6.2/02
Report	Bramby-Gunary J., 2013a, Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) - Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Seedling Emergence and Seedling Growth Test,

	Report No: ACE-12-182, AgroChemex Ltd, Manningtree, United Kingdom. (Syngenta File No. A18385B_10003)
Guideline(s):	OECD Guideline for the Testing of Chemicals. Guideline 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (July 2006).
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	Not relevant

Executive Summary

A pre-emergent application of A18385B plus adjuvant adigor A12127R, at rates up to 500 g A18385B per hectare resulted in ER_{50} values ranging from 2.44 to >500 g A18385B/ha. *Raphanus sativus* (radish) was the most sensitive species, with an ER_{50} of 2.44 g A18385B/ha based on foliar dry weight with 95% confidence limits of 2.02 to 2.93 g A18385B/ha.

Materials

Test material	A18385B Prosulfuron/Dicamba/Nicosulfuron WG (4/40/10)
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.32% w/w Dicamba: 41.0% w/w Nicosulfuron: 10.5% w/w
Description:	Brown granules
Stability of test compound:	Stable under standard conditions.
Reanalysis/expiry date:	30 September 2014
Treatments	
Test concentrations:	0.2, 0.6, 1.8, 5.4, 16.2, 48.6, 145.8, 500 g A18385B/ha
Control:	Water only
Spray volume:	200L/ha ± 10%
Adjuvant:	Adigor (A12127R) (mixture of emulsified fatty acid esters on basis of oleic acid methylester). The ratio of A18385B to A12127R was 1: 3 (A18385B: A12127R). Results are expressed in terms of the test item containing the adjuvant in this ratio
Application method:	Mardrive cabinet track sprayer with 8004E TeeJet flat fan nozzle
Test organisms	
Species:	*Avena sativa* (oat) *Lolium perenne* (ryegrass) *Oryza sativa* (rice) *Beta vulgaris* (sugar beet) *Brassica napus* (oilseed rape) *Cucumis sativa* (cucumber) *Daucus carota* (carrot) *Lactuca sativa* (lettuce) *Lycopersicon esculentum* (tomato) *Raphanus sativus* (radish)
Test soil:	Sandy loam mixed as follows: 20 litres of sterile loam + 10 litres of sand. The soil was determined to consist of 75% w/w sand (2.00 – 0.063mm), 8% silt w/w (0.063 – 0.002 mm), 17% w/w clay (<0.002 mm). The organic carbon content was 1.3% w/w.
Test design	
Test vessels:	Non-porous plastic pots (8 x 8 x 8 cm) placed in saucers filled with enough water to ensure that the pots were kept moist at all times
Sampling interval:	Plants were assessed at 7, 14 or 15 and 21 days after 50% emergence in controls

	for emergence, mortality and visual phytotoxicity. Biomass and height were assessed at test termination
Replication:	Five pots per treatments, four plants per pot
Duration:	21 days after 50% emergence in the controls
Environmental conditions	
Test temperature:	8.1 – 23.4ºC (Mean; 18.2ºC)*
Humidity:	51.4 – 100% (Mean; 76.7%)*
Soil pH:	7.4
Lighting:	Ambient lighting was supplemented by sodium vapour lamps giving at least a 16 hour day. The mean ambient light intensity for the study period was 6.0 kilo lux (Kl), and the maximum intensity was 38.5 Kl

*The temperature fell below the range specified in the study plan on a few occasions and the humidity rose above the range specified in the study plan on a few occasions; however the plants were healthy and grew well. This is a minor deviation, which had no impact on the study.

Study Design and Methods

Experimental dates: 11th October 2012 to 26th November 2012
Planted seeds of three monocot species (*Avena sativa, Lolium perenne and Oryza sativa*) and seven dicot species (*Beta vulgaris, Brassica napus, Cucumis sativus, Daucus carota, Lactuca sativa, Lycopersicon esculentum* and *Raphanus sativus*) were sprayed with a series of eight test concentrations of A18385B alongside a control (water only). Nominal test concentrations used in the definitive test for all test species ranged from 0.2 to 500 g A18385B per hectare.
For the seedling emergence and growth test, the test seeds were sown directly into the pots. The surface applications of the test treatments were made immediately after sowing. The pots were allowed to dry and then transferred to a greenhouse.
Observations were made 7, 14 or 15 and 21 days after 50% emergence in controls to document seedling emergence, mortality and visual phytotoxicity. Plant height was recorded at the final assessment. Plant condition was described by noting the presence or absence of possible signs of phytotoxicity such as chlorosis, leaf distortion and stunting. Each plant was then assigned a numerical score that described the plant condition. This was a scale from 0 to 100% - a subjective or qualitative assessment that determines whether damage is absent (0%), slight (1 – 39%), moderate (40 – 69%), severe (70 – 99%) or all plants dead (100%). A score of 10 does not mean that 10% of the plant is showing the effect (e.g. chlorosis), merely that the severity of the effect (e.g. chlorosis) is very slight.
The growth of emerged seedlings was evaluated at the end of the test (21 days after 50% emergence in controls) by assessing the height and biomass of seedlings. Plant biomass was estimated by measuring the total dry weight of the shoots within each replicate. Seedling height was measured with a ruler to the nearest whole centimetre from the surface of the soil to the tip of the tallest leaf. Dead or non-emerged seedlings were assigned a height of 0 cm. Seedlings were then clipped at soil level; the shoots of all living seedlings within a replicate were placed in a labelled paper container, dried in an oven, and weighed as a group. Mean seedling height and replicate biomass were determined for each treatment group containing living seedlings at test termination.

Results and Discussion

Statistical analyses were used to evaluate effects of test substance application on plant emergence, height, biomass, and survival. The descriptive statistics for calculating Analysis of Variance Means were Least Significant Difference with 5% significance level. The 50% effect rate (ER_{50}) values were calculated using audited mean values of final height, final emergence and dry weight pre-treatment using simple probit-maximum likelihood estimation method with 95% confidence level. The NOER is the highest concentration at which no statistically significant adverse effect was observed ($p<0.05$) when compared to the control.
The NOER and ER_{50} for biomass, height and emergence for each of the ten test species are presented in the tables below:

Table A 33 Effect Rates of A18385B + A12127R on final emergence

Species	Final emergence (g A18385B/ha)		
	ER_{50}	**95% confidence limits**	**NOER**
Monocots			
***Avena sativa* (Oat)**	>500	N/A*	500
***Lolium perenne* (Ryegrass)**	>500	N/A*	145.8
***Oryza sativa* (Rice)**	>500	N/A*	500
Dicots			
***Beta vulgaris* (Sugar beet)**	>500	N/A*	500
***Brassica napus* (Oilseed rape)**	85.5	66.0, 114	5.4
***Cucumis sativus* (Cucumber)**	>500	N/A*	500
***Daucus carota* (Carrot)**	>500	N/A*	500
***Lactuca sativa* (Lettuce)**	>500	N/A*	500
***Lycopersicon esculentum* (Tomato)**	>500	N/A*	500
***Raphanus sativus* (Radish)**	>500	N/A*	500

N/A* = not applicable

Table A 34 Effect Rates of A18385B + A12127R on 21-Day Biomass and Final Height

Species	Biomass (g A18385B/ha)			Final Height (g A18385B/ha)		
	ER_{50}	**95% confidence limits**	**NOER**	**ER50**	**95% confidence limits**	**NOER**
Monocots						
***Avena sativa* (Oat)**	>500	N/A*	500	>500	N/A*	145.8
***Lolium perenne* (Ryegrass)**	32.0	26.2, 39.5	16.2	72.8	62.3, 85.7	5.4
***Oryza sativa* (Rice)**	51.1	40.6, 65.8	16.2	227	170, 315	48.6
Dicots						
***Beta vulgaris* (Sugar beet)**	10.6	9.47, 11.9	1.8	14.4	12.8, 16.3	1.8
***Brassica napus* (Oilseed rape)**	2.49	2.19, 2.84	0.6	7.95	7.04, 8.98	0.6
***Cucumis sativus* (Cucumber)**	23.8	19.1, 30.1	1.8	71.6	61.1, 84.7	1.8
***Daucus carota* (Carrot)**	13.1	11.2, 15.3	5.4	40.3	35.3, 46.3	5.4
***Lactuca sativa* (Lettuce)**	12.4	10.8, 14.3	5.4	39.3	34.6, 44.8	5.4
***Lycopersicon esculentum* (Tomato)**	5.18	4.45, 6.02	5.4	36.4	30.5, 43.7	5.4
***Raphanus sativus* (Radish)**	2.44	2.02, 2.93	1.8	3.65	2.87, 4.59	1.8

*N/A = not applicable

Validity criteria

The validity criteria for the test were met:

- There was at least 70% emergence in the controls
- The control seedlings did not exhibit any phytotoxic effects
- The mean survival of the emerged control seedlings was at least 90%
- The environmental conditions were identical for all the tested species
-

Conclusions

A pre-emergent application of A18385B plus adjuvant A12127R, at rates up to 500 g A18385B per hectare resulted in ER_{50} values ranging from 2.44 to >500 g A18385B/ha. *Raphanus sativus* (radish) was the most sensitive species, with an ER_{50} of 2.44 g A18385B/ha based on foliar dry weight with 95% confidence limits of 2.02 to 2.93 g A18385B/ha.

(Bramby-Gunary J, 2013a)

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.6.4 KCP 10.6.4 Semi-field and field tests on non-target plants

The following higher tier field vegetative vigour study with A18385B is new as it has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba. It was also not evaluated at zonal level for last authorization of A18385B.

Comments of zRMS:	The study was conducted to modifaied OECD guideline 227 and according to the principles of GLP. In the definitive test all the validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6.4/01
Report	Dickinson R.A. (2015): Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Vegetative Vigour Test in a higher tier field study. Report No ACE-14-062 AgroChemex Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF, United Kingdom Syngenta file No A18385B_10378
Guideline(s):	The methodology employed was based on OECD guideline Test No. 227 and was adapted for a field situation.
Deviations:	Not applicable
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Executive Summary

A foliar application of A18385B, at rates up to 81 g a.i. per hectare (equivalent to 243 ml a.i./ha) resulted in ER_{50} values ranging from 14.7 to >81 g/ha.5 species were exposed to a negative control and 5 application rates of the test substances. The most sensitive species tested was tomato with a Day 25 ER_{50} value of 14.7 g A18385B/ha for foliar fresh weight.

Materials

Test material	A18385B Prosulfuron/Dicamba/Nicosulfuron
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.38 % w/w (43.8 g/kg) Dicamba: 41.1% w/w (411 g/kg)

	Nicosulfate: 10.5 % w/w (105 g/kg)
Description:	Brown solid
Stability of test compound:	Stable under test conditions.
Reanalysis/expiry date:	September 2015
Adjuvant:	Adigor (A12127R)
Treatments	
Test concentrations:	1, 3, 9, 27, and 81 g /ha (equivalent to 3, 9, 27, 81, and 243 ml /ha) (spacing factor of 3)
Control:	Water
Spray volume:	200 mL /ha
Application method:	Hand boom sprayer
Test organisms	
Species:	*Brassica napus* (Oilseed rape) *Daucus carota* (Carrot) *Lactuca sativa* (lettuce) *Lycopersicon esculentum* (tomato) *Raphanus sativus* (Radish)
Test soil:	Clay loam
Test design	
Test facility type:	The study was carried out in the field at Aldhams Farm Research station.
Plots:	Plots were either 4 x 1.5 m or 3 x 2 m giving a total area of 6 m2. Treated and untreated plots were separated by at least 1 m. Plots for oilseed rape and lettuce were covered with nets to minimise damage by pests. Example plot diagrams are given in Appendix 5.
Sampling interval:	Days 7, 8, 10, 14, 15, 24, 25, 26, 29, 35, 37, and 39 depending on species all were assessed at least 3 times
Replication:	Five plots per treatment
Duration:	39 days
Environmental conditions	
Average air temperature:	May: -1.3 to 25.7°C June: 5.1 to 27.8 °C July: 5.1to 31.7°C August: 6.6 to 26.9°C September: 4.5 to 27.7°C
Precipitation (monthly sum):	May: 110 mm June:21.3 mm July: 55.6 mm August: 56.1 mm September: 18.0 mm

Study Design and Methods

Experimental dates: 20 May to 05 September 2014
Young plants of 5 species (*Brassica napus*, *Daucus carota*, *Lactuca sativa*, *Lycopersicon esculentum* and *Raphanus sativus*) were sprayed with a series of five test concentrations of A18385B. Nominal test concentrations used in the definitive test for all test species ranged from 1 to 81 g of formulated product per hectare.
On each sampling occasion, plants were sampled from the 3 middle rows of a 1 m section of a 1.5 m sub plot of each plot. The samples were not taken from <0.25 m of each end of the plots. The numbers of plants sampled from each plot were counted.
The sampled plants in one treatment plot were cut at soil level and then weighed (in g). This procedure was repeated for all the treatment plots in the five replicates of a species. Dead plants were not sampled.
Observations were made on days 7, 8, 10, 14, 15, 24, 25, 26, 29, 35, 37, and 39 depending on species all

were assessed at least 3 times with Oilseed rape, Carrot, Lettuce and Tomato being sampled 4 times over the test period.
Biomass (fresh weight) per plant was determined at the final two assessments. Biomass data were used to calculate NOER and ER_{50} values expressed in g A118385/ha for each species.

Results and Discussion

Statistical analysis was carried out by AgroChemex using Agriculture Research Manager (ARM) 8.0 software.

The descriptive statistics for calculating Analysis of Variance (AOV) Means using ARM 8.0 software were Least Significant Difference (LSD) with 5% significance level.
Significant differences in mean fresh weight between treatments are indicated by an asterisk after the mean values ($p \leq 0.05$, LSD).
The 50% Effect Rate (ER_{50}) values were calculated using mean values of fresh weight per treatment and ARM 8.0 software using simple probit – maximum likelihood estimation method with 95% confidence level. The mean values for each treatment group were transformed in ARM then compared to the untreated control.
The No Observed Effect Rate (NOER) was the highest rate of the test item at which no adverse effect was observed. In this test, the rate corresponding to the NOER, had no statistically significant adverse effect ($p \leq 0.05$) when compared with the control.

The NOER and ER_{50} for each of the species are presented in table below:

Table A 35 Effect Rates of A18385B on the vegetative vigour of plant species

Test species (Common name)	**Day No.**	**Fresh weight ER_{50} (g A18385B/ha)**	**Fresh weight NOER (g A18385B/ha)**
Oilseed rape	**24**	*	*
	37	25.0	9
Carrot	**24**	65.0	27
	37	63.1	27
Lettuce	**26**	36.8	9
	35	>81	27
Tomato	**25**	14.7	9
	39	33.6	9
Radish	**15**	ND	9
	29	ND	9

Validity criteria

The test was considered valid based on the following criteria:

- The environmental conditions were identical for all the tested species
- Control seedlings did not exhibit visible phytotoxic effects, plants exhibited only normal variation in growth and morphology for that particular species;

Conclusions
The most sensitive species tested was tomato with a Day 25 ER_{50} value of 14.7 g A18385B/ha for foliar fresh weight.

(Dickinson R.A. 2015)

The following higher tier field seedling emergence study with A18385B is new as it has not been evaluated as part of the EU assessment of prosulfuron, nicosulfuron and dicamba. It was also not evaluated at zonal level for last authorization of A18385B.

Comments of zRMS:	The study was conducted to modifaied OECD guideline 208 and according to the principles of GLP. In the definitive test all the validity criteria were met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6.4/02
Report	Dickinson R.A. (2015a): Prosulfuron/Dicamba/Nicosulfuron WG (A18385B) plus Adigor (A12127R) – Evaluation of the Phytotoxicity to Non Target Terrestrial Plant Seedling Emergence and Seedling Growth in a higher tier field study. Report No ACE-14-061 AgroChemex Ltd., Aldhams Farm Research Station, Dead Lane, Lawford, Manningtree, Essex, CO11 2NF, United Kingdom Syngenta file No A18385B_10377
Guideline(s):	The methodology employed was based on OECD guideline Test No. 208 and was adapted for a field situation.
Deviations:	Not applicable
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Executive Summary

A pre-emergent field application of A18385B, at rates up to 81 g per hectare (equivalent to 234 ml per hectare) resulted in ER_{50} values ranging from 18.0 to >81 g/ha. Three species were exposed to a water control and 5 treatment concentrations. *Lycopersicon esculentum* (tomato) was the most sensitive species, with a 37 day ER50 of 19.3 g/ha based on biomass.

Materials

Test material	A18385B Prosulfuron/Dicamba/Nicosulfuron
Lot/Batch #:	SMU2BP004
Actual content of active ingredients:	Prosulfuron: 4.38 % w/w (43.8 g/kg) Dicamba: 41.1% w/w (411 g/kg) Nicosulfuron: 10.5 % w/w (105 g/kg)
Description:	Brown solid
Stability of test compound:	Stable under test conditions.
Reanalysis/expiry date:	September 2015
Treatments	
Test concentrations:	1, 3, 9, 27, and 81 g /ha (equivalent to 3, 9, 27, 81, and 243 ml /ha) (spacing factor of 3)
Control:	Water
Adjuvent:	A12127R (Adigor)

Spray volume:	200 mL /ha
Application method:	hand boom sprayer
Test organisms	
Species:	*Brassica napus* (Oilseed rape) *Raphanus sativus* (Radish) *Lycopersicon esculentum* (tomato)
Test soil:	Sandy loam and clay loam
Test design	
Test facility type:	Field at Aldhams Farm Research station.
Plots:	3 x 2 m, Treated and untreated plots were separated by at least 1 m. Plots for oilseed rape and radish were covered with nets to minimise damage by pests.
Sampling interval:	7, 14, 23/26, and 37/39 days after treatment
Replication:	Five plots per treatment
Duration:	39 days
Environmental conditions	
Average air temperature:	July: 5.1to 31.7°C August: 6.6 to 26.9°C September: 4.5 to 27.7°C October: 2.7 to 22.4 °C November: -1.1 to 18.8 °C
Precipitation (monthly sum):	July: 55.6 mm August: 56.1 mm September: 18.0 mm October: 15.0 mm November: 6.30 mm

Study Design and Methods

Experimental dates: 09 May to 17 November 2014
Planted seeds of three species (*Brassica napus*, *Raphanus sativus* and *Lycopersicon esculentum*) were sprayed with a series of five test concentrations of A18385B. Nominal test concentrations used in the definitive test for all test species ranged from 1 to 81 g of formulated product per hectare. The number of emerged seedlings was determined at test termination.
For assessments where no sampling for fresh weights occurred the number of plants in each control plot was counted. At sampling, the number of plants sampled was counted for each plot.
Oilseed rape and radish; the number of plants emerged in the 3 middle rows of the control plots were counted and once a minimum of 30 plants per m2 (based on the number of seed sown) was obtained this was considered as day 0 for the growth assessments. At each assessment interval the number of plants emerged in the 3 middle rows of each control plot was counted and expressed as a percentage of the expected. The emergence of the three middle rows in each treated plot was then estimated based on the mean emergence by the controls. At the final assessment (day 39) the number of plants in the remaining sub plot were counted and emergence was estimated based on the day 26 emergence records.
Tomato; the number of plants emerged in the 3 middle rows of the control plots were counted and once a minimum of 50% emergence (based on seeds sown) was obtained this was considered as day 0 for the growth assessments. Emergence was expressed as a percentage of the seeds sown or seeds/seedling remaining (at the day 37 assessment).
The tomato assessment differed from the oilseed rape and radish assessment. This was because the original study methodology required a 70% emergence of the controls for the study to be valid. However after the initial attempts to obtain this were unsuccessful it was necessary to change the study design to a minimum amount of emergence per m2 based on commercial and experimental practices. As the tomato phase of the study was complete and the sowing was within that accepted for commercial outdoor growing, the study was considered acceptable.
The plants were assessed for emergence and visual phytotoxicity expressed as a percentage of healthy untreated control plants.

Results and Discussion

Statistical analysis was carried out by AgroChemex using Agriculture Research Manager (ARM) 8.0 software.

The descriptive statistics for calculating Analysis of Variance (AOV) Means using ARM 8.0 software were Least Significant Difference (LSD) with 5% significance level.
The 50% Effect Rate (ER_{50}) values were calculated using mean values of fresh weight per treatment and ARM 8.0 software using simple probit – maximum likelihood estimation method with 95% confidence level. The mean values for each treatment group were transformed in ARM then compared to the untreated control.
The No Observed Effect Rate (NOER) was the highest rate of the test item at which no adverse effect was observed. In this test, the rate corresponding to the NOER, had no statistically significant adverse effect ($p \leq 0.05$) when compared with the control.
The LOER, NOER, and ER_{50} for each of the species are presented in table below:

Table A 36 Effect Rates of A18385B on Fresh weight

Species	**Fresh weight Day 23/26 (g/ha)**			**Fresh weight Day 37/39 (g/ha)**		
	LOER	**NOER**	**ER_{50}**	**LOER**	**NOER**	**ER_{50}**
Brassica napus (Oilseed rape)	81	27	>81	81	27	>81
Raphanus sativus (Radish)	81	27	>81	81	27	>81
Lycopersicon esculentum (tomato)	27	9	18.0	27	9	19.3

Samples taken on day 26 and 39 for oilseed rape and radish, samples taken on day 23 and 37 for the tomato

Validity criteria

The study is considered valid as:

- Untreated control plants did not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, and wilting, leaf and stem deformation) and the plants exhibited only normal variation in growth and morphology for that particular species.
- Environmental conditions for a particular species were comparable.
- The mean untreated control plant emergence was at least 30 plants per 1 m^2 (oilseed rape and radish).
- There were no emergence validity criteria for tomato.

Conclusions

The most sensitive species tested was tomato with an ER_{50} value for foliar fresh weight of 18.0 g A18385B/ha on day 23 and 19.3 g A18385B/ha on day 37.

(Dickinson R.A, 2015a)

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data