

European Union



**Draft Renewal Assessment Report prepared according to the Commission Regulation
(EU) N° 1107/2009**

FLUFENACET

**Volume 3 – Annex B.9 (PPP) – Flufenacet + Diflufenican 600 SC
ECOTOXICOLOGY**

RMS: Poland

Co-RMS: France

**Summary, evaluation and assessment of the data and information examined and the list of
studies relied upon, annotated as to the period(s) for which the particular studies are
to be protected**

August 2016

Version History

When	What
January 1998	Initial DAR
April 2000	Addendum Ecotoxicology
April 2003	Flufenacet final Addendum Ecotoxicology
August 2016	DRAR

Table of contents

B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISK FOR NON-TARGET SPECIES	5
B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES	9
B.9.1.1. Effects on birds	9
B.9.1.1.1. Toxicity endpoints for birds	9
B.9.1.2. Effects on terrestrial vertebrates other than birds	12
B.9.1.2.1. Toxicity endpoints for terrestrial other than birds	12
B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES	14
B.9.2.1. Risk assessment for birds	15
B.9.2.2. Risk assessment for terrestrial vertebrates other than birds	34
B 9.3. EFFECTS ON AQUATIC ORGANISM	42
B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae macrophytes	48
B.9.3.1.1. Acute toxicity to fish	48
B.9.3.1.2. Acute toxicity to aquatic invertebrates	48
B.9.3.1.3. Effects on algal growth	49
B.9.3.1.4. Effects on aquatic macrophytes	52
B.9.3.2. Additional long term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organism.	57
B.9.3.3. Further testing on aquatic organisms	57
B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISM	57
B.9.5. EFFECTS ON ARTHROPODS	103
B.9.5.1. Effects on bees	113
B. 9.5.1.1. Contact and oral acute toxicity	113
B.9.5.1.2. Other toxicity test on adult bees and bee larvae	118
B.9.5.2. Effects on non-target arthropods other than bees	118
B.9.5.2.1. Toxicity data	118
B 9.5.2.1.1. Standard laboratory testing for non-target arthropods	120
B 9.6. RISK ASSESSMENT FOR ARTHROPODS	141
B 9.6.1. Risk assessment for honeybees	141
B.9.6.2. Risk assessment for non-target arthropods other than bees	151
B.9.7. EFFECTS ON NON TARGET SOIL MESO-AND MACROFAUNA	157
B.9.7.1. Earthworms	157
B.9.7.1.1. Toxicity data	157
B.9.7.2. Effects on non-target soil meso-and macrofauna (other than earthworms)	184
B.9.7.2.1. Toxicity data	184
B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO-AND MACROFAUNA	192
B.9.8.1. Risk assessment for earthworms	192
B. 9.8.2. Risk assessment for non-target soil meso- and macrofauna (other than earthworms)	196
B 9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION	201
B 9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION	205
B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS	207
B.9.11.1. Summary of screening data	207
B.9.11.2. Testing on non-target plants	207
B.9.11.3. Extended laboratory studies on non-target plants	251
B.9.11.4. Semi-field and field tests on non-target plants	251
B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS	252
B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)	261

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISM (FLORA AND FAUNA)	261
B.9.15. REFERENCES RELIED ON	262

B.9. ECOTOXICOLOGY DATA AND ASSESSMENT OF RISK FOR NON-TARGET SPECIES

Flufenacet is a herbicide that was authorised for the use in the EU by its inclusion into the Annex I of the Council Directive 91/414/EEC (Commission Directive 2003/84/EC of 25 September 2003) in 2003 as entry No. 65. That authorisation entered into force from 1st January 2004 and was due to expire on the 31st December 2013. When the Directive 91/414/EEC was repealed by Council Regulation 1107/2009 of 21st October 2009, the authorisation of flufenacet in the EU was granted by its listing, as entry No. 65, in the Part A of the Annex to the Commission Implementing Regulation (EC) 540/2011, expiring on 31st December 2013. That authorisation period was further extended to 31st October 2016 by means of the Commission Regulation (EC) No. 823/2012 of 14th September 2012.

The evaluation was based on the Draft Assessment Report prepared by the Rapporteur Member State – France, in August 1997 and Addenda to it on the basis of the documentation submitted by the Applicant – Bayer Crop Sciences, identified in course of the evaluation as a sole Applicant for flufenacet. In support of the inclusion of flufenacet into the Annex I of the Council Directive 91/414/EEC a Review report for the active substance flufenacet (Flufenacet 7469/VI/98-final, 3 July 2003) was issued, summarising the results of the evaluation and providing the EU-agreed List of the EndPoints for this active substance.

For the purpose of the current evaluation, aimed on the renewal of the approval of flufenacet in the EU, the Applicant provided a Dossier consisting of, in section B.9. – Ecotoxicology, old studies, already evaluated for the previous approval for use of flufenacet in the EU, and new studies, updating the dataset for flufenacet.

This renewal assessment report (RAR) contains summaries of studies on flufenacet, which were not available at the time of the Annex I inclusion under Directive 91/414/EEC and were, therefore, not evaluated during the first EU review of this compound. In addition, all studies, which were already submitted for the Annex I inclusion under Directive 91/414/EEC, were re-evaluated according to the current valid test guidelines and were summarized in the RAR (study title is greyed out).

Studies which were submitted for the first EU peer-review of the active substance flufenacet but are no longer a data requirement according to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) are briefly summarized (text in italic).

For the first inclusion of flufenacet into the Annex I the Applicant proposed the representative GAP comprising following uses:

- in Maize (Corn) to suppress annual grass weeds, pre-emergence, once per season in application rate 480 – 600 g/ha, in Northern and Southern European Countries;
- in Soybean and Sunflower, to suppress annual grass weeds, pre-emergence, once per season in application rate 480 – 600 g/ha, in Southern European Countries;
- in Winter Cereals (wheat, barley, rye and triticale), to suppress annual grass weeds, early post-emergence in autumn (at 2nd leaf stage of grass weeds), once per season in application rate 120 – 240 g/ha, in Northern and Southern European Countries; in Northern and Southern Europe.

The representative formulation was FOE 5043 WG 60, containing 60% of flufenacet.

For the present evaluation the Applicant proposed the revised representative GAP, limited the intended uses to those suppressing annual weeds in cereals, pre-emergence and early post emergence (range of BBCH 00-22) in autumn and at early spring.

It shall be noted that, unlike for the previous assessment, the Applicant proposed the representative formulation code named DFF+FFA SC 600, containing 400 g/L flufenacet and 200 g/L diflufenican. The proposed trade names are Herold SC (to be used in both Northern and Southern European countries), Fosburi (to be used only in NE climatic zone) and Firebird (to be used in SE climatic zone).

For the Annex I listing process of diflufenican also the formulation Flufenacet + Diflufenican SC 600 (DFF+FFA SC 600, Herold SC 600) was submitted as representative formulation. Hence, some formulation studies (e.g. on non-target arthropods and non-target terrestrial plants) were already evaluated during this Annex I listing process.

With the present RAR only flufenacet is under evaluation and not the mixing partner diflufenican. Hence, missing studies on diflufenican according to regulation (EC) 1107/2009 do not influence the evaluation of the active ingredient under consideration. In most cases studies on the mixture formulation will be available.

Ecotoxicological endpoints used in the risk assessment for active substance-flufenacet were derived from studies with the formulated product – representative formulation Flufenacet + Diflufenican SC 600, the active substance flufenacet and the metabolites listed in the residue definition for risk assessment.

In some cases, a solo formulation of flufenacet, Flufenacet 500 SC (containing 500 g a.s./L), Flufenacet 60 WG (containing 600 g a.s./L), Flufenacet 508.8 SC (containing 508.8 g s.a./L) were used to address the intrinsic toxicity of flufenacet.

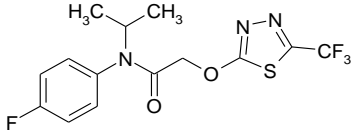
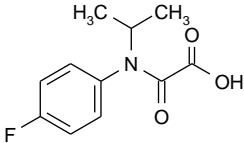
That new representative GAP, used in the evaluation in the area of ecotoxicology risk assessment, is presented below in the table B.9.0-1. For clarity reasons the application rates for the second active substance of the EU-representative formulation – diflufenican, were not reported in this table.

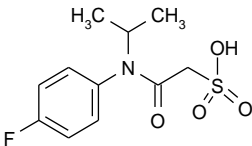
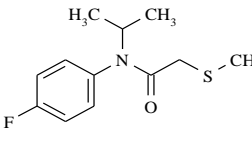
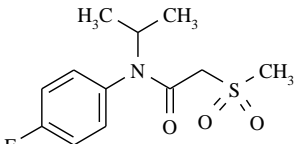
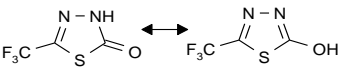
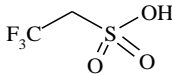
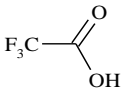
Table B.9.0-1: The proposed updated representative GAP for flufenacet.

Region	Crop	Product name	Data on application						
			Type of application	Number of Applic.	Interval between applications	Application time		Application rate - flufenacet [g/ha]	Spray volume [L/ha]
						Period	Crop's growth stage (BBCH)		
North EU	Cereals (winter wheat, winter barley, winter rye)	Herold SC (400 g/L flufenacet + 200 g/L diflufenican)	Foliar spraying	1	Not applicable – single application	Early post-emergence, Autumn only;	10-13	240	200 – 400
South EU	Cereals (wheat, winter barley)	Fosburi (400 g/L flufenacet + 200 g/L diflufenican)	Foliar spraying	1	Not applicable – single application	Early post-emergence	11-13	240	80 – 400
North EU	Cereals (winter wheat, winter barley, winter rye)	Firebird (400 g/L flufenacet + 200 g/L diflufenican)	Foliar spraying	1	Not applicable – single application	Pre-emergence and early post-emergence	00-22	120	200 – 400
South EU	Cereals (wheat, barley)	Herold SC (400 g/L flufenacet + 200 g/L diflufenican)	Foliar spraying	1	Not applicable – single application	Early post-emergence	11-13	160	200 – 400

Metabolites which require ecotoxicological assessment are listed in the table B.9.0-2.

Table B.9.0-2: Flufenacet and its metabolites which require ecotoxicological assessment.

No.	Structure, Report Name	Synonyms, Codes, Chemical Name	Synonyms, Codes, Chemical N
a.s.	 <p>flufenacet (active substance)</p>	FOE 5043, AE F133402, BCS-AB27364 CAS: N-(4-fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy] acetamide	Soil, water, sediment, air
M01	 <p>FOE oxalate</p>	FOE 5043-oxalate FOEOXALATE, FOEACID OXALATE AE 0841913 BCS-AB16305 flufenacet-oxalate CAS: Acetic acid, 2-[(4-fluorophenyl)(1-methylethyl) amino]-2-oxo-	Soil (major), water and sediment (minor) Goat, hen Corn, soybean, cotton, wheat; Rotational crops: kale, turnip, wheat

No	Structure, Report Name	Synonyms, Codes, Chemical N	Synonyms, Codes, Chemical N
M02 ^a	 <p>FOE sulfonic acid</p>	FASO3H AE 0841914 KTS 9465 (sodium salt) BCS-AZ23374 (sodium salt) WAK 6222 (acid) ethanesulfonic acid sodium salt CAS: sodium salt: (2-[(4-fluorophenyl)(1-methylethyl)amino]-2-oxo-ethanesulfonic acid sodium salt)	Soil (major), water and sediment (minor)
			Rat
			Corn, soybean, wheat, potato, cotton; Rotational crops: kale, turnip, whea
M05	 <p>FOE methylsulfide</p>	WAK 7825 FAMS BCS-CP38571 IUPAC: N-(4-fluorophenyl)-N-isopropyl-2-(methylsulfanyl)acetamide (generated by ICS Naming)	Water and sediment (major)
			Corn
			Goat, hen
M07	 <p>FOE methylsulfone</p>	FAMS02 FOE methyl-sulfone BCS-CO62475 IUPAC: N-(4-fluorophenyl)-N-isopropyl-2-(methylsulfonyl)acetamide (generated by ICS Naming)	Soil (major), water and sediment (minor)
			Corn, soybean; Rotational crops: kale wheat
			Rat
M09	 <p>FOE-thiadone (keto-enol tautomers)</p>	Thiadone TH HWH 4343 BCS-AA41715 IUPAC: 5-(trifluoromethyl)-1,3,4-thiadiazol-2(3H)-one, 5-(trifluoromethyl)-1,3,4-thiadiazol-2-ol (generated by ICS Naming)	Soil (major), water and sediment (major)
			Rat (detected as aglycon and as glucuronide (M24) and oxalyl-acetate conjugates (M26); Goat, hen 4)
			Corn, soybean; Rotational crops: transient metabolite as it is detected as N-glucoside (M25) and N-malonyl-alanine conjugate (M3)
M44 ^a	 <p>FOE 5043-trifluoroethanesulfonic acid</p>	TFESA BCS-CU62474 (sodium salt) IUPAC: 2,2,2-trifluoroethanesulfonic acid	Soil (major),
M45	 <p>trifluoroacetic acid</p>	TFA AE C502988 (acid) AE1046319 (sodium salt) BCS-AZ56567 (sodium salt) IUPAC: trifluoroacetic acid	Soil (major)
			Soil (aerobic & anaerobic) Rotational crops: turnip, Swiss chard, wheat (main metabolite in all rotated crops)
			Rat

B.9.1. EFFECTS ON BIRDS AND OTHER TERRESTRIAL VERTEBRATES**B.9.1.1. Effects on birds****B.9.1.1.1. Toxicity endpoints for birds**

The acute, dietary and reproductive toxicity studies with flufenacet have been performed with mallard duck and bobwhite quail. Studies were already submitted for the first EU peer-review of the active substance flufenacet. Two new acute studies one for Canary (*Serinus canaria*) and another one for Mallard duck (*Anas platyrhynchos*) with the active substance - flufenacet were submitted for the renewal of the EU peer-review. The study summaries are provided under point B.9.1.1 of Volume 3 (CA).

A summary of the toxicity of flufenacet to birds is given in table B.9.1.1.1-1.

Table B.9.1.1.1-1: Summary of toxicity flufenacet to birds.

Test species	Test design	Ecotoxicological endpoint			Reference
Bobwhite quail (<i>Colinus virginianus</i>)	acute, oral	LD ₅₀	1608	mg a.s./kg bw	██████ (1992) M-003866-01-1
Mallard duck (<i>Anas platyrhynchos</i>)		LD ₅₀	> 2000	mg a.s./kg bw	██████ (1997) M-003851-01-1
Canary (<i>Serinus canaria</i>)		LD ₅₀	434	mg a.s./kg bw	██████ 2013 M-468210-01-1 KCA 8.1.1.1/03
Bobwhite quail (<i>Colinus virginianus</i>)	5-day dietary	LC ₅₀ LDD ₅₀	> 5317 > 755	ppm mg a.s./kg bw/d	██████ (1994) M-003859-0 -1
Mallard duck <i>Anas platyrhynchos</i>		LC ₅₀ LDD ₅₀	> 4970 > 949	ppm mg a.s./kg bw/d	██████ (1993) M-003864-01-1
Bobwhite quail <i>Colinus virginianus</i>)	22-weeks feeding, reproduction	NOEC	441	ppm	██████ (1994) M-003861-01-1
		NOEL	34	mg a.s./kg bw/d	
Mallard duck (<i>Anas platyrhynchos</i>)	21-weeks feeding, reproduction	NOEC	88	ppm	██████ (1994) M-003858-01-1
		NOEL	9.4	mg a.s./kg bw/d	

In the bold: Endpoints used in the regulatory risk assessment

Diflufenican:

The results of the studies carried out with the mixing partner diflufenican are summarised in the Table B.9.1.1.1-2.

Table B.9.1.1.1-2: Critical avian toxicity endpoints for diflufenican.

Test substance	Test species	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84	
		LD ₅₀	>2150 mg as/kg bw
Diflufenican	'Bird' acute, oral		
	Bobwhite quail, reproduction	NO(A)EL	91.84 mg as/kg bw/d

Toxicity of the formulated product

Toxicity studies for birds with formulated product DFF+FFA 600 SC is not available.

According to recommendations given in the EFSA GD 2009, step 1 in Appendix 2, the acute surrogate LD₅₀ is calculated for the mixture of active substances with known toxicity assuming additivity according to following equation:

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

With:

$X(a.s._i)$ = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(a.s._i)$ must be 1)

$LD_{50}(a.s._i)$ = acute toxicity value for active substance [i]

The active substance content of the formulation DFF+FFA 600 SC is 32.5% flufenacet and 16.4% diflufenican, making up a total of 48.9% product. The Table B.9.1.1.1-3 and B.9.1.1.1-4 shows the calculation of the predicted LD₅₀ (mix) of flufenacet and diflufenican when mixed in these proportions (step 1 in Appendix 2 to the EFSA GD 2009).

Table B.9.1.1.1-3: Avian LD_{mix} for Flufenacet and diflufenican when combined as DFF+FFA SC 600.

	Flufenacet	Diflufenican
Content in the formulation DFF+FFA 600 SC	32.5%	16.4%
Fraction in the a.s. mixture	32.5 / 48.9 = 0.67	16.4 / 48.9 = 0.33
LD ₅₀ of a.s. [mg/kg bw]	434*	2150*
Fraction / LD ₅₀	0.67 / 434 = 0.0015	0.33 / 2150 = 0.00015
Sum	0.00165	
1/ sum = predicted LD ₅₀ (mix)	606 mg mix/kg bw	

*based on the lowest LD₅₀ values: for Canary (Flufenacet) and for Mallard duck (Diflufenican, EFSA Scientific Report (2007) 122, 1-84)

The surrogate value of LD₅₀ for (mix) is estimated to be 606 mg mix/kg bw.

EFSA guidance (2009) recommended (STEP 1 in Appendix B) to check tox per fraction. Calculation of the “tox per fraction” provides information if one active ingredient dominates the acute toxicity of a formulation so that further assessments of the mixture toxicity would not be indicated.

Based on the guidance provided in EFSA (2009) for comparison of single active substance and mixture toxicity in terms of potential risk, the following “tox per fraction” results were obtained for flufenacet:

According to this formula the sum of the active substances must be 1.

Hence for the formulation DFF+FFA 600 SC we considered a fraction of 1. The calculation of the deviation below was performed by „tox per fraction a.s.“/ (“tox per fraction mix”/100)) – 100 = deviation in %.

The result gives the deviation of the “a.s. tox per fraction” in % from the “mix tox per fraction”; the “flufenacet tox per fraction” of 648 is 106.9 % of the “mix tox per fraction” of 606 for DFF + FFA, resulting in a deviation of +7% for flufenacet from the “mix tox per fraction”.

Table B.9.1.1.1-4: The deviation of the a.s.tox per fraction in % from the mix tox per fraction.

Substance	Toxicity	Fraction	Tox per fraction	Deviation
Birds				
Mix DFF + FFA	LD ₅₀ mix = 606	1	606	
FFA	LD ₅₀ = 434	0.67	648	+7%
DFF	LD ₅₀ = 2150	0.33	6515	+1075%

¹ The calculation provided by The Applicant

For birds the deviation of the tox per fraction for flufenacet from the tox per fraction of the mixture was below 10%, indicating that flufenacet contributes to more than 90% to the toxicity of the formulation. Therefore, the risk for mixture is covered by flufenacet. An assessment of mixture toxicity is not indicated.

B.9.1.2. Effects on terrestrial vertebrates other than birds**B.9.1.2.1. Toxicity endpoints for terrestrial other than birds**

Flufenacet has been tested for potential acute and long-term toxicity for rat, mouse and rabbit.

The new acute, dietary and developmental studies to mammals were submitted for trifluoroacetate metabolite (TFA, M45) for the renewal of active substance flufenacet. All studies were evaluated in the Section Toxicology.

The key endpoints from these studies are presented in the Table B.9.1.2.1-1 and Table B.9.1.2.1-2.

Table B.9.1.2.1-1: Toxicity of flufenacet to mammals.

Test species	Study	Toxicity endpoints		Reference
Flufencet				
Rat	Acute oral	LD ₅₀	♂1611 ♀589 mg a.s.g bw ¹	<div></div> (1993) M-004865-02-1 and M-004865-02-1
Rat	Two-generation reproduction	NOAEL	500 ppm 37.4 mg a.s./kg bw/d ² <u>Parental:</u> There was compound related reduction in body weight for P generation females during the pre mating phase in the P and F generation adults increased absolute and relative liver weights and histopathological changes in the liver at 37.4 mg a.s./kg bw. No effects on reproduction and litter parameters.	<div></div> (1995) M-004984-03-1

Bold values: Endpoints used for TER calculations for screening Step and Tier 1.

1) The endpoint included in the LoEPs (2017), Section Toxicology.

2) The ecotoxicological relevant NOAEL was discussed in the Vol.3 (CA), B9, under Point B.9.1.2.2.

Table B.9.1.2.1-2: Toxicity of metabolite TFA to mammals.

Test species	Study	Toxicity endpoints		Reference
TFA				
Rat	acute oral	LD ₅₀	> 2000 mg met /kg bw	██████████ (2013) M-444479-01-1
Rat	90 days dietary	NOAEL _{ecotox}	1600 ppm ¹ (♂ 98, ♀ 123 mg met/kg bw/d) Based on the lack of both body weight decrease and other toxic effects at 1600 ppm The liver weight ↑	██████████ (2007) M-283994-01-1 KCA 5.8.1/27 Evaluated by Diesing (2014)

Bold values: Endpoints used for TER calculation

¹ Ecotoxicological relevant NOAEL of 98 mg met/kg bw derived from administered dose of 1600 ppm (evaluated by Diesing, 2014, M477154-01-1, please refer to Vol. 3. B.9 (CA), Point B.9.1.2.2.)

Diflufenican**Table B.9.1.2.1-3: Toxicity of mixing partner diflufenican to mammals**

Test substance	Test species	EU agreed endpoints (EFSA Scientific Report (2007) 122, 1-84)	
Diflufenican	Rat acute, oral	LD ₅₀	> 5000 mg a.s./kg bw
	Rat reproduction	NOAED	35.5 mg a.s./kg bw/d

Toxicity data for mammals exposed to DFF+FFA 600 SC are presented in the Table B.9.1.2.1-4 below.

Table B.9.1.2.1-4: Toxicity for representative formulation DFF+FFA 600 SC

Test species	Test design	Ecotoxicological endpoint [mg product/kg bw]	Reference
Rat	acute, oral	500 < LD ₅₀ < 2000	██████████, 2002 M-055334-01-1

In addition, based on the recommendations given in the EFSA GD 2009, step 1 in Appendix 2, the acute surrogate LD₅₀ was calculated for the mixture of active substances with known toxicity, assuming additivity according to following equation :

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

With:

X(a.s._i) = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(a.s._i)$ must be 1)

LD₅₀(a.s._i) = acute toxicity value for active substance [i]

The active substance content of the formulation DFF+FFA 600 SC is 32.5% flufenacet and 16.4% diflufenican, making up a total of 48.9% product. Table B.9.1.2.1-5 shows the calculation of the predicted LD₅₀ (mix) of flufenacet and diflufenican when mixed in these proportions (step 1 in Appendix 2 to the EFSA GD 2009).

Table B.9.1.2.1-5: Mammals LD_{mix} for Flufenacet and Diflufenican when combined as DFF+FFA SC 600

	Flufenacet	Diflufenican
Content in the formulation DFF+FFA 600 SC	32.5%	16.4%
Fraction in the a.s. mixture	32.5 / 48.9 = 0.67	16.4 / 48.9 = 0.33
LD ₅₀ of a.s. [mg/kg bw]	589*	5000*
Fraction / LD ₅₀	0.67 / 589 = 0.0011	0.33 / 5000 = 0.000066
Sum		
1/ sum = predicted LD ₅₀ (mix)	833 mg /kg bw	

*on based the lowest LD₅₀ values for rats (Flufenacet) and (Diflufenican, EFSA Scientific Report (2007) 122, 1-84)

Calculation of the “tox per fraction” provides information if one active ingredient dominates the acute toxicity of a formulation so that further assessments of the mixture toxicity would not be indicated.

Based on the guidance provided in EFSA GD for birds and mammals (2009) for comparison of single active substance and mixture toxicity in terms of potential risk, the following “tox per fraction” results were obtained for flufenacet: According to this formula the sum of the active substances must be 1. Hence for the formulation DFF+FFA we considered a fraction of 1.

The calculation of the deviation below was performed by „tox per fraction a.s.“/ (“tox per fraction mix”/100)) – 100 = deviation in %. The result gives the deviation of the “a.s. tox per fraction” in % from the “mix tox per fraction”; the “flufenacet tox per fraction” of is 105.53 % of the “mix tox per fraction” of 833 for DFF + FFA, resulting in a deviation of +5.5 % for flufenacet from the “mix tox per fraction”.

Table B.9.1.2.1-6: Deviation of the tox fraction.

Substance	Toxicity	Fraction	Tox per fraction	Deviation
Mix DFF & FFA 600 SC	LD _{50 mix} = 833	1	966	
FFA (Flufenacet)	LD ₅₀ = 589	0.67	879	+5.5%
DFF (Di-flufenican)	LD ₅₀ = 5000	0.33	15151.5	+1818,9%

For mammals the deviation of the tox per fraction for flufenacet from the tox per fraction of the mixture was below 10%, indicating that flufenacet contributes to more than 90% to the toxicity of the formulation. Therefore, the risk for mixture is covered by flufenacet. An assessment of mixture toxicity is not indicated.

B.9.2. RISK ASSESSMENT FOR BIRDS AND OTHER TERRESTRIAL VERTEBRATES

The risk assessment for birds and mammals was conducted according to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (EFSA Journal 2009;7 (12): 1438).

Intended application pattern

The use pattern for this formulation is summarised in Table B.9.2-1.

Table B.9.2-1: The use pattern of DFF+FFA 600 SC.

Crop	Timinig of application BBCH	Number of application	Maximum rate [L product/ha]	Maximum application rate, individual treatment [g a.s./ha]*
Winter cereals	00-22	1	0.3	120
Winter cereals	11-13	1	0.4	160
Winter cereals	10-13	1	0.6	240

* a.s.-Flufenacet

B.9.2.1. Risk assessment for birds**Toxicity**

The active substance endpoints used in the risk assessment for birds are shown in the Table B.9.1.1.1-1.

The lowest value of LD₅₀ 434 mg a.s./kg bw from the acute toxicity study for Canary (*Serinus canary*) was used in the acute risk assessment. The values of LC₅₀ derived from the short-term dietary toxicity studies performed with *Colinus virginianus* and Mallard duck were above the highest tested rate, and determination of an exact LC₅₀ value was not possible. Available data do not indicate that flufenacet would be more toxic to birds via dietary route of exposure.

Consideration of reproductive endpoints for flufenacet used in the risk assessment

The LD₅₀/10 is considered as an endpoint in the reproductive assessment to take into account 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 (see Appendix J – Phase-specific approach for reproductive risk assessment of EFSA Guidance Document). For the screening assessment, the lowest of either the LD₅₀/10 or the NOEL from the avian reproduction studies should be considered as worst-case toxicity endpoint. For flufenacet, the LD₅₀/10 (43.4 mg a.s./kg bw/day) exceeds the NOEL of 9.4 mg a.s./kg bw/day from the reproductive study for mallard duck. Consequently, the NOEL is used as relevant worst-case endpoint in the risk assessment.

Flufenacet metabolites

In plants flufenacet is degraded via glutathione conjugation resulting in metabolites containing either the fluorophenyl acetamide-or the thiadone-moiety of the parent compound.

Plant metabolite studies in wheat performed in Section Residues (please refer to Volume 3. CA, Section B.7) indicated two plant metabolites which exceed 10% of radioactive residues in edible crop parts in wheat: FOE-oxalate (M01), with fluorophenyl moiety and trifluoroacetate (TFA, M45) which is formed by break-down of thiadone (M09).

For these metabolites no toxicity endpoints to birds are available.

The weight of evidence approach for these metabolites was performed by RMS based on information on toxicity of these metabolites provided in the Section Toxicology. Additionally for RMS's request Applicant performed the relevant calculations of exposure of TFA metabolite to birds based on residue studies carried out in primary and rotation crops.

Exposure

Exposure of birds was calculated according to following equations:

$$\text{DDDA [mg/kg bw]} = \text{application rate [kg/ha]} \times \text{shortcut value (SV)} \times \text{MAF}_{90}$$

$$\text{DDDLT [mg/kg bw]} = \text{application rate [kg/ha]} \times \text{shortcut value (SV)} \times \text{ftwa} \times \text{MAF}_{\text{mean}}$$

Where:

DDD daily dietary dose

FIR/bw food intake rate related to body weight

Ftwa time weighted average factor (long-term considerations; default value: 0.53)

MAF₉₀ Multiple Application Factor for acute risk assessment, 90th percentile

MAF_{mean} Multiple Application Factor for long-term risk assessment, mean

Evaluation is performed in a stepwise approach, starting from screening step which considers worst case exposure assumptions and enables identification of substances that do not require further consideration.

Acute risk assessment

Screening step

At the screening step evaluation is performed for “indicator species”, which due to its size and feeding habits, is considered to have higher exposure than other species that occur in a particular crop at particular time.

The lowest toxicity endpoint LD₅₀ of 434 mg a.s./kg b.w. was used in the acute risk assessment.

Relevant birds indicator species identified in EFSA Guidance Document for Birds and Mammals (2009) together with respective SV values are presented in Table B.9.2.1-1.

Table B.9.2.1-1: Representative avian indicator species and relevant shortcut values for the risk assessment at screening level.

Crop group	Indicator species	Shortcut value	
		Acute	Long-term
Bare soil	Small granivorous	24.7	11.4
Cereals	Small omnivorous bird	158.8	64.8

The screening step crop grouping relevant to the uses of DFF+FFA SC 600 (expressed as kg flufenacet/ha) are performed in the Table B.9.2.1-2.

Table B.9.2.1-2: Screening step crop grouping relevant of flufenacet in DFF+FFA SC 600 formulation.

Crop group	Indicator species	Use pattern		
		Rate (kg a.s./ha)	No. of applications	App. Interval (days)
Bare soil <10 BBCH	Small granivorous	0.120	1	N/A
Cereals	Small omnivorous bird	0.240	1	N/A
		0.160		
		0.120		

The risk assessment was performed for all proposed uses of DFF+FFA SC 600 to winter cereals.

In the screening step it is assumed that animals satisfy all their dietary needs in the treated area feeding on only one food type and do not avoid contaminated food. Therefore, avoidance, fraction of diet obtained in the treated area and fraction of food type in the diet are all set to 1.

The acute risk to birds is assessed by calculation of an acute Toxicity: Exposure ratio (TER_A) according to the following equation:

$$TER_A = \frac{LD_{50} [mg/kg bw]}{DDD [mg/kg bw]}$$

The daily dietary dose (DDD) and TER values for the relevant indicator species for acute exposure to flufenacet following the proposed use of DFF+FFA SC 600 are given in Table B.9.2.1-3 below.

Table B.9.2.1-3: Screening step – estimates of acute exposure and risk to flufenacet following application of DFF+FFA SC 600 in cereals.

Crop group	Indicator Species	SVs	App. rate (kg a.s./ha)	MAF	DDD	LD ₅₀ (mg a.s./kg bw)	TER _A	Trigger value
Flufenacet								
Bare soil	small granivorous	24.7	0.120	1	2.96	434	146.62	≥10
Cereals	small omnivorous bird	158.8			19.05		22.78	
Cereals	small omnivorous bird	158.8	0.160	1	25.40		17.08	
Cereals	small omnivorous bird	158.8	0.240	1	38.1		11.39	

The TER_A values are greater than the trigger of 10, indicating low acute risk to birds from flufenacet following application of DFF+FFA SC 600 at all proposed label rates.

Long-term risk assessment

Screening step

The screening step crop grouping relevant to the use of DFF+FFA SC 600 are given in Table B.9.2.1-1 above.

The lowest toxicity endpoint NOEL of 9.4 mg a.s./kg bw was used in the long-term risk assessment.

Long-term risk is assessed by comparing the long-term DDD with the worst case NOEL from the reproduction studies, expressed as daily dietary dose, to give a long-term Toxicity:Exposure Ratio (TER_{LT}):

$$TER_{LT} = \frac{NOEC [mg/kg bw/day]}{DDD [mg/kg bw/day]}$$

The daily dietary dose (DDD) and TER values for the relevant indicator species for long-term exposure to flufenacet following the proposed use of DFF+FFA SC 600 are given in Table B.9.2.1-4 below.

Table B.9.2.1-4: Screening step – estimates of long-term exposure and risk to flufenacet following application of DFF+FFA SC 600 in cereals.

Crop group	Indicator Species	SVm	App. rate (kg a.s./ha)	MAF	f _{wa}	DDD	NOEL (mg a.s./kg bw/day)	TER _{LT}	Trigger value
Flufenacet									
Bare soil	small granivorous bird	11.4	0.120	1	0.53	0.72	9.4	13.05	≥5
Cereals	small omnivorous bird	64.8		1	0.53	4.12		2.28	
Cereals	small omnivorous bird	64.8	0.160	1	0.53	5.49		1.71	
Cereals	small omnivorous bird	64.8	0.240	1	0.53	8.24		1.14	

Note: TER shown in bold falls below the relevant trigger.

TER_{LT} value is above the trigger of 5 only for small granivorous bird for use in cereals after pre-emergence application at rate 120 g a.s./ha. Therefore, for a small omnivorous bird further consideration is needed for all proposed uses of DFF+ FFA SC 600.

Therefore, the tier 1 risk assessment was performed for all proposed uses of DFF+FFA 600 SC.

Long –term risk assessment

Tier 1 risk assessment

Relevant birds generic species identified in EFSA GD for Birds and Mammals (2009) together with respective SV values are presented in Table B.9.2.1-5.

Table B.9.2.1-5: Representative avian indicator species and relevant shortcut values for the risk assessment at screening level.

Crop group	Generic focal species	Representative species	Shortcut value
			Long-term
Bare soil	Small omnivorous bird “lark”	Woodlark (<i>Lullula arborea</i>)	8.2
	Small insectivorous bird “wagtail”	Yellow wagtail (<i>Motacilla flava</i>)	5.9
Cereals	Small omnivorous bird “lark”	Woodlark (<i>Lullula arborea</i>)	10.9
	Large herbivorous bird “goose”	Pink-footed goose (<i>Anser brachyrhynchos</i>)	16.2

The daily dietary dose (DDD) and TER values for the relevant indicator species for long-term exposure to flufenacet following the proposed use of DFF+FFA SC 600 are given in Table B.9.2.1-6 below.

Table B.9.2.1-6: Tier 1 – estimates of long-term exposure and risk to flufenacet following application of DFF+ FFA SC 600 in cereals.

Crop grouping/growth stage	Generic focal species	SVm	App.rate (kg a.s./ha)	MAF	f _{twa}	DDD	NOEL (mg a.s./kg bw/day)	TER _{LT}	Trigger
Flufenacet									
Cereals BBCH 10-29	Small omnivorous birds “lark”	10.9	0.120	1	0.53	0.70	9.4	13.42	≥5
Cereals BBCH 10-29	Large herbivorous bird “goose” Pink-foot goose	16.2				1.03		9.12	
Cereals BBC 10-29	Small omnivorous birds “lark”	10.9	0.160	1	0.53	0.92		10.21	
Cereal BBCH 10-29	Large herbivorous bird “goose” Pink-foot goose	16.2				1.37		6.86	
Cereals BBCH 10-29	Small omnivorous birds “lark”	10.9	0.240	1	0.53	1.39		6.76	
Cereals BBCH 10-29	Large herbivorous bird “goose” Pink-foot goose	16.2				2.06		4.56	

All TER_{LT} values are above the relevant trigger of 5, except the large herbivorous birds – Pink-foot goose for use in winter cereals at rate 1 x 240 g a.s./ha.

The Applicant as a refinement of the risk assessment for large herbivorous bird “goose” proposed to use the refined f_{twa} value based on the DT₅₀ geomean value for flufenacet obtained from trials on cereals carried out using winter cereals at the growth stage BBCH 24-25. The study is not compliant with the proposed EU representative GAP, the crop’s growth stage being later than that assumed in the GAP for use in Winter Cereals at application rate 240 g Flufenacet/ha (BBCH 10-13). Additionally, it should be indicated that application of DFF+FFA SC 600 in the evaluated study occurred at spring (March–April) when the plants were in mid–tillering stage. At the end of the study the growth stage was 30-31 BBCH (see CA, Vol 3. B).

The increase in biomass of winter cereals growing at spring, when starting from the growth stage BBCH 24-25, may be faster comparing to the would-be increase of the same parameter for the same crop but in autumn, when the crop is at the growth stage of BBCH 10-12. That may result in the dilution of the residue of uptaken Flufenacet. However, that dilution is not expected to be significant.

Therefore, small difference in biomass and tested growth stages does not represent a principal deficiency of the study that would justify a complete rejection of the data. In cereals at early growth stages (until end of tillering) plant size (biomass) is not considered to represent the most crucial parameter for the decline of residues.

The following facts support this statement are as follows:

- At the study start (day 0 after application) active substance concentration on the plant are related only to the use rate (compound per ha) and thus are independent from the actual growth stage

- The process of residue decline (DT₅₀) is independent from the concentration at start of the study
- It is correct that plant growth may lead to a kind of, residue dilution ‘but the sampling intervals were rather short in this study (one to two days until day 5), the contribution from this effect is rather limited.
- More than the growth stage at the study start, actual temperatures during the study period have the potential to influence plant growth. It is noted that the mean temperatures during sampling period of this study (~6 to ~11°C in mid March to early April) are considered to be representative also for the climatic conditions that can be found at autumn uses in October to November.

In RMS’s opinion the longest DT₅₀ value of **5.101** days should be used in calculation of ftwa to cover uncertainties related to differences between the growth stages indicated in the representative GAP – BBCH 10-13, and tested – BBCH 24-25 for Winter cereals and application rate 240 g Flufenacet/ha, as well as those resulting from the small data set (results available only for four sites Germany, Belgium, Netherlands and for single replicates within sites).

Therefore, RMS proposes the refinement based on the longest DT₅₀ value, a flufenacet specific time-weighted average factor (f_{TWA}) for cereals calculated using the following formula from Appendix H of the EFSA Guidance Document:

$$f_{TWA} = \frac{1 - e^{-kt}}{kt}$$

Where: k = ln(2)/DT₅₀ (rate constant)

t = averaging interval.

Using a standard averaging interval of 21 days, a DT₅₀ of 5.101 days corresponds to a 21-day time-weighted-average factor (f_{TWA}) of 0.3302. Using the refined f_{TWA} value of 0.3302, the long-term risk assessment for „large herbivorous birds” is presented in Table B.9.2.1-7 below.

Table B.9.2.1-7: Refined reproductive risk (TER_{LT}) to large herbivorous birds using DT₅₀ data from flufenacet residue trails in cereals.

Crop grouping/ growth stage	Generic focal species	Shortcut value (mg a.s./kg bw/day)	App. rate (kg a.s./ha)	MAF	f _{TWA}	DDD (mg a.s./kg bw/day)	NOEL (mg a.s./kg bw/day)	TER _{LT}	Trigger value
Cereals Early (shoots)	Large herbivorous bird “goose” Pink-foot goose	16.2	0.24	1	0.33	1.28	9.4	7.34	≥5

Considering the refined ftwa value of 0.3302, based on the longest DT₅₀ days value, the TER_{LT} value is above the Annex VI trigger of 5 indicating acceptable long-term risk for birds.

Comments RMS:

The flufenacet is intended to be used as an early post-emergence herbicide in winter cereals at maximum application rate of 1 x 240 g a.s./ha only in autumn, clearly outside the breeding season of birds, the exposure of some individuals which would occur, is expected to be negligible.

RMS is of the opinion that the value of 4.56 is very close to the threshold value of 5 and for this reason may be considered acceptable. The approach of the refinement of the long-term risk assessment for large herbivorous bird „, geese”, considering the refined ftwa value of 0.3302, based on the longest DT₅₀ of 5.101 days value for flufenacet in cereal, resulting in TER_{LT} value above the Annex VI trigger of 5, should be decided at MS level.

The risk assessment for metabolites

There are no toxicity endpoints for two metabolites – FOE oxalate and TFA, to birds.

For this reason, the RMS performed the assessment using the weight-of-evidence approach, which is presented below:

The weight-of-evidence based assessment for metabolites, carried out by the RMS

In plants flufenacet is degraded via glutathione conjugation resulting in metabolites containing either the fluorophenyl acetamide-or the thiadone-moiety of the parent compound.

Plant metabolite studies in wheat (please refer to Volume 3 (CA) B.7, Point 7.2.1.3) indicated two plant metabolites which exceed 10% of radioactive residues in edible crop parts in wheat: FOE-oxalate (M01), with fluorophenyl moiety and trifluoroacetate metabolite (TFA, M45) with is formed by break-down of thiadone (M09). FOE oxalate (M1) revealed to be a major metabolite in wheat forage, straw, hay and grain components. It proved to be predominant in wheat grain. This metabolite containing fluorophenyl acetamide moiety and was included in the plant residue definition. The studies examining the toxicity of this metabolite for the livestock (lying hen, lactating goats, rats) and its residues in these organism showed that FOE Oxalate is not a product of metabolism of Flufenacet. Therefore, the sole route of exposure to it is with diet. Hen metabolism study performed for lying hens, summarized in section Residues in this RAR (please refer to Volume 3, B.7, Point. B 7.2.2.1, ██████████ 1995, ██████████) showed that FOE Oxalate, when administered to the test animals with food was minimally absorbed. The absorbed FOE Oxalate was not metabolized and the tissue analysis showed that it was retained predominantly in liver. It may be assumed that from there it would be depurated with urine. Low levels of that compound in muscles and fat indicate that FOE Oxalate taken up with food will not cumulate in the organism.

At the same time it shall be indicated that these results were obtained for FOE Oxalate administered at the level 5 mg/kg body weight, corresponding to approximately 350 times of exposure of poultry.

That indicates that the risk to wild bird resulting from the consumption of food containing that compound is expected to be minimal, if not negligible.

From the available data it was not possible to derive the toxicity endpoint, but RMS is of the opinion that, on the basis of the evaluation presented above there is no need for doing that.

The trifluoroacetate (TFA) metabolite is a plant and a soil metabolite of several plant protection products and a metabolite of other chemicals.

The toxicity endpoint for this metabolite for birds is not available. For this reason, RMS considered all available information of toxicity of this metabolite for mammals and poultry studies.

The metabolite TFA is formed in the rat metabolism study at up to 10% of the parent. Therefore, it is expected to have been formed in other mammalian toxicity studies and as a result, the risk assessment for this metabolite will be covered by the risk assessment for the active substance.

The acute toxicity endpoint LD₅₀ for mammals for trifluoroacetate metabolite was estimated to be > 2000 mg met./kg b.w. higher than the value of LD₅₀ equal 589 mg a.s./kg b.w. for estimated for parent compound-flufenacet.

It can be concluded that the rat toxicity study gives evidence that this metabolite is not toxic to mammals, which could be used to help address the risk to birds.

In addition, a study in laying hens with oral doses of (1-¹⁴C) TFA – metabolite, was conducted and evaluated in the Section Residues (please refer to Volume 3 (CA), B.7, Point B 7.2.2.1.2., [REDACTED] R., 2013). In this study six hens were orally dosed (by gavage) once daily for 14 consecutive days with an actual dose of ca. 0.5 mg (1-¹⁴C)TFA per kg body weight. In relation to daily food consumption, the administered dose corresponds to 7.84 mg TFA/dry weight. No toxic symptoms in laying hens were observed at an application dose of 0.5 mg met/kg b.w.

The Applicant was asked by RMS to compare the TFA concentration found in the wheat metabolism study after application of flufenacet to winter cereals according to proposed GAP to those tested in a hen TFA metabolism study. The calculations performed by the Applicant are presented below:

Applicant's assessment

- **Concentrations of TFA found in a wheat metabolism study according to application rate of 270 g a.s./ha**

With flufenacet a wheat metabolism study (label: [thiadiazole-5-¹⁴C]) was conducted at an application rate of 270 g/ha which is close to the supported critical GAP of 240 g/ha.

The study with the (thiadiazole-5-¹⁴C) Flufenacet at 270 g a.s./ha shows that trifluoroacetate metabolite (TFA) is found in samples of straw and grain at harvest (84 days after application), however not in green material (forage) collected 4 days after application.

The concentration of TFA metabolite found in wheat metabolism study, evaluated also in the section Residues in this RAR (Bogartz R, Miebach D, 2013, M-444475-01) is presented in the Table B.9.2.1-8 below.

Table B.9.2.1-8: The concentration of TFA metabolite found in a wheat metabolism study.

Commodity	Day after application	Content TFA ¹ [mg/kg]
forage	4	none
hay (adjusted for fresh green material)*	56	0.527 (0.15)
straw	84	0.577
grain	84	0.219

*Hay as feeding item for ruminants need to be adjusted to the fresh weight since the fresh cereal plants are only accessible to the birds in the field (forage 25% dry matter, hay 88% dry matter cf. OECD guidance on residues in livestock) --> $88/25 = 3.52$ (i.e. 1 kg hay corresponds to 3.52 kg fresh material)

If 1 kg hay contains 0.527 mg TFA this corresponds to 0.150 mg TFA/kg fresh green material.

¹conversion Factor 0.314 (as the metabolism report present residue data "expresses as parent compound" a conversion to actual TFA level was considered).

- **Dose level of TFA tested in the hen metabolism study**

Six hens were orally dosed (by gavage) once daily for 14 consecutive days with an actual dose of ca. 0.5 mg [1-¹⁴C]TFA) per kg body weight. In relation to the daily feed consumption, the level of the administered dose corresponds to ~7.84 mg TFA/kg dry feed. In comparison to the measured environmental TFA concentration in commodities that could be consumed by birds, the (corresponding) feed concentration tested in the hen metabolism study was distinctly higher, in particular by a factor of 52 for green plant material (7.84 mg TFA/0.15 mg) and by a factor of 35 (7.84/0.219) for wheat grain.

Dose levels: The measured environmental TFA concentrations result in the following exposure levels for the woodlark:

- Herbal food with an intake rate of 2.26: 0.34 mg/kg bw/day
- Grain food with an intake rate of 0.23: 0.05 mg/kg bw/day

A dose level of 0.5 mg TFA/kg bw/day was administered in the hen metabolism study.

RMS conclusion:

Taking into account that exposure of metabolite TFA to poultry did not result in toxic effects after oral dosing at a rate 0.5 mg met/kg b.w. it is also expected that this metabolite will not be toxic for birds. In addition, the acute LD₅₀ value of this metabolite for mammals shows less toxicity in comparison to parent compound. In RMS opinion, the toxicity of TFA metabolite for birds could be covered by toxicity of active substance. For that reason the risk assessment provided for active substance - flufenacet, covers the risk assessment for the TFA metabolite.

Based on evaluation concern on FOE-oxalate metabolite the acute risk to birds resulting from the consumption of food containing that compound is expected to be minimal, if not negligible.

The lack of chronic/reproductive effects cannot be deduced from the short term study conducted with laying hens. However, taking into account that FOE-oxalate metabolite does not bioaccumulate and is quickly excreted a chronic exposure of birds can be considered negligible.

Rotation crop

TFA residues were also identified in plant material in a confined rotational crop study.

In response to the request of RMS the Applicant presented the results of one study on metabolism of [thiadiazole-5-¹⁴ C] Flufenacet in confined rotational crops, which was evaluated, also in the Section Residues (please refer to Volume 3 (CA), B.7. Point B.7.6.1, Bongartz R, Klankers, 2012, M-443538-01-1) and compared the TFA concentration found in the rotation crops after an application of 903 g a.s./ha to those tested in a hen TFA metabolism study.

A summary of Applicant's assessment is presented below.

- **Concentrations of TFA found in rotated crops after application rate of 903 g a.s./ha**

Using [thiadiazole-5-¹⁴C]-labelled flufenacet for plant metabolism studies, the amount of TFA residues in plant material was characterised in a confined rotational crop study. Flufenacet was applied at a rate of 903 g a.s./ha to bare soil and wheat (cereal crop), turnip (root crop) and Swiss chard (leafy crop) were sown 30 days, 142 days and 317 days after application.

The following concentrations of TFA were found:

Table B.9.2.1-9: The concentration of TFA metabolite found in a rotation crop study.

Crop	Appl. Type	Appl. rate [g as/ha]	Commodity	TFA residue [mg TFA-Na/kg]		
				PBI 30 days [#]	PBI 142 days	PBI 317 days
wheat	pre-planting	903	Grain	1.085	2.854	0.478
			Straw	1.518	3.446	1.499
			Hay	1.274	3.069	1.396
			Forage	0.550	0.862	0.539
turnip	pre-planting	903	Root	0.188	0.072	0.032
			Leaves	2.408	1.323	0.371
Swiss chard	pre-planting	903	leaves, mature	1.184	1.103	0.736
			leaves, intermed.*	2.106	0.730	1.785

[#] PBI: plant back interval, growth stage (50% of final leaf mass); * intermediate growth stage

In wheat forage and grain the highest TFA concentrations were found after a plant back interval of 142 days: 0.862 mg/kg in forage and 2.854 mg/kg in grain. Already after a plant back interval of 30 days TFA concentration were highest in turnip leaves (2.408 mg/kg) and in immature Swiss chard leaves (2.106 mg/kg).

- **Dose level of TFA tested in the hen metabolism study**

Six hens were orally dosed (by gavage) once daily for 14 consecutive days with an actual dose of ca. 0.5 mg [1-¹⁴C] TFA per kg body weight. In relation to the daily feed consumption, the level of the administered dose corresponds to ~7.84 mg TFA/kg dry feed.

TFA concentrations: In comparison to the TFA concentration in commodities that could be consumed by birds, the feed concentration tested in the hen metabolism study was higher, in particular by a factor of 2.7 for wheat grain, by a factor of 9.1 for wheat forage, by a factor of 3.2 for turnip leaves and by a factor of 3.7 for immature Swiss chard leaves.

Dose levels: The measured TFA concentrations result in the following exposure levels for a small and a large bird (woodlark and goose).

Table B.9.2.1-10: The measured TFA concentrations result in the following exposure levels for a small and a large bird (woodlark and goose).

Species	Commodity	TFA (mg/kg)	Feed intake rate	Daily Dietary Dose (mg/kg bw/day)
Woodlark (28.5 g)	Wheat grain	2.854	0.23	0.66
	Wheat forage	0.862	2.26	1.95
	Turnip leaves	2.408		5.44
	Swiss chard	2.106		4.76
Goose (2645 g)	Wheat forage	0.862	0.3	0.26
	Turnip leaves	2.408		0.72
	Swiss chard	2.106		0.63

A dose level of 0.5 mg TFA/kg bw/day was administered in the hen metabolism study. For a small granivorous bird the estimated dietary dose would be marginally higher than this dose level. A strictly herbivorous small bird would exceed the dose level tested in the hen study by a factor of 4 when wheat forage was considered; the factor would be 10 for turnip leaves and Swiss chard. With residues found in wheat forage the dose level estimated for the large herbivorous bird would be lower than the one used in the hen study but it would be slightly higher for turnip leaves and Swiss chard.

RMS conclusion:

The presented exposure assessment of birds to TFA as residues of Flufenacet in plants was an unrealistic case due to the fact that the application rate of Flufenacet used in the study – 903 g a. s./ha, was almost four times higher than the highest application rate proposed in the EU-representative GAP for this assessment – 240 g a. s./ha. In addition, the exposure estimates to reflect worst-case scenarios as 100% food intake on previously treated fields has been taken into account. Therefore, the exposure from residues in rotated crops to birds obtained from this study is only additional information and was presented for transparency of evaluation.

Risk to birds through drinking water

According to the EFSA Guidance Document for Birds and Mammals (2009), two scenarios need to be considered for assessing the risk via the consumption of drinking water.

The **leaf scenario** is relevant for birds taking water that is collected in leaf whorls after application of a pesticide to a crop and subsequent rainfall or irrigation. This scenario applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract birds. Since none of the proposed crop uses falls into these categories, the leaf scenario does not apply to the use of DFF+FFA SC 600.

The **puddle scenario** is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This scenario is relevant for all intended uses of DFF+FFA 600 SC and should therefore be assessed.

The predicted environmental concentration in puddles is calculated as follows:

$$PEC_{\text{puddle}} = \frac{AR/10}{1000(w + K_{oc} \times s)}$$

Where:

AR application rate (g/ha); divisor of 10 to achieve rate in mg/m²
 w 0.02 (pore water term; volume)
 s 0.0015 (soil term: volume, density, organic carbon content)

When multiple spray applications are considered, a MAF based on the DT₅₀ in soil (single first order kinetics, geometric mean as used for PEC_{gw} and PEC_{sw}) may be applied to achieve the effective application rate AR_{eff}.

$$AR_{\text{eff}} = AR \times MAF_m = AR \times \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

Where:

k ln(2)/DT₅₀ (rate constant)
 n number of applications
 i application interval (d)

According to the EFSA Guidance Document for Birds and Mammals (2009), 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/day) 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). For flufenacet, the mean value of K_{FOC} is determined as 245.9 g/mL (please refer to Volume 3, (CA) Section B.8).

Table B.9.2.1-11: Ratios of effective application rate to endpoints for flufenacet following the use of DFF+FFA SC 600 in cereals.

Intended use	App. Rate* (g a.s./ha)	MAF	AREff (g a.s./ha)	LD ₅₀ (mg a.s./kg bw)	Ratio of AREff to LD ₅₀	NOEL (mg a.s./kg bw/day)	Ratio of AREff to NOEL	Ratio trigger
Cereals	240	1	240*	434	0.55	9.4	25.53	≤50

*The maximum application rate

The resulting ratio is clearly below the trigger value of 50 indicating that the acute and long-term risk to birds via the consumption of drinking water can be considered acceptable without further calculations.

Effects of secondary poisoning:

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log P_{OW} greater than 3 have potential for bioaccumulation. Only flufenacet has a log P_{OW} > 3 and therefore, based on the low log P_{OW} value, the risk assessment from bioaccumulation of that substance to fish-eating and worm-eating birds is required. For none of the metabolites of flufenacet the log P_{OW} exceed the trigger value of 3 (Table B.9.2.1-12), therefore, the risk from bioaccumulation has not to be assessed for those metabolites.

Table B.9.2.1-12: Log Pow values for flufenacet and its metabolites.

Substance	log P _{ow}
Flufenacet	3.5
FOE oxalate (M01)	0.80
	pH-dependent
	-2.0 (pH 5)
	-2.2 (pH 7)
FOE sulfonic acid (M02)	-2.4 (pH 9)
	Not pH-dependent
FOE methylsulfide (M05)	-2.72
	2.6 (pH 5)
	2.6 (pH 7)
FOE methylsulfone (M07)	2.6 (pH 9)
	1.7 (pH 5)
	1.7 (pH 7)
FOE-Thiadone (M09)	1.7 (pH 9)
	pH-dependent
	1.92 (pH 4.3)
	0.62 (pH 7)
FOE 5043-trifluoroethanesulfonic acid (M44)	-0.90 (pH 9.4)
	pH-dependent
	-3.0 (pH 5)
	-2.95 (pH 7)
trifluoroacetic acid (TFA) (M45)	-3.16 (pH 9)
	pH-dependent
	-2.5 (pH 5)
	-2.6 (pH 7)
	-2.8 (pH 9)

*Section 2.7 “Partition coefficient n-octanol/water” in the CA

Food chain from earthworm to earthworm-eating birds

For the effects of secondary poisoning on earthworm-eating birds, the dry soil approach was followed as recommended by the EFSA Guidance Document. According to this approach, the bioconcentration factor for the earthworm is calculated as follows:

$$BCF_{earthworm} = \frac{0.84 + 0.012 P_{ow}}{f_{oc} \times K_{oc}}$$

With:

K_{OC}= organic carbon adsorption coefficient (agreed by e-fate : 245.9 mL/g (arithmetic mean) for flufenacet)

f_{oc} = organic carbon content of soil (take 0.02 as a default value)

K_{ow} = calculated from log P_{ow} of 3.5 for flufenacet

BCF_{earthworm} = (0.84 + 0.012 x 3162.28) / 0.02 x 245.9= 7.9

The estimated residues in earthworms are then calculated as:

$$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$$

The time window used for PECs is 21 days.

According to information provided in e – fate section the following worst case PEC_{soil} values for flufenacet are:

- 0.320 mg a.s./kg dws, for application rate of 240 g a.s./ha
- 0.2133 mg a.s./kg dws, for application rate of 160 g a.s./ha
- 0.1600 mg a.s./kg dws for application rate of 120 g a.s./ha

The PEC_{earthworm} is then converted to daily dose by multiplying with 1.05 and compared with the relevant long-term NOEL. The multiplier is based on a 100g bird eating 104.6 g worms per day (generic indicator species earthworm eater: blackbird).

The calculation of the long-term Toxicity to Exposure Ratio (TER) depends on the selection of the suitable endpoint and is defined as follows:

Long-term risk: $TER_{LT} = NO(A)EL [mg \text{ a.s./kg bw/d}] / DDD_{LT}$

Table B.9.2.1-13: Estimates of exposure and risk to flufenacet through bioconcentration in earthworms following the application of DFF+FFA SC 600 in cereals.

Log P _{ow}	K _{oc} (mL/g)	BCF _{worm}	PEC _{soil} (mg/kg)*	PEC _{worm}	DDD	NOEL (mg a.s./kg bw/day)	TER	Trigger
3.5	245.9	7.9	0.320 ¹	2.53	2.65	9.4	3.54	≥5
			0.2133 ²	1.68	1.77		5.31	
			0.160 ³	1.26	1.32		7.12	

¹application rate 1 x 0.240 kg a.s./ha, ² application rate 1 x 0.160 kg a.s./ha, ³application rate dose 1 x 0.120 kg a.s./ha

* max PEC_{soil}

The TER is below the Annex VI trigger of 5 for application rate - 1 x 240 g a.s./ha, indicating the needs for further refinement. For the remaining uses of DFF+FFA SC 600 the risk is considered acceptable.

Thus, the 21 d PEC_{sTWA} was used to refine the exposure through bioconcentration of flufenacet in earthworms following the application of DFF+FFA SC 600 at rate 1 x 240 g a.s./ha.

Table B.9.2.1-14: Estimates of exposure and risk to flufenacet through bioconcentration in earthworms following the application of DFF+FFA SC 600 in cereals.

Log P _{ow}	K _{oc} (mL/g)	BCF _{worm}	21 d twa PEC _{soil} (mg/kg)	PEC _{worm}	DDD	NOEL (mg a.s./kg bw/day)	TER	Trigger
3.5	245.9	7.9	0.282	2.23	2.35	9.4	4.0	≥5

The Applicant provided further refinement of exposure to focal species – blackbird, using the following argumentation (cited word by word from the Applicant's dossier):

The typical diet of blackbirds consists of fruit and invertebrates. According to the PSD Bird Bible (Buxton et al. 1998) the highest proportion of earthworms in blackbird diet observed is 87% (nestlings in spring). Therefore a PD of 0.87 is used in the risk assessment. Since fruit will not be gathered from the treated field this part of the diet can be excluded from the DDD calculation.

Open cereal fields at the time of application of the highest rate of DFF+FFA SC 600 (BBCH 10-13) do not appear to meet the feeding habit requirements of the species (needs some trees and bushes). This limited overlap of habitat conditions of cereal fields with the requirements of blackbirds would be one of the main reasons why the proportion of blackbirds observed on arable fields is below 2% as reported by Seitz (1989).

Refinement of the exposure for earthworm-eating birds is presented in the Table B.9.2.1-15 below:

Table B.9.2.1-15: Refined estimates of exposure and risk to flufenacet through bioconcentration in earthworms following the application of DFF+FFA SC 600 in cereals.

Log Pow	K _{oc} (mL/g)	BCF _{worm}	21 d twa PEC _{soil} (mg/kg)	PEC _{worm}	PD	DDD	NOEL (mg a.s./kg bw/day)	TER	Trigger
3.5	245.9	7.9	0.282	2.23	0.87	2.04	9.4	4.6	≥5

The TER value for the blackbird did not exceed the trigger of 5 for the long-term exposure when using a refined proportion of earthworms in the diet.

In the next Step of the refinement the following argumentation was given by the Applicant:

It is well known that agricultural operations heavily influence the earthworm communities in arable soil under cereal fields. The earthworm biomass on arable fields is usually only about 25% of the earthworm biomass under grass (Scheffer / Schachtschnabel 1998). Soil moisture is the an important factors limiting the availability of earthworms on cereal fields (Edwards & Lofty 1972). In dry and warm conditions, earthworms will rarely venture close to the soil surface. Earthworms will only be available for very short time on the surface on the day after heavy rainfall, but this does not lead to long-term exposure and is thus not relevant to a long-term risk assessment for flufenacet.

Therefore, cereal fields at BBCH 10-13 are obviously no attractive feeding ground when searching for earthworms. The reasons are probably related to a combination of low earthworm abundance and low earthworm availability, linked to the structure and traits of cereal fields.

RMS does not agree with the argumentation given by Applicant concerning the low attractivity of cereals' fields to blackbird. Even assuming lower abundance of earthworms in cereals' fields, the risk for earthworm-eating birds could not be excluded.

In addition, RMS verified and corrected the PD value for blackbird presented by the Applicant.

According to the Bird Bible (Buxton *et al.* 1998), the maximal earthworm content in diet is 78% not 87%. According to the EFSA guidance (2009), the DDD must be calculated on the basis of the complete diet of the focal species.

The residues in soil invertebrates other than earthworms must also be considered in the calculation.

The Bird Bible (Buxton *et al.* 1998) reported for blackbird diets that may be synthesized as:

1. 78% earthworms and 22% other invertebrates (April-May: nestling diet), OR
2. 43% earthworms and 57% other invertebrates (June; nestling diet), OR
3. 2% vegetable materials, 42% earthworms and 56% other invertebrates (no seasonal data; nestling)

The diet of the backbird in the first option of the refinement of the risk consists of 78% earthworms and 22% other invertebrates. It is relevant for nestlings, for the period April-May and winter oilseed rape as a crop.

Based on these assumptions calculations of food energy of total mixed diet, of the food intake rate (FIR) and TER values based on the refined, were all carried out in line with the recommendations of the relevant EFSA Guidance for B&M, 2009. They are presented below in two tables – Table B.9.2.1-16 for the food energy of the total mixed diet and FIR, and Table B.9.2.1-17 for TER.

Table B.9.2.1-16: Calculation of food energy of total mixed diet and the food intake rate (FIR) in the first diet composition for blackbird.

Species	Food type	% in diet wet wt	kJ/g wet weight	kJ/g wet weight adjusted AE	Energy share (kJ) per 1 g mixed diet	Wet weight(g) of food types consumed	Wet weight(g) of food types consumed	FIR/bw
Passerines (Turdus merula-Blackbird)	Ground-dwelling invertebrates (w/o interception)	22%	7.082	5.383	1.1842	19.64	19.64	0.17
	Soil invertebrates	78%	2.972	2.259	1.7619	69.62	69.62	0.62
					sum	Wet wt (g) of whole diet to achieve DEE	Wet wt (g) of whole diet to achieve DEE	
					2.9461	89.26	89.26	

Log a=1.032, log b 0.676, DEE(KJ/d)=262.955

Table B.9.2.1-17: TER value based on the refined FIR/bw, RUD (90% th) and PEC_{worm} for blackbird.

Species	FIR/bw	Food type	Wet wt (g) of food types consumed	Predefined RUD	User-defined RUD	IF (FOCUS)	MAF	DDD
Passerines (Turdus merula-Blackbird)	0.17	Ground-dwelling invertebrates (w/o interception)	19.64	13.8		-	0.53	0.303
	0.62	Soil invertebrates	69.62		PEC _{worm} x app.rate= 2.22 mg a.s./kg x 0.24 kg a.s./ha =9.3	-	-	1.37
								sum
								1.67
							TER	5.6

Considering other invertebrates as ground dwelling invertebrates without interception' using 90th percentile RUD and PEC_{worm} of 2.22, the first diet gave a worst TER of 5.6.

Therefore, the risk assessment to vermivorous birds following the intended use of the DFF+ FFA SC 600 is considered acceptable. The second option of the refinement of the risk consists of 43% earthworms and 57% other invertebrates. It is relevant for nestlings, for the period June and Winter Oilseed Rape as a crop. Based on these assumptions calculations of food energy of total mixed diet, of the food intake rate (FIR) and TER values based on the refined, were all carried out in line with the recommendations of the relevant EFSA Guidance for B&M, 2009. They are presented below in two tables – Table B.9.2.1-18 for the food energy of the total mixed diet and FIR, and Table B.9.2.1-19 for TER.

Table B.9.2.1-18: Calculation of food energy of total mixed diet and the food intake rate (FIR) in the second diet composition for blackbird.

Species	Food type	% in diet wet wt	kJ/g wet weight	kJ/g wet weight adjusted AE	Energy share (kJ) per 1 g mixed diet	Wet weight(g) of food types consumed	Wet weight(g) of food types consumed	FIR/bw
Passerines (Turdus merula-Blackbird)	Ground-dwelling invertebrates (w/o interception)	57%	7.082	5.383	3.0681	37.11	37.11	0.33
	Soil invertebrates	43%	2.972	2.259	0.9713	27.99	27.99	0.25
					Sum	Wet wt (g) of whole diet to achieve DEE	Wet wt (g) of whole diet to achieve DEE	
					4.039	65.10	65.10	

Log a=1.032, log b 0.676, DEE(KJ/d)=262.955

Table B.9.2.1-19: TER value based on the refined FIR/bw, RUD (90th) and PEC_{worm} for blackbird.

Species	FIR/bw	Food type	Wet wt (g) of food types consumed	Predefined RUD	User-defined RUD	IF (FOCUS)	MAF	DDD
Passerines (Turdus merula-Blackbird)	0.33	Ground-dwelling invertebrates (w/o interception)	37.11	13.8		-	0.53	0.573
	0.25	Soil invertebrates	27.99		PEC _{worm} x app.rate= 2.22 mg a.s./kg x 0.24 kg a.s./ha =9.3	-	-	0.551
								sum
								1.24
							TER	8.4

Considering other invertebrates as ground dwelling invertebrates without interception' using 90th percentile RUD and PEC_{worm} of 2.22, the second diet gave a TER of 8.4.

Therefore, the risk assessment to vermivorous birds following the intended use of DFF+FFA SC 600 is considered acceptable.

The third option of the refinement of the risk consists of 2% vegetables materials, 42% earthworms and 42% other invertebrates. It is relevant for nestlings, no seasonal data. Based on these assumptions calculations of food energy of total mixed diet, of the food intake rate (FIR) and TER values based on the refined, were all carried out in line with the recommendations of the relevant EFSA Guidance for B&M, 2009 they are presented below in two tables – Table B.9.2.1-20 for the food energy of the total mixed diet and FIR, and Table B.9.2.1-21 for TER.

Table B.9.2.1-20: Calculation of food energy of total mixed diet and the food intake rate (FIR) in the third diet composition for blackbird.

Species	Food type	% in diet wet wt	kJ/g wet weight	kJ/g wet weight adjusted AE	Energy share (kJ) per 1 g mixed diet	Wet weight(g) of food types consumed	Wet weight(g) of food types consumed	FIR/bw
Passerines (Turdus merula-Blackbird)	Grass and cereals	2%	4.154		3.157	1.31	1.31	0.01
	Ground-dwelling invertebrates (w/o interception)	56%	7.082	5.383	3.0143	36.57	36.57	0.32
	Soil invertebrates	42%	2.972	2.259	0.9487	27.43	27.43	0.24
					Sum	Wet wt (g) of whole diet to achieve DEE	Wet wt (g) of whole diet to achieve DEE	
					4.0261	65.31	65.31	

Log a=1.032, log b 0.676, DEE(KJ/d)=262.955

Table B.9.2.1-21: TER value based on the refined FIR/bw, RUDs and PEC_{worm} for blackbird.

Species	FIR/bw	Food type	Wet wt (g) of food types consumed	Predefined RUD	User-defined RUD	IF (FOCUS)	MAF	DDD
Passerines (Turdus merula-Blackbird)	0.01	Grass and cereals	1.31	102.3	-	-	0.53	0.149
	0.17	Ground-dwelling invertebrates (w/o interception)	36.57	13.8	-	-	0.53	0.565
	0.62	Soil invertebrates	27.43		PEC _{worm} x app.rate= 2.22 mg a.s./kg x 0.24 kg a.s./ha =9.3	-	-	0.540
								sum
								1.254
							TER	7.5

Considering grass and cereals and other invertebrates as ground dwelling invertebrates without interception' using 90th percentile RUD and PEC_{worm} of 2.22, the third diet gave a TER of 7.5.

Therefore, the risk assessment to vermivorous birds following the intended use of DFF+FFA SC 600 is considered acceptable.

Food chain from fish to fish-eating birds

A bioconcentration factor in fish of 71.4 was determined for flufenacet.

According to the EFSA Guidance Document for Birds and mammals (2009), the residues in fish are estimated based on this bioconcentration factor and the following formula:

$$PEC_{fish} = PEC_{water} \times TWA \times BCF$$

Where PEC_{water} is the highest PEC value for surface water as determined in the fate & behaviour section (Volume 3, Section B.8).

According to information provided in e – fate section the following worst case PEC_{sw} values for flufenacet are:

- 0.0624 mg a.s./kg dws, for application rate of 240 g a.s./ha
- 0.0416 mg a.s./kg dws, for application rate of 160 g a.s./ha
- 0.0312 mg a.s./kg dws, for application rate of 120 g a.s./ha

The residue in fish is converted to daily dose by multiplying with 0.159, and compared with the relevant long-term NOAEL. The multiplier is based on a 1000 g bird eating 159 g fresh fish per day.

Following formula was used in TER calculations:

$$\text{TER} = \text{NO(A)EL [mg as/kg bw/d]} / \text{DDD}$$

Table B.9.2.1-22 shows the TER value for the risk after exposure to flufenacet for fish-eating birds.

Table B.9.2.1-22: Estimates of exposure and risk to flufenacet through bioconcentration in fish following the application of DFF+FFA SC 600 in cereals.

BCF _{fish}	PEC _{water} (mg/L)*	PEC _{fish}	DDD	NOEL (mg a.s./kg bw/day)	TER	Trigger
71.4	0.0624 ¹	4.45	0.71	9.4	13.23	≥5
	0.0416 ²	2.97	0.47		20.0	
	0.0312 ³	2.22	0.35		26.85	

¹ application rate 1 x 0.240 kg a.s./ha, ² application rate 1 x 0.160 kg a.s./ha, ³ application rate 1 x 0.120 kg a.s./ha

* max PEC_{sw} (STEP1)

The TER value is above the trigger of 5, indicating that risk to fish-eating birds following use of DFF+FFA SC 600 is considered acceptable.

Baits: Concentration of active substance in bait in mg/kg

The formulation DFF+FFA SC 600 is intended for use as a foliar spray, and therefore this information is not required.

Pellets, granules, prills of treated seed

The formulation DFF+FFA SC 600 is intended for use as a foliar spray, and therefore this information is not required.

Amount of active substance in or on each item

The formulation DFF+FFA SC 600 is intended for use as a foliar spray, and therefore this information is not required.

Proportion of the active substance LD₅₀ per 100 items and per gram of items

The formulation DFF+FFA SC 600 is intended for use as a foliar spray, and therefore this information is not required.

Size and shape of pellet or prill

The formulation DFF+FFA SC 600 is intended for use as a foliar spray, and therefore this information is not required.

B.9.2.2. Risk assessment for terrestrial vertebrates other than birds

The risk assessment for effects on terrestrial vertebrates other than birds has been updated according to the latest EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009).

Toxicity

The active substance endpoints used in the risk assessment for mammals are shown in the Table B.9.1.2.1-1.

The lowest LD₅₀ value of 589 mg a.s./kg bw from the acute toxicity study in rat was used in the acute risk assessment. A study for the representative formulation DFF+FFA SC 600 was performed. In addition, the acute toxicity of DFF+FFA SC 600 to mammals was calculated assuming dose additivity of the single active substance in the formulation. These calculations showed that the compound driving the toxicity of formulation was Flufenacet, hence the toxicity of that compound covers the toxicity of the representative formulation.

To address the long-term toxicity of Flufenacet the Applicant proposed use the NOAEL_{ecotox} = 37.4 mg/kg b.w., obtained in two-generations study for rat. RMS agrees with Applicant proposal of NOAEL_{ecotox}.

The justification for selection of that value is presented under the relevant data point in the document (please refer to Volume 3, B.9 (CA), under Point B.9.1.2.2).

Flufenacet metabolites

In plants flufenacet is degraded via glutathione conjugation resulting in metabolites containing either the fluorophenyl acetamide- or the thiadone-moiety of the parent compound.

Plant metabolite studies in wheat (please refer to Volume 3 (CA) Section B7) indicated two plant metabolites which exceed 10% of radioactive residues in edible crop parts in wheat: FOE-oxalate (M01), with fluorophenyl moiety and trifluoroacetate metabolite (TFA, M45) which is formed by break-down of thiadone (M09).

FOE Oxalate

In the studies performed on mammals – rats and lactating goats (representing livestock) it was demonstrated that FOE Oxalate was not a product of metabolism of Flufenacet. It shall be therefore stated that, as it was in case of mammals, the sole route of exposure to it was via food.

FOE Oxalate was demonstrated not to be metabolized within the body once absorbed from the digestive track. At the same time it shall be indicated that the mean level of absorption was low; 70% was removed with faeces and 28% of the administered dose with urine. The elimination was fast as it occurred within 24 hours after uptake of the contaminated food. The accumulation of FOE Oxalate within the tissues was not observed. These results were obtained for the dose level of 1 mg/kg body weight (in studies with rats).

The detailed assessment can be found in Section Toxicology (please refer to Volume 3 (CA), Section B6, Point B.6.1.2, [REDACTED]). That indicates that the risk to wild mammals resulting from the consumption of food containing that compound is expected to be minimal, if not negligible.

Therefore the risk assessment for that metabolite was not performed as it was not necessary, the substance is rapidly depurated from the body and the absorption from the digestive track is minimal.

TFA

For the metabolite TFA a full reproduction toxicity study was not available. Two dietary studies ([REDACTED] (2005, 2007) and one developmental study for rats ([REDACTED] 2010) evaluated in the Section Toxicology, were indicated by the Applicant as the basis for estimating NOEL_{ecotox} for mammals from the exposure to TFA metabolite.

RMS agrees with Applicant proposal of NOEL_{ecotox} value of 98 mg TFA/kg b.w., which is based on the lack of both decrease body weight and toxicity effects to mammal at this application rate as the relevant endpoint to be used in the long-term risk assessment to mammals for TFA. The justification for selection of that value is presented under the relevant data point in the Volume 3, B.9 (CA), under Point B.9.1.2.2.

Exposure

Exposure of mammals was calculated according to following equations:

$$\begin{aligned}\text{DDDA [mg/kg bw]} &= \text{application rate [kg/ha]} \times \text{shortcut value (SV)} \times \text{MAF}_{90} \\ \text{DDDLT [mg/kg bw]} &= \text{application rate [kg/ha]} \times \text{shortcut value (SV)} \times \text{ftwa} \times \text{MAF}_{\text{mean}}\end{aligned}$$

Where:

DDD	daily dietary dose
FIR/bw	food intake rate related to body weight
Ftwa	time weighted average factor (long-term considerations; default value: 0.53)
MAF ₉₀	Multiple Application Factor for acute risk assessment, 90th percentile
MAF _{mean}	Multiple Application Factor for long-term risk assessment, mean

Evaluation is performed in stepwise approach, starting from screening step which considers worst case exposure assumptions and enables identification of substances that do not require further consideration.

Acute risk assessment

Screening step

At the screening step evaluation is performed for “indicator species”, which due to its size and feeding habits, are considered to have higher exposure than other species that occur in a particular crop at particular time.

Relevant mammals indicator species identified in EFSA GD for Birds and Mammals (2009) together with respective SV values are presented in Table B.9.2.2-1.

Table B.9.2.2-1: Representative mammal indicator species and relevant shortcut values for the risk assessment at screening level.

Crop	Indicator species	Short cut value	
		Acute	Long- term
Bare soil	Small granivorous mammals	14.4	6.6
Cereals	Small herbivorous mammals	118.4	48.3

The screening step crop grouping relevant to the uses of DFF+FFA SC 600 (expressed as kg flufenacet/ha) are performed in Table B.9.2.2-2.

Table B.9.2.2-2: Screening step crop grouping relevant for the use of flufenacet in DFF+FFA SC 600.

Crop group	Indicator Species	Use pattern		
		Rate (kg a.s./ha)	No. of applications	App. interval (days)
Bare soil	Small granivorous mammal	0.120	1	N/A
Cereals	Small herbivorous mammal	0.240	1	N/A
		0.160		
		0.120		

In the screening step it is assumed that animals satisfy all their dietary needs in the treated area feeding on only one food type and do not avoid contaminated food. Therefore, avoidance, fraction of diet obtained in the treated area and fraction of food type in the diet are all set to 1.

The acute risk to mammal is assessed by calculation of an acute Toxicity: Exposure ratio (TER_A) according to the following equation:

$$TER_A = \frac{LD_{50} [mg/kg bw]}{DDD [mg/kg bw]}$$

The daily dietary dose (DDD) and TER values for the relevant indicator species for acute exposure to flufenacet and its metabolite–TFA following the proposed use of DFF+FFA SC 600 are given in Table B.9.2.2-3 below.

Table B.9.2.2-3: Screening step – estimates of acute exposure risk to flufenacet TFA metabolite following application of DFF+FFA SC 600 in cereals.

Crop group	Indicator species	SVs	App. rate (kg a.s./ha)	MAF	DDD (mg /kg bw)	LD ₅₀ (mg /kg bw)	TER _A	Trigger
Flufenacet								
Bare soil	small granivorous mammal	14.4	0.120	1	1.73	589	340.46	≥10
Cereals	small herbivorous mammal	118.4		1	14.20		41.47	

Cereals	small herbivorous mammal	118.4	0.160	1	18.94		31.09	
Cereals	small herbivorous mammal	118.4	0.240	1	28.4		20.73	
TFA								
Bare soil	small granivorous mammal	14.4	0.0377*	1	0.54	>2000	3703.70	≥10
Cereals	small herbivorous mammal	118.4		1	4.46		448.43	
Cereals	small herbivorous mammal	118.4	0.0502*	1	5.94		336.70	≥10
Cereals	small herbivorous mammal	118.4	0.0754*	1	8.93		223.96	

*corrected for molecular weight of TFA (114.02g/mol, i.e. 31.4% of the parent flufenacet). Additionally, a formation of 100% TFA from flufenacet was assumed.

Based on the screening assessment, the acute TER values of the different exposure scenarios for flufenacet and its metabolite TFA following the proposed use of DFF+FFA SC 600 are above the Annex VI trigger value of 10, indicating an acceptable acute dietary risk to mammals.

Long- term risk assessment

Screening step

The long-term risk to mammals was assessed from long-term TER values, calculated according to the following equation

$$TER_{LT} = \frac{NOEC [mg/kg \text{ bw/day}]}{DDD [mg/kg \text{ bw/day}]}$$

The daily dietary dose (DDD) and TER values for the relevant indicator species for long-term exposure to flufenacet and its metabolite following the proposed use of DFF+FFA SC 600 are given in Table B.9.2.2-4 below.

Table B.9.2.2-4: Screening step – estimates of long-term exposure and risk to flufenacet and its metabolite TFA following application of DFF+FFA SC 600 in cereals.

Crop group	Indicator species	Shortcut value _m	App. rate (kg a.s./ha)	MAF	f _{tw}	Long-term DDD	NOEL (mg a.s./kg bw/day)	TER _{LT}	Trigger
Flufenacet									
Bare soil	Small granivorous mammal	6.6	0.120	1	0.53	0.42	37.4	89.04	≥5
Cereals	Small herbivorous	48.3				3.07		12.18	

Crop group	Indicator species	Shortcut value _m	App. rate (kg a.s./ha)	MAF	f _{wa}	Long-term DDD	NOEL (mg a.s./kg bw/day)	TER _{LT}	Trigger
	mammal								
Cereals	Small herbivorous mammal	48.3	0.160	1	0.53	4.09		9.14	
Cereals	Small herbivorous mammal	48.3	0.240	1	0.53	6.14		6.09	
TFA									
Bare soil	Small granivorous mammal	6.6	0.0377*	1	0.53	0.13	98	753.84	≥5
Cereals	Small herbivorous mammal	48.3				0.96		102.08	
Cereals	Small herbivorous mammal	48.3	0.0502*	1	0.53	1.28		76.56	
Cereals	Small herbivorous mammal	48.3	0.0754*	1	0.53	1.93		50.77	

*Corrected for molecular weight of TFA (114.02g/mol, i.e. 31.4% of the parent flufenacet. Additionally, a formation of 100% TFA from flufenacet was assumed.

Note: TER shown in bold falls below the relevant trigger

Based on the screening assessment, the long-term TER values of the different exposure scenarios for flufenacet and its metabolite TFA following the proposed use of DFF+FFA SC 600 are above the Annex VI trigger value of 5, indicating an acceptable long-term risk to mammals.

Risk to mammals through drinking water

There are two scenarios provided in the EFSA Guidance Document for Birds and Mammals (2009) for assessing the risk via the consumption of drinking water.

The *leaf scenario* is not deemed relevant for small mammals.

The *puddle scenario* is relevant for mammals taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of DFF+FFA SC 600 a pesticide to a crop or bare soil. This scenario is relevant for all intended uses of and should therefore be assessed.

The predicted environmental concentration in puddles is calculated as follows:

$$PEC_{\text{puddle}} = \frac{AR/10}{1000(w + K_{oc} \times s)}$$

Where:

AR application rate (g/ha); divisor of 10 to achieve rate in mg/m²

w 0.02 (pore water term; volume)

s 0.0015 (soil term: volume, density, organic carbon content)

When multiple spray applications are considered, a MAF based on the DT₅₀ in soil (single first order kinetics, geometric mean as used for PEC_{gw} and PEC_{sw}) may be applied to achieve the effective application rate AR_{eff}.

$$AR_{\text{eff}} = AR \times MAF_m = AR \times \frac{1 - e^{-nki}}{1 - e^{-ki}}$$

Where:

k $\ln(2)/DT_{50}$ (rate constant)

n number of applications

i application interval (d)

According to the EFSA Guidance Document for Birds and Mammals (2009) 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/day) does not exceed 50 in the case of less sorptive substances ($K_{OC} < 500$ L/kg) or 3000 in the case of DFF+FFA SC 600 more sorptive substances ($K_{OC} \geq 500$ L/kg). For flufenacet the mean K_{FOC} is determined as 245.9 (please refer to Volume 3, (C.A), Section B 8).

Table B.9.2.2-7: Ratios of effective application rate to endpoints for flufenacet following the use of DFF+FFA SC 600 in cereals.

Intended use	App. rate (g a.s./ha)	MAF	AR _{eff} (ga.s/ha)	LD ₅₀ (mg a.s./kg bw)	Ratio of AR _{eff} to LD ₅₀	NOAEL (mg a.s./kg bw/day)	Ratio of AR _{eff} to NOEL	Ratio trigger
cereals	240	1	240*	589	0.40	37.4	6.41	50

*The worst case scenario covers all remained uses

The resulting ratio is clearly below the trigger value of 50 indicating that the acute and long term risk to mammals via the consumption of drinking water can be considered acceptable without further calculations.

Effects of secondary poisoning

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) substances with a log P_{OW} greater than 3 have potential for bioaccumulation. Only flufenacet has a log $P_{OW} > 3$ (3.5) and therefore, based on the low log P_{OW} values, the risk assessment from bioaccumulation of that substance to fish-eating and worm-eating birds is required.

However, for none of the metabolites of flufenacet, the log P_{OW} exceed the trigger value of 3 (Table B.9.2.2-8). Therefore, the risk from bioaccumulation has not to be assessed for metabolites of flufenacet.

Table B.9.2.2-8: Log Pow values for flufenacet and its metabolites

Substance	log P_{ow}
Flufenacet	3.5
FOE oxalate (M01)	0.80
	pH-dependent
	-2.0 (pH 5)
	-2.2 (pH 7)
FOE sulfonic acid (M02)	-2.4 (pH 9)
	Not pH-dependent
	- 2.72

FOE methylsulfide (M05)	2.6 (pH 5) 2.6 (pH 7) 2.6 (pH 9)
FOE methylsulfone (M07)	1.7 (pH 5) 1.7 (pH 7) 1.7 (pH 9)
FOE-thiadone (M09)	pH-dependent 1.92 (pH 4.3) 0.62 (pH 7) - 0.90 (pH 9.4)
FOE 5043-trifluoroethanesulfonic acid (M44)	pH-dependent -3.0 (pH 5) -2.95 (pH 7) -3.16 (pH 9)
trifluoroacetic acid (TFA) (M45)	pH-dependent -2.5 (pH 5) -2.6 (pH 7) -2.8 (pH 9)

*Section 2.7 "Partition coefficient n-octanol/water" in the CA

Food chain from earthworm to earthworm-eating mammals

For the effects of secondary poisoning on earthworm-eating mammals, the dry soil approach was followed as recommended by the EFSA Guidance Document for Birds and Mammals (2009). According to this approach, the bioconcentration factor for the earthworm is calculated as follows:

$$BCF_{earthworm} = \frac{0.84 + 0.012 P_{OW}}{f_{OC} \times K_{OC}}$$

With:

K_{OC} = organic carbon adsorption coefficient ((agreed by e-fate : 245.9 mL/g (arithmetic mean) for flufenacet

f_{OC} = organic carbon content of soil (take 0.02 as a default value)

$$BCF_{earthworm} = (0.84 + 0.012 \times 3162.28) / 0.02 \times 245.9 = 7.9$$

According to information provided in e – fate section the following worst case PEC_{soil} values for flufenacet are:

- 0.320 mg a.s./kg dws, for application rate of 240 g a.s./ha
- 0.2133 mg a.s./kg dws, for application rate of 160 g a.s./ha
- 0.160 mg a.s./kg dws, for application rate of 120 g a.s./ha

The estimated residues in earthworms are then calculated as:

$$PEC_{earthworm} = PEC_{soil} \times BCF_{earthworm}$$

The $PEC_{earthworm}$ is then converted to daily dose by multiplying with 1.28, and compared with the relevant long-term NOAEL. The multiplier is based on a 10g mammal eating 12.8 g worms per day.

The calculation of the long-term Toxicity to Exposure Ratio (TER) depends on the selection of the suitable endpoint and is defined as follows:

$$\text{Long-term risk: } TER_{LT} = NO(A)EL \text{ [mg a.s./kg bw/d]} / DDD_{LT}$$

Table B.9.2.2-9 shows the TER value for the risk after exposure to flufenacet for earthworm-eating mammals.

Table B.9.2.2-9: Estimates of exposure and risk to flufenacet through bioconcentration in earthworms following the application of DFF+FFA SC 600 in cereals.

Log Pow	K _{oc} (mL/g)	BCF _{worm}	PEC _{soil} (mg/kg)	PEC _{worm}	DDD	NOAEL (mg a.s./kg bw/day)	TER	Trigger
3.5	245.9	7.9	0.320 ¹	2.53	3.23	37.4	11.57	≥5
			0.2133 ²	1.68	2.15		17.39	
			0.160 ³	1.26	1.61		23.22	

¹ application rate 1 x 0.240 kg a.s./ha, ² application rate 1 x 0.160 kg a.s./ha, ³ application rate 1 x 0.120 kg a.s./ha

The TER exceeds the trigger of 5, indicating an acceptable risk.

Food chain from fish to fish-eating mammals

A bioconcentration factor in fish of 71.4 was determined for flufenacet (please refer to Volume 3, (C.A), Section B.9).

According to the EFSA Guidance Document for Birds and Mammals (2009), the residues in fish are estimated based on this bioconcentration factor and the following formula:

$$PEC_{fish} = PEC_{water} \times TWA \times BCF$$

Where PEC_{water} is the highest PEC value for surface water

According to information provided in e – fate section (please refer to Volume 3, (CA) Section 8) the following worst case PEC_{sw} values for flufenacet are:

- 0.0624 mg a.s./kg dws, for application rate of 240 g a.s./ha
- 0.0416 mg a.s./kg dws, for application rate of 160 g a.s./ha
- 0.0312 mg a.s./kg dws for application rate of 120 g a.s./ha

The residue in fish is converted to daily dose by multiplying with 0.142, and compared with the relevant long-term NOAEL. The multiplier is based on a 3000 g mammal eating 425 g fresh fish per day.

Table B.9.2.2.10- shows the TER value for the risk after exposure to flufenacet for fish-eating mammals.

Table B.9.2.2-10: Estimates of exposure and risk to flufenacet through bioconcentration in fish following the application of DFF+FFA SC 600 in cereals.

BCF _{fish}	PEC _{water} (mg/L)	PEC _{fish}	DDD	NOAEL (mg a.s./kg bw/day)	TER	Trigger
71.4	0.0624 ¹	4.45	0.63	37.4	59.36	≥5
	0.0416 ²	2.97	0.42		89.04	
	0.0312 ³	2.22	0.32		116.87	

¹ application rate 1 x 0.240 kg a.s./ha, ² application rate, 1 x 0.160 kg a.s./ha, ³ application rate 1 x 0.120 kg a.s./ha
max PEC_{sw} (STEP 1)

In conclusion: The acute and long-term risk of flufenacet is considered acceptable for mammals following the intended uses of DFF+FFA SC 600 in winter cereals.

Acceptance of bait, granules or treated seed (palability testing)

No study on palability with the formulation was performed.

As DFF+FFA SC 600 is intended for use as a spray, the acceptance of baits, granules and treated seed is not relevant.

Supervised cage/field trials

Supervised cage/field trial with the formulation were not performed, since low risk to mammals indicates that further studies are not required.

B. 9.2.3. Other data on effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

Not required according to 1107/2009.

B 9.3. EFFECTS ON AQUATIC ORGANISMS

Toxicity of flufenacet, its aquatic metabolites and the representative formulation DFF +FFA SC 600 to aquatic organisms was tested in numerous laboratory studies. Summaries of derived endpoints are provided in tables B.9.3-1 (active substance), B.9.3-2 (metabolites) and B.9.3-3 (formulated product).

Summaries of studies performed with the active substance and metabolites are presented in Volume 3 (CA)

B.9. Section Ecotoxicology.

Table B.9.3-1: Toxicity of Flufenacet to aquatic organism.

Organism	Test substance	Endpoint (type of the test)	Value (mg a.s./L)	Reference
Fish				
Oncorhynchus mykiss (Rainbow trout)	Flufenacet	96 h LC ₅₀ (static-renewal, mortality)	5.84 mm	██████████ (1995) M-002379-01-1
Lepomis macrochirus (Bluegill sunfish)	Flufenacet	96 h LC ₅₀ (static-renewal, Mortality)	2.13 mm	██████████ (1995) M-002378-01-1

Organism	Test substance	Endpoint (type of the test)	Value (mg a.s./L)	Reference
Cyprius carpio	Flufenacet	96 h LC ₅₀ (static-renewal, mortality)	10-12 mm >sat.con	██████████ (2010) M-361666-03-1
<i>Cyprinodon variegatus</i> (Sheepshead Minnow)	Flufenacet	96 h LC ₅₀ (static-renewal, mortality)	3.31 mm	██████████ ██████████ (1994) M-002422-01-1
<i>Oncorhynchus mykiss</i> (Rainbow trout)	Flufenacet	97-day NOEC (flow-trough, ELS study, growth)	0.334 mm	██████████ (1995) M-002357-01-1
<i>Cyprinodon variegatus</i> (Sheepshead Minnow)	Flufenacet	35-day NOEC (flow-through, ELS study, growth)	0.049 mm	██████████ ██████████ ██████████ (2013) M-464909-01-1
<i>Pimephales promelas</i> (Fathead minnow)	Flufenacet	279-day NOEC (flow-through, FFLC study, growth)	0.138 mm	██████████ C.V. (2002) M-082934-01-1
Aquatic invertebrates				
<i>Daphnia magna</i> (Waterflea)	Flufenacet	48 h EC ₅₀ (static, moratlity)	30.9 mm	Bowers L.M (1994) M-003805-01-1
<i>Americamysis bahia</i> Mysid shrimp	Flufenacet	96 h LC ₅₀ (flow-through, mortality)	5.6 mm	Claude M.B., at al (2013)
<i>Crassostrea virginica</i> Eastern oyster	Flufenacet	96 h LC ₅₀ (mortality), 96 h EC ₅₀ (shell growth)	>13.9 mm 12.6 mm	Wheat & Evans (1993) M-002427-01-1
<i>Hyalella azteca</i>	Flufenacet	96 h LC ₅₀ (static, mortality)	2.45 mm	Bowers L.M. (1995) M-002374-01-1
<i>Daphnia magna</i> Waterflea	Flufenacet	21-day NOEC (static-renewal, reproduction)	3.26 mm	Gagliano & Bowers (1994)
<i>Americamysis bahia</i> (Mysid shrimp)	Flufenacet	NOEC 28 d (flow-through, reproduction)	0.221 mm	Claude, M.B. et al. (2013) M-452207-01-1
Sediment dwelling organism				
<i>Chironomus riparius</i>	Flufenacet	28 d NOEC (developmental rate) Static test	5 nom	Bruns E. (2010) M-372857-01-1
Algae				
Green algae <i>Pseudokirchneriella subcapitata</i>	Flufenacet	96 h E _r C ₅₀ NOE _r C 72 h E _y C ₅₀ NOE _y C (static)	0.00315 im <0.00064 im 0.001783 im 0.00064 im	Bowers L.M, (1995) Dorgerloh M M-086475-01-1
Green algae <i>Pseudokirchneriella subcapitata</i>	Flufenacet	72 h E _r C ₅₀ NOE _r C 72 h E _y C ₅₀ NOE _y C (static test)	0.0212 geom 0.000138geom 0.00538 geom 0.000138geom	Bruns E. (2010) M-363891-03-1

Organism	Test substance	Endpoint (type of the test)	Value (mg a.s./L)	Reference
Pseudokirchneriella subcapitata (Green algae)	Flufenacet	96 h E _r C ₅₀ NOEC (Static test)	0.00645 nom. < 0.00225 nom	Anderson, J. P. E. (1997) M-002343-01-1
Green algae Pseudokirchneriella subcapitata	Flufenacet	72/96 h Geomean	E _r C ₅₀ 0.00755 ¹	Bruns E (2010) M-363891-03-1 Dorgerloh M M-086475-01-1 Anderson, J. P. E. (1997) M-002343-01-1
Green algae Desmodesmus subspicatus	Flufenacet	72 h E _r C ₅₀ NOE _r C 72 h E _y C ₅₀ NOE _y C (static test)	0.675 geom 0.0084 geom 0.07696 geom 0.0084 geom	Bruns E. (2011) M-415813-01-1
Chlorella vulgaris Green algae	Flufenacet	72 h E _r C ₅₀ NOE _r C 72 h E _y C ₅₀ NOE _y C (static test)	11.1 nom 0.98 nom 3.71 nom 0.98 nom	Bruns E. (2011) M-416169-01-1
Blue algae Synechococcus leopoliensis	Flufenacet	72 h E _r C ₅₀ NOE _r C 72 h E _y C ₅₀ NOE _y C (static test)	>10 nom 0.307 nom >10 nom 0.096 nom	Bruns E. (2011) M-415814-01-1
Blue-green algae Anabaena flos-aquae	Flufenacet	96h E _r C ₅₀ 96h E _y C ₅₀ NOE _r C NOE _y C	>53.2 mm 26.65 mm 3.77 mm <1.930 mm	Hugens& Alexaner (1993) M-002423-01-1
Freshwater diatom Navicula pelliculosa	Flufenacet	96h E _r C ₅₀ 96h E _y C ₅₀ NOE _r C NOE _y C (statitc test)	5.044 mm 2.13 mm 1.120 mm 1.120 mm	Bowers, L. M.; Dobbs, M. G. (1995) M-002355-01-1 ²
Chlamydomonas terricola	Flufenacet	216 h E _r C ₅₀ NOE _r C 216 h E _y C ₅₀ NOE _y C	0.657 nom 0.096 nom 0.332 nom 0.096 nom	Sobczyk H (2011) M-418627-01-1
Aquatic macrophyte				
Lemna gibba Duckwed	Flufenacet	7-day E _r C ₅₀ (frond no) NOE _r C 7-day E _r C ₅₀ (frond area) NOE _r C 7 -day E _y C ₅₀ (frond no) NOE _y C 7 -day E _y C ₅₀ (frond area) NOE _y C	0.0161 nom 0.000658 nom 0.0139 nom 0.000658 nom 0.00763 nom 0.00658 nom 0.006824 nom 0.000658 nom	Bruns (2013) M-451198-01-1

Organism	Test substance	Endpoint (type of the test)	Value (mg a.s./L)	Reference
Lemna gibba Duckweed	Flufenacet	Peak exposure: one or two 24-h- peaks; total test duration 14 d	No inhibition >50% up to 0.126 mg a.s./L peak E ₁ C ₅₀ >0.126 mg/L nom	Bruns (2013) M-452567-01-1 2 3

mm = mean measured concentration, n..... nominal concentration, im.... initial measured concentration, geom geometric mean measured concentration

values in bold were used in the risk assessment

¹geometric mean value E₁C₅₀ of three laboratory studies with Pseudokirchneriella subcapitata (Dorgerloh M, 1998: M-086475-01-1, Bruns E., 2010: M-363891-03-1 and Anderson, J. P. E., 1997: M-002343-01-1)

² The study is not fully reliable and was considered as supportive.

³The study is not considered in the current risk assessment. The study is valid it may be used in the refined risk assessment for macrophytes only if:

- Further evidence is provided that rooted macrophytes are not more sensitive to flufenacet than Lemna sp.
- The peak exposure design of the study covers the peaks observed in the FOCUS scenarios.

Table B.9.3-2: Toxicity of metabolites of flufenacet to aquatic organism

Organism	Test substance	Endpoint (type of the test)	Value (mg a.s./L)	Reference
Fish				
Oncorhynchus mykiss (Rainbow trout)	FOE sulfonic acid	96 h LC ₅₀ (static-renewal, mortality)	>86.7 nom	██████████ (1995) M-004932-01-1
Oncorhynchus mykiss (Rainbow trout)	FOE-Thiadone	96 h LC ₅₀ (static, mortality)	9.1 mm	██████████ (1998) M-005388-01-1
Lepomis Macrochirus (Bluegill)	FOE-Thiadone	96 h LC ₅₀ (static, mortality)	18.6 mm	██████████ (1999) M-009214-01-1
Sheepshead minnow (Cyprinodon variegatus)	FOE-Thiadone	96 h LC ₅₀ (static, mortality)	15.3 mm	██████████ (1999) M-005388-01-1
Brachydanio rerio (Zebra fish)	TFA	96 h LC ₅₀ (static, mortality)	>1200 nom	██████████ (1992) M-247889-01-1
Brachydanio rerio (Zebra fish)	TFA	144 h EC ₅₀ (embryo acute, static)	700 nom 3000 nom	██████████ (2013) M-462660-01-1 ^a
Aquatic invertebrates				
Daphnia magna (Waterflea)	FOE sulfonic acid	48 h EC ₅₀ (static, motatlity)	>87.3 nom	Heimbach F., (1995) M-004930-01-1
Daphnia magna (Waterflea)	FOE-Thiadone	48 h EC ₅₀ (static, mortlity)	31.7 mm	Bowers L.M.&C.VLam (1998) M-005390-01-1
Mysidopsis bahia	FOE-Thiadone	96 h LC ₅₀ (Flow-through, mortality)	>15.1 mm	Palmer, S. J.; Krueger, H. O. (1998) M-005110-01-1
Crassostrea virginica Eastern oyster	FOE-Thiadone	96 h LC ₅₀ (Flow-through, mortality)	22 mm	Palmer S.J.& Krueger H. (1998) M-005108-01-1

Organism	Test substance	Endpoint (type of the test)	Value (mg a.s./L)	Reference
Daphnia magna (Waterflea)	TFA	48 h LC ₅₀ (Static, mortality)	>1200 nom	Groeneveld et al. (1992) M-247890-01-1
Algae				
Green algae Pseudokirchneriella subcapitata	FOE oxalate	72 h ErC ₅₀ NOE _r C 72 h EbC ₅₀ NOE _b C (static test)	>100 nom >100 nom >100 nom >100 nom	Bruns E. (2009) M-358823-011
Green algae Pseudokirchneriella subcapitata	FOE methylsulfide	72 h ErC ₅₀ NOE _r C 72 h EbC ₅₀ NOE _b C (static test)	83.8 nom 10 nom 30.5 nom 10 nom	Dogerlorh M. (1998) M-002341-01-1
Green algae Pseudokirchneriella subcapitata	FOE methylsulfone	72 h ErC ₅₀ NOE _r C (static test)	>10 nom >10 nom	Bruns E. (2010) M-364591-01-1
Green algae Desmodesmus subspicatus	FOE sulfonic acid	72 h ErC ₅₀ 72 h EbC ₅₀ NOE _b C NOE _r C (static test)	>86.7 >86.7 ≥86.7 ≥86.7	Anderson (1995) M-004931-01-1
Green algae Pseudokirchneriella subcapitata	TFA	72 h ErC ₅₀ 72 h EyC ₅₀ NOE _r C NOE _y C (static test)	192.48 nom 4.19 nom 0.36 nom <0.36 nom	Groeneveld et al. (1992) M-247820-01-1
Green algae Pseudokirchneriella Subcapitata	TFA	72 h ErC ₅₀ 72 h EbC ₅₀ NOE _{b,r} C (static test)	>1.2 nom >1.2 nom 0.12	Berends & Molenaar (1993) M-247818-02-1
Green algae Scenedesmus supspicatus	TFA	EC ₅₀ (static test)	>120 nom	Berends , Keetelaar-Jansen, van Dijk (1995) M-247825-01-1 ¹
Green algae (various species)	TFA	ErC ₅₀ (static test)	>112 nom to >2400 nom	Berends (1996) M-247818-02-1 ^a
Green algae Pseudokirchneriella subcapitata	FOE 5043- (Trifluoroethane sulfonic acid)	96h ErC ₅₀ 96h EyC ₅₀ NOE _r C NOE _y C (static test)	>100 nom >100 nom >100 nom >100 nom	Bruns E. (2012) M-444217-01-1
Green algae Pseudokirchneriella subcapitata	FOE Thiadone	72 h ErC ₅₀ 72 h NOE _r C 72 h EbC ₅₀ 72 h NOE _b C (static test)	15.0 nom 2.10 nom 4.10 nom 0.66 nom	Hall, A. T.; Lam, C. V., (1999) M-009214-01-1 ¹
Aquatic macrophyte				
Lemna gibba Duckweed	FOE oxalate	7-day ErC ₅₀ (frond no) NOE _r C 7-day ErC ₅₀ (frond area) NOE _r C	>100 nom 50 nom >100 nom >100 nom	Bruns E. (2009) M-358823-01-1

Organism	Test substance	Endpoint (type of the test)	Value (mg a.s./L)	Reference
		(static test)		
Lemna gibba Duckweed	FOE methylsulfide	7- day E _r C ₅₀ (frond no) NOE _r C 7-day E _r C ₅₀ (frond area) NOE _r C 7-day E _y C ₅₀ (frond no) NOE _y C 7-day E _y C ₅₀ (frond area) NOEC (static test)	125.30 nom 29.60 nom 106.0 nom 13.2 nom 65.02 nom 13.20 nom 61.97 nom 29.60 nom	Bruns E. (2010) M-393709-01-1
Lemna gibba Duckweed	FOE methylsulfone	7- day E _r C ₅₀ (frond no) NOE _r C 7 -day E _r C ₅₀ (frond area) NOE _r C (static test)	> 100 nom >100 nom >100 nom ≥100 nom	Bruns E. (2010) M-369703-01-1
Lemna gibba Duckweed	TFA	7-d E _r C ₅₀ (frond no) 7d NOEC 7 -d E _y C ₅₀ (frond no) NOEC	1990 nom 300 nom 768.6 nom 600 nom	Smyth et al. (1993) M-247900-01-1
<i>Lemna gibba</i> , <i>Myriophyllum</i> <i>spicatum</i> <i>Myriophyllum</i> <i>sibiricum</i>	TFA	7d EC ₅₀ 14 EC ₅₀ 14 EC ₅₀ (static test)	618.3 (wet mass) 222.1 (root length) 357.1 (wet mass)	Hanson & Solomon, (2004) M-455787-01-1 ^a
Lemna minor Duckweed	FOE 5043- Thiadone	7- day E _r C ₅₀ (frond no) NOE _r C 7 day (frond area) NOE _r C 7- day E _y C ₅₀ (frond no) NOE _y C 7 -day (frond area) NOEC (static test)	20.80 nom <1.25 nom 18.32 nom 5 nom 9.86 nom <1.25 nom 8.68 nom <1.25 nom	Bruns E. (2010) M-393718-01-3
Lemna gibba Duckweed	FOE 5043 (Trifluoroethane sulfonic acid)	7 -day E _r C ₅₀ (frond no) NOE _r C 7 -day E _r C ₅₀ (frond area) NOE _r C 7 -day E _y C ₅₀ (frond no) NOE _y C 7- day E _y C ₅₀ (frond area) NOE _y C (static test)	> 10 nom >10 nom >10 nom >10 nom >10 nom >10 nom >10 nom >10 nom	Weyers (2013) M-445884-01-1

mm = mean measured concentration, ... nom= nominal concentration

Values in bold were used in the risk assessment

¹ The study is not fully reliable but can be used as supportive information indicating that metabolite is clearly less toxic than active substance.

^a The study is considered as additional information only

Table B.9.3-3: Toxicity of representative formulaion to aquatic organism.

Organism	Test substance	Endpoint (type of the test)	Value (µg product/L)	Reference
Lemna gibba Duckwed	DFF+FFA 600 SC	7 d E _r C ₅₀ (frond no) 7 d NOE _r C ₅₀ (frond no) 7 d E _b C ₅₀ (dry weight) 7 d NOE _b C ₅₀ (dry weight) (static test)	307 nom 20 nom 258 nom 40 nom	Dorgerloh M., Sommer H., (2001)* M-073160-01-1
Pseudokirchneriella Subcapitata	DFF+FFA 600 SC	72 h E _r C ₅₀ 72 h E _b C ₅₀ 72 h NOE _b C 72 h NOE _r C (static test)	6.63 nom 2.42 nom <0.938 nom <0.938 nom	Dorgerloh M., Sommer H., (2001)* M-073137-01-1*

* The endpoints will not be used in the risk assessment until the Applicant reanalyses the results of the study using the measured initial concentrations.

B.9.3.1. Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

B.9.3.1.1. Acute toxicity to fish

For the representative formulation DFF+FFA SC 600 studies on fish were not performed.

It is considered acceptable as Lemna sp. and algae were clearly the most sensitive species to active substance (by more than a factor 100). Therefore, in accordance with European Commission (2002b) the studies for aquatic plants and algae were performed for formulation.

B.9.3.1.2. Acute toxicity to aquatic invertebrates

Acute toxicity to Daphnia magna

For representative formulation DFF+FFA SC 600 studies on fish were not performed.

It is considered acceptable because of the Lemna sp. and algae were clearly the most sensitive species to active substance (by more than a factor 100). Therefore, in accordance with European Commission (2002b) the studies for only aquatic plants and algae were performed for formulation.

B.9.3.1.3. Effects on algae growth**B.9.3.1.3.1. FOE 5043 & Diflufenican SC 600 – Influence on the Growth of Green Algae, *Selenastrum capricornutum*.**

Reference:	FOE 5043 & Diflufenican SC 600 – Influence on the Growth of Green Algae, <i>Selenastrum capricornutum</i> .
Author(s), year:	Dorgerloh, M., Sommer, H., 2001
Report/Doc. number:	Study No: E 323 1937-8, Report No: DOM 20073, Reference BCS No: M-073137-01-1
Guideline(s):	OECD 201 (March 1984), ISO 8692, ASTM E 1218
GLP:	Yes

<u>Test substance:</u>	FOE 5043 & Diflufenican SC 600, Formulation no: 07205/0024(0006). Content of active substances: 32.3% (401.5 g/L) Flufenacet and 14.4% Diflufenican (217.0 g /L).
Test species:	<i>Selenastrum capricornutum</i> .
Number of organisms:	10,000 cells/mL, 3 replicate vessels per test level and 6 replicate vessels per control
Type of test, duration:	Static test, 72 hours
<u>Applied concentrations:</u>	
Nominal:	Control (0), 0.938, 1.88, 3.75, 7.5, 15 and 30 µg test item/L
Test conditions:	
Water quality:	Two nutrient media according to OECD guideline
Temperature:	21.5-24.0°C (mean, 23°C)
pH:	7.81-8.31 (0 h), 8.10-8.71 (72 h)
Incubation:	Continuous illumination, 6496-7392 (mean 6888 Lux)
Test parameters:	Cell numbers were estimated photometrically. For this purpose, samples of treated, inoculated culture medium were placed in 5 cm cuvettes and the extinctions were determined at a wave length of 578 nm using a single-beam-photometer. To detect possible alterations in algae cells that might influence extinction measurements, such as unusual cell size, samples from each flask were examined under a microscope at a magnification of 400 times. Samples were analysed (HPLC-MS/MS) for the actual concentration of flufenacet present in the test medium of all treatment levels and controls on Day 0 and Day 3.

Findings:

Analytical data: The quantities of flufenacet found at the beginning of the test (day 0) in reference to the nominal concentrations were 45 to 178 % (average 103 %). The quantities of FOE 5043 found in the two lowest test levels were inconstant. The Applicant explained this by handling mistake which did not influence

the results, because the ErC_{50} is mainly based on higher test levels of this study. The quantities of FOE 5043 found at the end (day 3) were 62 to 99 % (average 84 %).

RMS stated that the explanation seems plausible, and proved it by calculation using programme Curve Export Professional 2.3.6.

The original tables from the study report are presented below.

Table 7a. Comparison of nominal and analytically determined concentrations of FOE 5043 in treated cultures at Day 0.

Nominal Concentration in µg test item (FOE 5043)/L	Actual Concentration (µg FOE 5043/L) ²			%
	1 Determination	2 Determination	Average	
0.938 (0.302)	0.551	0.525	0.538	178
1.88 (0.605)	0.272	0.276	0.274	45
3.75 (1.21)	1.08	1.12	1.10	91
7.50 (2.42)	2.30	2.39	2.34	97
15.0 (4.83)	4.78	4.84	4.81	100
30.0 (9.66)	10.2	9.94	10.1	104

Average: 103 %

Table 7b. Comparison of nominal and analytically determined concentrations of FOE 5043 in treated cultures at Day 3.

Nominal Concentration in µg test item (FOE 5043)/L	Actual Concentration (µg FOE 5043/L) ³			%
	1 Determination	2 Determination	Average	
0.938 (0.302)	0.261	0.232	0.246	82
1.88 (0.605)	0.367	0.378	0.372	62
3.75 (1.21)	1.05	1.01	1.03	85
7.50 (2.42)	2.03	2.03	2.03	84
15.0 (4.83)	4.65	4.63	4.64	96
30.0 (9.66)	9.65	9.40	9.52	99

Average: 84 %

Statistic:

The 0 - 72 h EC_{50} for biomass (E_bC_{50}) and for algal growth rate (ErC_{50}) were calculated using probit analyses after Finney (1952), and the slopes of the regression lines were calculated following Litchfield and Wilcoxon (1949). Calculations were carried out using commercial software (Ratte, 1993-1998).

The NOEC's and LOEC's were calculated by an analysis of variance Dunnett's-Test. The effect threshold was calculated as geometric mean between NOEC and LOEC.

Findings:

Morphological effects:

No morphological change in algae was observed from 3.75 µg product /L (after 72 h) and following tested concentration including and up to 30 µg product /L.

Table B.9.3.1.3.1-1: Effects of on FOE 5043 & Diflufenican SC 600 SC to Pseudokirchneriella subcapitata.

DFF+FFC 600 SC (µg /L) Nominal	Mean cell number per ml (0-72h) x10 ⁴	Biomass		Growth rate per day	
		Area under the curve (0-72h)	% inhibition relative to the control	Average specific growth rate (0- 72h)	% inhibition of growth rate compared to control
				µ	%
Control	66.35	1500	-	1.42	-
0.938	43.42	1044*	30.4	1.28*	9.9
1.88	44.59	1097*	26.9	1.29*	9.3
3.75	9.81	393*	73.8	0.78*	45.2
7.50	4.53	231*	84.6	0.53*	62.8
15	3.66	192*	87.2	0.46*	67.8
30	3.04	147*	90.2	0.39*	72.3

At start 10000 cell/mL

*Statistically significant from control (one-side Dunnet's test, $p \leq 0.05$)

Growth inhibition values based on nominal concentration obtained with DFF+FFA 600 SC on Selenastrum capricornutum were as follows:

72 h $E_r C_{50} = 6.63 \mu\text{g product/L}$

72 h $E_b C_{50} = 2.42 \mu\text{g product/L}$

$NOE_r C < 0.938 \mu\text{g product/L}$

$NOE_b C < 0.938 \mu\text{g product/L}$

RMS comments:

The study was conducted according to the OECD 201 test guideline (2006). In general the study is in line with the stated test guideline and all validity criteria are met. According to the validity criteria given in the test guideline 201 (2006) the biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72 hour test period. In the study the cell density increased by a factor of 66.5. The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2, 2-3) in the control must not exceed 35%. The mean coefficient of variation was determined to be 29.6 %.

The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed 7%. The mean coefficient of variation for the whole test period was 2.75 %.

Measured concentrations are not in the $\pm 20\%$ range of nominal concentrations for the two lowest treatments at day 0. Since the 2 lowest treatments induced significant effects on the average specific growth rate of algae, the deviation between the actual and nominal concentrations may affect the endpoint calculations.

Therefore, the results of the study should be reanalyzed by Applicant on the basis of measured initial concentrations.

Agreed endpoints:

72 h E_rC_{50} = 6.63 µg product/L, (based on nominal concentration)

72 h E_bC_{50} = 2.42 µg product/L, (based on nominal concentration)

72 h NOE_rC < 0.938 µg product /L, (based on nominal concentration)

72 h NOE_bC < 0.938 µg product /L, (based on nominal concentration)

The endpoints will not be used in the risk assessment until the Applicant reanalyses the results of the study using the measured initial concentrations.

B.9.3.1.4. Effects on aquatic macrophytes**B.9.3.1.4.1. FOE 5043 & Diflufenican SC 600- Toxicity (7 days) to *Lemna gibba* G3 in a Static Test.**

Reference:	FOE 5043 & Diflufenican SC 600 Toxicity (7 days) to <i>Lemna gibba</i> G3 in a Static Test.
Author(s), year:	Dorgerloh, M., Sommer, H., 2001
Report/Doc. number:	Study No: DOM 20074, Reference BCS No: M-073160-01-1
Guideline(s):	OECD 221, Revised Draft Document (October 2000)
GLP:	Yes

Material and methods:

Test substance:	FOE 5043 & Diflufenican SC 600 (HEROLD® SC 600) an SC formulation of Flufenacet (405.3 g/L) and Diflufenican (204.5 g/L), Formulation-No.: 07205/0024 (0006).
Test species:	<i>Lemna gibba</i> G3
Source:	Horticulture Crops Quality Laboratory, U.S. Department of Agriculture, Beltsville, MD 20705, U.S.A.
Growth medium:	Steinberg's nutrient medium slightly modified as recommended by DIN/ISO
Growth chamber:	Glass dishes were used with a diameter of 10 cm and a height of 6 cm (total volume of approx. 200 mL) These test vessels were covered with lids of glass to permit gas exchange and illumination under sterile conditions to the greatest possible extent.
Inoculum:	The inoculum of <i>Lemna gibba</i> G3 used to begin the test was taken from a less than 10-day old sterile stock culture. Three plants, consisting of four fronds each (for a total of 12 fronds) were aseptically added to each test vessel using a sterile pincer.

Number of organism:	Three replicate vessels per test level and 3 replicate vessels per control. 12 fronds per vessel,
Type of test, duration:	Static, 7 days
<u>Applied concentrations:</u>	
Nominal:	0 (control), 10.0, 20.0, 40.0, 80.0, 160, 320 and 640 µg test item/L
<u>Test conditions:</u>	
Water quality:	Steinberg's nutrient medium (slightly modified by DIN/ISO)
Temperature:	24 ±2°C
pH:	Test start: 4.87-5.57
	Test end: 5.61-6.18
Light regime:	Continuous illumination of 6496-6608 Lux
Test parameters:	The temperature was determined by a continuous measurement in one additional incubated glass vessel filled with the same amount of de-ionised water as in the test vessels. Temperature was recorded hourly.
	The pH was measured on the days 0 and 2, 5, 7 in all test level and the control.
	Counting of fronds: so as to eliminate subjective decisions on frond maturity, every frond visible projecting beyond the edge of the parent frond was counted.
	Dry weight was measured only on day 7.
Analytical data:	Samples were analyzed for the actual concentrations of Flufenacet and Diflufenican present in the test medium with exception of the two lowest concentrations of Diflufenican and additionally in the control on day 0 and day 7. Test solutions were analysed using HPLC technique.
Observation:	Visual observations were made on study days 2, 5, and 7.
	Plant frond numbers and total frond area of plants were determined on days for this study.
Statistics:	Growth data, based on (a) average-growth rates of frond number and (b) dry weights (biomass), respectively, were used to conduct the following statistical analyses:
	- t-test to determine if controls can be pooled
	- chi-square test to determine the normality of the data set and,
	- Bartlett's test for homogeneity of variances
	Data which did not fit a normal distribution were analyzed by the non-parametric Kruskal-Wallis'test and Dunn's Multiple Comparison test to determine if there was a significant difference between the control and treatment groups.

This growth data, without previous data transformation were analyzed according to the following statistical methods:

- the Dunnett's test,
- the Bonferroni's t-test,
- the Tukey's test and
- the Williams' test.

Statistical analyses were conducted using a PC based computer program with conclusions of statistical significance based on a 95 percent confidence level ($\alpha=0.05$). The EC_{50} value with 95%-confidence intervals and slope was calculated by using a computer program which estimated the EC_{50} using one of three statistical techniques: moving average, binomial probability or probit analysis.

Findings:

Analytical measurements:

Based on analytical findings of FOE 504 (flufenacet) 3 in all test levels on day 0 between 44 and 100 % (average 74 %) of nominal were found.

On day 7 there were analytical findings between 38 and 92 % (average 67 %) of nominal.

Based on analytical findings of Diflufenican in all test levels (except the two lowest test concentrations, which were below the limit of quantification of the analytical method) on day 0 between 73 and 91 % (average 82 %) of nominal were found.

On day 7 there were analytical findings between 54 and 69 % (average 62 %) of nominal. These results of both active substances show a slight decrease under static test conditions.

Data on final frond number, growth rate and % inhibition of average growth rate and dry weight are summarized in the tables below.

Table B.9.3.1.4-1: Fronds number and % inhibition of average growth rate of total frond number.

Nominal concentration (µg product/L)	Frond number			
	Final frond Number at day 7 (mean)	Average growth (0-7days)	% inhibition of average growth rate of fronds number	Visual observation
control	83	0.276	-	-
10	92	0.291	-5.1	-
20	80	0.271	2.2	-
40	72	0.256	7.6*	Slight chlorosis on Day 5
80	37	0.162	41.6*	Slight chlorosis on Day 2-7
160	28	0.122	55.8*	Slight chlorosis 2d; middle to strong chlor. day 5 + 7.
320	27	0.116	58.2*	Slight-middle chlorosis 2d, middle to strong chlor. Day 5 + 7
640	27	0.114	58.8*	Slight-middle chlorosis 2d, middle to strong chlor., day 5 + 7

* Statistically significant to control (Dunnett's and Williams $\alpha=0.05$)**Table B.9.3.1.4-2: Dry weight on Day 7 and % inhibition relative to control. Biomass.**

Nominal concentration (µg product./L)	Biomass (dry weight)	
	Dry weight on Day 7 (g)	% inhibition relative to control
Control	0.0086	-
10	0.0107	-28
20	0.0084	3.2
40	0.0075	14.9
80	0.0057*	39.7
160	0.0052*	46.9
320	0.0043*	58.2
640	0.0047*	53.7

* Statistically significant to control (Dunnett's and Williams $\alpha=0.05$)

Observed visual effect: Morphological effects (chlorosis) were observed at the test concentration including and up 40 µg product/L .

Conclusion:

Growth inhibition values based on nominal concentration with on Lemna gibba G3 were as follows:

0-7 d Frond number growth rate:	$E_rC_{50} = 307 \mu\text{g product/L}$
	$NOE_rC = 20 \mu\text{g product/L}$
	$E_rC_{10} = 10 \text{ product/L}$
	$EC_{25} = 5 \mu\text{g product/L}$
	0-7 d $E_rC_{90} > 640 \text{ product/L}$
0-7 d Dry weight:	$E_bC_{50} = 258 \mu\text{g product/L}$
	$NOE_bC = 40 \mu\text{g product/L}$
	$LOEC = 80 \mu\text{g product/L}$
	$E_bC_{10} = 6.29 \text{ product/}$
	$E_bC_{50} = 1.78 \text{ product/}$
	0-7 d $E_bC_{90} > 640 \text{ product/L}$

Comments RMS:

The study was conducted according to OECD test guideline 221 (2000). According to the validity criteria given in the OECD test guideline (2006) the doubling time of the frond number in the control should be less < 2.50 day, corresponding to approx. a seven-fold increase in seven days and an average specific growth rate of 0.275 per day.

In this study the mean doubling time of the frond number in the control was 2.500 day and the mean growth rate was 0.276.

The mean factor of frond number measured in the control between 0 and 7 days, was 6.9.

In RMS opinion the slight deviations from validity criteria will not significantly influence of study results.

Hence, the study is considered acceptable.

However, measured concentrations are not in the $\pm 20\%$ range of nominal concentrations at day 0. **Given these deviations and the inconsistencies in the observed effects, the results of the study should be reanalyzed by Applicant on the basis of measured initial concentrations.**

Agreed endpoints:

0-7 d Frond number growth rate:	$E_rC_{50} = 307 \mu\text{g product/L}$
	$NOE_rC = 20 \mu\text{g product/L}$
0-7 d Dry weight:	$E_bC_{50} = 258 \mu\text{g product/L}$
	$NOE_bC = 40 \mu\text{g product/L}$

The endpoints will not be used in the risk assessment until the Applicant reanalyses the results of the study using the measured initial concentrations.

B.9.3.2. Additional long term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms.

Additional long-term and chronic toxicity studies on aquatic organisms performed with formulation were not required.

B.9.3.3. Further testing on aquatic organisms

In view of the risk assessment indicating acceptable risk to aquatic organisms, further testing on aquatic organisms is not required.

B.9.4. RISK ASSESSMENT FOR AQUATIC ORGANISMS

The risk assessment is based on the current Guidance Document „Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters”, EFSA Panel on Plant Protection Products and their Residues(PPR) 23 European Food Safety Authority (EFSA), Parma, Italy”, on the risk assessment for aquatic organisms.

The toxicity endpoints of flufenacet and its metabolites used in the risk assessment are summarised in the Table B.9.3-1 and the Table B.9.3-2.

Exposure**Surface water**

Exposure of aquatic organisms to flufenacet and its relevant metabolites was estimated according to FOCUS requirements by calculation of predicted concentrations in surface water (PEC_{sw}) and sediment (PEC_{sed}) with consideration of intended use pattern as well as relevant degradation data and properties of particular compounds. With regard to metabolites, predicted concentrations were calculated for major aquatic metabolites as well as for soil metabolites, which may migrate to surface water as a result of run-off or drainage events. PEC_{sw} and PEC_{sed} values were calculated in a stepwise approach from Step 1 (worst case) to Step 4.

Maximum exposure of aquatic organisms was calculated for applications to cereals for the following scenarios:

- autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence
- spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence
- autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence
- spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence
- autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence

Details of calculations of predicted concentrations in surface water and sediment are presented in Section Fate and Behaviour (please refer to Volume 3, (PPP) Section B 8).

Summary of worst case PEC_{sw} values for the active substance and metabolites taken into account in the aquatic risk assessment is presented in tables B.9.4-1 to B.9.4-6 below:

Table B. 9.4-1: Summary of maximum FOCUS Step 1 and Step 2 values for flufenacet .

Compound	FOCUS Step	Scenario	Winter cereals 1x120 g s.a./ha	Winter cereals 1 x160 g s.a./ha	Winter cereals 1x240 g s.a./ha
			max PEC _{sw} (µg a.s./L)	max PEC _{sw} (µg a.s./L)	max PEC _{sw} ^a (µg a.s./L)
Flufenacet	STEP 1	Not relevant	31.227 ^a 31.277 ^s	41.636 ^a 41.636 ^s	62.454
	STEP 2	Northern Europe	13.797 ^a 6.057 ^s	18.395 ^a 8.076 ^s	27.593
		Southern Europe	11.217 ^a 11.217 ^s	14.956 ^a 14.956 ^s	22.433

^a autumn application in winter cereals, ^s spring application in winter cereals

Table B.9.4-2: Summary of maximum FOCUS Step 1 and Step 2 values for metabolites of flufenacet.

Compound	FOCUS Step	Scenario	Winter cereals 1x120 g s.a./ha	Winter cereals 1 x160 g s.a./ha	Winter cereals 1x240 g s.a./ha ^a
			max PEC _{sw} (µg a.s./L)	max PEC _{sw} (µg a.s./L)	max PEC _{sw} (µg a.s./L)
FOE Oxalate	STEP 1	Not relevant	6.466 ^a 6.466 ^s	8.622 ^a 8.622 ^s	12.933
	STEP 2	Northern Europe	2.540 ^a 1.038 ^s	3.386 ^a 1.384 ^s	5.079
		Southern Europe	2.039 ^a 2.039 ^s	2.719 ^a 2.719 ^s	4.078
FOE Sulfonic acid	STEP 1	Not relevant	7.941 ^a 7941 ^s	10.588 ^a 10.588 ^s	15.882
	STEP 2	Northern Europe	3.748 ^a 1.515 ^s	4.997 ^a 2.020 ^s	7.500
		Southern Europe	3.004 ^a 3.004 ^s	4.005 ^a 4.005 ^s	6.007
FOE Methylsulfone	STEP 1	Not relevant	1.896 ^a 1.896 ^s	2.528 ^a 2.528 ^s	3.792
	STEP 2	Northern Europe	0.944 ^a 0.412 ^s	1.259 ^a 0.549 ^s	1.888
		Southern Europe	0.767 ^a 0.756 ^s	1.022 ^a 1.022 ^s	1.533
FOE Methylosulfide	STEP 1	Not relevant	0.084 ^a 0.084 ^s	0.111 ^a 0.111 ^s	0.167
	STEP 2	Northern Europe	0.084 ^a 0.084 ^s	0.111 ^a 0.111 ^s	0.167
		Southern Europe	0.084 ^a 0.084 ^s	0.111 ^a 0.111 ^s	0.167
FOE Thiadone	STEP 1	Not relevant	1.464 ^a 1.464 ^s	1.952 ^a 1.952 ^s	2.928
	STEP 2	Northern Europe	0.543 ^a 0.468 ^s	0.724 ^a 0.624 ^s	1.086
		Southern Europe	0.518 ^a 0.518 ^s	0.691 ^a 0.691 ^s	1.036
FOE-5043 Trifluoroethane sulfonic acid	STEP 1	Not relevant	1.084 ^a 1.084 ^s	1.445 ^a 1.445 ^s	2.168
	STEP 2	Northern Europe	0.352 ^a 0.141 ^s	0.469 ^a 0.188 ^s	0.703
		Southern Europe	0.281 ^a 0.281 ^s	0.375 ^a 0.375 ^s	0.563

Compound	FOCUS Step	Scenario	Winter cereals 1x120 g s.a./ha	Winter cereals 1 x160 g s.a./ha	Winter cereals 1x240 g s.a./ha ^a
			max PEC _{sw} (µg a.s./L)	max PEC _{sw} (µg a.s./L)	max PEC _{sw} (µg a.s./L)
TFA Trifluoroacetic acid	STEP 1	Not relevant	10.228 ^a 10.228 ^s	13.638 ^a 13.638 ^s	20.457
	STEP 2	Northern Europe	5.100 ^a 2.040 ^s	6.800 ^a 2.720 ^s	10.200
		Southern Europe	4.080 ^a 4.080 ^s	5.444 ^a 5.438 ^s	8.160

^a autumn application in winter cereals, ^s spring application in winter cereals

Table B. 9.4-3: Summary of maximum FOCUS Step 3 values for flufenacet.

Compound	FOCUS Step	Winter cereals 1x120 g s.a./ha		Winter cereals 1 x160 g s.a./ha		Winter cereals 1x240 g s.a./ha
		Spring application max PEC _{sw} (µg a.s./L)	Autumn application max PEC _{sw} (µa.s.g/L)	Spring application max PEC _{sw} (µa.s.g/L)	Autumn application max PEC _{sw} (µa.s.g/L)	Autumn application max PEC _{sw} (µa.s.g/L)
Flufenacet	D1 ditch	0.846	2.680	1.129	4.328	6.543
	D1 stream	0.629	1.672	0.838	2.699	4.082
	D2 ditch	1.702	3.227	2.412	3.957	6.199
	D2 stream	1.111	2.021	1.574	2.480	3.882
	D3 ditch	0.760	0.758	1.014	1.010	1.514
	D4 pond	0.0267	0.398	0.0357	0.756	1.168
	D4 stream	0.572	0.658	0.763	1.081	1.647
	D5 pond	0.0289	0.560	0.0387	0.766	1.170
	D5 stream	0.614	0.710	0.818	0.946	1.420
	D6 ditch	0.756	2.764	1.009	3.732	5.693
	R1 pond	0.0687	0.0609	0.0913	0.0797	0.116
	R1 stream	0.764	2.800	1.021	3.790	5.811
	R3 stream	1.080	3.783	1.450	4.980	7.641
	R4 stream	0.501	1.167	0.668	3.957	5.980

^a autumn application in winter cereals, ^s spring application in winter cereals

TableB. 9.4-4: Summary of maximum FOCUS Step 4, 10 m-meter buffer zone for flufenacet.

Compound	FOCUS Step	Buffer zone for [10 m]	Winter cereals 1x120 g s.a./ha		Winter cereals 1 x160 g s.a./ha		Winter cereals 1x240 g s.a./ha
			Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)
Flufenacet	D1 ditch	SD	0.194	2.680	0.259	4.328	6.543
	D1 stream	SD	0.163	1.672	0.218	2.699	4.082
	D2 ditch	SD	1.702	3.227	2.412	3.957	6.199
	D2 stream	SD	1.111	2.021	1.574	2.480	3.882
	D3 ditch	SD	0.109	0.109	0.146	0.145	0.218
	D4 pond	SD	0.0169	0.394	0.0224	0.750	1.159
	D4 stream	SD	0.111	0.550	0.148	1.081	1.674
	D5 pond	SD	0.0190	0.556	0.0255	0.761	1.163

Compound	FOCUS Step	Buffer zone for [10 m]	Winter cereals 1x120 g s.a./ha		Winter cereals 1 x160 g s.a./ha		Winter cereals 1x240 g s.a./ha
			Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)
	D5 stream	SD	0.120	0.579	0.160	0.812	1.249
	D6 ditch	SD	0.114	2.764	0.153	3.732	5.693
	R1 pond	SD	0.0318	0.0283	0.0422	0.0370	0.0543
	R1 stream	SD+RO	0.347	1.354	0.464	1.697	2.602
	R3 stream	SD+RO	0.493	1.728	0.662	2.246	3.446
	R4 stream	SD+RO	0.217	0.527	0.287	1.786	2.699

Table B.9.4-5: Summary of maximum FOCUS Step 4, 20 m-meter buffer zone for flufenacet.

Compound	FOCUS Step	Buffer zone [20 m]	Winter cereals 1x120 g s.a./ha		Winter cereals 1 x160 g s.a./ha		Winter cereals 1x240 g s.a./ha
			Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)
Flufenacet	D1 ditch	SD	0.169	2.680	0.232	4.328	6.543
	D1 stream	SD	0.121	1.672	0.168	2.699	4.082
	D2 ditch	SD	1.702	3.227	2.412	3.957	6.199
	D2 stream	SD	1.111	2.021	1.574	2.480	3.882
	D3 ditch	SD	0.0569	0.0567	0.0756	0.0754	0.113
	D4 pond	SD	0.0114	0.391	0.0153	0.747	1.154
	D4 stream	SD	0.0577	0.550	0.0769	1.081	1.674
	D5 pond	SD	0.0135	0.554	0.0183	0.758	1.159
	D5 stream	SD	0.0626	0.579	0.0836	0.812	1.249
	D6 ditch	SD	0.0622	2.764	0.0834	3.732	5.693
	R1 pond	SD	0.0178	0.0158	0.0237	0.0208	0.0310
	R1 stream	SD+RO	0.182	0.652	0.0243	0.883	1.354
	R3 stream	SD+RO	0.259	0.907	0.348	1.173	1.799
	R4 stream	SD+RO	0.114	0.275	0.150	0.933	1.410

Table B. 9.4-6: Summary of maximum FOCUS Step 4, 10 m-meter buffer zone, VFS-mod.

Compound	FOCUS Step	Buffer zone for	Winter cereals 1x120 g s.a./ha		Winter cereals 1 x160 g s.a./ha		Winter cereals 1x240 g s.a./ha
			Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)
Flufenacet	D1 ditch	SD	0.194	2.680	0.259	4.328	6.543
	D1stream	SD	0.163	1.672	0.218	2.699	4.082
	D2 ditch	SD	1.702	3.227	2.412	3.957	6.199
	D2stream	SD	1.111	2.021	1.574	2.480	3.882
	D3 ditch	SD	0.109	0.109	0.146	0.145	0.218
	D4 pond	SD	0.0169	0.394	0.0224	0.750	1.159
	D4stream	SD	0.111	0.550	0.148	1.081	1.674
	D5 pond	SD	0.0190	0.556	0.0255	0.761	1.163
	D5stream	SD	0.120	0.579	0.160	0.812	1.249
	D6 ditch	SD	0.114	2.764	0.153	3.732	5.693
	R1 pond	SD	0.0164	0.0164	0.0218	0.0218	0.0326

Compound	FOCUS Step	Buffer zone for	Winter cereals 1x120 g s.a./ha		Winter cereals 1 x160 g s.a./ha		Winter cereals 1x240 g s.a./ha
			Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Spring application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)	Autumn application max PEC _{sw} (µg/L)
	R1stream	SD+RO	0.0971	0.0969	0.129	0.129	0.194
	R3stream	SD+RO	0.337	1.275	0.452	0.181	0.272
	R4stream	SD+RO	0.0971	0.0975	0.129	0.128	0.192

Groundwater

Since groundwater may become surface water, potential exposure of aquatic organisms via groundwater should be also considered. Groundwater simulations carried out for flufenacet and its relevant soil metabolites according to FOCUS requirements demonstrated that concentrations of compounds in groundwater is exceed the threshold concentration of 0.1 µg/L for five metabolites. The only one metabolite TFA, for which maximum calculated PEC_{gw} is greater than maximum Step 1 PEC_{sw} value was considered in the aquatic risk assessment. As a worst case PEC_{gw} values was used in the risk assessment instead the PEC_{sw}.

Table B.9.4-7: Concentration of metabolite TFA considered in aquatic risk assessment

Compound	Max PEC _{sw}	Max PC _{gw}	PEC _{sw} considered in aquatic risk assessment
TFA	20.457	22.426	22.426

Exposure of aquatic organisms to remaining compounds via groundwater is considered negligible and the risk assessment is thus deemed not necessary.

Risk assessment

The potential acute and long-term risk to aquatic organisms resulting from exposure to flufenacet and its metabolites was assessed by comparison of the maximum PEC_{sw} values calculated at particular FOCUS Steps with relevant toxicity endpoints presented in Tables B.9.3-1 and B.9.3-2.

Based on the representative most sensitive endpoint values and the PEC_{sw} values (highest values selected as worst case), the TER-values have been calculated, based on the following equations:

$$TER_A = LC_{50} \text{ or } EC_{50} / PEC_{sw}, TER_{Lt} = E_r C_{50} / PEC_{sw}$$

The risk is considered acceptable, if the TER_a values for fish and invertebrates are >100, and the TER_{lt} values >10. Effects to aquatic organisms from exposure to the metabolites of flufenacet were tested for the algae and aquatic macrophytes under consideration of the high sensitivity of the parent compound -flufenacet to these taxonomic groups. In addition, the studies for fish and aquatic invertebrates were performed for FOE sulfonic acid, FOE-Thiadone, Trifluoroacetic acid (TFA) metabolites and were taken into consideration in the risk assessment.

The toxicity of formulation DFF+FFA 600 SC were performed for the most sensitive to Flufenacet groups algae and aquatic plants.

RMS comments:

The endpoints obtained from the formulation's studies will not be used in the risk assessment until the Applicant reanalyses the results of the studies using the measured initial concentrations.

In addition, the Applicant is kindly request to perform the calculation of mixture toxicity according to the recommendations given in the AGD, 2013.

Acute risk assessment to fish

For flufenacet the risk assessment for fish was performed for two species – one freshwater fish species and one saltwater fish species. The freshwater fish species used was the most sensitive one - *Lepomis macrochirus* (Bluegill sunfish), while the representant of saltwater fish was - *Cyprinodon variegatus* (Sheepshead Minnow).

The acute toxicity studies to fish for metabolite FOE sulfonyl acid, FOE-Thiadone and for TFA were evaluated in this RAR and were taken into consideration in the risk assessment.

No studies with metabolites FOE-oxalate, FOE 5043 trifluoroethane sulfonic acid, FOE methylsulfone and FOE methylsulfide were submitted. However, under consideration that the most sensitive groups of aquatic organisms were identified algae and macrophytes acute toxicity studies with fish are not considered necessary.

The risk assessment for metabolites were limited to the most sensitive species tested.

The acute risk assessment for fish are presented in the Table B.9.4-8 below.

Table B.9.4-8: TER_A values for fish exposed to flufenacet and its metabolites.

Compound	Species	LC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw}	TER _A	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Lepomis macrochirus (Bluegill sunfish)	2130	Step 1	31.227	68.21	>100
			Step 2 NE	13.797	154.38	
			Step 2 SE	11.217	189.90	
	Cyprinodon variegatus (Sheepshead Minnow)	3310	Step 1	31.227	105.99	
FOE-Sulfonic acid	Oncorhynchus mykiss (Rainbow trout)	>86 700	Step 1	7.941	>10918.02	
FOE-Thiadone	Oncorhynchus mykiss (Rainbow trout)	9100	Step 1	1.464	6215.84	
TFA	Brachydanio rerio (Zebra fish)	>1200000	Step 1	10.228	>117325	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Lepomis macrochirus (Bluegill sunfish)	2130	Step 1	31.277	68.21	>100
			Step 2 NE	6.057	351.66	
			Step 2 SE	11.217	189.90	
	Cyprinodon variegatus (Sheepshead Minnow)	3310	Step 1	31.227	105.99	
FOE	Oncorhynchus mykiss	>86700	Step 1	7.941	>10918.02	

Compound	Species	LC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw}	TER _A	Trigger
Sulfonic acid	(Rainbow trout)					
FOE-Thiadone	Oncorhynchus mykiss (Rainbow trout)	9100	Step 1	1.464	6215.84	
TFA-	Brachydanio rerio (Zebra fish)	>1200 000	Step 1	10.228	>117325	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Lepomis macrochirus (Bluegill sunfish)	2130	Step 1	41.636	51.15	>100
			Step 2NE	18.395	115.79	
			Step 2SE	14.956	142.41	
	Cyprinodon variegatus (Sheepshead Minnow)	3310	Step 1	41.636	79.49	
			Step 2NE	18.395	179.94	
			Step 2SE	14.956	221.31	
FOE Sulfonic acid	Oncorhynchus mykiss (Rainbow trout)	>86700	Step 1	10.588	>8188.51	
FOE-Thiadone	Oncorhynchus mykiss (Rainbow trout)	9100	Step 1	1.952	4661.88	
TFA	Brachydanio rerio (Zebra fish)	>120 0000	Step 1	13.638	>87989.44	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Lepomis macrochirus (Bluegill sunfish)	2130	Step 1	41.636	51.15	100
			Step 2 NE	8.076	263.75	
			Step 2 SE	14.956	142.41	
	Cyprinodon variegatus (Sheepshead Minnow)	3310	Step 1	41.636	79.49	
			Step 2 NE	8.076	409.85	
			Step 2 SE	14.956	221.31	
FOE Sulfonic acid	Oncorhynchus mykiss (Rainbow trout)	>86 700	Step 1	10.588	>8188.51	
FOE-Thiadone	Oncorhynchus mykiss (Rainbow trout)	9100	Step 1	1.952	4661.88	
TFA- Trifluoroacetic acid	Brachydanio rerio (Zebra fish)	>1200 000	Step 1	13.638	>879894.44	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Lepomis macrochirus (Bluegill sunfish)	2130	Step 1	62.454	34.10	100
			Step 2 NE	27.593	77.19	
			Step 2 SE	22.433	94.94	
	Cyprinodon variegatus (Sheepshead Minnow)	3310	Step 1	62.454	52.99	
			Step 2 NE	27.593	119.95	
			Step 2 SE	22.433	147.55	

Compound	Species	LC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw}	TER _A	Trigger
FOE Sulfonic acid	Oncorhynchus mykiss (Rainbow trout)	>86 700	Step 1	15.882	54590.01	
FOE-Thiadone	Oncorhynchus mykiss (Rainbow trout)	9100	Step 1	2.928	3107.92	
TFA	Brachydanio rerio (Zebra fish)	>1200000	Step 1	22.426 ¹	>53509.31	

1) max PEC_{gw}
values in bold indicate unacceptable risk

All TER_A values calculated for metabolites with consideration of worst case exposure assumptions for all proposed uses are far above the trigger of 100 indicating acceptable acute risk to fish.

TER_A value calculated for flufenacet with consideration of Step 1 PEC_{sw} value was below the trigger of 100. Acceptable acute risk to fish could be concluded for Step 2 exposure estimates for all proposed uses in winter cereals except the maximum application rate - 240 g a.s./ha. Acceptable acute risk to fish for the most sensitive species *Lepomis macrochirus* could be, however concluded for Step 3 exposure estimates.

The TER_A calculations based on FOCUS Step 3 are presented in the Table B.9.4-9.

Table B. 9.4-9: Acute toxicity exposure ratios (TER_A) for fish

Compound	Species	LC ₅₀ (µg a.s./L)	FOCUS STEP 3	Max PEC _{sw} (µg a.s./L)	TER _A	Trigger
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	<i>Lepomis macrochirus</i> (Bluegill sunfish)	2130	D1 ditch	6.543	325.53	≥100
			D1 stream	4.082	521.80	
			D2 ditch	6.199	343.60	
			D2 stream	3.882	548.68	
			D3 ditch	1.514	1406.86	
			D4 pond	1.168	1823.63	
			D4 stream	1.647	1293.26	
			D5 pond	1.170	1820.51	
			D5 stream	1.420	1500.00	
			D6 ditch	5.693	374.19	
			R1 pond	0.116	18 362	
			R1 stream	5.811	366.54	
			R3 stream	7.641	278.75	
			R4 stream	5.980	356.18	

Acute TER values calculated for fish exposed after application of flufenacet according to recommendations are clearly above the respective triggers demonstrating acceptable acute risk to fish.

In addition, RMS presented the alternative approach to the acute risk assessment, performed according to the recommendations given in AGD, 2013, based on the usage of geometric mean recommended when the results from more than one species are available. The use of geometric mean - LC₅₀ of 4505 µg a.s./L, determined for four fish species (Oncorhynchus mykiss with LC₅₀ of 5840 µg a.s./L, Lepomis macrochirus with LC₅₀ of 2130 µg a.s./L, Cyprinus carpio with 10000 µg a.s./L and Cyprinodon variegatus with LC₅₀ of 3310 µg a.s./L), is considered relevant (endpoints derived using the same methodology and criteria and the difference between the lowest endpoint and geomean being lower than the factor of 10, in this particular case being 2).

The TER_A calculations based on FOCUS Step 1 are presented in the Table B.9.4.1-10.

Table B.9.4-10: TER_A values for fish exposed to flufenacet based on the geomean LC₅₀ value.

Compound	Species	LC ₅₀ geomean (µg a.s./L)	FOCUS STEP	Max PEC _{sw} (µg a.s./L)	TER _A	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Fish	4505	Step 1	31.227	144.03	>100
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Fish	4505	Step 1	31.277	144.03	>100
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Fish	4505	Step 1	41.636	108.19	>100
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Fish	4505	Step 1	41.636	108.19	100
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Fish	4505	Step 1	62.454	72.13	100
			Step 2 NE	27.593	163.26	
			Step 2 SE	22.433	200.82	

* values in bold indicate unacceptable risk

Acute TER_A values calculated for fish based on geomean LC₅₀ value, exposed after application of flufenacet are clearly above the respective triggers demonstrating acceptable acute risk to fish.

Acute risk assessment to aquatic invertebrates:

Acute toxicity studies for flufenacet with four aquatic invertebrate species were submitted. The most sensitive species were observed to be the species Hyalella azteca with LC₅₀ of 2.45 mg a.s./L, followed by Americamysis bahia and Crassostrea virginica. Among all aquatic invertebrates tested the Daphnia magna was the less sensitive species to flufenacet with EC₅₀ of 30.9 mg a.s./L. No studies with metabolites FOE-oxalate, FOE 5043 trifluoroethane sulfonic acid, FOE methylosulfone and FOE methylosulfide were submitted. However, under consideration that the most sensitive groups of aquatic organism were identified algae and macrophytes acute toxicity studies with fish are not considered necessary for these metabolites.

For FOE sulfonic acid, FOE-Thiadone and TFA the study for Daphnia magna were performed.

In case of, FOE-Thiadone three studies for aquatic invertebrates were evaluated.

Due to that Daphnia magna was less sensitive to flufenacet the risk assessment was also performed for most sensitive aquatic invertebrates.

Acute risk assessment for aquatic invertebrates is presented in table B.9.4-10 below:

Table B.9.4-10: TER_A values for aquatic invertebrates exposed to flufenacet and its metabolites for autumn and spring application to winter cereals.

Compound	Species	EC ₅₀ /LC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw}	TER _A	Trigger	
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence							
Flufenacet	Daphnia magna	309000	Step 1	31.227	9895.28	≥100	
	Americamysis bahia	5600	Step 1	31.227	179.33		
	Crassostrea virginica	12600	Step 1	31.227	403.49		
	Hyalella azteca	2450	Step 1	31.227	78.45		
			Step 2 NE	13.797	175.57		
			Step 2 SE	11.217	218.41		
FOE sulfonic acid	Daphnia magna	>87 300	Step 1	7.941	>10993.57		
FOE-Thiadone	Daphnia magna	31700	Step 1	1.464	21653.00		
	Mysidiopsis bahia	>15100	Step 1	1.464	>10314.20		
	Crassostrea virginica	22 000	Step 1	1.464	15027.32		
TFA	Daphnia magna	>1200000	Step 1	10.228	>117324.99		
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence							
Flufenacet	Daphnia magna	309000	Step 1	31.277	9879.46	≥100	
	Americamysis bahia	5600	Step 1	31.277	179.04		
	Crassostrea virginica	12600	Step 1	31.277	402.85		
	Hyalella azteca	2450	Step 1	31.277	78.33		
			Step 2 NE	6.057	404.49		
			Step 2 SE	11.217	218.41		
FOE sulfonic acid	Daphnia magna	>87 300	Step 1	7.941	>10993.57		
FOE-Thiadone	Daphnia magna	31700	Step 1	1.464	21653.00		
	Mysidiopsis bahia	>15100	Step 1	1.464	>10314.20		
TFA	Daphnia magna	>1200000	Step 1	10.228	>117324.99		
Autumn use in winter cereals at rate 1x 160 g a.s./ha, post emergence							
Flufenacet	Daphnia magna	309000	Step1	41.636	7421.46		≥100

Compound	Species	EC ₅₀ /LC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw}	TER _A	Trigger
	Americamysis bahia	5600	Step 1	41.636	134.49	
	Crassostrea virginica	12600	Step 1	41.636	302.62	
	Hyaella azteca	2450	Step 1	41.636	58.84	
			Step 2 NE	18.395	133.18	
			Step 2 SE	14.956	163.81	
FOE sulfonic acid	Daphnia magna	>87 300	Step1	10.588	>8245.18	
FOE-Thiadone	Daphnia magna	31700	Step 1	1.952	16239.75	
	Mysidiopsis bahia	>15100	Step 1	1.952	>7735.65	
	Crassostrea virginica	22 000	Step 1	1.952	11270.49	
TFA	Daphnia magna	>1200000	Step 1	13.638	>87989.44	
Spring use in winter cereals at rate 1x 160 g a.s./ha, post emergence						
Flufenacet	Daphnia magna	309000	Step1	41.636	7421.46	≥100
	Americamysis bahia	5600	Step 1	41.636	134.49	
	Crassostrea virginica	12600	Step 1	41.636	302.62	
	Hyaella azteca	2450	Step 1	41.636	58.84	
			Step 2 NE	8.076	303.36	
			Step 2 SE	14.956	163.81	
FOE sulfonic acid	Daphnia magna	>87 300	Step1	10.588	>8245.18	
FOE-Thiadone	Daphnia magna	31700	Step 1	1.952	16239.75	≥100
	Mysidiopsis bahia	>15100	Step 1	1.952	>7735.65	
	Crassostrea virginica	22 000	Step 1	1.952	11270.49	
TFA	Daphnia magna	>1200000	Step 1	13.638	>87989.44	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Daphnia magna	309000	Step 1	62.454	4947.64	≥100
	Americamysis bahia	5600	Step 1	62.454	89.66	
			Step 2 NE	27.593	202.95	
			Step 2 SE	22.433	249.63	
	Crassostrea virginica	12600	Step 1	62.454	201.74	
	Hyaella azteca	2450	Step 1	62.454	39.22	

Compound	Species	EC ₅₀ /LC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw}	TER _A	Trigger
			Step 2 NE	27.593	88.79	
			Step 2 SE	22.433	109.21	
FOE Sulfonic acid	Daphnia magna	>87 300	Step 1	15.882	>5496.78	
FOE-Thiadone	Daphnia magna	31700	Step 1	2.928	10826.50	
	Mysidiopsis bahia	>15100			>5157.10	
	Crassostrea virginica	22 000			7513.66	
TFA	Daphnia magna	>1200 000	Step 1	22.426 ¹	>53509.31	

1) max PEC_{gw}
values in bold indicate unacceptable risk

All TER_A values calculated for metabolites with consideration of worst case exposure assumptions are far above the trigger of 100 indicating acceptable acute risk to aquatic invertebrates.

TER_A value calculated for flufenacet with consideration of Step 2 PEC_{sw} value at rate 240 g a.s./ha for autumn use to winter cereals and the lowest toxicity endpoint for *Hyalella azteca* was below the trigger of 100. Acceptable acute risk to this species could be, however concluded for Step 3 exposure estimates.

The TER_A calculations based on FOCUS Step 3 are presented in the Table B.9.4-11.

Table B. 9.4-11: Acute toxicity exposure ratios (TER_A) for *Hyalella azteca* based on worst case scenario PEC_{sw} from STEP 3.

Compound	Species	LC ₅₀ (µg a.s./L)	FOCUS STEP 3	Max PEC _{sw} (µg a.s./L)	TER _A	Trigger
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	<i>Hyalella azteca</i>	2450	D1 ditch	6.543	374.44	≥100
			D1stream	4.082	600.19	
			D2 ditch	6.199	395.22	
			D2stream	3.882	631.11	
			D3 ditch	1.514	1618.22	
			D4 pond	1.168	2097.60	
			D4stream	1.647	1487.55	
			D5 pond	1.170	2094.01	
			D5stream	1.420	1725.35	
			D6 ditch	5.693	430.35	
			R1 pond	0.116	21120.68	
			R1stream	5.811	421.61	
			R3stream	7.641	320.63	

Compound	Species	LC ₅₀ (µg a.s./L)	FOCUS STEP 3	Max PEC _{sw} (µg a.s./L)	TER _A	Trigger
			R4stream	5.980	409.69	

All TER_A values calculated for flufenacet with consideration of calculation STEP 3 PEC_{sw} are far above the trigger of 100 indicating acceptable acute risk to aquatic invertebrates.

In addition, RMS presented the alternative approach to the acute risk assessment, performed according to the recommendations given in AGD, 2013, based on the usage of geometric mean recommended when results from more than one species are available. The use of geometric mean - EC₅₀ of 7512 µg a.s./L, determined for three aquatic invertebrates species (*Daphnia magna* with EC₅₀ of 30900 µg a.s./L, *Americamysis bahia* with LC₅₀ of 5600 µg a.s./L and *Hyalella azteca* with LC₅₀=2450 µg a.s./L, is considered relevant (endpoints derived from the same criteria and the difference between the lowest endpoint and geomean being lower than factor of 10, in this particular case being 3).

The TER_A calculations based on FOCUS Step 1 are presented in the Table B.9.4.1-12.

Table B.9.4-12: TER_A values for aquatic invertebrates exposed to flufenacet based on the geomean EC₅₀.

Compound	Species	EC ₅₀ /LC ₅₀ geomean (µg a.s./L)	FOCUS STEP	Max PEC _{sw} (µg a.s./L)	TER _A	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Aquatic invertebrates	7512	Step 1	31.227	240.17	>100
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Aquatic invertebrates	7512	Step 1	31.277	240.17	>100
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Aquatic invertebrates	7512	Step 1	41.636	180.63	>100
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Aquatic invertebrates	7521	Step 1	41.636	180.63	>100
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Aquatic invertebrates	7512	Step 1	62.454	120.28	>100

All TER_A values calculated for flufenacet with consideration of calculation STEP 1 PEC_{sw} and geomean EC₅₀ value are far above the trigger of 100 indicating acceptable acute risk to aquatic invertebrates.

Long- term risk for fish:

For flufenacet the lowest chronic fish NOEC of 49 µg/L for the saltwater fish species *Cyprinodon variegatus* was used in the risk assessment.

Long-term risk assessment for fish is presented in the Table B.9.4-13 below.

Table B.9.4-13 TER_{LT} values for fish exposed to flufenacet for spring and autumn application in winter cereals.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Cyprinodon variegatus	49	Step 1	31.227	1.56	≥10
			Step 2NE	13.797	3.55	
			Step 2SE	11.217	4.36	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	Step 1	31.277	1.56	≥10
			Step 2NE	6.057	8.08	
			Step 2SE	11.217	4.36	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	Step 1	41.636	1.17	≥10
			Step 2NE	18.395	2.66	
			Step 2SE	14.956	3.27	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	Step 1	41.636	1.17	≥10
			Step 2NE	8.076	6.06	
			Step 2SE	14.956	3.27	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	Step 1	62.454	0.78	≥10
			Step 2NE	27.593	1.77	
			Step 2SE	22.433	2.18	

*values in bold indicate unacceptable risk

All TER_{LT} values calculated for flufenacet with consideration of Step 1 and Step 2 PEC_{sw} values for all proposed uses in winter cereals were below the trigger of 10. Therefore, a refined risk assessment based on FOCUS Step 3 were performed and presented in the Tables B.9.4-14.

Table B.9.4-14: TER_{LT} values for fish exposed to flufenacet for autumn and spring application to winter cereals.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence						
Flufenacet	Cyprinodon variegatus	49	D1 ditch	2.68	18.28	≥10
			D1 stream	1.672	29.30	
			D2 ditch	3.227	15.20	
			D2 stream	2.021	24.25	
			D3 ditch	0.758	64.65	
			D4 pond	0.398	123.11	
			D4 stream	0.658	74.50	
			D5 pond	0.56	87.50	
			D5 stream	0.71	69.01	
			D6 ditch	2.764	17.72	
			R1 pond	0.0609	804.6	
			R1 stream	2.8	17.5	
			R3 stream	3.783	12.95	
			R4 stream	1.167	42.00	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	D1 ditch	0.846	58.00	≥10
			D1 stream	0.629	77.90	
			D2 ditch	1.702	28.80	
			D2 stream	1.111	44.10	
			D3 ditch	0.760	64.50	
			D4 pond	0.0267	1835.20	
			D4 stream	0.572	85.70	
			D5 pond	0.0289	1695.50	
			D5 stream	0.614	79.80	
			D6 ditch	0.756	64.81	
			R1 pond	0.0687	713.30	
			R1 stream	0.764	64.40	
			R3 stream	1.080	45.40	
			R4 stream	0.501	97.80	
Autumn use to winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	D1 ditch	4.328	11.32	≥10
			D1 stream	2.699	18.15	
			D2 ditch	3.957	12.38	
			D2 stream	2.480	19.75	
			D3 ditch	1.010	48.51	
			D4 pond	0.756	64.81	

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D4 stream	1.081	45.32	
			D5 pond	0.766	63.96	
			D5 stream	0.946	51.79	
			D6 ditch	3.732	13.13	
			R1 pond	0.0797	614.80	
			R1 stream	3.790	12.92	
			R3 stream	4.980	9.83	
			R4 stream	3.957	12.38	
Spring use to winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	D1 ditch	1.129	43.40	≥10
			D1 stream	0.838	58.50	
			D2 ditch	2.412	20.31	
			D2 stream	1.574	31.13	
			D3 ditch	1.014	48.32	
			D4 pond	0.0357	1372.55	
			D4 stream	0.763	64.22	
			D5 pond	0.0387	1266.15	
			D5 stream	0.818	59.90	
			D6 ditch	1.009	48.60	
			R1 pond	0.0913	536.70	
			R1 stream	1.021	48.0	
			R3 stream	1.450	33.80	
			R4 stream	0.668	73.40	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	D1 ditch	6.543	7.48	≥10
			D1 stream	4.082	12.00	
			D2 ditch	6.199	7.90	
			D2 stream	3.882	12.62	
			D3 ditch	1.514	32.36	
			D4 pond	1.168	41.95	
			D4 stream	1.647	29.75	
			D5 pond	1.170	41.88	
			D5 stream	1.420	34.50	
			D6 ditch	5.693	8.60	
			R1 pond	0.116	422.41	
			R1 stream	5.811	8.43	
			R3 stream	7.641	6.41	
			R4 stream	5.980	8.19	

*values in bold indicate unacceptable risk

All TER_{LT} values calculated for flufenacet with consideration of STEP 3 PEC_{sw} values for spring application in winter cereals for all proposed rates were above of the trigger of 10, indicated acceptable long-term risk for fish.

TER_{LT} values calculated for flufenacet with consideration of STEP 3 PEC_{sw} values for autumn application to winter cereals were below the trigger of 10 for the following scenarios:

- R3 (stream) for application rate 1 x 160 g a.s./ha
- D1 (ditch), D2 (ditch), D6 (ditch) R1 (stream), R3 (stream) and R4 (stream) for application rate 1 x 240 g a.s./ha.

Therefore, further refinement was performed using FOCUS Step 4 PEC_{sw} with 10 m FOCUS buffer zone.

Table B.9.4-15: TER_{LT} values for fish exposed to flufenacet with 10 m FOCUS buffer zone for autumn application to winter cereals.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 4 (10 m buffer zone)	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	R3 stream	2.246	21.81	≥10
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	D 1 ditch	6.543	7.48	≥10
			D 2 ditch	6.199	7.90	
			D6 ditch	5.693	8.60	
			R1 stream	2.602	18.83	
			R3 stream	3.446	14.21	
			R4 stream	2.699	18.15	

TER_{LT} values calculated for flufenacet with consideration of STEP 4 PEC_{sw} values with 10 m FOCUS buffer zone for R3 scenario at rate 1x 160 g a.s./ha and for R1, R3 and R4 scenarios at rate 1x 240 g a.s./ha for autumn use in winter cereals were above the trigger of 10, indicated acceptable long-term risk for fish.

For D1 (ditch), D2, (ditch) and D6 (ditch) scenarios further refinement was performed using FOCUS STEP 4 PEC_{sw} with 20 m buffer zone.

Table B.9.4-16: TER_{LT} values for fish exposed to flufenacet with 20 m FOCUS buffer zone for autumn application to winter cereals.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 4 (20 m buffer zone)	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Cyprinodon variegatus	49	D1 (ditch)	6.543	7.48	≥10
			D2 (ditch)	6.199	7.90	
			D6 ditch)	5.693	8.60	

TER_{LT} values calculated for flufenacet with consideration of STEP 4 PEC_{sw} values with 20 m FOCUS buffer zone for autumn use in winter cereals for D1 (ditch), D2 (ditch) and D6 (ditch) scenarios were still below the trigger of 10, indicated needs to further refinement.

Therefore, the refinement of long-term risk assessment for fish was based on the geomean NOEC- 131 µg a.s./L. The use of geometric mean determined for three fish species (Oncorhynchus mykiss with NOEC of 334 µg a.s./L, Pimephales promelas with NOEC of 138 of µg a.s./L and Cyprinodon variegates with NOEC with 49 µg a.s./L, is considered relevant (endpoints derived using the criteria and the difference between the lowest endpoint and geomean being lower than the factor of 10, in this particular case being 3).

The TER_{LT} calculations based on FOCUS Step 1-3 are presented in the Table B.9.4.17 and the Table B.9.4.1-18.

Table B.9.4-17 TER_{LT} values for fish exposed to flufenacet based on the geomean NOEC and PEC_{sw} calculation in FOCUS Step 1-2 for spring and autumn application in winter cereals.

Compound	Species	NOEC geomean (µg a.s./L)	FOCUS Step	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Fish	131	Step 1	31.227	4.18	≥10
			Step 2 NE	13.797	9.49	
			Step 2 SE	11.217	11.67	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Fish	131	Step 1	31.277	4.18	≥10
			Step 2NE	6.057	21.62	
			Step 2SE	11.217	11.67	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Fish	131	Step 1	41.636	3.14	≥10
			Step 2NE	18.395	7.12	
			Step 2SE	14.956	8.75	

Compound	Species	NOEC geomean (µg a.s./L)	FOCUS Step	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Fish	131	Step 1	41.636	3.14	≥10
			Step 2NE	8.076	16.22	
			Step 2SE	14.956	8.75	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Fish	131	Step 1	62.454	2.09	≥10
			Step 2NE	27.593	4.74	
			Step 2SE	22.433	5.83	

Table B.9.4-18: TER_{LT} values for fish exposed to flufenacet for autumn and spring application to winter cereals.

Compound	Species	NOEC geomean (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence						
Flufenacet	Fish	131	D1 ditch	2.68	48.88	≥10
			D1 stream	1.672	78.34	
			D2 ditch	3.227	40.59	
			D2 stream	2.021	64.81	
			D3 ditch	0.758	172.821	
			D4 pond	0.398	329.14	
			D4 stream	0.658	199.08	
			D5 pond	0.56	233.92	
			D5 stream	0.71	184.50	
			D6 ditch	2.764	47.39	
			R1 pond	0.0609	2151.06	
			R1 stream	2.8	46.78	
			R3 stream	3.783	34.62	
			R4 stream	1.167	112.25	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Fish	131	D1 ditch	0.846	154.84	≥10
			D1 stream	0.629	208.26	
			D2 ditch	1.702	76.96	
			D2 stream	1.111	117.91	
			D3 ditch	0.760	172.36	
			D4 pond	0.0267	4906.36	
			D4 stream	0.572	229.02	
			D5 pond	0.0289	4532.87	
			D5 stream	0.614	213.35	

Compound	Species	NOEC geomean (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D6 ditch	0.756	173.28	
			R1 pond	0.0687	1906.84	
			R1 stream	0.764	171.46	
			R3 stream	1.080	121.29	
			R4 stream	0.501	261.47	
Autumn use to winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	variegatus Fish	131	D1 ditch	4.328	30.26	≥10
			D1 stream	2.699	48.53	
			D2 ditch	3.957	33.10	
			D2 stream	2.480	52.82	
			D3 ditch	1.010	129.70	
			D4 pond	0.756	173.28	
			D4 stream	1.081	121.18	
			D5 pond	0.766	171.01	
			D5 stream	0.946	138.47	
			D6 ditch	3.732	35.10	
			R1 pond	0.0797	1643.66	
			R1 stream	3.790	34.56	
			R3 stream	4.980	26.30	
			R4 stream	3.957	33.10	
Spring use to winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Fish	131	D1 ditch	1.129	116.03	≥10
			D1 stream	0.838	156.32	
			D2 ditch	2.412	54.31	
			D2 stream	1.574	83.22	
			D3 ditch	1.014	129.19	
			D4 pond	0.0357	3669.46	
			D4 stream	0.763	171.69	
			D5 pond	0.0387	3385.01	
			D5 stream	0.818	160.14	
			D6 ditch	1.009	129.83	
			R1 pond	0.0913	1434.83	
			R1 stream	1.021	128.30	
			R3 stream	1.450	90.34	
			R4 stream	0.668	196.10	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Fish	131	D1 ditch	6.543	20.02	≥10

Compound	Species	NOEC geomean (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D1 stream	4.082	32.09	
			D2 ditch	6.199	21.13	
			D2 stream	3.882	33.74	
			D3 ditch	1.514	86.52	
			D4 pond	1.168	112.15	
			D4 stream	1.647	79.53	
			D5 pond	1.170	111.96	
			D5 stream	1.420	92.25	
			D6 ditch	5.693	23.01	
			R1 pond	0.116	29.31	
			R1 stream	5.811	22.54	
			R3 stream	7.641	17.14	
			R4 stream	5.980	21.90	

*values in bold indicate unacceptable risk

All TER_{LT} values calculated for flufenacet with consideration of calculation STEP 3 PEC_{sw} and geomean NOEC value are above the trigger of 10 indicating acceptable long-term risk to fish.

Long - term risk for aquatic invertebrates

For flufenacet the lowest chronic toxicity endpoint NOEC of 221 µg a.s./L was obtained for Mysisidopsis bahia species. Therefore, this endpoint was used in the risk assessment.

Long-term risk assessment is presented in the Table B.9.4-19 below.

Table B.9.4-19: TER_{LT} values for Mysisidopsis bahia exposed to flufenacet.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Mysidopsis bahia	221	Step 1	31.227	7.06	≥10
			Step 2NE	13.797	16.01	
			Step 2SE	11.217	19.70	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Mysidopsis bahia	221	Step 1	31.277	7.06	≥10
			Step 2NE	6.057	36.48	
			Step 2SE	11.217	19.70	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Mysidopsis	221	Step1	41.636	5.30	≥10

Compound	Species	NOEC (µg a.s./L)	FOCUS Step	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
	bahia		Step 2NE	18.395	12.01	
			Step 2SE	14.956	14.77	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Mysidopsis bahia	221	Step 1	41.636	5.30	≥10
			Step 2NE	8.076	27.36	
			Step 2SE	14.956	14.77	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Mysidopsis bahia	221	Step 1	62.454	3.53	≥10
			Step 2NE	27.593	8.00	
			Step 2SE	22.433	9.85	

*values in bold indicate unacceptable risk

TER_{LT} values calculated for flufenacet with consideration of Step 2 PEC_{sw} values and the lowest toxicity endpoint were above the trigger of 10, except the maximum application rate 1 x 240 g a.s./ha.

Therefore, the TER calculations based on FOCUS Step 3 for application rate 1 x 240 g a.s./ha were conducted in the Table below:

Table B. 9.4.1-20: Chronic toxicity exposure ratios (TER_{LT}) for aquatic invertebrates based on worst case scenario PEC_{sw} from STEP 3.

Compound	Species	NOEC (µg s.a./L)	FOCUS STEP 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence						
Flufenacet	Mysidopsis bahia	221	D1 ditch	6.543	33.77	≥10
			D1stream	4.082	54.14	
			D2 ditch	6.199	35.65	
			D2stream	3.882	56.92	
			D3 ditch	1.514	145.97	
			D4 pond	1.168	189.21	
			D4stream	1.647	134.18	
			D5 pond	1.170	188.88	
			D5stream	1.420	155.63	
			D6 ditch	5.693	38.81	
			R1 pond	0.116	1905.17	
			R1stream	5.811	38.03	
			R3stream	7.641	28.92	
			R4stream	5.980	36.95	

TER_{LT} value calculated for flufenacet with consideration of Step 3 PEC_{sw} value for maximum application rate 1 x 240 g a.s./ha was above the trigger of 10 which indicated acceptable long-term risk to aquatic invertebrates.

In addition, RMS presented the long-term risk assessment for aquatic invertebrates based on the geomean NOEC of 847 µg a.s./L. The use of geometric mean of two species (*Daphnia magna* with NOEC of 3260 µg a.s./L and *Americamysis bahia* with NOEC₅₀ of 221 µg a.s./L) is considered relevant (endpoints derived from the same criteria and the difference between the lowest endpoint and geomean being lower than factor of 10, in this particular case being 4).

The TER_{LT} calculations based on FOCUS Step 1 are presented in the Table B.9.4.1-21

Table B.9.4-21 TER_{LT} values for aquatic invertebrates exposed to flufenacet based on the geomean NOEC and PEC_{sw} calculation in FOCUS Step 1 for spring and autumn application in winter cereals.

Compound	Species	NOEC geomean (µg a.s./L)	FOCUS Step	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Aquatic invertebrates	849	Step 1	31.227	27.18	≥10
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Aquatic invertebrates	849	Step 1	31.277	27.14	≥10
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Aquatic invertebrates	849	Step 1	41.636	20.39	≥10
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Aquatic invertebrates	849	Step 1	41.636	20.39	≥10
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Aquatic invertebrates	849	Step 1	62.454	13.59	≥10

All TER_{LT} values calculated for flufenacet with consideration of calculation STEP 1 PEC_{sw} and NOEC geomean values are below the trigger of 10 indicating needs to further refinement.

Risk assessment for sediment dwelling organisms

One toxicity study for *Chironomus riparius* was performed (in water-spiked) with NOEC=5 mg a.s./L, based on emergence.

Long - term risk assessment for sediment dwellers is presented in Table B.9.4-22 below:

Table B.9.4-22: TER_{LT} values for sediment dwelling organism exposed to flufenacet.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence						
Flufenacet	<i>Chironomus riparius</i>	5000	Step 1	31.227	160.11	≥10

Compound	Species	NOEC (µg a.s./L)	FOCUS Step	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Chironomus riparius	5000	Step 1	31.227	160.11	≥10
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Chironomus riparius	5000	Step 1	41.636	120.08	≥10
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Chironomus riparius	5000	Step 1	41.636	120.08	≥10
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Chironomus riparius	5000	Step 1	62.454	80.05	≥10

Risk assessment for flufenacet based on endpoint derived from water spiked study demonstrated acceptable long-term risk to sediment dwelling organisms already for Step 1 PEC_{sw}.

Risk assessment for algae

Following the Aquatic Guidance Document (AGD) „Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters”, EFSA Panel on Plant Protection Products and their Residues (PPR) 23 European Food Safety Authority (EFSA), Parma, Italy”, on the risk assessment for aquatic organisms, growth rate is the preferred endpoint to be used in the risk assessment since it is more robust considering varying test conditions. Biomass endpoints should not be used as direct use of the biomass concentrations without logarithmic transformation can not be applied to an analysis of results from a system in exponential growth. Yield is only included for cases where specific regulatory requirements in some countries may need to be fulfilled. Taking into account that recommendation given in the AGD (2013), the risk assessment was based on growth rates for the most sensitive to flufenacet alga species—*Selenastrum carpicornutum*.

The geometric mean value $E_rC_{50} = 7.55 \mu\text{g s.a./L}$, based on the results obtained from three laboratory studies for *Pseudokirchinella subcapitata* with 96 h $E_rC_{50} = 3.15 \mu\text{g s.a./L}$, 96 h $E_rC_{50} = 6.45 \mu\text{g s.a./L}$ and 72 h $E_rC_{50} = 21.20 \mu\text{g s.a./L}$ was proposed by RMS to be used in the risk assessment.

To derive that value RMS used the data from two standard studies and from the non-standard study. In case of two of them, having the lower endpoints (96 h $E_rC_{50} = 3.15 \mu\text{g s.a./L}$ from one of the standard studies, and 96 h $E_rC_{50} = 6.45 \mu\text{g s.a./L}$ from the non-standard study, but with the study design similar to what recommends the OECD Guideline No. 201) one of the validity criteria was not met, rendering the studies of limited, although sufficient, reliability. In the other newly submitted study, determined toxicity endpoint was much higher (72 h $E_rC_{50} = 21.20 \mu\text{g s.a./L}$) in comparison to the two former studies.

As all three studies are considered acceptable for the same species, the RMS decided to calculate the geometric mean E_rC_{50} value.

The risk assessment for algae is presented in the Table B 9.4-23 below:

Table B. 9.4-23: TER_{LT} values for *P. subcapitata* exposed to flufenacet and its metabolites.

Compound	Species	ErC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw}	TER _A	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence						
Flufenacet	P.subcapitata	7.55	Step 1	31.227	0.24	≥10
			Step 2 NE	13.797	0.54	
			Step 2 SE	11.217	0.67	
FOE oxalate	P.subcapitata	>100 000	Step 1	6.466	>15465.51	
FOE methylsulfide	P.subcapitata	83 800	Step 1	0.084	997619.04	
FOE methylsulfone	P.subcapitata	>10 000	Step 1	1.896	>5274.26	
FOE-Sulfonic acid	P.subcapitata	>86700	Step 1	7.941	>10918.02	
TFA	P.subcapitata	>1200	Step 1	10.228	>117.32	
FOE-Trifluoroethane sulfonic acid	P.subcapitata	>100 000	Step 1	1.084	>92250.92	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post-emergence						
Flufenacet	P.subcapitata	7.55	Step 1	31.277	0.24	≥10
			Step 2 NE	6.057	1.24	
			Step 2 SE	11.217	0.67	
FOE oxalate	P.subcapitata	>100 000	Step 1	6.466	>15465.51	
FOE methylsulfide	P.subcapitata	83 800	Step 1	0.084	997619.04	
FOE methylsulfone	P.subcapitata	>10 000	Step 1	1.896	>5274.26	
FOE-Sulfonic acid	P.subcapitata	>86700	Step 1	7.941	>10918.02	
TFA	P.subcapitata	>1200	Step 1	10.228	>117.32	
FOE Trifluoroethane sulfonic acid	P.subcapitata	>100 000	Step 1	1.084	>92250.92	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post-emergence						
Flufenacet	P.subcapitata	7.55	Step 1	41.636	0.18	≥10
			Step 2	18.395	0.41	
			Step 2	14.956	0.50	
FOE oxalate	P.subcapitata	>100 000	Step 1	8.622	>11598.23	
FOE methylsulfide	P.subcapitata	83 800	Step 1	0.111	754954.95	
FOE methylsulfone	P.subcapitata	>10 000	Step1	2.528	3955.69	
FOE-Sulfonic acid	P.subcapitata	>86700	Step1	10.588	8188.51	
TFA	P.subcapitata	>1200	Step 1	13.638	87.98	
FOE-Trifluoroethane sulfonic acid	P.subcapitata	>100 000	Step 1	1.445	69204.15	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post-emergence						
Flufenacet	P.subcapitata	7.55	Step 1	41.636	0.18	≥10
			Step 2NE	8.076	0.93	
			Step 2 SE	14.956	0.50	
FOE oxalate	P.subcapitata	>100 000	Step 1	8.622	>11598.23	
FOE methylsulfide	P.subcapitata	83 800	Step 1	0.111	754954.95	
FOE methylsulfone	P.subcapitata	>10 000	Step 1	2.528	>3955.69	
FOE-Sulfonic acid	P.subcapitata	>86700	Step1	10.588	8188.51	
TFA	P.subcapitata	1200	Step 1	13.638	>87.98	
FOE-Trifluoroethane sulfonic acid	P.subcapitata	>100 000	Step 1	1.445	>69204.15	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	Step 1	62.454	0.12	

Compound	Species	E _r C ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw}	TER _A	Trigger
				27.593	0.27	≥10
				22.433	0.33	
FOE oxalate	P.subcapitata	>100 000	Step 1	12.933	>7732.15	
FOE methylsulfide	P.subcapitata	83 800	Step 1	0.167	501796.40	
FOE methylsulfone	P.subcapitata	>10 000	Step 1	3.792	>2637.13	
FOE-Sulfonic acid	P.subcapitata	>86700	Step 1	15.882	5459.01	
TFA	P.subcapitata	>1200	Step 1	22.426 ¹	>53.50	
FOE-Trifluoroethane sulfonic acid	P.subcapitata	>100 000	Step 1	2.168	>46125.46	

¹ max PEC_{gw}
values in bold indicate unacceptable risk

All TER_{LT} values calculated for metabolites of flufenacet with consideration of worst case exposure assumptions are far above the trigger of 10 indicating acceptable chronic risk to algae. However, the TER_{LT} values for flufenacet obtained by FOCUS 2 calculations for spring and autumn applications to winter cereals are lower than the Annex VI trigger value of 10 and needs further refinement.

Therefore, the TER calculations based on FOCUS Step 3 were conducted for all proposed uses of flufenacet.

STEP 3

Table B. 9.4-24: Chronic toxicity exposure ratios (TER_{LT}) for algae based on worst case scenario PEC_{sw} from STEP 3.

Compound	Species	E _r C ₅₀ (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	2.680	2.81	≥10
			D1 stream	1.672	4.51	
			D2 ditch	3.227	2.33	
			D2 stream	2.021	3.73	
			D3 ditch	0.758	9.96	
			D4 pond	0.398	18.96	
			D4 stream	0.658	11.47	
			D5 pond	0.560	13.48	
			D5 stream	0.710	10.63	
			D6 ditch	2.764	2.73	
			R1 pond	0.0609	123.97	
			R1 stream	2.800	2.69	
			R3 stream	3.783	1.99	
			R4 stream	1.167	6.46	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	0.846	8.92	≥10
			D1 stream	0.629	12.0	
			D2 ditch	1.702	4.43	
			D2 stream	1.111	6.79	
			D3 ditch	0.760	9.93	
			D4 pond	0.0267	282.77	

Compound	Species	ErC ₅₀ (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D4 stream	0.572	13.19	≥10
			D5 pond	0.0289	261.24	
			D5 stream	0.614	12.29	
			D6 ditch	0.756	9.98	
			R1 pond	0.0687	109.89	
			R1 stream	0.764	9.98	
			R3 stream	1.080	6.99	
			R4 stream	0.501	15.06	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	4.328	1.74	≥10
			D1 stream	2.699	2.79	
			D2 ditch	3.957	1.90	
			D2 stream	2.480	3.04	
			D3 ditch	1.010	7.47	
			D4 pond	0.756	9.98	
			D4 stream	1.081	6.98	
			D5 pond	0.766	9.85	
			D5 stream	0.946	7.98	
			D6 ditch	3.732	2.02	
			R1 pond	0.0797	94.73	
			R1 stream	3.790	1.99	
			R3 stream	4.980	1.51	
			R4 stream	3.957	1.89	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	1.129	6.68	≥10
			D1 stream	0.838	9.00	
			D2 ditch	2.412	3.13	
			D2 stream	1.574	4.79	
			D3 ditch	1.014	7.44	
			D4 pond	0.0357	211.48	
			D4 stream	0.763	9.89	
			D5 pond	0.0387	195.09	
			D5 stream	0.818	9.22	
			D6 ditch	1.009	7.48	
			R1 pond	0.0913	82.69	
			R1 stream	1.021	7.39	
			R3 stream	1.450	5.20	
			R4 stream	0.668	11.30	
Autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	6.543	1.15	≥10
			D1 stream	4.082	1.84	
			D2 ditch	6.199	1.21	
			D2 stream	3.882	1.94	
			D3 ditch	1.514	4.98	
			D4 pond	1.168	6.46	
			D4 stream	1.647	4.58	
			D5 pond	1.170	6.45	
			D5 stream	1.420	5.31	

Compound	Species	ErC ₅₀ (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D6 ditch	5.693	1.32	
			R1 pond	0.116	65.08	
			R1 stream	5.811	1.29	
			R3 stream	7.641	0.98	
			R4 stream	5.980	1.26	

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B. 9.4-25: The FOCUS STEP 3 scenarios, for which the P.subcapitata TER_{LT} needs further refinement.

Scenario STEP 3	Winter cereals				
	Autumn use 120 g s.a./ha	Autumn use 160 g a.s./ha	Autumn use 240 g a.s./ha	Spring use 120 g a.s./ha	Spring use 160 g a.s./ha
D1 ditch	D1 ditch	D1 ditch	D1 ditch	D1 ditch	D1 ditch
D1 stream	D1 stream	D1 stream	D1 stream	-	D1 stream
D2 ditch	D2 ditch	D2 ditch	D2 ditch	D2 ditch	D2 ditch
D2 stream	D2 stream	D2 stream	D2 stream	D2 stream	D2 stream
D3 ditch	D3 ditch	D3 ditch	D3 ditch	D3 ditch	D3 ditch
D4 pond	-	D4 pond	D4 pond	-	-
D4 stream	-	D4 stream	D4 stream	-	D4 stream
D5 pond	-	D5 pond	D5 pond	-	-
D5 stream	-	D5 stream	D5 stream	-	D5 stream
D6 ditch	D6 ditch	D6 ditch	D6 ditch	D6 ditch	D6 ditch
R1 pond	-	-	-	-	-
R1 stream	R1 stream	R1 stream	R1 stream	R1 stream	R1 stream
R3 stream	R3 stream	R3 stream	R3 stream	R3 stream	R3 stream
R4 stream	R4 stream	R4 stream	R4 stream		-

- No further refinement is needed

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 3 maximum PEC_{sw} values for flufenacet, the TER_{LT} values for algae meet the required trigger of 10, except the scenarios given in the Table B.9.4-24. For those scenarios, P.subcapitata TER needs further refinement.

Therefore, the PEC_{sw} values for flufenacet were further refined at FOCUS Step 4 using 10-metres- and 20-metres-wide FOCUS buffer zones for mitigation of Spray Drift and Run-off. As an additional refinement step in Run-off mitigation were performed the calculations in VFS-mod assuming the 10-metres wide buffer zone for reducing migration with Spray Drift (D and R) scenarios and Run-off (for R scenarios only).

STEP 4 – 10-metres wide FOCUS buffer zone for mitigation of Spray Drift and Run-off

TER_{LT} values for relevant scenarios based on worst case scenario PEC_{sw} from STEP 4 with 10 meter buffer zone FOCUS was presented in the Table B.9.4-26.

Table B. 9.4-26: Chronic toxicity exposure ratios (TER_{LT}) for *P.subcapitata* based on worst case scenario PEC_{sw} from STEP 4 with 10 meter buffer zone FOCUS.

Compound	Species	E _r C ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	2.680	2.81	≥10
			D1 stream	1.672	4.51	
			D2 ditch	3.227	2.33	
			D2 stream	2.021	3.73	
			D3 ditch	0.109	69.26	
			D6 ditch	2.764	1.84	
			R1 stream	1.354	6.94	
			R3 stream	1.728	4.36	
			R4 stream	0.527	14.32	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	0.194	38.91	≥10
			D2 ditch	1.702	4.43	
			D2 stream	1.111	6.79	
			D3 ditch	0.109	69.26	
			D6 ditch	0.114	66.22	
			R1 stream	0.347	21.75	
			R3 stream	0.493	15.31	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	4.328	1.74	≥10
			D1 stream	2.699	2.79	
			D2 ditch	3.957	1.90	
			D2 stream	2.480	3.04	
			D3 ditch	0.145	52.00	
			D4 pond	0.750	10.06	
			D4 stream	1.081	6.98	
			D5 pond	0.761	9.92	
			D5 stream	0.812	9.29	
			D6 ditch	3.732	2.02	
			R1 stream	1.697	4.44	
			R3 stream	2.246	3.36	
			R4 stream	1.786	4.22	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	0.259	29.15	≥10
			D1 stream	0.218	34.63	

Compound	Species	E _r C ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D2 ditch	2.412	3.13	
			D2 stream	1.574	4.79	
			D3 ditch	0.146	51.71	
			D4 stream	0.148	51.01	
			D5 stream	0.160	47.18	
			D6 ditch	0.153	49.34	
			R1 stream	0.464	16.27	
			R3 stream	0.662	11.40	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	6.543	1.15	≥10
			D1 stream	4.082	1.84	
			D2 ditch	6.199	1.21	
			D2 stream	3.882	1.94	
			D3 ditch	0.218	34.63	
			D4 pond	1.159	6.51	
			D4 stream	1.674	4.51	
			D5 pond	1.163	6.49	
			D5 stream	1.249	6.04	
			D6 ditch	5.693	1.32	
			R1 stream	2.602	2.90	
			R3 stream	3.446	2.19	
			R4 stream	2.699	2.79	

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B. 9.4-27: The FOCUS STEP 4 scenarios with 10 m buffer, for P.subcapitata which the TER_{LT} needs further refinement.

Autumn use 120 g s.a./ha	Spring use 120 g s.a./ha	Autumn use 160 g a.s./ha	Spring use 160 g a.s./ha	Autumn use 240 g a.s./ha
D1 ditch	D 2 ditch	D1 ditch	D2 ditch	D1 ditch
D1 stream	D2 stream	D1 stream	D2 stream	D1 stream
D2 ditch		D2 ditch	-	D2 ditch
D2 stream	-	D2 stream	-	D2 stream
		D4 stream		D4 pond
		D5 pond		D4 stream
		D5 stream		D5 pond
D6 ditch	-	D6 ditch	-	D5 stream
	-		-	D6 ditch
	-	R1 stream	-	R1 stream
R1 stream	-	R3 stream	-	R3 stream
R3 stream	-	R4 stream	-	R4 stream

- No further refinement is needed

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 4 PEC_{sw} values for flufenacet calculated assuming 10-metres buffer zone for mitigation of Spray Drift in all D scenarios and Spray Drift and Run-off in all R scenarios (for Run-off the adequate reduction factors given by FOCUS L&M Guideline for 10-12 –metres wide vegetated buffer zone were used), the TER_{LT} values for algae meet the required trigger of 10, except the scenarios given in the Table B.9.4-27. For those scenarios, *P.subcapitata* TER needs further refinement.

As a result, PEC_{sw} values for flufenacet for autumn application to winter cereals were further refined using the FOCUS Step 4 with 20 meter buffer zone FOCUS.

STEP 4 – 20-metres wide FOCUS buffer zone for mitigation of Spray Drift and Run-off

TER_{LT} values for relevant scenarios based on worst case scenario PEC_{sw} from STEP 4 with 20 meter buffer zone FOCUS was presented in the Table B.9.4-28.

Table B. 9.4-28: Chronic toxicity exposure ratios (TER_{LT}) for *P.subcapitata* based on worst case scenario PEC_{sw} from STEP 4 with 20 meter buffer zone.

Compound	Species	ErC ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	2.680	2.81	≥10
			D1 stream	1.672	4.51	
			D2 ditch	3.227	2.33	
			D2 stream	2.021	3.73	
			D6 ditch	2.764	2.73	
			R1 stream	0.652	11.57	
			R3 stream	0.907	8.32	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D2 ditch	1.702	4.43	≥10
			D2 stream	1.111	6.79	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	4.328	1.74	≥10
			D1 stream	2.699	2.79	
			D2 ditch	3.957	1.90	
			D2 stream	2.480	3.04	
			D5 pond	0.758	9.96	
			D5 stream	0.812	9.29	
			D6 ditch	3.732	2.02	
			D4 stream	1.081	6.98	
			R1 stream	0.883	8.55	
			R3 stream	1.173	6.43	

Compound	Species	E _r C ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			R4 stream	0.993	7.60	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D2 ditch	2.412	3.13	≥10
			D2 stream	1.574	4.79	
Autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	D1 ditch	6.543	1.15	≥10
			D1 stream	4.082	1.84	
			D2 ditch	6.199	1.21	
			D2 stream	3.882	1.94	
			D4 pond	1.154	6.54	
			D4 stream	1.674	4.51	
			D5 pond	1.159	6.51	
			D5 stream	1.249	6.04	
			D6 ditch	5.693	1.32	
			R1 stream	1.354	5.57	
			R3 stream	1.799	4.19	
			R4 stream	1.41	5.35	

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B. 9.4-29: The FOCUS STEP 4 scenarios with 20 m buffer, for P.subcapitata which the TER_{LT} needs further refinement.

Autumn use 120 g s.a./ha	Spring use 120 g s.a./ha	Autumn use 160 g a.s./ha	Spring use 160 g a.s./ha	Autumn use 240 g a.s./ha
D1 ditch	D2 ditch	D1 ditch	D2 ditch	D1 ditch
D1 stream	D2 stream	D1 stream	D2 stream	D1 stream
D2 ditch	-	D2 ditch	-	D2 ditch
D2 stream	-	D2 stream	-	D2 stream
D6 ditch	-	D5 pond	-	D6 ditch
-	-	D5 stream	-	D4 pond
R3 stream	-	D6 ditch	-	D4 stream
-	-	D4 stream	-	D5 pond
-	-	R1 stream	-	D5 stream
-	-	R3 stream	-	R1 stream
-	-	R4 stream	-	R3 stream
-	-	-	-	R4 stream

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 4 PEC_{sw} values for flufenacet calculated assuming 20-metres buffer zone for mitigation of Spray Drift in all D scenarios and Spray Drift and Run-off in all R scenarios (for Run-off the adequate reduction factors given by FOCUS

L&M Guideline for 20 –metres wide vegetated buffer zone were used), the TER_{LT} values for algae meet the required trigger of 10, except the scenarios given in the Table B.9.4-29.

The further refinement is required for these scenarios.

At the same time it shall be pointed out that in case of the scenarios D4-pond, D4-stream, D5-pond, D5-stream and D6-ditch, when the required trigger value for TER_{LT} was not met for applications in autumn, the risk may still be negligible. That is due to the fact that for those scenarios the predominant identified migration route was drainage occurring shortly after application and late in autumn and in winter, when algae are in dormant stage. It was also noticed that for these scenarios the concentrations of Flufenacet above the level of the safe PEC_{SW} values were obtained for the period between December and the end of February or the first days of March on the latest. Similar statement with regard to the temporal occurrence of modelled concentrations of Flufenacet in SW bodies may be drawn for all R scenarios.

Overall conclusion on algae:

Autumn Uses:

The general conclusions drawn from the risk assessment for algae presented above, are provided below, individually for each use listed in the EU-representative GAP.

In case of the post-emergence use in Winter cereals in autumn, at application rate **240 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream, D4 pond, D4 stream, D5 pond, D5 stream, D6 ditch, R1 stream, R3 stream and R4 stream. In case of scenarios D4 pond, D4 stream, D5 pond, D5 stream and D6 ditch RMS noticed that although safe PEC_{SW} values were not reached at STEP 4, it may be stated that the risk for algae was not substantial due to the fact that they occurred late in autumn and in winter, when algae are in dormant period from December to February; also in none of these scenarios the PEC_{SW} values above the safe level were observed later than in the first days of March. Also in case of all R scenarios for which safe PEC_{SW} value was not reached, similar conclusion may be drawn.

The only safe scenario identified within that use was D3, assuming 10-metres wide buffer zone for mitigation of Spray Drift.

Therefore for that use it may be stated that one safe scenario was identified – D3 scenario assuming 10-metres wide non-spray buffer zone to mitigate the Spray drift.

In case of the post-emergence use in Winter cereals in autumn, at application rate **160 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream, D4 stream, D5 pond, D5 stream, D6 ditch, R1 stream, R3 stream and R4 stream. In case of scenarios D4 stream, D5 pond, D5 stream and D6 ditch RMS noticed that

although safe PEC_{SW} values were not reached at STEP 4, it may be stated that the risk for algae was not substantial due to the fact that they occurred late in autumn and in winter, when algae are in dormant period from December to February; also in none of these scenarios the PEC_{SW} values above the safe level were observed later than in the first days of March. Also in case of all R scenarios for which safe PEC_{SW} value was not reached, similar conclusion may be drawn.

The only safe scenario identified within that use was D3, assuming 10-metres wide buffer zone for mitigation of Spray Drift.

Therefore for that use it may be stated that one safe scenario was identified – D3 scenario assuming 10-metres wide non-spray buffer zone to mitigate the Spray drift.

In case of the post-emergence use in Winter cereals in autumn, at application rate **120 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream, D6 ditch and R3 stream. In case of scenario D6 ditch RMS stated that although safe PEC_{SW} values were not reached at STEP 4, it may be stated that the risk for algae was not substantial due to the fact that they occurred late in autumn and in winter, when algae are in dormant period from December to February; also in none of these scenarios the PEC_{SW} values above the safe level were observed later than in the first days of March. Also in case of R3 scenario similar conclusion may be drawn.

The safe scenarios identified within that use were D4 and D5 – all three already at STEP 3 (hence no buffer zone needed to be implemented), D3 ditch assuming 10-metres wide non-spray buffer zone to mitigate Spray Drift, R1 assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off and R4 assuming 10-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off.

Therefore, for that use it may be stated that the following safe scenarios were identified: scenarios D4 and D5 without any buffer zone (safe PEC_{SW} values were obtained already at STEP 3), D3 ditch assuming 10-metres wide non-spray buffer zone to mitigate Spray Drift, R1 scenario assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off and R4 scenario assuming 10-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off.

SPRING USE

The general conclusions drawn from the risk assessment for algae presented above, are provided below, individually for each use listed in the EU-representative GAP.

In case of the post-emergence use in Winter cereals at spring, at application rate **160 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D2 ditch and D2 stream

In case of scenarios D4 pond, D5 pond, R1 pond and R4 stream safe PEC_{SW} values were obtained already at STEP 3 (so no buffer needed).

In case of the following scenarios: D1 ditch, D1 stream, D3 ditch, D4 stream, D5 stream and D6 ditch, safe PEC_{SW} values were obtained at STEP 4 after implementation of the 10-metres wide non-spray buffer zone for mitigation of the Spray Drift;

For scenarios R1 stream and R3 stream safe PEC_{SW} values were obtained at STEP 4 after implementation of 10-metres wide vegetated buffer zone for mitigation of the Spray Drift and Run-off.

Therefore for that application pattern only for scenario D2 safe use was not demonstrated. In case of scenario R4 it was demonstrated that implementation of the buffer zone mitigating Spray Drift and/or Run-off was not necessary. 10-metres wide non-spray buffer zone was demonstrated to be necessary to obtain safe PEC_{SW} values for scenarios D1, D3, D4, D5, D6, R1 and R3. In case of scenarios R1 and R3 that buffer zone has to be vegetated in order to mitigate the Run-off.

In case of the post-emergence use in Winter cereals at spring, at application rate **120 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D2 ditch and D2 stream

In case of scenarios D1 stream, D4 pond, D4 stream, D5 pond and D5 stream, R1 pond, and R4 stream safe PEC_{SW} values were obtained already at STEP 3.

In case of the scenarios D1 ditch, D3 ditch and D6 ditch safe PEC_{SW} values were obtained at STEP 4 after implementation of the 10-metres wide non-spray buffer zone for mitigation of the Spray Drift;

For scenarios R1 stream and R3 stream safe PEC_{SW} values were obtained at STEP 4 after implementation of 10-metres wide vegetated buffer zone for mitigation of the Spray Drift and Run-off.

Therefore for that application pattern only for scenario D2 safe use was not demonstrated. In case of scenarios D4, D5 and R4 it was demonstrated that implementation of the buffer zone mitigating Spray Drift and/or Run-off was not necessary. 10-metres wide non-spray buffer zone was demonstrated to be necessary to obtain safe PEC_{SW} values for scenario D1, D3, D6, R1 and R3. In case of scenarios R1 and R3 that buffer zone has to be vegetated in order to mitigate the Run-off.

As an additional refinement step RMS carried out the calculations using the VFS-mod option to mitigate run-off. The selected vegetated buffer zone was 10-metres wide. The same width of the buffer zone was used in function of the non-spray buffer zone for simultaneous mitigation of the Spray Drift.

The results of the calculations are presented below in the table B.9.4-30. Only those scenarios are listed, for which a safe use was not obtained at STEP 4 assuming the maximum admissible reduction factors set by FOCUS.

As VFS-mod is a tool specific only for mitigating Run-off, and hence for R scenarios, in the table below only the results obtained for R scenarios, where relevant, are presented.

Table B. 9.4-30: Chronic toxicity exposure ratios (TER_{LT}) for based on worst case scenario PEC_{sw} from 10-metres buffer zone, VFS-mod.

10-metres buffer zone, VFS-mod.						
Compound	Species	ErC ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	P.subcapitata	7.55	R3 stream	1.275	5.92	≥10
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	R1 stream	0.129	58.52	≥10
			R3 stream	0.181	41.71	
			R4 stream	0.128	58.98	
Autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence						
Flufenacet	P.subcapitata	7.55	R1 stream	0.194	38.91	≥10
			R3 stream	0.272	27.75	
			R4 stream	0.192	39.32	

Based on the results presented above it may be stated that the use of the VFS-mod for R scenarios assuming the 10-metres wide vegetated buffer zone resulted in reaching safe uses in all scenarios of concern in autumn post emergence use in winter cereals at 240 g Flufenacet/ha and 160 g Flufenacet/ha. In case however of autumn pre emergence use in winter cereals at 120 g/ha for R3 scenario a wider buffer zone than determined using VFS-mod will be needed to demonstrate the safe use also for that scenario and application pattern.

Risk assessment for Lemna gibba

In the documentation provided by the Applicant two studies were available – the old study, submitted for the previous authorization of Flufenacet in the EU, and the new study, submitted specifically for the purpose of the current evaluation. RMS verified both studies and found them acceptable, stating at the same time that due to the deficiencies identified in course of the assessment the old study shall be considered only as indicative and should not to be used to derive the regulatory toxicity endpoints.

The old study by [Huges and Alexander; 1993] was carried out in line with the FIFRA Guideline 123-2 and the 14-days endpoint of 2.43 µg a.s./L (previous EU-agreed endpoint), based on frond counts solely, was calculated. In 1998, Dorgerloh repeated calculations to obtain a 7-day ErC₅₀ = 31.8 µg/L, based on frond count and nominal concentrations of the test item. The additional calculations, performed on the RMS's request in line with the current standards, resulted in a 7-day ErC₅₀ = 25.92 µg a.s./L, based on frond count and mean measured concentrations (arithmetic mean) of the test item. However, the study by [Hughes & Alexander; 1993] was indicated by the Applicant to be not valid according to current guidelines (OECD 221). RMS identified that as a deficiency not having an impact on the validity of the study, but not enabling any of

the determined 7-days ErC₅₀ to be used as fully reliable regulatory endpoint as a second endpoint like frond dry weight or frond area had not been determined.

Therefore, to address this data requirement, the Applicant submitted a new 7-day Lemna study by [Bruns; 2013]. In this study two parameters, frond number and frond area, were assessed as required by the currently valid OECD 221 guideline. The determined endpoint relevant for risk assessment – the 7-day ErC₅₀ based on growth rates of frond area was by more than a factor of 2 lower than the one recalculated by Dorgerloh (1998) out of the 14-day study. In addition the OECD guideline 221 states that growth related endpoints should be used for risk assessment purposes to allow comparison of sensitivity of different species. As in addition the no observed effect concentrations (NOECs) from both studies reveal that the test organisms were of equal sensitivity (0.44 and 0.658 µg/L from the old and new study, respectively) it is considered justified that new fully valid and according to current state of the science performed 7-day Lemna-study supersedes the old 14-day Lemna study where the endpoint is based solely on the frond counts. Consequently the risk assessment will be performed using the new 7-day ErC₅₀ of 13.9 µg a.s./L based on growth rate.

The risk assessment based on the the new 7-day ErC₅₀ of 13.9 µg a.s./L based on growth rate is presented below in tables B.9.4-31 – B.9.4-37.

Table B. 9.4-31: TER_{Lt} values for Lemna gibba exposed to flufenacet and its metabolites.

Compound	Species	ErC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw} (µg/L)	TER _A	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Lemna gibba	13.9	Step1	31.227	0.44	≥10
			Step 2NE	13.797	1.00	
			Step 2SE	11.217	1.24	
FOE oxalate		>100 000	Step1	6.466	>15465.51	
FOE methylsulfide		106 000	Step1	0.084	1261904.76	
FOE methylsulfone		>100 000	Step1	1.896	>52742.61	
TFA		1990 000	Step1	10.228	194563.94	
FOE-Thiadone		18 320	Step1	1.464	12513.66	
FOE-Trifluoroethane sulfonic acid		>10 000	Step1	1.084	>9225.09	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	Step1	31.277	0.44	≥10
			Step2 NE	6.057	2.30	
			Step2 SE	11.217	1.23	
FOE oxalate		>100 000	Step 1	6.466	>15465.51	
FOE methylsulfide		106 000	Step 1	0.084	1261904.76	
FOE methylsulfone		>100 000	Step1	1.896	>52742.61	
TFA		1999 000	Step1	10.228	194563.94	
FOE-Thiadone		18.320	Step 1	1.464	12513.66	
FOE-Trifluoroethane sulfonic acid		>10 000	Step 1	1.084	>9225.09	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	Step 1	41.636	0.33	≥10
			Step 2NE	18.395	0.76	
			Step 2SE	14.956	0.93	
FOE oxalate		>100 000	Step 1	8.622	11598.23	
FOE methylsulfide		106 000	Step1	0.111	>954954.95	
FOE methylsulfone		>100 000	Step1	2.528	>39556.69	
TFA		1990 000	Step1	13.638	145918.82	
FOE-Thiadone		18 320	Step1	1.952	9385.24	

Compound	Species	ErC ₅₀ (µg/L)	FOCUS STEP	Max PEC _{sw} (µg/L)	TER _A	Trigger
FOE-Trifluoroethane sulfonic acid		>10 000	Step1	1.445	>6920.41	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufnacet	Lemna gibba	13.9	Step 1	41.636	0.33	
			Step 2 NE	8.076	1.72	
			Step 2 SE	14.956	0.93	
FOE oxalate		>100 000	Step1	8.622	>11598.23	
FOE methylsulfide		106 000	Step1	0.111	954954.95	
FOE methylsulfone		>100 000	Step1	2.528	>39556.69	
TFA		1990 000	Step1	13.638	145918.82	
FOE-Thiadone		18 320	Step1	1.952	9385.24	
FOETrifluoroethanesulfonic acid		>10 000	Step1	1.445	>6920.41	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	Step 1	62.454	0.22	10
				27.593	0.50	
				22.433	0.62	
FOE oxalate		>100 000	Step 1	12.933	>7732.16	
FOE methylsulfide		106 000	Step 1	0.167	634730.53	
FOE methylsulfone		>100 000	Step 1	3.792	>26371.30	
TFA		1990 000	Step 1	22.426 ¹	88736.28	
FOE-Thiadone		18 320	Step 1	2.928	6256.83	
FOE-Trifluoroethane sulfonic acid		>10 000	Step 1	2.168	>4612.54	

1) max PEC_{gw}

values in bold indicate unacceptable risk

All TER_{LT} values calculated for metabolites of flufenacet with consideration of worst case exposure assumptions are far above the trigger of 10 indicating acceptable chronic risk to Lemna gibba.

However, the TER_{LT} values for flufenacet obtained by FOCUS 2 calculations are lower than the Annex VI trigger value of 10 for all proposed uses in winter cereals and needs further refinement.

Table B. 9.4-32: Chronic toxicity exposure ratios (TER_{LT}) for Lemna gibba based on worst case scenario PEC_{sw} from STEP 3.

Compound	Species	ErC ₅₀ (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	2.680	5.19	≥10
			D1 stream	1.672	8.31	
			D2 ditch	3.227	4.31	
			D2 stream	2.021	6.88	
			D3 ditch	0.758	18.34	
			D4 pond	0.398	34.92	
			D4 stream	0.658	21.12	
			D5 pond	0.560	24.82	
			D5 stream	0.710	19.58	
			D6 ditch	2.764	5.03	
			R1 pond	0.0609	228.24	
			R1 stream	2.800	4.96	
			R3 stream	3.783	3.67	
R4 stream	1.167	11.91				
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	0.846	16.43	≥10
			D1 stream	0.629	22.10	

Compound	Species	ErC ₅₀ (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D2 ditch	1.702	8.17	
			D2 stream	1.111	12.51	
			D3 ditch	0.760	18.29	
			D4 pond	0.0267	520.60	
			D4 stream	0.572	24.30	
			D5 pond	0.0289	480.97	
			D5 stream	0.614	22.64	
			D6 ditch	0.756	18.39	
			R1 pond	0.0687	202.333	
			R1 stream	0.764	18.19	
			R3 stream	1.080	12.87	
			R4 stream	0.501	27.74	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	4.328	3.21	≥10
			D1 stream	2.699	5.15	
			D2 ditch	3.957	3.51	
			D2 stream	2.480	5.60	
			D3 ditch	1.010	13.76	
			D4 pond	0.756	18.39	
			D4 stream	1.081	12.86	
			D5 pond	0.766	18.15	
			D5 stream	0.946	14.69	
			D6 ditch	3.732	3.72	
			R1 pond	0.0797	174.40	
			R1 stream	3.790	3.67	
			R3 stream	4.980	2.79	
			R4 stream	3.957	3.51	

*values in bold indicate unacceptable risk

Table B. 9.4-32. Chronic toxicity exposure ratios (TER_{LT}) for Lemna gibba based on worst case scenario PEC_{sw} from STEP 3 (continued).

PEC _{sw} from TER 3 (continued)						
Compound	Species	E _r C ₅₀ (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	1.129	12.31	≥10
			D1 stream	0.838	16.59	
			D2 ditch	2.412	5.76	
			D2 stream	1.574	8.83	
			D3 ditch	1.014	13.71	
			D4 pond	0.0357	389.36	
			D4 stream	0.763	18.22	
			D5 pond	0.0387	359.17	
			D5 stream	0.818	16.99	
			D6 ditch	1.009	13.78	
			R1 pond	0.0913	152.25	
			R1 stream	1.021	13.61	
			R3 stream	1.450	9.59	
			R4 stream	0.668	20.81	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	6.543	2.12	
			D1 stream	4.082	3.41	

Compound	Species	E _r C ₅₀ (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D2 ditch	6.199	2.24	≥10
			D2 stream	3.882	3.58	
			D3 ditch	1.514	9.18	
			D4 pond	1.168	11.90	
			D4 stream	1.647	8.44	
			D5 pond	1.170	11.88	
			D5 stream	1.420	9.79	
			D6 ditch	5.693	2.44	
			R1 pond	0.116	119.83	
			R1 stream	5.811	2.39	
			R3 stream	7.641	1.82	
			R4 stream	5.980	2.32	

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B. 9.4-33: The FOCUS STEP 3 scenarios in winter cereals, for which the TER_{LT} Lemna gibba needs further refinement.

Scenario STEP 3	Winter cereals				
	Autumn use 120 g s.a./ha	Spring use 120 g a.s./ha	Autumn use 160 g a.s./ha	Spring use 160 g a.s./ha	Autumn use 240 g a.s./ha
D1 ditch	D1 ditch	-	D1 ditch	-	D1 ditch
D1 stream	D1 stream	-	D1 stream	-	D1 stream
D2 ditch	D2 ditch	D2 ditch	D2 ditch	D2 ditch	D2 ditch
D2 stream	D2 stream	-	D2 stream	D2 stream	D2 stream
D3 ditch	-	-	-	-	D3 ditch
D4 pond	-	-	-	-	-
D4 stream	-	-	-	-	D4 stream
D5 pond	-	-	-	-	-
D5 stream	-	-	-	-	D5 stream
D6 ditch	D6 ditch	-	D6 ditch	-	D6 ditch
R1 pond	-	-	-	-	-
R1 stream	R1 stream	-	R1 stream	-	R1 stream
R3 stream	R3 stream	-	R3 stream	R3 stream	R3 stream
R4 stream	-	-	R4 stream	-	R4 stream

- no further refinement is needed

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 3 maximum PEC_{sw} values for flufenacet, the TER_{LT} values for Lemna gibba meet the required trigger of 10, except the scenarios given in the Table B.9.4-33. For these scenarios Lemna sp. TER needs further refinement.

Therefore, the PEC_{sw} values for flufenacet were further refined at FOCUS Step 4 using 10-metres- and 20-metres-wide FOCUS buffer zones for mitigation of Spray Drift and Run-off. As an additional refinement step in Run-off mitigation were performed the calculations in VFS-mod assuming the 10-metres wide buffer zone for reducing migration with Spray Drift (D and R) scenarios and Run-off (for R scenarios only).

FOCUS STEP 4 -10-metres wide FOCUS buffer zone for mitigation of Spray Drift and Run-off

TER_{LT} values for relevant scenarios based on worst case scenario PEC_{sw} from STEP 4 with 10 meter buffer zone FOCUS were presented in the Table B.9.4-34

Table B. 9.4-34: Chronic toxicity exposure ratios (TER_{LT}) for Lemna gibba based on worst case scenario PEC_{sw} from STEP 4 with 10 meter buffer zone.

Compound	Species	E _r C ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	2.680	5.19	≥10
			D1 stream	1.672	8.31	
			D2 ditch	3.227	4.31	
			D2 stream	2.021	6.88	
			D6 ditch	2.764	5.03	
			R1 stream	1.354	10.27	
			R3 stream	1.728	8.04	
Spring in winter cereals at rate 1 x 120 g a.s./ha, post-emergence						
Flufenacet	Lemna gibba	13.9	D2 ditch	1.702	8.17	≥10
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	4.328	3.21	≥10
			D1 stream	2.699	5.15	
			D2 ditch	3.957	3.51	
			D2 stream	2.480	5.60	
			D6 ditch	3.732	3.72	
			R1 stream	1.697	8.19	
			R3 stream	2.246	6.19	
			R4 stream	1.786	7.78	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D2 ditch	2.412	5.76	≥10
			D2 stream	1.574	8.83	
			R3 stream	0.662	21.00	
Autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	6.543	2.12	≥10
			D1 stream	4.082	3.41	
			D2 ditch	6.199	2.24	
			D2 stream	3.882	3.58	
			D6 ditch	5.693	2.44	
			D3 ditch	0.218	63.76	
			D4 stream	1.674	8.30	
			D5 stream	1.249	11.13	
			R1 stream	2.602	5.34	
			R3 stream	3.446	4.03	
			R4 stream	2.699	5.15	

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B.9.4-35: The FOCUS STEP 4 scenarios with 10 m buffer, for which the TER_{LT} Lemna gibba needs further refinement.

Scenario STEP 4	Autumn use 120 g s.a./ha	Spring use 120 g s.a./ha	Winter cereals		
			Autumn use 160 g a.s./ha	Spring use 160 g s.a./ha	Autumn use 240 g a.s./ha
D1 ditch	D1 ditch	D2 ditch	D1 ditch	D2 ditch	D1 ditch
D1 stream	D1 stream	-	D1 stream	D2 stream	D1 stream
D2 ditch	D2 ditch	-	D2 ditch	-	D2 ditch
D2 stream	D2 stream	-	D2 stream	-	D2 stream
D6 ditch	D6 ditch	-	D6 ditch	-	D6 ditch
D4 stream	-	-	-	-	D4 stream
R1 stream	-	-	R1 stream	-	R1 stream
R3 stream	R3 stream	-	R3 stream	-	R3 stream
R4 stream	-	-	R4 stream	-	R4 stream

- No further refinement is needed

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 4 with 10 meter buffer zone PEC_{sw} values for flufenacet, the TER_{LT} values for Lemna gibba meet the required trigger of 10, except the scenarios given in the Table B.9.4-35. For these scenarios Lemna gibba TER needs further refinement. As a result, PEC_{sw} values for flufenacet for autumn and spring application to winter cereals were further refined using the FOCUS Step-4 with 20 meter buffer zone FOCUS.

STEP 4 – 20-metres wide FOCUS buffer zone for mitigation of Spray Drift and Run-off

Table B. 9.4-36: Chronic toxicity exposure ratios (TER_{LT}) for Lemna gibba based on worst-case scenario PEC_{sw} from STEP 4 with 20 meter buffer zone.

PEC _{sw} from STEP 4 with 20 meter buffer zone.						
Compound	Species	E _r C ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	2.680	5.19	≥10
			D1 stream	1.672	8.31	
			D2 ditch	3.227	4.31	
			D2 stream	2.021	6.88	
			D6 ditch	2.764	5.03	
			R3 stream	0.907	15.33	
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post-emergence						
Flufenacet	Lemna gibba	13.9	D2 ditch	1.702	8.17	≥10
Autumn use in winter cereals at rate 1x 160 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D1 ditch	4.328	3.21	≥10
			D1 stream	2.699	5.15	
			D2 ditch	3.957	3.51	
			D2 stream	2.480	5.60	

Compound	Species	ErC ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µga.s./L)	TER _{LT}	Trigger
			D6 ditch	3.732	3.72	
			R1 stream	0.883	15.74	
			R3 stream	1.173	11.85	
			R4 stream	0.933	14.90	
Spring use in winter cereals at rate 1x 160 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	D2 ditch	2.412	5.76	≥10
			D2 stream	1.574	8.83	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet Flufenacet	Lemna gibba	13.9	D1 ditch	6.543	2.12	≥10
			D1 stream	4.082	3.41	
			D2 ditch	6.199	2.24	
			D2 stream	3.882	3.58	
			D4 stream	1.674	8.30	
			D6 ditch	5.693	2.44	
			R1 stream	1.354	10.27	
			R3 stream	1.799	7.73	
R4 stream	1.410	9.86				

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B.9.4-37: The FOCUS STEP 4 scenarios with 20 m buffer, for which the TER_{LT} Lemna gibba needs further refinement.

Scenario STEP 4	Autumn use 120 g s.a./ha	Spring use 120 g s.a./ha	Winter cereals		
			Autumn use 160 g a.s./ha	Spring use 160 g s.a./ha	Autumn use 240 g a.s./ha
D1 ditch	D1 ditch	D2 ditch	D1 ditch	D2 ditch	D1 ditch
D1 stream	D1 stream	-	D1 stream	D2 stream	D1 stream
D2 ditch	D2 ditch	-	D2 ditch	-	D2 ditch
D2 stream	D2 stream	-	D2 stream	-	D2 stream
D6 ditch	D6 ditch	-	D6 ditch	-	D6 ditch
D4 stream	-	-	-	-	D4 stream
R1 stream	-	-	-	-	-
R3 stream	-	-	-	-	R3 stream
R4 stream	-	-	-	-	R4 stream

- No further refinement is needed

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 4 PEC_{sw} values for flufenacet calculated assuming 20-metres buffer zone for mitigation of Spray Drift in all D scenarios and Spray Drift and Run-off in all R scenarios (for Run-off the adequate reduction factors given by FOCUS L&M Guideline for 10-20 –metres wide vegetated buffer zone were used, the TER_{LT} values for algae meet the required trigger of 10, except the scenarios given in the Table B.9.4-37.

For those scenarios, Lemna gibba TER needs further refinement.

At the same time it shall be pointed out that in case of the scenarios D4-pond, D4-stream, D5-pond, D5-stream and D6-ditch, when the required trigger value for TER_{LT} was not met for applications in autumn, the risk may still be negligible. That is due to the fact that for those scenarios the predominant identified migration route was drainage occurring shortly after application and late in autumn and in winter, when both algae and *Lemna* sp. are in dormant stage. It was also noticed that for these scenarios the concentrations of Flufenacet above the level of the safe PEC_{SW} values were obtained for the period between December and the end of February or the first days of March on the latest.

Similar statement with regard to the temporal occurrence of modelled concentrations of Flufenacet in SW bodies may be drawn for all R scenarios

These conclusions were based on the thorough analysis of the TOXSWA concentration profiles in water phase presented in graphical form in the Appendixes 3-5 of the document Vol. 3 B.8. CP of this RAR. Also these results were analysed using the E-PAT 1.0 tool (results not presented in the RAR).

Overall conclusion:

Lemna gibba

Autumn Uses:

The general conclusions drawn from the risk assessment for *Lemna gibba* presented above are given, are provided below, individually for each use listed in the EU-representative GAP.

In case of the post-emergence use in Winter cereals in autumn, at application rate **240 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream, D4 stream, D6 ditch, R3 and R4. In case of scenarios D4 stream and D6 ditch RMS noticed that although safe PEC_{SW} values were not reached at STEP 4, it may be stated that the risk for *Lemna* spp. was not substantial due to the fact that they occurred late in autumn and in winter, when *Lemna* sp. in dormant period from December to February; also in none of these scenarios the PEC_{SW} values above the safe level were observed later than in the first days of March. Also in case of all R scenarios for which safe PEC_{SW} value was not reached, similar conclusion may be drawn.

The safe scenarios identified within that use were: D3 ditch assuming 10-metres wide buffer zone for mitigation of Spray Drift, D4 pond already at STEP 3 (hence no buffer zone needed to be implemented), D5 pond already at STEP 3 (hence no buffer zone needed to be implemented), D5 stream assuming 10-metres wide buffer zone for mitigation of Spray Drift, R1 pond already at STEP 3 (hence no buffer zone needed to be implemented), R1 stream assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off;

Therefore, for that use it may be stated that three safe scenarios were identified: for the D3 scenario assuming 10-metres wide non-spray buffer zone to mitigate the Spray drift, for D5 scenario assuming 10-metres wide non-spray buffer zone to mitigate Spray Drift and R1 assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off.

In case of the post-emergence use in Winter cereals in autumn, at application rate **160 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream, and D6 ditch. In case of scenario D6 ditch RMS noticed that although safe PEC_{SW} values were not reached at STEP 4, it may be stated that the risk for *Lemna* spp. was not substantial due to the fact that they occurred late in autumn and in winter, when *Lemna* sp. was in dormant period from December to February; also in none of these scenarios the PEC_{SW} values above the safe level were observed later than in the first days of March.

The safe scenarios identified within that use were: D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream – all them already at STEP 3 (hence no buffer zone needed to be implemented), as well as R1 pond already at STEP 3 (hence no buffer zone needed to be implemented), R1 stream assuming 20-metres wide vegetated buffer zone for mitigation of Spray Drift and Run-off, R3 stream assuming 20-metres wide vegetated buffer zone for mitigation of Spray Drift and Run-off and R4 stream assuming 20-metres wide vegetated buffer zone for mitigation of Spray Drift and Run-off

Therefore for that use it may be stated that the following safe uses were identified: for scenarios D3, D4 and D5 without any buffer zone (safe PEC_{SW} values were obtained already at STEP 3) as well as R1, R3 and R4 scenarios assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off.

In case of the post-emergence use in Winter cereals in autumn, at application rate **120 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream and D6 ditch. In case of scenario D6 ditch RMS noticed that although safe PEC_{SW} values were not reached at STEP 4, it may be stated that the risk for *Lemna* spp.e was not substantial due to the fact that they occurred late in autumn and in winter, when *Lemna* sp. is in dormant period from December to February; also in none of these scenarios the PEC_{SW} values above the safe level were observed later than in the first days of March

The safe scenarios identified within that use were D3 ditch, D4 (pond and stream), D5 (pond and stream), R1 pond and R4 stream – all they already at STEP 3 (hence no buffer zone needed to be implemented), as well as R1stream assuming 10-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off and R3 assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off

Therefore for that use it may be stated that the following safe uses were identified: for scenarios D3, D4, D5 and R4 without any buffer zone (safe PEC_{SW} values were obtained already at STEP 3) as well as R1 scenario assuming 10-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off and R3 scenario assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off.

SPRING USE

The general conclusions drawn from the risk assessment for *Lemna gibba* presented above are given, are provided below, individually for each use listed in the EU-representative GAP.

In case of the post-emergence use in Winter cereals at spring, at application rate **160 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D2 ditch and D2 stream;

In case of scenarios D1 ditch, D1 stream, D3 ditch, D4 pond, D4 stream, D5 pond and D5 stream, R1 pond, R1 stream and R4 stream safe PEC_{sw} values were obtained already at STEP 3.

For scenario R3 stream safe PEC_{sw} values were obtained at STEP 4 after implementation of 10-metres wide vegetated buffer zone for mitigation of the Spray Drift and Run-off.

Therefore for that application pattern only for scenarios D2 safe use was not demonstrated. In case of scenario R3 it was demonstrated that it was necessary to implement 10 meter buffer zone mitigating Spray Drift and Run-off. In all remaining scenarios identified as returning the safe PEC_{sw} values it was demonstrated that the implementation of any mitigation measures was not necessary as the assessment was finalised at Step 3.

In case of the post-emergence use in Winter cereals at spring, at application rate **120 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenario D2 ditch.

In case of scenarios D1 ditch, D1 stream, D2 stream, D3 ditch, D4 pond, D4 stream, D5 pond and D5 stream, D6 ditch, R1 pond, R1 stream, R3 stream and R4 stream safe PEC_{sw} values were obtained already at STEP 3. For that application pattern assessment at STEP 4 was not performed.

Therefore for that application pattern only for scenario D2 safe use was not demonstrated. In case of scenarios D1, D3, D4, D5, D6, R1, R3 and R4 it was demonstrated that implementation of the buffer zone mitigating Spray Drift and/or Run-off was not necessary.

As an additional refinement step RMS carried out the calculations using the VFS-mod option to mitigate run-off. The selected vegetated buffer zone was 10-metres wide. The same width of the buffer zone was used in function of the non-spray buffer zone for simultaneous mitigation of the Spray Drift.

The results of the calculations are presented below in the table B.9.4-38. In it are listed only those scenarios, for which safe PEC_{sw} values were not obtained at STEP 4 assuming the maximum admissible reduction factors set by FOCUS.

As VFS-mod is a tool specific only for mitigating Run-off, and hence for R scenarios, in the table below only the results obtained for R scenarios, where relevant, were presented.

Table B. 9.4-38: Chronic toxicity exposure ratios (TER_{LT}) for Lemna gibba based on worst case scenario PEC_{sw} from 10-metres buffer zone, VFS-mod.

Compound	Species	ErC ₅₀ (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	R1 stream	0.129	107.75	≥10
			R3 stream	0.181	79.80	
			R4 stream	0.128	108.60	
Autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence						
Flufenacet	Lemna gibba	13.9	R1 stream	0.194	71.64	≥10
			R3 stream	0.272	51.10	
			R4 stream	0.192	72.40	

Based on the results presented above it may be stated that the use of the VFS-mod for R scenarios assuming the 10-metres wide vegetated buffer zone resulted in reaching safe PEC_{sw} values in all scenarios of concern in autumn post-emergence use in Winter cereals at 160 g a.s./ha and 240 g Flufenacet/ha.

The Applicant as a refinement provided the risk assessment based on microcosm study (Foekema 1997, see CA, Vol 3., B9). This study was re-evaluated by RMS and was considered valid in case of Macrophytes (Lemna sp. and Elodea sp.). The results of the study suggest that rooted plants are more sensitive than Lemna sp.

Therefore, RMS would like to propose the NOEC_{macrophytes} of 6 µg a.s./L value with the Assessment Factor of 5 to refine the risk for aquatic macrophytes, including Lemna sp and Elodea sp.

In addition, the peak exposure study for Lemna sp. with ErC₅₀ > 126 µg a.s./L to refine the risk assessment for R scenarios (Bruns 2013, see CA, Vol 3, B9) was performed.

RMS is of the opinion that although the study is valid it may be used in the refined risk assessment for macrophytes only if:

- Further evidence is provided that rooted macrophytes are not more sensitive to flufenacet than Lemna sp.
- The peak exposure design of the study covers the peaks observed in the FOCUS scenarios.

Therefore, the study will not be used in the current risk assessment.

Risk assessment for Macrophytes including Lemna sp. and Elodea sp.

Consequently the risk assessment will be performed using the new NOEC_{macrophytes} = 6 µg a.s./L obtained from Microcosm study (see Foekema 1997, CA, Vol 3., B9) with AF of 5 and it is presented below in tables B.9.4-39 – B.9.4-46.

Table B. 9.4-39: TER_{LT} values for Macrophytes exposed to flufenacet.

Compound	Species	NOEC (µg a.s./L)	FOCUS STEP	Max PEC _{sw} (µg a.s./L)	TER _A	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	Step1	31.227	0.19	≥5
			Step 2NE	13.797	0.43	
			Step 2SE	11.217	0.53	

Compound	Species	NOEC (µg a.s./L)	FOCUS STEP	Max PEC _{sw} (µg a.s./L)	TER _A	Trigger
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Microcosm	6	Step1	31.277	0.19	≥5
	(Lemna sp +		Step2 NE	6.057	0.99	
	Elodea sp.)		Step2 SE	11.217	0.53	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm	6	Step 1	41.636	0.14	≥5
	(Lemna sp +		Step 2NE	18.395	0.32	
	Elodea sp.)		Step 2SE	14.956	0.40	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm	6	Step 1	41.636	0.14	≥5
	(Lemna sp +		Step 2 NE	8.076	0.74	
	Elodea sp.)		Step 2 SE	14.956	0.40	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Microcosm	6	Step 1	62.454	0.09	≥5
	(Lemna sp +			27.593	0.21	
	Elodea sp.)			22.433	0.26	

values in bold indicate unacceptable risk

TER_{LT} values for flufenacet obtained by FOCUS 1-2 calculations are lower than trigger value of 5 for all proposed uses in winter cereals and needs further refinement.

Table B. 9.4-40: Chronic toxicity exposure ratios (TER_{LT}) for Macrophytes based on worst case scenario PEC_{sw} from STEP 3.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	2.680	2.23	≥5
			D1 stream	1.672	3.58	
			D2 ditch	3.227	1.85	
			D2 stream	2.021	2.96	
			D3 ditch	0.758	7.91	
			D4 pond	0.398	15.07	
			D4 stream	0.658	9.11	
			D5 pond	0.560	10.71	
			D5 stream	0.710	8.45	
			D6 ditch	2.764	2.17	
			R1 pond	0.0609	98.52	
			R1 stream	2.800	2.14	
			R3 stream	3.783	1.58	
R4 stream	1.167	5.14				
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	0.846	7.09	≥5
			D1 stream	0.629	9.53	
			D2 ditch	1.702	3.52	
			D2 stream	1.111	5.40	
			D3 ditch	0.760	7.89	
			D4 pond	0.0267	224.71	
			D4 stream	0.572	10.48	
			D5 pond	0.0289	207.61	

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			D5 stream	0.614	9.77	
			D6 ditch	0.756	7.93	
			R1 pond	0.0687	87.33	
			R1 stream	0.764	7.85	
			R3 stream	1.080	5.55	
			R4 stream	0.501	11.97	
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	4.328	1.38	≥5
			D1 stream	2.699	2.22	
			D2 ditch	3.957	1.51	
			D2 stream	2.480	2.41	
			D3 ditch	1.010	5.94	
			D4 pond	0.756	7.93	
			D4 stream	1.081	5.55	
			D5 pond	0.766	7.83	
			D5 stream	0.946	6.34	
			D6 ditch	3.732	1.60	
			R1 pond	0.0797	75.28	
			R1 stream	3.790	1.58	
			R3 stream	4.980	1.20	
			R4 stream	3.957	1.51	

*values in bold indicate unacceptable risk

Table B. 9.4-31. Chronic toxicity exposure ratios (TER_{LT}) for Macrophytes based on worst case scenario PEC_{sw} from STEP 3 (continued).

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	1.129	5.31	≥5
			D1 stream	0.838	7.15	
			D2 ditch	2.412	2.48	
			D2 stream	1.574	3.81	
			D3 ditch	1.014	5.91	
			D4 pond	0.0357	168.06	
			D4 stream	0.763	7.86	
			D5 pond	0.0387	155.03	
			D5 stream	0.818	7.33	
			D6 ditch	1.009	5.94	
			R1 pond	0.0913	65.71	
			R1 stream	1.021	5.87	
			R3 stream	1.450	4.13	
			R4 stream	0.668	8.98	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	6.543	0.91	≥5
			D1 stream	4.082	1.46	
			D2 ditch	6.199	0.96	
			D2 stream	3.882	1.54	
			D3 ditch	1.514	3.96	
			D4 pond	1.168	5.13	
			D4 stream	1.647	3.64	
			D5 pond	1.170	5.12	
			D5 stream	1.420	4.22	
			D6 ditch	5.693	1.05	

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 3	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			R1 pond	0.116	51.72	
			R1 stream	5.811	1.03	
			R3 stream	7.641	0.78	
			R4 stream	5.980	1.00	

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B. 9.4-41: The FOCUS STEP 3 scenarios in winter cereals, for which the TER_{LT} Macrophytes needs further refinement.

Scenario STEP 3	Winter cereals				
	Autumn use 120 g s.a./ha	Spring use 120 g a.s./ha	Autumn use 160 g a.s./ha	Spring use 160 g a.s./ha	Autumn use 240 g a.s./ha
D1 ditch	D1 ditch	-	D1 ditch	-	D1 ditch
D1 stream	D1 stream	-	D1 stream	-	D1 stream
D2 ditch	D2 ditch	D2 ditch	D2 ditch	D2 ditch	D2 ditch
D2 stream	D2 stream	-	D2 stream	D2 stream	D2 stream
D3 ditch	-	-	-	-	D3 ditch
D4 pond	-	-		-	-
D4 stream	-	-		-	D4 stream
D5 pond	-	-	-	-	-
D5 stream	-	-	-	-	D5 stream
D6 ditch	D6 ditch	-	D6 ditch	-	D6 ditch
R1 pond	-	-	-	-	-
R1 stream	R1 stream	-	R1 stream	-	R1 stream
R3 stream	R3 stream		R3 stream	R3 stream	R3 stream
R4 stream	-	-	R4 stream	-	R4 stream

- no further refinement is needed

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 3 maximum PEC_{sw} values for flufenacet, the TER_{LT} values for Macrophytes meet the required trigger of 10, except the scenarios given in the Table B.9.4-41. For these scenarios Macrophytes TER needs further refinement.

Therefore, the PEC_{sw} values for flufenacet were further refined at FOCUS Step 4 using 10-metres- and 20-metres-wide FOCUS buffer zones for mitigation of Spray Drift and Run-off. As an additional refinement step in Run-off mitigation were performed the calculations in VFS-mod assuming the 10-metres wide buffer zone for reducing migration with Spray Drift (D and R) scenarios and Run-off (for R scenarios only).

FOCUS STEP 4 -10-metres wide FOCUS buffer zone for mitigation of Spray Drift and Run-off

TER_{LT} values for relevant scenarios based on worst case scenario PEC_{sw} from STEP 4 with 10 meter buffer zone FOCUS were presented in the Table B.9.4-42.

Table B. 9.4-42: Chronic toxicity exposure ratios (TER_{LT}) for Macrophytes based on worst case scenario PEC_{sw} from STEP 4 with 10 meter buffer zone.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	2.680	2.23	≥5
			D1 stream	1.672	3.58	
			D2 ditch	3.227	1.85	
			D2 stream	2.021	2.96	
			D6 ditch	2.764	2.17	
			R1 stream	1.354	4.43	
			R3 stream	1.728	3.47	
Spring in winter cereals at rate 1 x 120 g a.s./ha, post-emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D2 ditch	1.702	3.52	≥5
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	4.328	1.38	≥5
			D1 stream	2.699	2.22	
			D2 ditch	3.957	1.51	
			D2 stream	2.480	2.41	
			D6 ditch	3.732	1.60	
			R1 stream	1.697	3.53	
			R3 stream	2.246	2.67	
			R4 stream	1.786	3.35	
Spring use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D2 ditch	2.412	2.48	≥5
			D2 stream	1.574	3.81	
			R3 stream	0.662	9.06	
Autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	6.543	0.91	≥5
			D1 stream	4.082	1.46	
			D2 ditch	6.199	0.96	
			D2 stream	3.882	1.54	
			D6 ditch	5.693	1.05	
			D3 ditch	0.218	27.52	
			D4 stream	1.674	3.58	
			D5 stream	1.249	4.80	
			R1 stream	2.602	2.30	
			R3 stream	3.446	1.74	

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
			R4 stream	2.699	2.22	

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B.9.4-43: The FOCUS STEP 4 scenarios with 10 m buffer, for which the TER_{LT} Macrophytes needs further refinement.

Scenario STEP 4	Autumn use 120 g s.a./ha	Spring use 120 g s.a./ha	Winter cereals		
			Autumn use 160 g a.s./ha	Spring use 160 g s.a./ha	Autumn use 240 g a.s./ha
D1 ditch	D1 ditch	D2 ditch	D1 ditch	D2 ditch	D1 ditch
D1 stream	D1 stream	-	D1 stream	D2 stream	D1 stream
D2 ditch	D2 ditch	-	D2 ditch	-	D2 ditch
D2 stream	D2 stream	-	D2 stream	-	D2 stream
					D4 stream
D6 ditch	D6 ditch	-	D6 ditch	-	D5 stream
D4 stream	-	-	-	-	D6 ditch
R1 stream	R1 stream	-	R1 stream	-	R1 stream
R3 stream	R3 stream	-	R3 stream	-	R3 stream
R4 stream	-	-	R4 stream	-	R4 stream

- No further refinement is needed

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 4 with 10 meter buffer zone PEC_{sw} values for flufenacet, the TER_{LT} values for Macrophytes meet the required trigger of 10, except the scenarios given in the Table B.9.4-43. For these scenarios Macrophytes TER needs further refinement. As a result, PEC_{sw} values for flufenacet for autumn and spring application to winter cereals were further refined using the FOCUS Step-4 with 20 meter buffer zone FOCUS.

STEP 4 – 20-metres wide FOCUS buffer zone for mitigation of Spray Drift and Run-off

Table B. 9.4-44: Chronic toxicity exposure ratios (TER_{LT}) for Macrophytes based on worst-case scenario PEC_{sw} from STEP 4 with 20 meter buffer zone.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 120 g a.s./ha, pre-emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	2.680	2.23	≥5
			D1 stream	1.672	3.58	
			D2 ditch	3.227	1.85	
			D2 stream	2.021	2.96	
			D6 ditch	2.764	2.17	
			R1 stream	0.652	9.20	
			R3 stream	0.907	6.61	

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µga.s./L)	TER _{LT}	Trigger
Spring use in winter cereals at rate 1 x 120 g a.s./ha, post-emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D2 ditch	1.702	3.52	≥5
Autumn use in winter cereals at rate 1x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	4.328	1.38	≥5
			D1 stream	2.699	2.22	
			D2 ditch	3.957	1.51	
			D2 stream	2.480	2.41	
			D6 ditch	3.732	1.60	
			R1 stream	0.883	6.79	
			R3 stream	1.173	5.11	
			R4 stream	0.933	6.43	
Spring use in winter cereals at rate 1x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D2 ditch	2.412	2.48	≥5
			D2 stream	1.574	3.81	
Autumn use in winter cereals at rate 1x 240 g a.s./ha, post emergence						
Flufenacet Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	D1 ditch	6.543	0.91	≥5
			D1 stream	4.082	1.46	
			D2 ditch	6.199	0.96	
			D2 stream	3.882	1.54	
			D4 stream	1.674	3.58	
			D5 stream	1.249	4.80	
			D6 ditch	5.693	1.05	
			R1 stream	1.354	4.43	
			R3 stream	1.799	3.33	
			R4 stream	1.410	4.25	

*values in bold indicate unacceptable risk

In the table below are listed all the scenarios for which the TER_{LT} value did not meet the trigger.

Table B.9.4-45: The FOCUS STEP 4 scenarios with 20 m buffer, for which the TER_{LT} Macrophytes needs further refinement.

Scenario STEP 4	Autumn use 120 g s.a./ha	Spring use 120 g s.a./ha	Winter cereals		
			Autumn use 160 g a.s./ha	Spring use 160 g s.a./ha	Autumn use 240 g a.s./ha
D1 ditch	D1 ditch	D2 ditch	D1 ditch	D2 ditch	D1 ditch
D1 stream	D1 stream	-	D1 stream	D2 stream	D1 stream
D2 ditch	D2 ditch	-	D2 ditch	-	D2 ditch
D2 stream	D2 stream	-	D2 stream	-	D2 stream
					D5 stream
D6 ditch	D6 ditch	-	D6 ditch	-	D6 ditch
D4 stream	-	-	-	-	D4 stream
R1 stream	-	-	-	-	R1 stream

Scenario STEP 4	Autumn use 120 g s.a./ha	Spring use 120 g s.a./ha	Winter cereals		
			Autumn use 160 g a.s./ha	Spring use 160 g s.a./ha	Autumn use 240 g a.s./ha
R3 stream	-	-	-	-	R3 stream
R4 stream	-	-	-	-	R4 stream

- No further refinement is needed

Based on a comparison of the results of the standard laboratory toxicity studies with FOCUS Step 4 PEC_{sw} values for flufenacet calculated assuming 20-metres buffer zone for mitigation of Spray Drift in all D scenarios and Spray Drift and Run-off in all R scenarios (for Run-off the adequate reduction factors given by FOCUS L&M Guideline for 10-20 –metres wide vegetated buffer zone were used, the TER_{LT} values for algae meet the required trigger of 10, except the scenarios given in the Table B.9.4-36.

For those scenarios, Macrophytes TER needs further refinement.

Overall conclusion

Macrophates including Lemna sp and Elodea sp.

Autumn Uses:

The general conclusions drawn from the risk assessment for Macrophytes including Lemna sp. and Elodea sp. presented above are given, are provided below, individually for each use listed in the EU-representative GAP.

In case of the post-emergence use in Winter cereals in autumn, at application rate **240 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream, D4 stream, D5 stream, D6 ditch, R1 stream, R3 stream and R4 stream.

The safe scenarios identified within that use were: D3 ditch assuming 10-metres wide buffer zone for mitigation of Spray Drift, D4 pond already at STEP 3 (hence no buffer zone needed to be implemented), D5 pond already at STEP 3 (hence no buffer zone needed to be implemented), R1 pond already at STEP 3 (hence no buffer zone needed to be implemented).

Therefore for that use it may be stated that one safe scenario was identified – D3 scenario assuming 10-metres wide non-spray buffer zone to mitigate the Spray drift.

In case of the post-emergence use in Winter cereals in autumn, at application rate **160 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream, and D6 ditch.

The safe scenarios identified within that use were: D3 ditch, D4 pond, D4 stream, D5 pond, D5 stream – all them already at STEP 3 (hence no buffer zone needed to be implemented), as well as R1 pond already at STEP 3 (hence no buffer zone needed to be implemented), R1 stream assuming 20-metres wide vegetated buffer zone for mitigation of Spray Drift and Run-off, R3 stream assuming 20-metres wide vegetated buffer

zone for mitigation of Spray Drift and Run-off and R4 stream assuming 20-metres wide vegetated buffer zone for mitigation of Spray Drift and Run-off.

Therefore for that use it may be stated that the following safe uses were identified: for scenarios D3, D4 and D5 without any buffer zone (safe PEC_{sw} values were obtained already at STEP 3) as well as R1, R3 and R4 scenarios assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off.

In case of the post-emergence use in Winter cereals in autumn, at application rate **120 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D1 ditch, D1 stream, D2 ditch, D2 stream and D6 ditch.

The safe scenarios identified within that use were D3 ditch, D4 (pond and stream), D5 (pond and stream), R1 pond and R4 stream – all they already at STEP 3 (hence no buffer zone needed to be implemented), and R1 stream, R3 stream assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off

Therefore for that use it may be stated that the following safe uses were identified: for scenarios D3, D4, D5 and R4 without any buffer zone (safe PEC_{sw} values were obtained already at STEP 3) and R1 and R3 scenarios assuming 20-metres wide vegetated buffer zone to mitigate Spray Drift and Run-off.

SPRING USE

The general conclusions drawn from the risk assessment for Macrophytes presented above are given, are provided below, individually for each use listed in the EU-representative GAP.

In case of the post-emergence use in Winter cereals at spring, at application rate **160 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenarios:

- D2 ditch and D2 stream;

In case of scenarios D1 ditch, D1 stream, D3 ditch, D4 pond, D4 stream, D5 pond and D5 stream, R1 pond, R1 stream and R4 stream safe PEC_{sw} values were obtained already at STEP 3.

For scenario R3 stream safe PEC_{sw} values were obtained at STEP 4 after implementation of 10-metres wide vegetated buffer zone for mitigation of the Spray Drift and Run-off.

Therefore for that application pattern only for scenarios D2 safe use was not demonstrated. In case of scenario R3 it was demonstrated that it was necessary to implement 10 meter buffer zone mitigating Spray Drift and Run-off. In all remaining scenarios identified as returning the safe PEC_{sw} values it was demonstrated that the implementation of any mitigation measures was not necessary as the assessment was finalised at Step 3.

In case of the post-emergence use in Winter cereals at spring, at application rate **120 g Flufenacet/ha**, after application of the highest admissible mitigation measures – buffer zone of 20 metres for mitigation of Spray Drift and Run-off safe use was not demonstrated in case of scenario D2 ditch.

In case of scenarios D1 ditch, D1 stream, D2 stream, D3 ditch, D4 pond, D4 stream, D5 pond and D5 stream, D6 ditch, R1 pond, R1 stream, R3 stream and R4 stream safe PEC_{sw} values were obtained already at STEP 3. For that application pattern assessment at STEP 4 was not performed.

Therefore, for that application pattern only for scenario D2 safe use was not demonstrated. In case of scenarios D1, D3, D4, D5, D6, R1, R3 and R4 it was demonstrated that implementation of the buffer zone mitigating Spray Drift and/or Run-off was not necessary.

As an additional refinement step RMS carried out the calculations using the VFS-mod option to mitigate run-off. The selected vegetated buffer zone was 10-metres wide. The same width of the buffer zone was used in function of the non-spray buffer zone for simultaneous mitigation of the Spray Drift.

The results of the calculations are presented below in the table B.9.4-46. In it are listed only those scenarios, for which safe PEC_{sw} values were not obtained at STEP 4 assuming the maximum admissible reduction factors set by FOCUS.

As VFS-mod is a tool specific only for mitigating Run-off, and hence for R scenarios, in the table below only the results obtained for R scenarios, where relevant, were presented.

Table B. 9.4-46: Chronic toxicity exposure ratios (TER_{LT}) for Macrophytes based on worst case scenario PEC_{sw} from 10-metres buffer zone, VFS-mod.

Compound	Species	NOEC (µg a.s./L)	FOCUS Step 4	Max PEC _{sw} (µg a.s./L)	TER _{LT}	Trigger
Autumn use in winter cereals at rate 1 x 160 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	R1 stream	0.129	46.51	≥5
			R3 stream	0.181	33.14	
			R4 stream	0.128	46.87	
Autumn use in winter cereals at rate 1 x 240 g a.s./ha, post emergence						
Flufenacet	Microcosm (Lemna sp + Elodea sp.)	6	R1 stream	0.194	30.92	≥5
			R3 stream	0.272	22.05	
			R4 stream	0.192	31.25	

Based on the results presented above it may be stated that the use of the VFS-mod for R scenarios assuming the 10-metres wide vegetated buffer zone resulted in reaching safe PEC_{sw} values in all scenarios of concern in autumn post-emergence use in Winter cereals at 160 g a.s./ha and 240 g Flufenacet/ha.

B.9.5. EFFECTS ON ARTHROPODS

B.9.5.1. Effects on bees

Acute and contact studies of flufenacet on bees were evaluated within the process of Annex I inclusion and were considered acceptable by RMS–France. However, the new studies for active substance flufenacet were submitted for the process of renewal. These studies were performed according to current OECD 213 and OECD 214 (1998) test guidelines. The toxicity endpoints obtained from these studies $LD_{50} > 109.2 \mu\text{g a.s./bee}$ and $LD_{50} > 100 \mu\text{g a.s./bee}$ were considered valid and appropriate for the risk assessment.

Regarding the toxicity data on the technical flufenacet please refer to Volume 3. (CA), Section B.9.

One new study with the representative formulation DFF+FFA SC 600 was submitted for the renewal and was summarised under point B.9.5.1.1.

B. 9.5.1.1. Contact and oral acute toxicity

Table B.9.5.1.1-1. Summary of toxicity of flufenacet and the formulation DFF+FFA 600 SC to bees and toxicity flufenacet only to bumble bees.

Organism	Test substance	Time scale	Endpoint	Toxicity value	Reference
Honeybee (<i>Apis mellifera</i>)	Flufenacet tech.	48 h acute oral and contact toxicity test	LD_{50} -oral LD_{50} -contact	$> 170.5 \mu\text{g a.s./bee}^1$ $> 194 \mu\text{g a.s./bee}^1$	Nengel (1995) M-004919-01-1
Honeybee (<i>Apis mellifera</i>)	Flufenacet tech.	48 h acute oral and contact toxicity test	LD_{50} -oral LD_{50} -contact	$> 109.2 \mu\text{g a.s./bee}^2$ $> 100 \mu\text{g a.s./bee}^2$	Schmitzer (2011) M-421687-01-1
Bumble bees	Flufenacet tech.	48 h acute contact	LD_{50} -contact	$> 100 \mu\text{g a.s./bee}$	Vergé (2014) M-478561-01-1
Honeybee (<i>Apis mellifera</i>)	DFF+FFA 600 SC	48 h acute oral and contact toxicity test	LD_{50} -oral LD_{50} -contact	$> 217.8 \mu\text{g product/bee}$ $> 200 \mu\text{g product/bee}$	Schmitzer & Sekine (2009) M-356881-01-1

In bold: values use in the risk assessment

¹ EU agreed endpoints (Flufenacet, 7469/VI/98-Final/ 3 July/2003)

² New endpoints for active substance-Flufenacet

B.9.5.1.1.1. Effects of diflufenican + flufenacet SC 600 (200+400) G (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory.

Reference:	Effects of diflufenican + flufenacet SC 600 (200+400) G (Acute Contact and Oral) on Honey Bees (<i>Apis mellifera</i> L.) in the Laboratory.
Author(s), year:	Schmitzer, S., Sekine, T., 2009.
Previous evaluation:	None; relevant for renewal application
Report/Doc. number:	IBACON Project No: 47502035, Reference No. M-356881-01-1
Guideline(s):	OECD Guideline 213 and 214 (1998)
GLP:	Yes

<u>Test substance:</u>	Diflufenican + Flufenacet SC 600 (200+400 g/L) G, diflufenican (AE F088657) 15.6 % w/w, 191.4 g/L, flufenacet (FOE 5043) 32.1 % w/w, 394.5 g/L. Batch ID: EV56001418, density 1.229 g/mL.
Reference:	Perfekthion (BAS152 11 1), Batch No: FRE-000627, 422 g dimethoate/l (analysed)
Wetting agent:	Adhäsit, 100 g/L Marlopon (nominal), Batch no.: 0150207
Test species:	<i>Apis mellifera</i> L., adult worker honeybees
Type of test:	Acute oral and contact toxicity test
Test cages:	In both test procedures the bees were kept in cages (width: 10 cm; depth: 5.5 cm; height: 8.5 cm). The front side of the cages were equipped with a glass sheet so that the bees could be observed. The bottom of the cages consisted of a perforated board, which guaranteed sufficient air supply for the test animals. The test cages were lined with filter paper.
<u>Number of organism:</u>	10 individuals in 5 replicates per test item dose level, controls and reference item.
Food:	Commercial ready-to-use syrup (Apiinvert; 30 % sucrose, 31 % glucose, 39 % fructose) ad libitum.
<u>Oral toxicity test:</u>	
Applied concentration:	Control: 50% (w/w) aqueous sucrose solution (50% tap water, 50% ready-to-use syrup) Test item: 200 µg product /bee (nominal) Test item 217.8 µg product /bee (measured) Reference item: 0.30, 0.20, 0.15, 0.10 µg dimethoate/bee (nominal) Reference item 0.30, 0.16, 0.08, 0.05 µg dimethoate/bee (measured)
Exposure route:	Aqueous stock solutions of the test item and reference item were prepared in such a way that they had the respective target concentration of the test item once they were subsequently mixed with sugar syrup at a ratio of 1 + 1. After mixing of these test solutions with ready-to-use sugar syrup (composition of the sugar component: 30 % saccharose, 31 % glucose, 39 % fructose) the final concentration of sugar syrup in the test item solutions

	<p>offered to the bees was 50 %. For the control water and sugar syrup was used at the same ratio (1 + 1).</p> <p>The test bees were starved for 20 minutes before they were fed with the solutions. After 1 hours, the feeding troughs were exchanged with clean feeders containing ready-to-use syrup and the retrieved containers re-weighed to determine the quantity of feed consumed.</p>
Test condition:	Temperature: 25°C, Relative humidity: 42 - 76 %, Darkness (except during observation).
Test parameter:	Mortality counts and checks for behavioural abnormalities (e.g. apathy, intensive cleaning, foodrefusal/vomiting, moving coordination problems,) were made after exposure for 4, 24 and 48 hours.
<u>Contact toxicity test:</u>	
Applied concentration:	Control: tap water with 0.5% Adhäsit (wetting agent to improve spreading of the test droplet on the water-repellent hairs on the thorax of bees) Test item: 200 µg a.s./bee (nominal) Reference item: 0.30, 0.20, 0.15, 0.10 µg dimethoate/bee (nominal)
Exposure:	A single 5 µL droplet of Diflufenican + Flufenacet SC 600 (200 + 400) G in an appropriate carrier (tap water + 0.5 % Adhäsit) was placed on the dorsal bee thorax. For the control one 5 µL droplet of tap water containing 0.5 % Adhäsit was used. The reference item was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.5 % Adhäsit).
Test condition:	Temperature: 25°C, Relative humidity: 42 - 76 %, Darkness (except during observation).
Test parameter:	Mortality counts and checks for behavioral abnormalities were made after exposure for 4, 24 and 48 h.
Statistic:	<p>The contact and oral LD₅₀ values of the test item were estimated according to moving average computation (Thomson and Weil, 1952). The software used to perform the statistical analysis was ToxRat Professional, Version 2.10, ToxRat Solutions GmbH, 2009.</p> <p>The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.</p>

Findings In the oral toxicity test the maximum nominal test level of Diflufenican + Flufenacet SC 600 (200+400) G: 200.0 µg product/bee, corresponded to an actual intake of 217.8 µg product/bee led to no mortality after 48 hours.

No mortality and behavioural effects occurred also in the control. No test item induced behavioural effects were observed at any time.

Effects of survival of the honeybees are presented in the Table B.9.5.1.1.1-1. and B.9.5.1.1.1-2.

Table B.9.5.1.1.1-1: Effects of Diflufenican + Flufenacet SC 600 (200+400 g/L) G on *Apis mellifera* following 48-h exposure in the acute toxicity test (average from 5 replicates per dosage).

Nominal dosage (consumed)	after 4 hours		after 24 hours		after 48 hours	
	mortality	behavioural abnormalities	mortality	behavioural abnormalities	mortality	behavioural abnormalities
	mean %	mean %	mean %	mean %	mean %	mean %
Test item [µg prod./bee] 200 (217.8)	0.0	0.0	0.0	0.0	0.0	0.0
Control (tap water+sugar solution)	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
0.33 (0.30)	90.0	10.0	98.0	2.0	100.0	0.0
0.16 (0.16)	24.0	62.0	96.0	0.0	96.0	0.0
0.15 (0.08)	4.0	4.0	48.0	0.0	60.0	0.0
0.10 (0.05)	0.0	0.0	8.0	0.0	8.0	0.0
Test substance 24/48h LD ₅₀ >218 µg prod./bee						
Reference: 24 h LD ₅₀ =0.10 µg a.s./bee (95% C.I.: 0.09-0.12 µg a.s./bee)						
48 h LD ₅₀ =0.10 µg a.s./bee (95% C.I: 0.08-0.12 µg a.s./bee)						

Contact toxicity test: At the end of the contact toxicity test (48 hours after application), there was 2.0 % mortality at 200.0 µg product/bee. No mortality and behavioural effects occurred in the control (water + 0.5 % Adhasit). No test item induced behavioural effects were observed at any time.

Table B. 9.5.1.1.1-2 : Effects of Diflufenican + Flufenacet SC 600 (200+400 g/L) G on *Apis mellifera* following 48-h contact exposure in the acute toxicity test (average from 5 replicates per dosage).

Nominal dose	after 4 hours		after 24 hours		after 48 hours	
	mortality	behavioural abnormalities	mortality	behavioural abnormalities	mortality	behavioural abnormalities
	mean %	mean %	mean %	mean %	mean %	mean %
Test item [µg prod./bee] 200.0	0.0	0.0	0.0	0.0	2.0	0.0
Control (tap water + wetting agent)	0.0	0.0	0.0	0.0	0.0	0.0

Nominal dose	after 4 hours		after 24 hours		after 48 hours	
	mortality	behavioural abnormalities	mortality	behavioural abnormalities	mortality	behavioural abnormalities
	mean %	mean %	mean %	mean %	mean %	mean %
Reference item [$\mu\text{g a.s./bee}$]						
0.30	4.0	26.0	92.0	2.0	92.0	0.0
0.20	0.0	0.0	84.0	0.0	90.0	0.0
0.15	0.0	0.0	42.0	2.0	60.0	2.0
0.10	0.0	0.0	0.0	6.0	18.0	2.0
Test substance 24/48h $\text{LD}_{50} > 200 \mu\text{g prod./bee}$						
Reference: 24 h $\text{LD}_{50} = 0.16 \mu\text{g a.s./bee}$ (95% C.I.: 0.14-0.18 $\mu\text{g a.s./bee}$)						
48 h $\text{LD}_{50} = 0.13 \mu\text{g a.s./bee}$ (95% C.I.: 0.11-0.16 $\mu\text{g a.s./bee}$)						

Conclusion: The LD_{50} (48 h) value was $> 217.8 \mu\text{g product/bee}$ in the oral toxicity test. The LD_{50} (48 h) value was $> 200.0 \mu\text{g product/bee}$ in the contact toxicity test.

Comment RMS:

All validity criteria according to the OECD guidelines 213 and 214 in the study was met.

The mean mortality of the controls in the oral and contact toxicity test was maximal 2 % ,which is in the line with the recommended maximum mortality of 10 % according to the OECD guidelines.

The 24 h LD_{50} values of the reference item (dimethoate) in the oral (24 h $\text{LD}_{50} = 0.10 \mu\text{g a.s./bee}$) and contact (24 h $\text{LD}_{50} = 0.16 \mu\text{g a.s./bee}$) toxicity tests were within the recommended range of 0.10 – 0.35 $\mu\text{g a.s./bee}$ (oral) and 0.10 – 0.30 $\mu\text{g a.s./bee}$ (contact), respectively.

Some deviations to the OECD guidelines were identified:

-The relative humidity in the oral and contact toxicity test is between 42 and 76% which is below the recommendations given in the OECD guidelines (relative humidity between 50 and 70%).

Based on the results of the oral and contact toxicity test the deviation of relative humidity is considered to have no impact on the honeybees.

- In the oral toxicity test the bees were starved for 20 minutes only and not for 2 hours as recommended in the OECD guideline. However, this may not have an effect on the results of the study considering the amount of consumed sugar solution.

-In the contact toxicity test a 5 μL droplet was used in deviation to the OECD guideline recommendation of a 1 μL droplet. This deviation is considered acceptable since a higher volume ensured a more reliable dispersion of the test item. The study is considered acceptable.

Agreed endpoints:

48 h $\text{LD}_{50} > 217.8 \mu\text{g product/bee}$ (oral toxicity), 48 h $\text{LD}_{50} > 200 \mu\text{g product /bee}$ (contact toxicity)

B.9.5.1.2. Other toxicity test on adult bees and bee larvae

According to the data requirements for active substances (Commission Regulation (EU) 283/2013) and/or plant protection products (Commission Regulation (EU) 284/2013) the chronic risk to adult honeybees has to be evaluated. However, no valid test guidelines are available to address this point. In the EFSA guidance document on risk assessment on honeybees (EFSA Journal 2013;11(7):3295) a study protocol (Appendix O) is given as support on how to perform a chronic oral toxicity test. The protocol is based on information from Decourtye et al. (2005), Suchail et al. (2001), Thompson H. (Food and Environment Research Agency, 2012) and CEB (2012). However, a the 10 day oral chronic toxicity study was submitted for the technical active substance flufenacet (please refer to Volume 3 (CA), Section B.9).

Table B.9.5.1.2-1: The chronic toxicity of flufenacet to adult bees and toxicity of to bee larva.

Organism	Test substance	Time scale	Endpoint	Toxicity Value	Reference
Chronic toxicity to adult bees (laboratory)					
Honeybee (<i>Apis mellifera</i>)	Flufenacet tech.	10 d chronic adult feeding study	LC ₅₀ NOEC	> 120 mg a.s./kg ≥ 120 mg a.s./kg (=4.42 µg a.s./bee/day)	Kling (2014) M-477339-01-1

In order to reveal whether flufenacet poses a risk to immature honey bee life stages, a bee brood feeding study has been conducted by following the provisions/method of Oomen P.A., de Ruijter, A. & van der Steen, J. (OEPP/EPPO Bulletin 22:613-616 (1992).

Bee brood test:**Table B.9.5.1.2-2: Toxicity of flufenacet and formulation DFF+FFC SC600 to bees**

Organism	Test substance	Time scale	Endpoint	Toxicity Value	Reference
Honeybee (<i>Apis mellifera</i>)	Flufenacet SC 508.8	21 day, bee brood feeding test .	No adverse effects on mortality, bee brood development (eggs, young larvae, old larvae, pupae) and colony development by feeding honey bee colonies sugar syrup with a flufenacet -concentration typical for/exceeding the concentration of flufenacet in the spray tank (1500 ppm)		Hecht-Rost (2012) M-456504-01-1

B.9.5.2. Effects on non-target arthropods other than bees**B.9.5.2.1. Toxicity data**

In the first Annex I listing process non-target arthropod data for a different formulation of flufenacet -FFA WG 60, were submitted and evaluated. The formulation FFA WG 60 is no longer considered to be the representative formulation, therefore only data on the new representative formulation Flufenacet + Diflufenican SC 600 (Herold SC 600) for the Annex I renewal process was evaluated in this RAR.

For the Annex I listing process of diflufenican also the formulation Flufenacet + Diflufenican SC 600 (DFF+FFA SC 600, Herold SC 600) was submitted as representative formulation. Hence, some formulation studies on non-target arthropods were already evaluated during this Annex I listing process of diflufenican.

The toxicity of Flufenacet + Diflufenican SC 600 (DFF + FFA 600 SC) to non-target arthropods has been investigated by carrying out Tier I glass plate tests on the parasitoid wasp *Aphidius rhopalosiphii* (Moll, M.; Buetzler, R. 2001) and the predatory mite *Typhlodromus pyri* (Goßmann, A. 2001).

Effects > 50% were found in T.pyri study, resulting in an LR₅₀ of 81.8 mL/ha, indicated needs to further refinement in the field. The extended laboratory studies (Tier 2) were generated on the *Typhlodromus pyri* (Chauzat, M.P., 2002), on green lacewing *Chrysoperla carnea* (Waibel, J.; 2009) under more realistic conditions, i.e. on treated leaves and on soil dwelling organism - *Aleochara bilineata* (Roehlig, U.; 2009) exposed to fresh residues of DFF+FFA SC 600 on natural soil.

In addition, an aged residue study has been conducted for DFF+FFA SC 600 with T. Pyri (Jans, D., 2009) to demonstrate the potential for recovery of this species. All study were considered valid and appropriate to use in the risk assessment.

The summary of the results are presented in the Table B.9.5.2-1-1.

Table B.9.5.2.1- 1: Flufenacet + Diflufenican SC 600: Ecotoxicological endpoints for arthropods other than bee.

Test species, references	Tested Formulation, study type, exposure	Ecotoxicological endpoint	
Typhlodromus pyri M-058604-01-1 Rep.No.: 9352063 Goßmann, A.; 2001	DFF+FFA SC 600	LR ₅₀ 81.8 mL prod./ha,	
	Laboratory, glass plates	Corr. Mortality [%]	Effect on Reproduction [%]
	22.5 mL prod./ha	1.9	1.3
	45 mL prod./ha	9.2	-12.5 ^A
	90 mL prod./ha	61.1	n.a.
	180 mL prod./ha	92.6	n.a.
Typhlodromus pyri M-034242-01-1 Rep.No.: 01TYBYL12 Chauzat, M.P.; 2002	DFF+FFA SC 600	LR ₅₀ 110.2 mL prod./ha,	
	Extended lab., exposure on detached bean leaves	ER ₅₀ >83.2 mL prod./ha,	
	9.9 mL prod./ha,	Corr. Mortality [%]	Effect on Reproduction [%]
	28.7 mL prod./ha,	0	4.4
	83.2 mL prod./ha,	0	13.3
	241.4 mL prod./ha,	17.1	-17.8 ^A
Typhlodromus pyri M-355238-01-1 Rep.Nr.: CW09/026 Jans, D.; 2009	DFF+FFA SC 600		
	Aged residues, spray deposits on maize plants, 1 appl. of 0.7 L/ha	Corr. Mortality [%]	Effect on Reproduction [%]
	Residues aged for 0 days:	98.9	n.a.
	Residues aged for 14 days:	87.1	n.a.
	Residues aged for 28 days:	9.5	8.4
Aphidius rhopalosiphii M-058618-01-1 Rep.No.: 9351001 Moll, M.; Buetzler, R.; 2001	DFF+FFA SC 600	LR ₅₀ > 700 mL prod./ha	
	Laboratory, glass plates	ER ₅₀ > 700 mL prod./ha	
		Corr. Mortality [%]	Effect on Reproduction [%]
	500 mL prod./ha,	0	9.0
	600 mL prod./ha,	2.0	14.0
	700 mL prod./ha	2.0	3.5

Test species, references	Tested Formulation, study type, exposure	Ecotoxicological endpoint		
Chrysoperla carnea M-352372-01-1 Rep.No.: CW09/010 Waibel, J.; 2009	DFF+FFA SC 600	LR ₅₀ > 600 mL prod./ha,		
	Extended lab., exposure on detached maize leaves	No effect on reproduction		
	Control	Corr. Mortality	Eggs/Female/Day	Hatching [%]
	30 mL prod./ha,	-	26.4	79.9
	63 mL prod./ha	0.0	24.1	81.4
	134 mL prod./ha,	7.7	23.9	80.7
	284 mL prod./ha,	2.6	27.5	83.4
Aleochara bilineata M-353760-01-1 Rep.No.: 09 10 48 027 A Roehlig, U.; 2009	600 mL prod./ha,	7.7	28.4	82.5
	600 mL prod./ha,	20.5	27.6	82.7
	DFF+FFA SC 600	ER ₅₀ > 600 mL prod./ha		
	Extended lab., spray deposits on soil (LUFA 2.1)	Effect on Reproduction [%]		
	60 mL prod./ha,	4.3		
	107 mL prod./ha,	-2.3 ^A		
	190 mL prod./ha,	1.7		
	337 mL prod./ha,	5.8		
	600 mL prod./ha,	7.9		

^A: A negative value indicates a higher reproduction rate in the treatment than in the control.

n.a.: not assessed

B 9.5.2.1.1. Standard laboratory testing for non-target arthropods

9.5.2.1.1.1. Effects of Flufenacet & Diflufenican SC 600 on the Parasitoid Aphidius rhopalosiphi in the Laboratory - Limit Test.

Reference:	Effects of Flufenacet & Diflufenican SC 600 on the Parasitoid Aphidius rhopalosiphi in the Laboratory - Limit Test.
Author(s), year:	Moll, M. & Bützler, R., 2001
Report/Doc. number:	Report No: IBACON Project 9351001, ReferenceBCS no. M-058618-011
Guideline(s):	IOBC/WPRS 1988, Mead-Briggs et al. 2000
GLP:	Yes

<u>Test substance:</u>	Flufenacet & Diflufenican SC 600. active ingredient: Flufenacet (FOE 5043), Diflufenican (DFF 200), formulation No.: 07205/0024 (0006), purity: 401.5 g/L Flufenacet, 217.0 g/L Diflufenican, (certificate analysis) Formulation density: 1.247 g/cm ³
Toxic reference:	Perfekthion (BAS 152 11 I), content of dimethoate: 417.5 g/L (analysed)
Test species:	Aphidius rhopalosiphi (Hymenoptera: Braconidae) adults, < 48 h old
Type of test:	Acute contact laboratory test
Number of organism:	<u>Exposure period:</u> 5 replicates with 10 adult wasps (7 females, 3 males) per replicate
	<u>Post-exposure period:</u> max. 15 replicates/treatment group: 1 female per unit
Application:	Control: deionised water Test item: - 700 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 281.05 g a.s./ha (FOE 5043) and 151.9 g a.s./ha (DFF)

	<p>- 600 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 240.9 g a.s./ha (FOE 5043) and 130.2 g a.s./ha (DFF)</p> <p>- 500 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 200.75 g a.s./ha FOE 5043 and 108.5 g a.s./ha (DFF)</p> <p>Treatments applied with a calibrated sprayer in 200 L water/ha</p>
Toxic reference:	0.3 mL Perfekthion/ha (corresponding to ~ 0.13 g a.s./ha taking into account a density of 1.077 g/cm ³ and an analyzed content of 417.5 g dimethoate/L).
Test units:	<p><u>Exposure units:</u></p> <p>2 treated glass plates, held apart by an untreated aluminium frame and held together with at least 2 clamps.</p> <p><u>Post-exposure units:</u></p> <p>Untreated pots with barley seedlings infested with host aphids of all developmental stages (<i>Rhopalosiphum padi</i>) enclosed within a clear polyacrylic cylinder with a hole (approximately 1.5-2.1 cm in diameter) closed with cotton wool for the introduction of the parasitoids and a gauze on the top; the soil surface of the flowerpot was covered with a thin layer of quartz sand.</p>
Exposure route, duration:	<p>Exposure period: 48 hours and 5 min.</p> <p>Post-exposure period:</p> <p>Parasitation period: 24 hours and 15 min. to 24 hours to 10 min.</p> <p>Post – parasitation period: 11 - 12 days</p> <p>(11 units of each treatment group were evaluated 11 days after parasitation and 4 units of each treatment group were evaluated 12 days after parasitation)</p>
Feeding:	A solution of fructose (25%) in small test tubes provided as source of food, ad libitum
Test condition:	<p>Temperature:</p> <p>20 - 23 °C (acclimatization period)</p> <p>20 - 23 °C (exposure period)</p> <p>20 - 24 °C (post-exposure period)</p> <p>Relative humidity:</p> <p>70 – 85% (acclimatization)</p> <p>70 – 85% (exposure period)</p> <p>63 - 68 % (post-exposure period)</p> <p>Light period: 16 h light/8 h dark</p> <p>Light intensity:</p> <p>1080 lux (acclimatization period)</p> <p>930–1020 lux (exposure period)</p> <p>4530- 15170 lux (post parasitation period)</p>

Test parameters:	<p>Mortality and behavioural abnormalities were assessed approx. 2, 24 and 48 h after introduction of the wasps to the test units.</p> <p>The parasitisation rate was determined at the end of the parasitisation phase by counting the number of mummies for each individual wasp.</p> <p>Reproduction phase was performed where the corrected mortality was $\leq 50\%$.</p>
Statistic:	<p>Mortality data were analyzed for significance using Fisher Exact Test.</p> <p>Reproduction was tested for normal distribution and homogeneity of variance using Kolmogoroff-Smirnov-Test ($\alpha = 0.05$) and Cochran-Test ($\alpha = 0.05$); because reproduction data were normally distributed and homogenous Dunnett-Test (multiple comparison, two-sided, $\alpha = 0.05$) was used. The software used to perform the statistical analysis was EASY ASSAY, Multiple Testing, © SPiRiT, Version 4.0 and SYSTAT Version.</p>

Findings:

After 48 hours exposure period no mortality was observed in the control as well as in all test item treatment groups. 100% mortality was observed in the reference item group. No abnormal behaviour was observed.

There was no statistically significant effect on reproduction up to and including 700 mL product/ha compared to the control and the reduction in reproduction was below the trigger value of 50% (3.5-14%).

Table B 9.5.2.1.1-1: Flufenacet & Diflufenican SC 600 effects of on *A. rhopalosiphi* following exposure on glass plates for 48 h under laboratory conditions.

Nominal application mL DFF+ FFA SC 600 /ha	Mortality		Parasitism efficiency		
	Mean ¹ ± SD	Control Corrected [%]	Parasitism rate ² [mummies/female] mean ± SD	Reproductive capacity %	% Reduction relative to control
Control	0.0	-	20.0 ± 11.5	-	-
500	0.0 ± 0.0	0.0	18.2 ± 12.6 ^{ns}	91	9
600	2.0 ± 4.5	2.0	17.2 ± 11.2 ^{ns}	86	14
700	2.0 ± 4.5	2.0	19.3 ± 11.7 ^{ns}	96.5	3.5

ns not statistically significant compared to control (Dunnett-Test, $\alpha = 0.05$)

1 Mean and standard deviation (SD) of the 5 replicates

2 Mean and standard deviation (SD) from 15 replicates each with 1 female

Conclusion:

48 h LR₅₀ > 700 mL Flufenacet & Diflufenican SC 600 /ha

48 h ER₅₀ > 700 mL Flufenacet & Diflufenican SC 600/ha

Comments RMS:

The study was conducted according to the IOBC test guidelines (Mead-Briggs et al., 2000, Mead-Briggs et al., 2009). The study is considered acceptable taking into account the validity criteria stated in the IOBC test guideline. The mortality of the adult wasps in the control was below 13% (being: 0%). For the fecundity assessments, wasps in the control group produced more than 5 mummies per female (being: mean 20 mummies per female). The number of wasps in the control producing no mummies was not more than two wasps (being: 0 wasps). The mortality observed in the toxic reference group (100% at ~ 0.13g a.s./ha) was in line with the IOBC test guideline.

Following deviation from the guideline was noted:

-Temperature temporarily was >23 °C, maximum temperature was 24 °C - in the post-exposure period at one day for approx. 5 hours (according to the test guideline the temperature should be at range 20 °C ± 3 °C). An indicated deviation is, however, considered as having no impact on the study results, since all validity criteria were met. The study is considered acceptable.

Agreed endpoints:

48 h LR₅₀ > 700 mL Flufenacet & Diflufenican SC 600 /ha

48 h ER₅₀ > 700 mL Flufenacet & Diflufenican SC 600 /ha

B 9.5.2.1.1.2. Effects of Flufenacet & Diflufenican SC 600 on the Predatory Mite**Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the Laboratory-Dose Response Design.**

Reference:	Effects of Flufenacet & Diflufenican SC 600 on the Predatory Mite Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the Laboratory-Dose Response Design.
Author(s), year:	A. Goßmann, 2001
Report/Doc. Number:	IBACON No: 9352063; 2001/MM/DD Referebce BCS No:
Guideline(s):	Blümel et al., 2000
GLP:	Yes

<u>Test substance:</u>	Flufenacet & Diflufenican SC 600, active ingredient: Flufenacet (FOE 5043), Diflufenican (DFF 200), formulation No.: 07205/0024 (0006), purity: 401.5 g/L Flufenacet, 217.0 g/L Diflufenican (according to certificate analysis). Density: 1.247 g/cm ³ .
Toxic reference:	Perfekthion (BAS 152 11 I), 417.5 g dimethoate/L
Test species:	Typhlodromus pyri about 2 days old (protonymphs not older than 1 day)
Number of organism:	3 replicates with 20 individuals per replicate
Application:	Control: deionised water Test item:

	<ul style="list-style-type: none"> - 22.5 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 0.14 g/L) - 45 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 0.28 g/L) - 90 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 0.56 g/L) - 180 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 1.12 g/L) - 360 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 2.24 g/L)
	Treatments applied with a calibrated sprayer in 200 L water/ha (correspond to 2 mg/cm ³)
	Toxic standard:
	- 8.0 mL in 200 L water/ha./ha corresponding to 40 uL Perfekthion EC/L
Test units:	Formed by two cover slides (glass, 24 x 60 mm) fixed by gluing small cover slides (glass, 20 x 20 mm) to both side ends with a barrier of sticky material to keep the mites on this test arena.
Test container:	Plastic trays (11 x 11 x 6 cm) half-filled with water, with a foam rubber and a glass-plate on top covered by tissue paper in contact with the water; the test units were placed on the tissue paper.
Exposure route, duration:	7 days, followed by a 7-day assessment of reproduction of the remaining mites
Feeding:	Pollen (mixture of pine and birch, 3:1) ad libitum
Test condition:	Temperature: 24-25 °C (acclimatization period) 23-25°C (exposure period) Relative humidity: 70 – 90% (acclimatization period) 60-90% (exposure period) Light regime: 16 h light /8 h dark Light intensity: 820 – 1320 lux (acclimatization and exposure period)
Test parameters:	Mortality: Mortality was recorded on day 3 and 7 after test initiation. Further 3 assessments were carried out with a maximum interval of 3 days up to day 14 (inclusive). Sex-ratio for reproduction testing at day 7 was 1 male: 5 females at the minimum except of unit 3 of the 45 mL/ha treatment, therefore 2 males from one and 2 males from unit 2 were transferred to unit 3.
	<u>Reproduction:</u>
	Number of eggs laid and number of live and dead juvenile stages per female was counted at day 7, day 10, day 12 and day 14 after test initiation in the control and two treatment groups with a 7 day mortality < 60%.

Statistic:

Mortality data were analyzed for significance by using Fisher exact-test (two-sided, $\alpha=0.05$). Reproduction data were tested for normal distribution and homogeneity of variance using R/s-Test ($\alpha = 0.05$) and Cochran-Test ($\alpha = 0.05$). Because reproduction data were not normally distributed and not homogeneous Student-t-test for non-homogeneous variances (multiple comparison, two-sided, $\alpha = 0.05$) was used.

The software used to perform the statistical analysis was

SYSSTAT 9 for Windows and EASY ASSAY, Multiple Testing, © SPiRiT, Version 4.0. The LR_{50} value based on the mortality after 7 days of (LR_{50}): exposure to the test item on the test organisms, its 95 % confidence limits and the χ^2 goodness of fit were estimated by applying Probit-Analysis, based on the corrected mortality data recorded after the first week of exposure.

The software used to perform the Probit-Analysis was EASY ASSAY, Critical Values.

Findings:

Not statistically significant lethal and reproduction effects on the predatory mite *T. pyri* were recorded up to 45 mL/ha Flufenacet & Diflufenican SC 600 in 200 L water/ha. Statistically significant acute lethal effects were observed at dosages of 90 mL /ha Flufenacet & Diflufenican SC 600/ha and higher (Fisher-exact-test, $\alpha = 0.05$).

The results for mortality and reduction in reproduction of *T.pyri* are given in the Table B 9.5.2.1.1.1.2-1 below.

Table B 9.5.2.1.1.2-1: Flufenacet & Diflufenican SC 600 effects of on *T.pyri* following exposure on glass plates for 7 days under laboratory conditions.

Nominal application mL Flufenacet & Diflufenican SC 600/ha	Mortality after 7 days		Reproduction (days 7-14)	
	Cumulative [%] \pm SD ¹	Control Corrected [%]	Mean number of eggs per female mean \pm SD	% Reduction number of eggs per female relative to control
Control	10 \pm 8.7	-	8.0	-
22.5	11.7 \pm 5.8	1.9	7.9 ^{ns}	1.3
45	18.3 \pm 15.3	9.2	9.0 ^{ns}	-12 ^A
90	65.0 \pm 26	61.1*	n.a	-
180	93.3 \pm 2.9	92.6*	n.a	-
360	100	100*	n.a	-
Toxic standard	100	100*	n.a.	-

* Statistically significant compared to control (Fisher test, $\alpha = 0.05$)

ns not statistically significant compared to control (Student-test, $\alpha = 0.05$)

¹ Number of dead mites including escaped mites

n.a .not assessed

A: A negative value indicates a higher reproduction rate in the treatment than in the control.

Conclusion:

The LR_{50} value was determined to be 81.8 mL Flufenacet & Diflufenican SC 600 /ha, (95% CI: 71.4-93.8 mL Flufenacet & Diflufenican SC 600/ha)

The reproduction was statistically not significant compared to control.

Comments RMS:

The study was conducted according to the IOBC test guideline (Blümel et al., 2000).

The study is considered acceptable taking into account the validity criteria stated in the IOBC test guideline (Blümel et al., 2000).

The mean mortality in the control treatment was below 20% on day 7 after treatment application (being: 10%). The mean number of eggs per female in the control (from day 7 to day 14) was ≥ 4 eggs/female (being: 8 eggs/ female). The mean mortality (control corrected) of protonymphs on day 7 exposed to the reference item was 100% and therefore in the recommended range between 50% and 100%.

RMS is of the opinion that the study is considered valid and acceptable.

Agreed endpoints:

7 LR₅₀ = 81.8 mL Flufenacet & Diflufenican SC 600 /ha

14 d ER₅₀ = > 45 mL Flufenacet & Diflufenican SC 600 /ha

B. 9.5.2.1.2. Extended laboratory studies**B. 9.5.2.1.2.1. The effects of Flufenacet & Diflufenican SC 600 on Typhlodromus pyri**

(Acari: Phytoseiidae) on natural substrate in laboratory (extended laboratory test).

Reference:	The effects of Flufenacet & Diflufenican SC 600 on Typhlodromus pyri (Acari: Phytoseiidae) on natural substrate in laboratory (extended laboratory test).
Author(s), year:	Chauzat, M.P., 2002
Report/Doc. number:	Report no: 01TYBYL12, Reference BCS no. M-034242-01-1
Guideline(s):	IOBC guideline (Blümel et al., 2000)
GLP:	Yes
Test substance:	Flufenacet & Diflufenican SC 600, Batch No. 07205/0024(0006), containing 406.52 g/L Flufenacet and 205.76 g/L Diflufenican (analysed), density: 1.247 g/mL
Toxic reference:	Danitol (100 g fenprothrin/L)
Test species:	Typhlodromus pyri, protonymphs 1 day old
Number of organism:	4 replicates with 20 individuals per replicate for each treatment group
Study duration	14 days
Application:	Control: deionized water
	Test item:
	9.9 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 4.0 g Flufenacet/ha and 2 g Diflufenican/ha)
	28.7 mL Flufenacet&Diflufenican SC 600/ha (corresponding to 11.7 g Flufenacet/ha and 5.9 g Diflufenican/ha)

83.2 mL Flufenacet&Diflufenican SC 600/ha
 (corresponding to 33.82 g Flufenacet/ha and 17.1 g Diflufenican/ha)
 241.4 mL Flufenacet&Diflufenican SC 600/ha
 (corresponding to 98.1g Flufenacet/ha and 49.7 g Diflufenican/ha)
 700 mL Flufenacet&Diflufenican SC 600/ha
 (corresponding to 284.6 g flufenacet/ha and 144 diflufenican/ha)
 Treatments applied with a calibrated sprayer in 200 L water/ha
 Toxic standard: 50 g fenpropathrin/L corresponding to 0.5 L product/ha.

Test units:

Test units consisted on detached secondary French bean leaves (Oxinel variety) with no stalk. A sticky barrier (Tangle-Trap Insect Trap Coating) enclosing an arena of 10-13 cm² area was applied on each leaf before treatment in order to prevent the mites from escaping. After the application, each leaf was placed on top of a tissue covered sponge, lower side upwards. Each sponge was placed in a plastic box filled with mineral water solution (commercial name "Ondine") closed with a mesh lid. A cotton wool pad covered the base of the stalk and the wet tissue covered sponge. A spot of walnut-apple (50:50) pollen was added on each test unit. Water supply was available to the test organism by the mean of a small cut in the leaf. The same individual test units were used for the mortality assessment and the subsequent fecundity evaluation.

Test condition:

Temperature: 25.5-26.3 °C (pre exposure period, 5 days before spraying)
 Temperature: 25.9-27 °C (exposure period)
 Relative humidity: 60-90% (pre exposure period, 5 days before spraying)
 Relative humidity: 43-78.2% (exposure period)
 Light regime: 16h light/8h dark (pre exposure period, 5 days before spraying)
 Light regime: 16 h light /8 h dark (exposure period)
 Light intensity: 1920-1950 Lux (exposure period)

Test parameters

Mortality assessments were carried out 1, 3 and 7 days after the application. The number of "alive", "dead", "trapped in the glue barrier", "trapped in the water" (in the cut in the leaf) and "escapees" were recorded in each test unit on each occasion. Fecundity assessments were carried out 7, 10, 12 and 14 days after the application in the test item rates were corrected mortality was equal or below 50%. In these cases sex-ratio was maintained to at least 1 male for 5 females in the test units on day 7, 10 and 12. If necessary, extra males originating from another replicate form the same treatment if possible or

from the *T. pyri* culture were added to the population. The number of "females", "males", "juveniles" and "eggs" were recorded in each test unit on each occasion.

Eggs and juveniles were removed from the test units on each occasion except for the last one.

Pollen was renewed 1, 3, 5, 7, 10 and 12 days after the application.

Statistic:

Mean mortality percentages (which included dead, escapees, trapped in the glue barrier, trapped in the water) were calculated for each treatment.

Corrected mortalities were also calculated using the Abbott's formula

Mortality and fecundity results were analysed by one way analysis of variance (ANOVA, $\alpha=0.05$) and a Tukey test ($\alpha=0.05$). The LR_{50} and the confidence interval were calculated from the test item mortalities found 7 days after mite introduction. The software "PROBIT" was used for these calculations.

Findings

No significant differences (Anova, Tukey test $\alpha=0.05$) in mortality rates and fecundity were found between the control and the test item treatment when applied at rates below or equal to 83.2 mL/ha.

Mortality in the control treatment was 12.5% and 100% in the toxic reference treatment 7 days after the application.

The result for mortality and reduction in reproduction of *T.pyri* is given in the table below:

Table B .9.5.2.1.2.1-1: Flufenacet & Diflufenican SC 600 effects of on *T.pyri* following exposure on leaf beans for 7-14 days under extended laboratory conditions.

Nominal application mL Flufenacet & Diflufenican SC 600/ha)	Mortality after 7 days		Fecundity		
	Mean 7 d mortality (%)	Control Correctd (%)	Mean cumulative number of eggs and juvenile per female]	Mean cumulative number of eggs and juvenile per female relative to control %	% Reduction of mean cumulative number of eggs and juvenile per female relative to control
Control	12.5	-	4.5	-	-
9.9 mL product/ha (4.0 g flufenacet/ha + 2.0 g diflufenican/ha)	7.5	0.0 ^B	4.3	95.6	4.4
28.7 mL product/ha (11.7 g flufenacet/ha + 5.9 g diflufenican/ha)	10.0	0.0 B	3.9	86.7	13.3
83.2 mL product/ha (33.82 g flufenacet/ha + 17.1 g diflufenican/ha)	27.5	17.1	5.3	117.8	-17.8 ^A
241.4 mL product/ha (98.1 g flufenacet/ha + 49.7 g diflufenican/ha)	95.0*	94.3*	n.a	n.a	n.a

Nominal application mL Flufenacet & Diflufenican SC 600/ha)	Mortality after 7 days		Fecundity		
	Mean 7 d mortality (%)	Control Corrected (%)	Mean cumulative number of eggs and juvenile per female]	Mean cumulative number of eggs and juvenile per female relative to control %	% Reduction of mean cumulative number of eggs and juvenile per female relative to control
700 mL product/ha (284.6 g flufenacet/ha + 144.0 g diflufenican/ha)	100*	100*	n.a	n.a.	n.a
Toxic standard	100*	-		-	n.a

^A Negative value indicates a higher reproduction rate in the treatment than in the control.

^B Corrected mortality was negative and thus corrected to 0%.

n.a not assessed

* Statistically significant compared to control (Annova, Tukey test $\alpha=0.05$)

Conclusion:

The 7 days LR₅₀ value for Typhlodromus pyri based on mortality was determined to be 110.2 mL Flufenacet & Diflufenican SC 600/ha.

No effects of reproduction above 50% was observed during the study.

Hence, the ER₅₀ value was estimated to be >83.2 mL prod./ha.

Comments RMS:

The study was conducted according to the IOBC test guideline (Blümel et al., 2000).

The study is considered acceptable taking into account the validity criteria given in the IOBC test guideline (Blümel et al., 2000). The mean mortality in the control treatment was below 20% on day 7 after treatment application (being: 12.5%). The mean number of eggs per female in the control (from day 7 to day 14) was ≥ 4 eggs/female (being: 4.5 eggs/ female). The mean mortality (control corrected) of protonymphs on day 7 exposed to the reference item was 100% and therefore was in the recommended range between 50% and 100%.

The following deviation from IOBC test guideline was noted:

- Relative humidity was below 60% seven times (down to 45.3%) during the study (60 % is recommended by the guideline)

An indicated deviation is however considered, as having no impact on the study results, since all validity criteria were met. The study is considered acceptable.

Agreed endpoints:

7 LR₅₀ = 110.2 mL Flufenacet & Diflufenican SC 600/ha

ER₅₀ > 83.2 mL Flufenacet & Diflufenican SC 600 /ha

B.9.5.2.1.2.1.2. Toxicity to the green lacewing *Chrysoperla carnea* Steph.(Neuroptera, Chrysopidae) using an extended laboratory test on *Zea mays* Flufenacet + Diflufenican SC 400 + 200 g/L.

Reference:	Toxicity to the green lacewing <i>Chrysoperla carnea</i> Steph. (Neuroptera, Chrysopidae) using an extended laboratory test on <i>Zea mays</i> Flufenacet + Diflufenican SC 400 + 200 g/L.
Author(s), year:	Waibel, J., 2009
Report/Doc. number:	Study No: CW09/010, Reference BCS no: M-352372-01-1
Guideline(s):	Vogt et al. (2000) modified, Candolfi et al. (2001)
GLP:	Yes
Test substance:	Flufenacet + Diflufenican SC 400 + 200 g/L. Batch No: EV56001418 Analysed content of active ingredient: Diflufenican 15.6% w/w (191.4 g/l), Flufenacet 32.1% w/w (394.5 g/L). The density: 1.229 g/mL
Test duration:	36 days
Toxic reference:	Dafane-L 40 (394.4 g dimethoate /L)
Test species:	Green lacewing, <i>Chrysoperla carnea</i> , 2 days old larvae
Number of organism:	40 replicates with 1 individuals per replicate for each treatment group.
Feeding:	Eggs of <i>Ephestia kuehniella</i> (during exposure) An artificial diet consisting of condensed milk, egg, egg yolk, honey, fructose, brewer's yeast, wheat germ and aqua dest. and supplied with water Through a cotton plug (during pre-reproduction and reproduction)
Application:	Control: deionised water Test item: - 30 mL Flufenacet&Diflufenican SC 600/ha - 63 mL Flufenacet&Diflufenican SC 600/ha - 134 mL Flufenacet&Diflufenican SC 600/ha - 284 mL Flufenacet&Diflufenican SC 600/ha - 600 mL Flufenacet&Diflufenican SC 600/ha Treatments applied with a calibrated sprayer in 200 L water/ha Toxic standard: 53.2 mL product/ha (corresponding to 21 g dimethoate/ha)
Study design:	<u>Exposure period:</u> After the application, an intact leaf disc of maize was laid on a wet cotton wool pad in a petri dish (9 cm diameter). A constant supply of deionised water from the bottom was assured. As individual test unit one steel ring (about 3 cm diameter and 3 cm high) was placed on the leaf. The top inner edge of the ring was coated with a lubricant (PTFE) in order to prevent the larvae from escaping. One larva was added to each test unit (approx. one hour after application). Once the larvae had pupated, the pupae were transferred to glass jars (closed with a gauze lid).

	<p><u>Post exposure period:</u></p> <p>All emerged adult individuals of the test series were kept in glass jars and fed with artificial diet water through a cotton plug.</p> <p>One week after the first egg laying in control the adult lacewings were sexed and transferred to cylinders of black paper (Ø = 10-12 cm; 15-17 cm high); all lacewings of one test group together. These were closed at both ends with gauze. After an egg laying period of 24 hours the adult lacewings were transferred into new glass jars and the cylinders with the gauze were put into plastic boxes.</p> <p>For the determination of the hatching rate all eggs (hatched and non hatched) on both the gauze and on the cylinder were counted.</p> <p>Two egg samples were taken within one week.</p>
Test condition:	<p>Temperature: 23.5-25.5 °C</p> <p>Relative humidity: 60 – 80%, with a short decline < 2 hours to 41%</p> <p>Light regime: 16 h light/8 h dark</p> <p>Light intensity: 1285-2830 Lux (mortality phase)</p> <p>Light intensity: 3080 – 3144 lux (reproduction phase)</p>
Test parameters:	<p>The mortality and development of the larvae was recorded at until pupation was completed and adults emerged.</p> <p>Assessments for pre-imaginal mortalities (cumulative sum of dead and missing organisms) were carried daily.</p> <p>The hatching rate (= fertility; HR) of the eggs is calculated as follows:</p> $HR (\%) = \text{Number of eggs hatched} / \text{total number of eggs} \times 100$
Statistic:	<p>Fisher's Exact Test (one side), p-value are adjusted according to Bonferroni-Holm -analysis of mortality data for significance.</p>
<u>Findings:</u>	<p>For the rates of 30, 134 and 600 mL product/ha the corrected mortality was 0, 2.6 and 20.5, respectively. For the rates of 63 and 284 ml product/ha it was 7.7% each. The mean number of eggs per female and day for the 30 mL product/ha rate was 24.1 with a hatching rate of 81.4%. For the rate 63 mL product/ha 23.9 eggs were laid with a hatching rate of 80.7%. The mean number of eggs for the 134 mL product/ha and 284 ml product/ha rates were 27.5 and 28.4, respectively with hatching rates of 83.4% and 82.5%. In the highest rate of 600 mL product/ha 27.6 eggs per female and day were laid with a hatching rate of 82.7%.</p>

Table B .9.5.2.1.2.1.2-1: The effects for mortality and reproduction of *Chrysoperla carnea* exposed to fresh-dried residues of Flufenacet & Diflufenican SC 600 in the laboratory condition.

Nominal application mL Flufenacet & Diflufenican SC 600/ha)	Mortality (%)	Control Corrected (%) ^a	Eggs per female /day	No. of fertile eggs/female/ day	Fertility (hatching rate in %)
Control	2.5		26.4	21.1	79.9
30	2.5	0.0	24.1	19.6	81.4
63	10.0	7.7	23.9	19.3	80.7
134	5.0	2.6	27.5	22.9	83.4
284	10.0	7.7	28.4	23.5	82.5
600	22.5	20.5*	27.6	22.7	82.7
Toxic reference	87.5	87.2	n.d.	n.d.	n.d.

^a Corrected mortality according to Schneider-Orelli

* Statistically significant compared to control (Fisher's Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm)

n.d. not determined

Conclusion:

The dose rates of 30, 63, 134 and 284 mL product/ha had no statistically significant influence on mortality. Only a statistically significant slight corrected mortality of 20.5% occurred at the highest dose rate of 600 mL product/ha. Due to that fact that the mortality in the highest treatment rate was <50% it was considered as not biological relevant.

There were no adverse effects of the test item on the reproductive performance at all rates tested. The LR₅₀ was empirically estimated to be > 600 mL product/ha.

Comments RMS:

The study was conducted according to the test guideline Vogt et al. (2000) modified, Candolfi et al. (2001).

The study is considered acceptable taking into account the validity criteria stated in this test guideline.

The control mortality was ≤ 20% (being 2.5%), the corrected mortality in the reference item was > 50% (being 87.2%), the average number of eggs per female per day in the control group was ≥ 15 (being 26.4) and the mean larval hatching rate in the control group was ≥ 70% (being 79.9%).

Hence, the RMS is of the opinion that the study is considered valid and acceptable for the use in the risk assessment.

Agreed endpoints:

LR₅₀ > 600 mL product/ha

ER₅₀ > 600 mL product/ha

B .9.5.2.1.2.1.3. Chronic toxicity (ER₅₀) of Diflufenican+Flufenacet SC 600 g/L to the rove beetle *Aleochara bilineata* GYLL. under extended laboratory conditions.

Reference:	Chronic toxicity (ER ₅₀) of Diflufenican+Flufenacet SC 600 g/L to the rove beetle <i>Aleochara bilineata</i> GYLL. under extended laboratory conditions.
Author(s), year:	Roehlig, U., 2009
Report/Doc. number:	Report No: 09 10 48 027 A, Reference BCS: no. M-353760-01-1
Guideline(s):	IOBC Guideline (GRIMM et al. 2000)
GLP:	Yes

Test substance:	Diflufenican + Flufenacet SC 600 g/L Analysed active ingredients: 15.6 % w/w (191.4 g/L) Diflufenican (AE F088657); 32.1 % w/w (394.5 g/L) Flufenacet (FOE 5043). Batch no: EV56001418. Density: 1.229 g/cm ³
Toxic reference:	Dimethoate EC 400 (BAS 152 11 I) 422.4 g dimethoate/l (analysed)
Test species:	Rove beetle, <i>Aleochara bilineata</i> GYLL (Coleoptera: Staphylinidae)
Age of organism:	Adults, 1-7 days old
Number of organism:	4 replicates with 20 individuals (10 females and 10 males, 10 pair) per replicate for each treatment group
Study duration:	65 days
Feeding:	Larvae of <i>Chironomus</i> spp. (frozen) approximately 1 hour after application and every 2-3 days depending on food consumption
Application:	Control: deionised water Test item: - 60 mL Flufenacet&Diflufenican SC 600/ha - 107 mL Flufenacet&Diflufenican SC 600/ha - 190 mL Flufenacet&Diflufenican SC 600/ha - 337 mL Flufenacet&Diflufenican SC 600/ha - 600 mL Flufenacet&Diflufenican SC 600/ha Treatments applied with a calibrated sprayer in 200 L water/ha Toxic standard: 1.5 L Dimethoate EC 400 /ha in 400 L water /ha (corresponding to 600 g dimethoate/ha)
Test cage:	<u>Exposure cage:</u> Plastic vessel (14 cm 8 cm high) covered with a lid of nylon gauze; filled with wet soil up to a height of about 5 cm (770 cm ³ soil/cage), corresponding to 1 kg dry soil moistened with deionised water to 35 % of WHC; side walls above the soil level treated with Fluon [®] . <u>Hatching cage:</u> 2 plastic cage (Bellaplast, 18.3 cm x 13.6 cm x 6.4 cm),

	one cage with gauze cover (mesh size 2 mm x 2 mm) and a sieve bottom with gauze (mesh size 2 mm x 2 mm), the other cage with intact bottom below for collecting the emerging beetles.
Test substrate:	LUFA 2.1 soil, batch no.: Sp 2.1 41 Corg: 0.81% pH: 5.1 CEC: 4 mval/100 g WHC: 33.2 g/100 g dry weight Particle size: <0.002 mm (clay) 3.0%, 0.002-0.05 mm (silt) 8.8% 0.05-2.0 mm (sand) 88.2% Soil type: sand, according to USDA classification
Test condition:	Temperature: 19-22 °C Relative humidity: 62 – 73%, with a short decline < 2 hours to 41% Light regime: 16 h light/8 h dark Light intensity: 1060 lux
Study design:	One day before treatment, moist sandy soil (LUFA 2.1) was filled into plastic vessels up to a height of about 5 cm. The test solutions were applied onto the substrate surface in an automatic application cabin (track sprayer: Schachtner Spray Lab). During the application the inner wall of the cage was protected by a removable aluminium collar. The test consisted of an exposure phase and a hatching phase. In the exposure phase, four replicates each with ten pairs of male and female adult beetles were exposed to treated substrate for 28 days. After air-drying of the spray residues (at room temperature for about 1 hour), the beetles were added impartially to each replicate of the test cages by placing them on the treated substrate after application, The beetles were fed approximately one hour after treatment application and then every 2 to 3 days depending on the food consumption. Food (thawed <i>Chironomid</i> larvae) was placed on the surface of the substrate. The cages were closed with gauze covers and incubated in a controlled environment test room. After 7, 14 and 21 days, approximately 500 onion fly pupae, <i>Delia antiqua</i> MEIGEN (Diptera; Anthomyiidae) were added and carefully mixed with the substrate of each test unit so that the pupae were distributed homogeneously within the test unit and completely covered with substrate. The number of pupae was determined by weight on each occasion.

Four weeks after test initiation, the exposure phase was terminated and the number of beetles alive and dead was recorded.

The substrate (containing the parasitized onion fly pupae) was allowed to dry for seven days (by removing the test unit lids). After this week, the pupae were removed from the substrate by a sieve (pore size: 2 mm x 2 mm) and placed into hatching cages (one such hatching cage for each exposure cage).

The test was terminated when in the control group no more *A. bilineata* adults had emerged from the onion fly pupae.

Test parameters:

Mortality (number of dead or alive introduced beetles) was assessed 4 weeks after application (these data are, however, not necessary for the evaluation of the test, it is only an additional information).

Reproduction (number of hatched beetles of F1 generation) was determined 5 weeks after application, thereafter daily up to the final assessment. The number of hatched beetles of the F1 generation was recorded over a period of 65 days. From these data the endpoint reproductive capacity was calculated.

Statistic:

BARTLETT's test was used to confirm variance homogeneity of the reproduction data followed by DUNNETT's multiple t-test, ($p \leq 0.05$; 1-sided). Statistical analysis was performed using ToxRat Professional 2.10 (RATTE, 2009).

Findings:

By the end of the reproduction phase (day 65) the mean number of hatched beetles per replicate in the control was 661 and the mean number of hatched beetles per introduced pupa in the control was 0.441.

The mean number of hatched beetles per replicate in the reference item group was reduced to 0.3 % compared to the control group.

Table B 9.5.2.1.2.1.3-1: The effects for Aleochara bilineata on reproduction of exposed to residues of Flufenacet & Diflufenican SC 600 in the laboratory condition.

Nominal application mL Flufenacet & Diflufenican SC 600/ha	Total number of hatched beetles of the F ₁ - generation per treatment group	Mean number of hatched beetles of the F ₁ -generation per replicate	Mean number of hatched beetles/host pupa	Parasitisation rate P (%)	Reduction of reproductive capacity (relative to control) R (%) ^{ns}
Control	2644	661	0.441	44.1	-
60	2530	633	0.422	42.2	4.3
107	2705	676	0.451	45.1	-2.3 ^A
190	2600	650	0.433	43.3	1.7
337	2490	623	0.415	41.5	5.8
600	2434	609	0.406	40.6	7.9
Dimethoate EC 400 1.5 L product /ha	8	2	0.0013	0.13	99.7

ns Not statistically significant differences between the control ((DUNNETT's multiple t-test, $p \leq 0.05$; 1- sided)

A a negative value indicates a higher reproduction rate in the treatment than in the control

Conclusion:

Statistical analysis of reproduction revealed no significant difference concerning the reproductive capacity between the control and all test item treatment groups.

Because of the reduction of reproductive capacity was below 50 % in all test item treatment groups the ER₅₀ is empirically estimated to exceed the highest tested application rate > 600 mL product/ha

Comments RMS:

The study was conducted according to the test guideline GRIMM et al. (2000).

The study is considered acceptable taking into account the validity criteria stated in test guideline GRIMM et al. (2000). Average number of hatched beetles of the F₁-generation in the control group was > 400 (being 661).

Reduction of the reproductive capacity in the reference item treatment group, relative to control > 50 % (being 99.7%).

Hence, the RMS is of the opinion that the study is considered acceptable.

Agreed endpoint:

ER₅₀ > 600 mL product/ha

B 9.5.2.1.2.1.4. Toxicity to the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test (under semi-field conditions aged residues on *Zea mays*) Flufenacet + Diflufenican SC 400 + 200 g/L.

Reference:	Toxicity to the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test (under semi-field conditions aged residues on <i>Zea mays</i>) Flufenacet + Diflufenican SC 400 + 200 g/L.
Author(s), year:	Jans D., 2009
Report/Doc. number:	Report No: CW09/026, Reference BCS no. M-355238-01-1
Guideline(s):	Blümel et al. (2000) modified, Candolfi et al. (2001)
GLP:	Yes

<u>Test substance:</u>	Flufenacet + Diflufenican SC 400 + 200 g/L, batch No: EV56001418 Diflufenican 15.6% w/w, (191.4 g/l), Flufenacet 32.1% w/w (394.5 g/L), density: 1.229 g/mL
Toxic reference:	Dafene L-40, 394.5 g dimethoate/L (analyzed)
Test species:	<i>Typhlodromus pyri</i> , predatory mite
Age of organism:	Protonymphs (less than 24 hours old)
Number of organism:	5 replicates, 20 protonymph per each replicate for test item, reference item and control
Study duration:	42 days
Acclimatization:	Eggs obtained from mass rearing at testing facility were incubated at condition similar to those used in the test Temperature: 20-25°C, Relative humidity: 60-80% Photoperiod: 16:8 h with a light intensity of >3000 Lux Feeding: mixture of apple: walnut
Application:	Control: deionised water Test item: 0.7 L product/ha in 400 L water Reference item: 0.1014 L product/ha in 400 L water , 40 g dimethoate/ha) (under semi-field conditions) 0.1014 L product/ha in 200 L water (under laboratory conditions, 40 g dimethoate/ha)
Test condition:	Laboratory condition: Temperature: 24-25.5°C Relative humidity: 65-75 %, with a short decline < 2 hours Light regime: 16 h light /8 h dark Light intensity: 584-1549 lux Semi-field condition: Temperature: 24-25.5°C Relative humidity: 65-75 %, with a short decline < 2 hours

Study design:

Light regime: 16 h light /8 h dark, 584-1549 lux

The test item at rate 0.7 L product/ha in 400 L water/ha and control (deionised water) were applied under semi-field condition on potted whole maize plants with rain protection (UV-permeable plexi glass).

Aging of the spray residues of the test item took place under natural semi-field conditions the whole study.

The reference item was applied once at rate 0.1014 L product/ha in 400 L water under semi-field conditions at the application day of the test item.

For the following bioassays the reference item was freshly applied at rate 0.1014 l product/ha in 200 L water/ha under laboratory condition on day 14 and 28, directly on maize leaves taken from untreated plants, which were stored until this time at outdoor condition.

After application or at the relevant interval afterwards one intact leaf randomized taken from different maize plants was cut and take under laboratory condition.

One unit consisted of a leaf disc, which was laid after application on a layer of wet filter paper on top of a water soaked floral foam.

A circle of insect glue (ø approx. 40 mm) was formed on the leaves.

Sets of such units were placed on a plastic tray such that the filter paper was constantly provided with deionised water.

On the day 0 approx. 0.5 to 1 hour after application the protonymphs were placed onto the plant surface by replicate of each treatment in the first bioassay and by test group in the second and third bioassay. The mites were transferred with a fine brush under a stereomicroscope and immediately afterwards examined to ensure they were undamaged and in good health. Then pollen (birch – pine mixture) was supplied as food and the units were kept under test conditions.

The water supply for the mites was ensured by sticking a pin into each of the leaves.

Mortality of 100 protonymphs for each treatment was assessed on several days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed. This assessment was done on day 1, 4 and 7 after exposure for the first bioassay started on the application day and the second bioassay started at day 14 after application.

For the last bioassay initiated at day 28 after application the mortality was assessed 1, 4, 7, 10, 12 and 14 days after exposure.

The reproduction rate of surviving mites was evaluated over the period of 7-14 days after treatment for the third bioassay started at day 28 after application by counting the total number of offspring (eggs and larvae)

produced. From these data the endpoints mortality (after 7 days) and effects on reproduction were calculated.

Test parameters: Mortality of 100 protonymphs for each treatment was assessed on several days after exposure by counting the number of living and dead mites. The number of escaped mites was calculated as the difference from the total number exposed. This assessment was done on day 1, 4 and 7 after exposure for the first bioassay started on the application day and the second bioassay started at day 14 after application.

For the last bioassay initiated at day 28 after application the mortality was assessed 1, 4, 7, 10, 12 and 14 days after exposure. The corrected mortality was obtained by comparing the values observed in the treated samples with those in the control samples, according to the formula of SCHNEIDER-ORELLI.

The reproduction rate of surviving mites was evaluated over the period of 7-14 days after treatment for the third bioassay started at day 28 after application by counting the total number of offspring (eggs and larvae) produced. From these data the endpoints mortality (after 7 days) and effects on reproduction were calculated.

Enviromental condition:

Weather data (temperature and relative humidity, under GLP) in the outdoor area were recorded at the site and the data on further parameters (hours of sunshine under non-GLPconditions) were taken from the records of BCS Global Biology Herbicides.

Statistic:

Mortality data were analyzed for significance by using Fisher's Exact Test (one-sided greater, $\alpha = 0.05$, Bonferroni-Holm. Reproduction data were tested by one way-Anova, Williams t-test. The computer program SAS (Version 9.1.3, 2002-2003) was used to perform the statistical analyse s.

Findings:

In this study 98.9% corrected mortality of the test item was found in the first bioassay started on DAA 0. A second bioassay was started 14 days after the application and still showed a corrected mortality of 87.1%.

A third bioassay was initiated on DAA 28 and resulted in a low corrected mortality of 9.5%. In this assay no statistically significant effects on reproduction occurred (8.4% reduction relative to control).

The effects of Flufenacet + Diflufenican SC 400 + 200 g/L tested on mortality and on reproduction after exposure of the mites to freshly applied and aged spray residues on excised maize leaf discs was presented in the Table B.9.5.2.1.2.1.4-1 below:

Table B.9.5.2.1.2.1.4 -1: The effects for T.pyri exposed to semi-field condition residues of Flufenacet & Diflufenican SC 600.

Test item	Flufenacet + Diflufenican SC 400 + 200 g/L (0.7 L product/ha)		
Test organism	Typhlodromus Pyri		
Exposure	Dried spray deposits on maize leaves (from treated maize plants)		
Start of bioassay	0 DAA ^a	14 DAA ^a	28 DAA ^a
	Mortality (%) after 7 days		
Control	12.0	7.0	5.0
Test item	99.0	88.0	14.0
Reference item	100.0	100.0	100.0
	Corrected mortality (%)		
Test item	98.9 ^b	87.1 ^b	9.5 ^b
Reference Item	100.0	100.0	100.0
	Reproduction		
	Number of eggs per female		
Control	-	-	7.5
Test item	-	-	6.9
	Reproduction rel. to control (%)		
Test item	-	-	8.4 ^c

a Days after application

b Statistically significant compared to control (Fisher's Exact test, one-sided)

c Not statistically significant compared to control (one-way ANOVA, Williams test, one-sided)

Conclusion:

The effects of Flufenacet+Diflufenican SC 400+200 G/L residues (aged under semi-field condition) on the survival of the predatory mite Typhlodromus pyri were determined after application of 0.7 l product/ha onto Zae mays. In this study 98.8% corrected mortality of the test item was found in the first bioassay started on DAA 0. A second bioassay was started 14 after the application and still showed a corrected mortality of 97%. A third bioassay was initiated on Day 28 and resulted a low corrected mortality of 28%. In this assay no statistically significant effects on reproduction occurred (8.4% reduction relative to control).

Comments RMS:

The study was conducted according to the test guidelines Blümel et al. (2000) modified, Candolfi et al. (2001). The study is considered acceptable taking into account the validity criteria stated for extended laboratory studies for T.pyri (Blümel et al. (2000).

In all three bioassays (DDA 0, 14 DDA, 28 DAA) the control mortality was below 20% and the mortality of the toxic reference group was 100%. The cumulated number of eggs per female for the reproduction assessment in the third bioassay was above 4 eggs per female (7.5 eggs/female after 28 days).

Conclusion:

In this study the mites have been exposed to fresh residues of DFF + FFA SC 600 and for residues aged for 14 and 28 days. Freshly dried residues of the test item resulted in 98.9% corrected mortality. A corrected

mortality of 87.1% was observed after an aging time of 14 days. An aging time of 28 days resulted in a low corrected mortality of 9.5% and no statistically significant effects on reproduction occurred (8.4% reduction relative to control).

The result of this aged residue study (with application rate of 0.7 L product/ha) demonstrated that after an aging time 28 days the residues have no impact on predatory mites.

B 9.6. RISK ASSESSMENT FOR ARTHROPODS

B 9.6.1. Risk assessment for honeybees

The risk assessment for effects on bees was conducted by Applicant according to the existing guidance in force at the time of the preparation and submission of the dossier namely the EU GD on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) and EPPO Standard PP 3/10.

The Applicant performed the required studies according to the Commission Regulation 283/2013 and 284/2013, both acute oral and contact toxicity studies (OECD 213 and 214) and 10 day oral chronic toxicity study. In addition, acute contact toxicity to adult bumble bees under laboratory condition was performed.

The colony feeding studies were performed with formulation of Flufenacet 508.8 SC (508.8 g flufenacet/L).

Exposure

The product DFF+FFA SC 600 is intended to be used as a foliar spray on winter cereals, with maximum application rate of 0.6 L product/ha corresponding to 240 g flufenacet/ha and 120 g diflufenican/ha, in a maximum of 1 application per year. Considering a density of Diflufenican + Flufenacet SC 600 of 1.251 g/mL (20°C) 600 mL product/ha corresponds to 750 g product/ha.

The maximum application rate 1 x 750 g product/ha applied in autumn early post-emergence covers the proposed use pattern of 1x 120 g a.s./ha and 1 x 160 g a.s./ha, respectively.

Hazard quotients were calculated for oral exposure (HQ_O) and contact exposure (HQ_C) for flufenacet and for formulation DFF+FFC SC 600.

A Hazard Quotient of less than 50 indicates a low risk to bees in the field.

Table B.9.6.1-1: Acute risk to adult bees from oral and contact exposure to flufenacet following the use of DFF+FFA 600 SC in cereals.

Test Species	Test substance	Exposure route	Application rate (g a.s./ha) ^a (g product/ha) ^b	LD ₅₀ (µg a.s./bee) (g product/ha)	HQ	Trigger value
Honeybee (<i>Apis mellifera</i>)	Flufenacet tech.	Oral	240	>109.2	<2.2	50
Honeybee (<i>Apis mellifera</i>)		Contact	240	>100	<2.4	
Honeybee (<i>Apis mellifera</i>)	DFF+FFA 600 SC	Oral	750 g product/ha	>217.8	<3.44	
		Contact		>200	<3.75	

^a a.s.- flufenacet only

^b g formulation/ha, density= 1.25 g/mL

The acute oral and contact HQ value for the active substance flufenacet, representative formulation DFF+ FFA SC 600 are below the trigger value of 50, indicating an acceptable risk to adult honey bees following the use in cereals.

The Applicant provided further risk assessment for bees taken into consideration the exposure to bees due to consumption of residues in pollen of cereals and flowering weeds.

Additional considerations of the risk assessment provided by Applicant:

Flufenacet can be considered as virtually non-systemic when early pre- or post-emergence spray application is done in winter cereals according to the studies of metabolism in plants (see CA 6.2 Metabolism, distribution and expression of residues, Section 6.2.1 Plants). In plant metabolism studies spray applied flufenacet result in negligible uptake and movement from the roots into the straw and grain samples. Consequently the theoretical exposure of bees to pollen containing residues of flufenacet due to early spray applications is considered to be negligible. In addition, winter cereals are not attractive to honey bees as they do not produce nectar so exposure for bees can only theoretically be to pollen.

Although flufenacet is virtually non-systemic and exposure via pollen of the target crop is considered to be negligible, the low risk to bees foraging on winter cereal flowers can be confirmed by applying the risk assessment principles for systemic products provided by EPPO Standard PP 3/10 (3). In this scheme, which has been validated using a range of substances, an extreme worst case exposure level of 1 mg a.s./kg is assumed as a screening level value for soil and seed treatments. This based on a survey of systemic insecticides where the 95th percentile concentration was 0.55 mg/kg. Consequently, residues of even systemic product are unlikely to ever exceed this screening level value and will more than cover the worst case theoretical exposure of bees to early applications of flufenacet. Consequently a risk assessment can be based on the worst case exposure in pollen of 1 mg/kg compared to the worse daily intake of pollen by bees and the acute and chronic endpoints for adult bees. It worth mentioning that this scheme represents an over conservative approach when applied for the case of flufenacet.

Risk to bees due to consumption of residues in pollen of cereals

Flufenacet residues in pollen are potentially negligible given the low plant-systemicity and cereals are not attractive to foraging honey bees. The honey bee is used here as representative species in a risk assessment. There are no requirements under Regulation (EC) No. 1107/2009 for a specific risk assessment for bees other than honey bees so this is provided only for illustrative purposes as supporting information of the low risk to bees.

Pollen exposure assessment

Information on the use and consumption of pollen as a food source by honey bees is provided by several authors (Simpson, 1955, Babendreier et al, 2004 and Rortais et al 2005). Pollen is the only natural protein source available to honey bees. Forager bee pollen consumption levels are negligible and the largest amounts are consumed by adult nurse bees in the colony. Consequently the risk to honey bees due to the consumption of pollen can be covered by considering the exposure to nurse bees. Pollen consumption levels for nurse honey bees are presented below:

Table B.9.6.1-2: Pollen consumption levels.

Type of bee	Pollen consumption	Notes
Honeybee	65 mg pollen/10 days	May consume up to 12 mg pollen in one day
Nurse bee	6.5 mg pollen /day	

For an estimate of the worst case 95th percentile residue present in pollen (and nectar) irrespective of application or seed loading rate EPPO 2010 considers that a concentration of 1mg a.s./kg (i.e.1 µg a.s./g) should be used for a screening level risk assessment. Based on this and pollen consumption rates the following realistic worst case risk assessment scenarios which cover the risk to bees due to the use of flufenacet as an early spray application for winter cereals are calculated.

Table B.9.6.1-3: Estimated worse case exposure levels.

Type of bee	Pollen consumption (g)	Residue level	Dose (µg/bee)
Honeybee	0.012 g	1 µg a.s./g	0.012 µg/bee
Nurse (acute risk)			
Honeybee	0.0065 g /day		0.0065 µg/bee/day
Nurse (chronic risk)			

Risk assessment for bees due to exposure to pollen

Using the appropriate endpoints the risk to bees due to the consumption of pollen containing residues of flufenacet is presented below. According to EPPO 2010 Toxicity Exposure Ratio trigger of 10 is applied to acute endpoints (oral LD₅₀) and No Observed Effect Daily Dose endpoints (NOEDD).

Table B.9.6.1-4: Flufenacet early pre- and post-emergence application: Systemic risk to bees via pollen consumption.

Type of bee	Risk	Endpoint	Exposure	Toxicity Exposure Ratio (TER)	EPPO (2010) Trigger
Honey bee (Nurse bee)	Acute*	LD ₅₀ >109.2 µg a.s./bee	0.012 µg/bee	9100	10
	Chronic	NOEDD:4.42 µg a.s./bee/day	0.0065 µg/bee/day	680	1

* Endpoints for technical material are used as exposure via pollen will not be to formulated product.

The calculated TER values are exceeding the triggers for the acute (10) and chronic (1) assessment indicating a high margin of safety based on a worst case assumption of exposure (i.e. above the 95th percentile) and exceed the EPPO 2010 triggers by several orders of magnitude.

Further considerations for the risk assessment

In addition to acute laboratory studies with adult honey bees, flufenacet was further subjected to topical and oral acute bumble bee testing. The studies did not reveal sensitivity differences between honey bee and bumble bee foragers.

Considerations on potential exposure to flowering weeds

The whole assessment presented below is based on the data submitted by the Applicant. RMS, after through examination, considers it valid. However, it shall be indicated that the assessment refers to autumn application. In RMS's opinion the EU-representative GAP lacked clarity, therefore spring applications in cereals at early growth stages cannot be excluded. Nevertheless, in the documents submitted by the Applicant it is indicated that the weeds attractive to bees are observed in fields on which cereals are grown at crop growth stages much higher than those indicated in the GAP. Therefore the assessment may be considered acceptable.

The Applicant's assessment is summarised below.

The potential exposure of honey bees to flowering weeds to an herbicide that is used during autumn at the pre-emergence of cereals is considered as low. The autumn period is in general of low activity for honey bee hives given the development phase of the colony which is getting prepared for the overwintering period. Even, in the rare case that the activity of foragers would be high in a period of days with mild temperatures during autumn, a recent publication (Maynard et al., 2015) demonstrates that the availability of flowering weeds in cereal fields at relevant application times for herbicides is minimal.

To analyse the presence of weeds in agricultural crops the available data has been extracted from the database for the crops cereals, sugar beet, and potatoes. As a conservative assessment, only the data in the control plots (i.e. no herbicide treatment) was considered to provide a worst-case situation.

All data originate from worldwide herbicide efficacy trials in cereals conducted between 2004 and 2014 has been compiled (Maynard, et al. 2015). The majority of the studies were carried out in Europe; however for completeness of the datasets trials performed outside Europe were also included. Information on weed species, weed growth stages (BBCH), weed diameter (cm), weed ground cover (%), and weed plants/m² were obtained. Each weed species per trial was recorded separately, thus there are several data set entries per trial.

In the analysis done for the data on cereal fields, flowering weeds exceeding 10% ground cover were only observed in 14 out of 2327 observations (i.e. 0.6%) and out of these 14 only one observation was possibly relevant under certain circumstances. Hence, exposure via flowering weeds is confirmed not to be a relevant route of exposure for bees.

In concluding remarks the Applicant however stated that low risk to bees foraging on flowering weeds can be confirmed with the results of the higher tier cage study OECD 75 (available during Peer review Commenting period).

Considerations on potential exposure to metabolites

Further the Applicant argued that the potential exposure to metabolites of flufenacet in bee relevant matrices (pollen of cereals or pollen and nectar of flowering weeds) is very unlikely and can be considered as negligible when taking into account the above argumentation on the low systemicity of flufenacet and the unlikely exposure via flowering weeds.

In concluding remarks the Applicant however stated that low risk to bees foraging either on flowering cereals or flowering weeds on can be confirmed with the results of the higher tier cage study OECD 75 (available during Peer review Commenting period).

RMS comments:

The risk assessment performed by Applicant according to GD on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002) and EPPO Standard PP 3/10 is considered acceptable. However, the risk assessment for bee brood development could be not finalized.

In course of evaluation of Bee brood study (Heccht-Rost, 20012, M-456504-01-1) performed by Applicant conducted according to Oomen (1992), RMS stated that there were discrepancies between the study protocol, numerical results and their graphical presentation. Additionally it was stated that there were no statistically significant differences between response in the negative control and the positive control (reference) in case of eggs and young larvae, although that was observed for the old larvae. RMS indicated that problem to the Applicant receiving the information that the study will be updated and in that form it should be ready for commenting period. At the same time the Applicant informed RMS about the new, ongoing semi-field study performed fully in line with the requirements of the current OECD 75 Guideline, which should also be made available during the commenting period. Therefore RMS would like to propose to include both studies into the assessment evaluating them during the peer-review stage of the assessment process.

In addition, the risk assessment for bees was provided by RMS based on the results on acute oral and acute contact studies for bees, (OECD 213 and 214 test guideline), acute contact study for bumble bees, 10 d oral chronic toxicity study for adults bees. Some additionally information supplied by the Applicant (presented in risk assessment above) was also used by RMS.

At the same time it should be noted that in the opinion of Applicant submitted as a statement (May, 2016), the risk assessment for bees for flufenacet should be based on EPPO scheme because the flufenacet application was submitted at the time 2014, when the New EFSA Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees), (EFSA Journal 2013;11 (7):3295) for bees was not enforced. At that time no valid protocols for some studies were available for the Applicant.

For clarity of evaluation the risk assessment for bees according to the document: „Pesticides Peer Review Expert Meeting 133 (September, 20) was provided in separate Point. B.9.6.1.1.

B.9.6.1.1. The risk assessment for bees provided according to recommendation given in the document: „Pesticides Peer Review Expert Meeting 133 (September, 2015)” in part of the - New EFSA Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees), (EFSA Journal 2013;11 (7):3295) published already in July 2013, but it has not come into force yet.

Acute adult toxicity:

The acute risk to honeybees from the use of DFF+FFA 600 SC was assessed using the maximum single application rate of 750 g product/ha correspond to 240 g flufenacet/ha and the LD₅₀ value (expressed as µg/bee) to calculate the Hazard Quotient (HQ) for contact exposure and ETR (exposure toxicity ratio) for oral exposure. The results are presented in the Table B.9.6.1.1-1 below.

Table B.9.6.1.1-1: The acute risk assessment for bees.

Test item	Route	LD ₅₀	Max. application rate g a.s./ha (g product/ha)	HQ/ETR	Trigger	Risk for adult bees acceptable?
Screening assessment						
Flufenacet tech.	Contact	>109.2	240	< 2.20	42	Yes
	oral	>100	240	<0.018	0.2	Yes
DFF+FFA 600 SC	Contact	>200	750 ¹	<3.75	42	Yes
	oral	>217.8	750 ¹	<0.026	0.2	Yes

¹ based on application rate of 600 mL product denc 1.251 g/mL

The hazard quotients and ETRs are below the relevant trigger values, indicating acceptable acute risk to adult bees.

Chronic adult toxicity

A chronic laboratory test with adult honey bees was provided for flufenacet. The results of the study were used for calculation of the Exposure Toxicity Ratio (ETR_{adult}) between the amount of residues that may be ingested by an adult bee in 1 day and the LDD₅₀ value.

The chronic exposure-toxicity ratio (ETR_{chronic adult oral}) is calculated using the following formula:

Chronic oral toxicity - screening step:

$$\text{ETR}_{\text{chronic adult oral}} = \text{AR} * \text{SV} / 10 \text{ d LDD}_5$$

With

AR...Application rate [kg a.s./kg]

SV...Short-cut value for the respective kind of application

LDD₅₀...Lethal dietary dose [µg a.s./bee/day]

Table B.9.6.1.1-2: Screenenig assessment for cereals.

Crop	Chronic LDD ₅₀	Max. application kg a.s./ha	Scenario	ETR _{adult}	Trigger	Risk for adult bees acceptable
Screening assessment						
Cereals	4.42	0.240	-	0.41	0.03	No
		0.160	-	0.276	0.03	No
		0.120	-	0.207	0.03	No

The ETR_{chronic adult oral} for the active substance is above the trigger value of 0.03 indicating a potential chronic risk to adult honey bees. Therefore, a refined chronic risk assessment (1st tier assessment) taking into account various exposure routes for each application rate of DFF+FFA SC 600 was provided below.

Chronic oral toxicity – 1 tier assessment

The chronic exposure-toxicity ratio (ETR_{chronic adult oral}) is calculated using the following formula:

$$\text{ETR}_{\text{chronic adult oral}} = \text{AR} * \text{Ef} * \text{SV} * \text{twa} / 10 \text{ d LDD}_{50}$$

With

AR...Application rate [kg a.s./ha]

Ef...Exposure factor

twa...Time weighted average

SV...Short-cut value for the respective kind of application

LDD₅₀...Lethal dietary dose [µg a.s./bee/day]

Table 9.6.1.1-3: Tier 1 assessment to bees.

rop	Chronic LDD ₅₀	Max. application kg a.s./ha	Scenario	ETR _{adult}	Trigger	Risk for adult bees acceptable
1st tier assessment						
Bare soil, crop attractive for pollen only	4.42	0.120	Treated crop	0.00	0.03	Yes
	4.42	0.120	weeds	0.01	0.03	Yes
	4.42	0.120	Field margin	0.00	0.03	Yes
	4.42	0.120	Adjacent crop	0.00	0.03	Yes
	4.42	0.120	Next crop	0.00	0.03	Yes
Cereals BBCH <10	4.42	0.120	Treated crop	0.00	0.03	Yes
	4.42	0.120	weeds	0.06	0.03	No
	4.42	0.120	Field margin	0.00	0.03	Yes
	4.42	0.120	Adjacent crop	0.00	0.03	Yes

rop	Chronic LDD ₅₀	Max. application kg a.s./ha	Scenario	ETR _{adult}	Trigger	Risk for adult bees acceptable
	4.42	0.120	Next crop	0.00	0.03	Yes
Cereals BBCH 10-29	4.42	0.120	Treated crop	0.02	0.03	Yes
	4.42	0.120	weeds	0.06	0.03	No
	4.42	0.120	Field margin	0.00	0.03	Yes
	4.42	0.120	Adjacent crop	0.00	0.03	Yes
	4.42	0.120	Next crop	0.00	0.03	Yes
Cereals BBCH 10-29	4.42	0.160	Treated crop	0.024	0.03	Yes
	4.42	0.160	weeds	0.075	0.03	No
	4.42	0.160	Field margin	0.0009	0.03	Yes
	4.42	0.160	Adjacent crop	0.0005	0.03	Yes
	4.42	0.160	Next crop	0.014	0.03	Yes
Cereals BBCH 10-29	4.42	0.240	Treated crop	0.036	0.03	No
	4.42	0.240	weeds	0.113	0.03	No
	4.42	0.240	Field margin	0.001	0.03	Yes
	4.42	0.240	Adjacent crop	0.00075	0.03	Yes
	4.42	0.240	Next crop	0.021	0.03	Yes

All the ETRchronic adult values in the first tier assessment are below the relevant trigger, indicating acceptable chronic risk to adult bees, except the following scenarios:

- cereals and scenario weeds at the rate 240 g a.s./ha
- scenario weeds at the rates 160 g a.s./ha and 120 g a.s./ha

Further consideration is needed.

Treated crop – cereals

Flufenacet can be considered as virtually non-systemic when early pre- or post-emergence spray application is done in winter cereals according to the studies of metabolism in plants (see CA 6.2 Metabolism, distribution and expression of residues, Section 6.2.1 Plants). In plant metabolism studies spray applied flufenacet result in negligible uptake and movement from the roots into the straw and grain samples.

Consequently the theoretical exposure of bees to pollen containing residues of flufenacet due to early spray applications is considered to be negligible. In addition the cereals are not attractive to honey bees as they not produce nectar so exposure for bees can only theoretically be to pollen.

In opinion of RMS growth stage of crops treated with Flufenacet – BBCH 10-13 (early growth stages in autumn) and BBCH 10-13 (early post emergence in the spring and in the autumn) practically excludes any

exposure of bees to the crop's pollen, because the cereals are not in flowering period. The potential exposure to metabolites of flufenacet in bee relevant matrices (pollen of cereals) is very unlikely and can be considered as negligible when taking into account the low systemicity of flufenacet.

The mainly possible exposure of bees to the pollen contaminated with Flufenacet may be related to the flowering weeds in cereal fields.

Considerations on potential exposure to flowering weeds

The whole assessment presented below is based on the data submitted by the Applicant. RMS, after thorough examination, considers it valid. However, it shall be indicated that the assessment refers to autumn application. In RMS's opinion the EU-representative GAP lacked clarity, therefore spring applications in cereals at early growth stages (10-13 BBCH) cannot be excluded. Nevertheless, in the documents submitted by the Applicant it is indicated that the weeds attractive to bees are observed in fields on which cereals are grown at crop growth stages much higher than those indicated in the GAP.

The Applicant's assessment is summarised below.

The potential exposure of honey bees to flowering weeds to an herbicide that is used during autumn at the pre-emergence of cereals is considered as low. The autumn period is in general of low activity for honey bee hives given the development phase of the colony which is getting prepared for the overwintering period. Even, in the rare case that the activity of foragers would be high in a period of days with mild temperatures during autumn, a recent publication (Maynard et al., 2015) demonstrates that the availability of flowering weeds in cereal fields at relevant application times for herbicides is minimal.

To analyse the presence of weeds in agricultural crops the available data has been extracted from the database for the crops cereals, sugar beet, and potatoes. As a conservative assessment only the data in the control plots (i.e. no herbicide treatment) was considered to provide a worst-case situation.

All data originate from worldwide herbicide efficacy trials in cereals conducted between 2004 and 2014 has been compiled (Maynard, et al. 2015). The majority of the studies were carried out in Europe; however for completeness of the datasets trials performed outside Europe were also included. Information on weed species, weed growth stages (BBCH), weed diameter (cm), weed ground cover (%), and weed plants/m² were obtained. Each weed species per trial was recorded separately, thus there are several data set entries per trial.

In the analysis done for the data on cereal fields, flowering weeds exceeding 10% ground cover were only observed in 14 out of 2327 observations (i.e. 0.6%) and out of these 14 only one observation was possibly relevant under certain circumstances. Hence, exposure via flowering weeds is confirmed not to be a relevant route of exposure for bees.

Further the Applicant argued that the potential exposure to metabolites of flufenacet in bee relevant matrices (pollen and nectar of flowering weeds) is very unlikely and can be considered as negligible when taking into account the low systemicity of flufenacet and the unlikely exposure via flowering weeds. In concluding remarks the Applicant however stated that low risk to bees foraging on flowering weeds can be confirmed with the results of the higher tier cage study OECD 75 (available during Peer review Commenting period).

Risk assessment for bumble bees

In addition to acute laboratory studies with adult honey bees, flufenacet was further subjected to topical acute bumble bee testing. The study did not reveal sensitivity differences between honey bee and bumble bee foragers.

Moreover, the the Hazard Quotient (HQ) between the application rate and the the acute contact laboratory LD₅₀ toxicity values for bumble bees was calculated:

$$HQ = AR/LD_{50}$$

Where

AR=application rate in kg a.s./ha

LD₅₀ is expressed in µg a.s./bee

Table B 9.1.1-4: Risk assessment for bumble bees.

Test item	Route	Oral LD ₅₀	Max. application rate g a.s./ha	Hazard quotient	Trigger	Risk for adults bees Acceptable?
Flufenacet tech.	Contact	>100	240	<2.40	7	Yes

The hazard quotients are below the trigger value of 50 for higher tier testing, indicating acceptable acute risk to adult bumble bees.

In addition the Applicant informed RMS about availability of the new acute oral toxicity study to bumble bees performed under laboratory condition.

Larval toxicity:

No laboratory acute larval toxicity test was submitted. In order to reveal whether flufenacet poses a risk to immature honey bee life stages, a bee brood feeding study has been conducted by following the provisions/method of Oomen P.A., de Ruijter, A. & van der Steen, J. (OEPP/EPPO Bulletin 22:613-616 (1992).

In course of evaluation of Bee brood feeding study (Hecch-Rost, 20012, M-456504-01-1) performed by Applicant conducted according to Oomen (1992), RMS stated that there were discrepancies between the study protocol, numerical results and their graphical presentation. Additionally it was stated that there were no statistically significant differences between response in the negative control and positive control (reference) in case of eggs and young larvae, although that was observed for the old larvae. RMS indicated that problem to the Applicant receiving the information that the study will be updated and in that form it should be ready for commenting period. At the same time the Applicant informed RMS about the new, ongoing semi-field study performed fully in line with the requirements of the current OECD 75 Guideline, which should also be made available during the commenting period.

Therefore RMS would like to propose to include both studies into the assessment evaluating them during the peer-review stage of the assessment process.

Conclusion: of RMS :

The acute risk assessment for adults bees is acceptable.

The chronic risk for adults and acute and chronic effects on colony survival and development, taking into account effects on honeybee larvae and honeybee behavior could be not finalized.

B.9.6.2. Risk assessment for non-target arthropods other than bees

The risk assessment for non-target arthropods other than bees was performed according to requirements of the ESCORT 2 Guidance Document (Candolfi, M.P. et al., 2000).

Exposure

Non-target arthropods living in the crop may be exposed to the formulation DFF +FFA SC 600 by direct over-spray during spray operations, or through contact with residues on plant and soil or in food items.

The in-field and off-field exposure of non-target terrestrial arthropods to the formulation DFF +FFA SC 600 following one application at 0.6 L/ha in winter cereals was estimated as recommended in the ESCORT II Guidance Document.

In-field

Within ESCORT II, the in-field exposure for non-target terrestrial arthropods is calculated as a Predicted Environmental Residue (PER) using the equation presented below:

$$PER_{in-field} = \text{application rate} \times MAF$$

The Multiple Application Factor (MAF) is a generic value which is used to take into account the potential build-up of applied substances between applications, based on the number of applications, the application interval and the DT₅₀ value. As only one application of Flufenacet + Diflufenican SC 600 is intended according to the GAP, a MAF values of 1 is used (See Appendix V of the ESCORT II Guidance Document).

In the risk assessment the highest application rate 0.6 L DDF + FFA SC 600 SC /ha, corresponding to 750 g product/ha (d=1.251 g /mL) and 240 g flufenacet/ha was used as the worst case exposure scenario.

The maximum predicted environmental residues (PER) occurring within the field after application of the formulation DFF+FFA S 600 are presented below.

Table B.9.6.2-1: In-field foliar and soil PER values for application of DFF+FFA SC 600.

Crop	Application rate (mL/ha)*	Number of applications	MAF	In-field PER (ml/ha)*
Winter cereals	600	1	1	600

*Maximum application rate

Off-field

The assessment of risk for areas immediately adjacent to the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to the formulation Flufenacet + Diflufenican SC 600 will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground. Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. Off-field foliar PER values were calculated from in-field foliar PER values in conjunction with drift values published by the BBA (2000) as shown in the following equation:

$$PER_{\text{off-field}} = \text{maximum foliar } PER_{\text{in-field}} \times \frac{\text{drift factor}}{\text{vegetation distribution factor}} \times \text{correction factor}$$

Drift factor: According to recommendations of the FOCUS surface water group, the overall 90th percentile probability is assumed to be a reasonable worst case scenario for drift estimation from a single application of a plant protection product. For the intended application of Flufenacet + Diflufenican SC 600 in cereals, the drift factor is derived from the category “field crops” with a drift value of 2.77% at 1 m.

Vegetation distribution factor: The model used to estimate spray drift was developed for drift onto a two-dimensional water surface, and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas.

Therefore, for 2-dimensional studies (glass plate or leaf disc), a vegetation distribution or dilution factor is incorporated into the equation when calculating foliar $PER_{\text{off-field}}$ values. From the ESCORT II Guidance Document a dilution factor of 10 is recommended. For 3-dimensional studies (spray treatment is applied onto whole plants), the vegetation distribution factor of 10 is not appropriate, as any dilution over the 3-dimensional vegetation surface is already accounted for in the study design.

Correction factor: For Tier I studies a correction factor of 10 is recommended by ESCORT 2 and will be used within this assessment.

In the risk assessment the highest application rate 0.6 L DFF+FFA SC 600/ha, corresponding to 750 g product/ha ($d=1.251 \text{ g/mL}$) and 240 g flufenacet/ha was used as the worst case exposure scenario.

The calculations of in-field PER were performed for the application rate expressed in mL product/ha.

Table B.9.6.2-2: Off-field foliar PER values for the application of the formulation DFF+FFA SC 600.

Use Pattern	Application rate (g a.s./ha)	Distance from edge of the crop (m)	MAF	Vdf	Corr. factor	MAF	Drift factor % drift/100	Off field PER (mL /ha)
Winter cereals	600	1	1	10	10	1	0.0277	16.62

Risk assessment

Tier 1 in-field risk assessment

The risk to non-target terrestrial arthropods exposed to flufenacet following the use of the formulation DFF+FFA SC 600 was assessed according to the ESCORT II Guidance Document.

In-field risk

The potential risk of the formulation in-field non-target terrestrial arthropods was assessed by calculating the hazard quotient (HQ), based on the Predicted Environmental Residue $PER_{in-field}$ and the toxicity value expressed as lethal rate LR_{50} , according to the following equation:

$$in\text{-}field\ HQ = \max.single\ application\ rate * MAF / LR_{50}$$

According to the ESCORT II Guidance Document, the HQ trigger value for laboratory toxicity data (Tier I) is 2.

In-field hazard quotient (HQ) was calculated for formulation Flufenacet + Diflufenican SC 600 using the maximum application rate in winter cereals of 0.6 L DFF+FFA SC 600 represents worst case and covers remaining intended uses (applications on winter cereals at rates 1x 0.3 L product /ha and 0.4 L product/ha).

The results of in-field risk assessment was performed in the Table B.9.6.2-3.

Table B.9.6.2-3: In-field risk to non-target terrestrial arthropods based on laboratory studies (Tier I) from exposure to the formulation DFF+ FFA SC 600 following the use in winter cereals.

Test substance	Test species	LR_{50} (mL/ha)	PER (mL/ha)	HQ	Trigger value
DFF+FFA SC 600	Typhlodromus pyri	81.8	600	7.30	2
	Aphidius rhopalosiphi	>700	600	0.85	

The results of the in-field tier 1 risk assessment for *Typhlodromus pyri* indicated that a higher tier in-field risk assessment is required. Extended laboratory studies have been conducted with *Typhlodromus pyri* and with two additional species *Chrysoperla carnea* and *Aleochara bilineata*.

The results from extended laboratory studies (Tier 2) with the standard species *T. pyri*, *Aleochara bilineata* and *Chrysoperla carnea* from exposure to the formulation DFF+FFA SC 600 following the use in winter cereals are presented in the Table B.9.2-4 below:

Table 9.6.2- 4: Ecotoxicological endpoints for arthropods other than beefor DFF+FFA SC600

Test species, references	Tested Formulation, study type, exposure	% Effects		
<i>Typhlodromus pyri</i> M-034242-01-1 Chauzat, M.P.; 2002	DFF+FFA SC 600 Extended lab., exposure on detached bean leaves 9.9 mL prod./ha, 28.7 mL prod./ha, 83.2 mL prod./ha, 241.4 mL prod./ha, 700 mL prod./ha,	Corr. Mortality [%]	Effect on Reproduction [%]	
		0	4.4	
		0	13.3	
		17.1	-17.8 ^A	
		94.3	n.a.	
		100	n.a.	
		LR ₅₀ 110.2 mL prod./ha, ER ₅₀ >83.2 mL prod./ha,		
<i>Chrysoperla carnea</i> M-352372-01-1 Waibel, J.; 2009	DFF+FFA SC 600 Extended lab., exposure on detached maize leaves Control 30 mL prod./ha, 63 mL prod./ha, 134 mL prod./ha, 284 mL prod./ha, 600 mL prod./ha,	Corr. Mortality	Eggs/Female/Day	Hatching [%]
		-	26.4	79.9
		0.0	24.1	81.4
		7.7	23.9	80.7
		2.6	27.5	83.4
		7.7	28.4	82.5
		20.5	27.6	82.7
		LR ₅₀ > 600 mL prod./ha ER ₅₀ > 600 mL prod./ha		
<i>Aleochara bilineata</i> M-353760-01-1 Roehlig, U.; 2009	DFF+FFA SC 600 Extended lab., spray deposits on soil (LUFA 2.1) 60 mL prod./ha, 107 mL prod./ha, 190 mL prod./ha, 337 mL prod./ha, 600 mL prod./ha,	Effect on Reproduction [%]		
		4.3		
		-2.3 ^A		
		1.7		
		5.8		
		7.9		
		ER ₅₀ >600 mL/prod./ha		

^A: A negative value indicates a higher reproduction rate in the treatment than in the control.

n.a.: not assessed

In - field refinement

Based on the results of the tier 1 in-field risk assessment extended laboratory studies were conducted for *T. pyri*, *C. carnea* and *A. bilineata*.

Table B.9.6.2-5: In-field risk to non-target terrestrial arthropods based on results from extended laboratory studies (Tier 2) with the standard species *T. Pyri* and two additional species *Aleochara bilineata* and *Chrysoperla carnea* from exposure to the formulation DFF+FFA SC 600 following the use in winter cereals.

Test species	In-field rate mL product/ha	LR ₅₀ , ER ₅₀ mL product/ha	Risk acceptable if	Refined assessment required ?
<i>Typhlodromus pyri</i>	600	>83.2	Effects <50%	Yes
<i>Chrysoperla carnea</i>	600	600	Effects <50%	No
<i>Aleochara bilineata</i>	600	600	Effects <50%	No

Under extended laboratory conditions *T. pyri* was found to be the most sensitive species.

In the 2D study done by Chauzat M.P in 2002, that was conducted on the natural substrate-deatched bean leaves, the 7 day LR₅₀ for *T.pyri* based on mortality was determined to be 110.2 mL/ha.

No effects on reproductions above 50% was observed including and up the highest tested rate of 83.2 mL /ha, resulting in an ER₅₀>83.2 mL/ha (lower rate than expected in- field habitas). The results of this study indicated potential harmful effects on *T.pyri* from the use of DFF+FFA SC 600 at maximum application rate of 600 mL product/ha. Consequently, an aged residue studies has been conducted for DFF+FFA SC 600 with *T. pyri* to demonstrate the potential for recovery. The study was conducted on potted maize plants with a single application rate of 700 mL product/ha (Jans, 2009). Aging of the spray residues of the test item on the potted maize plants took place under semi-field condition with rain protection during the whole study.

In this study the mites have been exposed to fresh residues of DFF + FFA SC 600 and to residues aged for 14 and 28 days. Freshly dried residues of the test item resulted in 98.9% corrected mortality. A corrected mortality of 87.1% was observed after an aging time of 14 days. An aging time of 28 days resulted in a low corrected mortality of 9.5% and no statistically significant effects on reproduction occurred (8.4% reduction relative to control). The results of this aged residue study (with application of 0.7 L product/ha) demonstrated that after an ageing time 28 days the residues have no impact on predatory mites. It can be concluded that, the potential for recovery is given for in field area and no acceptable adverse effects on non target arthropods have be expected in the in - field. In addition, an extended laboratory study with *Chrysoperla carnea* (Waibel I., 2009) has been conducted on the natural substrate – deatched maize leaves and indicated 20% corrected mortality for this species and no effects >50 % on fertility at highest tested rate of 600 mL product/ha.

For the second species –*Aleochara bilineata* the formulation DFF+FFA SC 600 has low effects on parasitation rate and reduction of reproductive capacity in comparison to the control. The highest reduction in capacity 7.9% was recored at the highest treatment rate of 600 mL product/ha. Therefore, the ER₅₀ value was estimated to be >600 mL product/ha. The one extended laboratory condition for *T. pyri* was performed resulting the ER₅₀ value of >83.2 ml product/ha and LR₅₀ value od 110 ml/ha, exceeding also maximum off field exposure.

It can be concluded that the risk to non-target arthropods in the in-field habitas is acceptable following the use of DFF+FFA SC 600 according to the proposed use pattern supported in this submission.

Off-field risk

The potential risk of the formulation to off-field non-target terrestrial arthropods was assessed by calculating the hazard quotient (HQ), based on the foliar Predicted Environmental Residue $PER_{off-field}$ and the toxicity value expressed as lethal rate LR_{50} , according to the following equation:

$$\text{off-field HQ} = \text{maximum single application rate} * \text{MAF} * (\text{drift factor} / \text{VDF}) * \text{correction factor} / LR_{50}$$

Correction factor: The ESCORT II Guidance Document recommends that a correction factor of 10 is applied for laboratory (Tier I) data, to account for the uncertainty with the extrapolation from *Typhlodromus pyri* and *Aphidius rhopalosiphii* as indicator species, to the species diversity expected in off-crop areas.

VDF (vegetation distribution factor) = 10

Hazard quotients off-field were calculated for the formulation following the use in winter cereals using the maximum application rate in winter cereals of 0.6 L DFF+FFA SC 600 represents worst case and covers remaining intended uses (the applications on winter cereals at rates 1 x 0.3 L product /ha and 1 x 0.4 L product/ha).

According to the ESCORT II Guidance Document, the HQ trigger value for laboratory toxicity data Tier I is 2.

Table B.9.6.2-6: Off-field risk to non-target terrestrial arthropods based on laboratory studies from exposure to the formulation DFF+FFA SC 600 following the use in winter cereals.

Test substance	Test species	LR ₅₀ mL product/ha	Vdf	Corr. factor	MAF	Off-field		Trigger value
						PER ^a mL product/ha	HQ	
DFF+FFA SC 600	<i>Typhlodromus pyri</i>	81.8	10	10	1	16.62	0.20	2
	<i>Aphidius rhopalosiphii</i>	>700	10	10	1	16.62	0.023	2

a spray drift - 2.77%

The off-field HQ values based on the laboratory studies (Tier 1) for non-target arthropods were below the Annex VI trigger values of 2 indicated acceptable risk off field.

Due to that fact that HQ in-field for *T.pyri* was above the trigger of 2, the additional extended lab studies (Tier 2) for *Chrysoperla carnea* and *Aleochara bilineata* were performed. The effects on reproduction were investigated in these studies. For *Chrysoperla carnea* no effects >50 % on fertility at highest tested rate of 600 mL product/ha were observed resulting an $ER_{50} > 600$ mL product/ha.

For the second species –*Aleochara bilineata* the highest reduction in capacity 7.9% was recored at highest treatment rate of 600 mL product/ha. The ER_{50} values > 600 mL product /ha estimated for both additional species were above the maximum $PER_{off-field}$ indicated an acceptable off risk.

Conclusion:

No unacceptable adverse effects on non-target arthropods in -field and off -field are to be expected from the use of DFF+FFA SC 600 according to the proposed use pattern.

B.9.7. EFFECTS ON NON TARGET SOIL MESO-AND MACROFAUNA**B.9.7.1. Earthworms****B.9.7.1.1. Toxicity data**

One study (Heimbach, 1997 see in CA, Vol 3, Section B9) on the reproductive toxicity of the active substance flufenacet (tested as Flufenacet WG 60) to earthworms was submitted for the first EU approval. The NOEC was estimated to be 3000 kg a.s./ha corresponding to 4 mg a.s./kg soil dw.

The new statistical analysis done by Kratz A. (1997) based on the original data from study Heimbach (1997) indicated the new endpoint to earthworm - NOEC=0.605 kg flufenacet/ha (based on growth).

However, the endpoint was considered not valid and was not used in the risk assessment by RMS.

The new standard laboratory study on the reproductive toxicity of representative formulation DFF+FFA SC 600 to earthworms was performed. In this study (Leicher T., 2010) the effects on growth and reproduction were determined in two runs of five different concentrations of formulation DFF+FFA 600 SC (the first run: 48, 85, 15.2, 27 and 48 mg product/ha, the second run with 0.8, 1.5, 2.6, 4.7 and 8.4 mg item/kg soil dw) incorporated into artificial soil, resulting in an NOEC_{reproduction}=2.6 mg product/kg dws.

Two field studies examined the influence on the population of earthworms.

One of them was the one-year earthworm field study with Flufenacet SC 500 (Leicher, 2008) applied on an arable field up to an application rate of 1.2 L/ha (600 g flufenacet/ha correspond to 0.438 mg flufenacet/kg dws). It was performed to examine the effect of the increasing application rate on the toxicity of flufenacet to natural earthworm population. Based on the results of that study it can be concluded that there was no long-term adverse effects, determined 5 and 11 months after application, in population of juvenile and adult earthworms resulting from application of 1.2 L Flufenacet 500 SC/ha.

Therefore, the determined NOAER = 1.2 L Flufenacet 500 SC/ha, corresponding to NOAER =0.438 mg flufenacet/kg soil dw (measured value). However it should be indicated that the study was not used because the most sensitive species to flufenacet - *Octolasion lacteum*, identified as such in another field study, was not tested. Therefore, the NOAER value of 0.438 mg flufenacet/kg dws was considered not appropriate to use in the risk assessment.

A one-year earthworm field study with the representative formulation DFF+ FFA SC 200+400 G was conducted in Southern Germany under field conditions, after one autumn application of Diflufenican SC 500A on bare soil, at a rate of 243.75 g diflufenican/ha (application 1), followed by once application of DFF+ FFA SC 200+400 G (diflufenican+flufenacet, application 2-DDA2): at different rates (0.6 L product/ha, 1.2 L product/ha and 1.8 L product/ha. Not statistically significant reduction in numbers and in biomass of total earthworms, total juveniles, total adults and single species occurred at any post treatment sampling (35, 183, 364 days) after application of the test item at rates of 0.6, 1.2 and 1.8 L/ha, following the plateau application of diflufenican at a rate of 243.77 g a.s/ha. However, it should be noted that biological significant effects (19-33%) could still be observed on the population *Octolasion lacteum* after 364 d at rates of 1.2. and 1.8 L/ha.

At rate of 0.6 L DFF+ FFA SC 200+400 G /ha biological significant but transient effects for this species were observed.

Therefore, **NOAER of 0.6 L DFF+ FFA SC 200+400 G /ha (leading to 0.203 mg flufenacet/kg soil dw)** was estimated from the study and this value was used in the risk assessment.

Studies with the soil metabolites such as: FOE oxalate, FOE sulfonic acid-Na-salt, FOE methylsulfone, TFA, FOE 5043-trifluoroethane sulfonic acid and FOE-Thiadone were conducted addressing the risk soil organisms including earthworms.

The study summaries for studies with the active substance flufenacet and the soil metabolites are provided in Volume 3, B.9 (CA)

A summary of the sub-lethal endpoint used in the present risk assessment is provided in Table B.9.7.1.1-1.

Table B 9.7.1.1-1: Sub-lethal ecotoxicological endpoints for flufenacet for earthworms.

Test Substance	Test species	Endpoint		Reference
FFA SC 500 (Flufenacet)	Earthworm field study	NOAER	1.2 L prod/ha 0.6 kg a.s./ha (0.438 mg a.s/kg dws) ¹	Leicher (2008) M-307211-01-1
FOE oxalate	Earthworm, reproduction (10% peat in test soil)	NOEC	≥100 mg p.m./kg dws	Leicher (2010) M-398163-01-1
FOE sulfonic acid-Na-salt	Earthworm, reproduction (5% peat in test soil)	NOEC	500 mg p.m./kg dws	Leicher (2009) M-358264-01-1
FOE methylsulfone	Earthworm, reproduction (5% peat in test soil)	NOEC	125 mg p.m./kg dws	Leicher (2010) M-362081-01-1
TFA	Earthworm, reproduction (10% peat in test soil)	NOEC	320 mg p. m./kg dws²	Luehrs (2005) M-251328-01-1
FOE trifluoroethane sulfonic acid	Earthworm, reproduction (5% peat in test soil)	NOEC	100 mg p.m./kg dws	Kratz (2012) M-436340-01-1
FOE-Thiadone	Earthworm, reproduction (5% peat in test soil).	NOEC	3.2 mg p.m.kg dws	Kratz (2012) M-442579-01-1

¹ The study not included because the most sensitive species to flufenacet - *Octolasion lacteum*, identified as such in another field study, was not tested. Therefore, the NOAER value of 0.438 mg flufenacet/kg dws was not used in the risk assesmet. (see C.A. Vol.3. B9)

² NOEC reduced to 320 mg /kg dws based on the effects on body weight in the concentration 1000 mg/kg

p.m: pure metabolite

in bold the values used in the risk assessment

The following sublethal study on earthworms performed with DFF+FFA SC 600 in the laboratory and one field study are provided in support of the assessment.

Table B 9.7.1.1-2: Endpoints for the representative formulation DFF+ FFA SC 600 used in the risk assessment.

Test substance	Test species	Endpoint		Reference
DFF + FFA SC 600	Earthworm, reproduction (5% peat in test soil)	NOEC	2.6 mg product/kg sdw 1.3 mg product/kg sdw*	Leicher (2010) M-362809-01-1
DFF + FFA SC 600	Earthworm field study	NOAER	0.6 L product /ha (0.203 mg flufenacet/kg soil dw)¹	Hamberger (2014) M-478092-01-1

*Endpoints corrected to allow for log $P_{ow} > 2$

¹ expressed as a.s.-flufenacet (measured value)
in bold the values used in the risk assessment

Earthworms

Studies on DFF+FFA 600 SC

B.9.7.1.1.1. Diflufenican + flufenacet SC 600 G: Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil with 5% peat.

Reference:	Diflufenican + flufenacet SC 600 G: Effects on survival, growth and reproduction on the earthworm <i>Eisenia fetida</i> tested in artificial soil with 5% peat.
Author(s), year:	Leicher, T., 2010
Report/Doc. number:	Report No: E 312 3772-5, Reference BCS no: M-362809-01-1
Guideline(s):	OECD 222: April 13, 2004, ISO 11268-2: 1998 (E)
GLP:	Yes

<u>Test substance</u>	Diflufenican + flufenacet SC 600 G content of a.s. (analysed): diflufenican: 191.4 g/L (15.6 % w/w); flufenacet: 394.5 g/L (32.1% w/w); Batch No: EV56001418, FAR 01403-00, density 1.229 g/mL
Test species:	Earthworm, <i>Eisenia fetida</i>
Number of organism:	8 replicates for control group, 4 replicates per test item concentration, each replicates with 10 individuals
Weight, age	The first test run: mean: 250-450 mg/worm, adults approximately 7 months. The second test run: 3 months old with a well developed clitellum
Type of test, duration	Laboratory sub-lethal test, 8 weeks (4 weeks adult mortality, 4 weeks juvenile development) for both test runs.
<u>Applied concentrations:</u>	
Nominal:	The first test run: 0 (control, quartz sand), 4.8, 8.5, 15.2, 27.0 and 48.0 mg test item/kg soil dw, incorporated into soil. The second test run: 0 (control, quartz sand), 0.8, 1.5, 2.6, 4.7 and 8.4 mg test item/kg soil dw, incorporated into soil.
Toxic standards	The first test run: Derosal flüssig (BAS 346F, 363 g carbendazim/L), tested at concentrations of 1.25, 2.5 and 5 mg a.s./kg dw soil

The second test run: Nutdazim 50 FLOW (Carbendazin 51.5%), tested at concentration 5 mg and 10 mg prod./kg dw soil

Test substrate

The first run:

Artificial soil, 5% sphagnum peat, 20 % kaolinite clay, 73.82 % industrial quartz sand, 0.18 % calcium carbonate, 1% dried ground cow manure (dried ground cow manure).

The artificial soil was prepared by mixing the dry components intensely in a laboratory mixer. Then, the soil was moistened with deionised water to reach a water content of 58 % of the maximum water holding capacity.

The second run:

Artificial soil, 5 % sphagnum peat, 20 % kaolinite clay, 73.7 % industrial quartz sand, 0.3 % calcium carbonate, 1% dried ground cow manure (dried ground horse manure).

The artificial soil was prepared by mixing the dry components intensely in a laboratory mixer. Then, the soil was moistened with deionised water to reach a water content of 42.7 % of the maximum water holding capacity.

Substrate/vassel:

In both runs of the study non-re-usable plastic boxes

(length x width x height *ca.* 16.5 cm x 12 cm x 6 cm, area approximately 200 cm²) were used as test vessels.

Each test vessel contained an amount of approximately 500 g dry weight artificial soil to obtain a depth of approximately 5 cm soil in the test vessels.

Temperature

The first and the second run tes : 20±2°C

Light regime

The first and the second test run:16 hours light/8 hours dark

Light intensity

The first run: 541- 583Lux

The second run: 520 -580 Lux

Water content:

The first test run:

Test start: 22.1 % (equivalent to 54.54 % of WHC)

Test end: 22.9% (equivalent of 56.52% of WHC)

The second test run:

Test start: 25.11 % mean soil moisture

Test end: 24.97 % mean soil measure

pH:

The first run:

Prior the test: 5.89

Test end: 6.51-6.61

The second test run;

Test start: 5.97 - 6.05

Test end: 5.99 - 6.0

Feeding:	Air-dried and finely ground animal manure; feeding interval was weekly during the first 4 weeks, weekly amount of manure (5 g) depended on the feeding activity. The off spring were fed only once at the start of the second week.
Test parameters:	<p>Temperature was recorded continuously during the whole test period. The moisture content of the artificial soil were determined prior to test start, at day application Day 0 and day 56 of the test. The pH measurements were made prior the test and the end on Day 56 of the study.</p> <p>Mortality of adults (assessed after 28 days), mean body weight of adults (measured at day 0 and after 28 days), morphological and behavioural changes of adults (observed at day 28) number of juvenile earthworms (counted after 8 weeks).</p>
Statistic:	The reproduction of the surviving test organisms per test vessel at the end of the study was compared to the control values. The homogeneity of variances of the data was checked by Cochran's test. The homogeneity hypothesis was accepted. The normal distribution of the data was tested by Kolmogorov-Smirnov test. The normality hypothesis of the data was accepted. The data were statistically evaluated by means of a Williams multiple sequential t-test. The statistical software package ToxRat Pro 2.10 (released February 19, 2009); (Ratte, 2001-2009) was used for the calculation.

Findings:

The first test run:

No mortality of adult earthworms was observed after 28 days of exposure at the control group and all test concentrations.

With respect to growth, the statistically significant different values for the growth relative to the control were observed at all test concentrations. Since there is no dose-response relationship these differences are not considered to be treatment related.

However, statistically significant different values for the number of juveniles relative to the control were observed at all test concentrations. It resulted that the $NOEC_{reproduction}$ was not achieved in the first test run. In addition, there was no significant difference in food consumption in treatments up to and including 48 mg test item/kg soil dry weight, when compared to the control.

All the treatment groups recorded complete consumption of food during the four weeks of exposure.

Effects on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days in the first test run are shown in the table B. 9.7.1.1.1-1 below:

Table B.9.7.1.1-1: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test in the first test run.

		Eisenia fetida				
		mg Diflufenican + Flufenacet SC 600 G /kg soil dw				
		1 st test run				
Exposure	Control	4.8	8.5	15.2	27.0	48.0
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0
Mean change of body weight of the adults from day 0 to day 28 [%] (Standard deviation)	+ 7.9 (± 3.5)	+ 14.7* (± 5.5)	+ 16.6* (± 5.7)	+18.4* (± 5.2)	+16.5* (± 5.9)	+12.6* (± 6.1)
Mean number of offspring per test vessel after 56 days (Standard deviation)	102.4 (± 15.3)	84.0** (± 15.7)	75.3** (± 7.8)	82.5** (± 18.5)	81.8** (± 14.8)	59.5** (± 14.8)
% reproduction compared to control	100	82.1**	73.5**	80.6**	79.9**	58.1**

* Mean value statistically significant different compared to the control (Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$, $p < 0.05$)

** Mean value statistically significant different compared to the control (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$, $p < 0.05$)

The second test run:

No mortality of adult earthworms was observed after 28 days of exposure at the control group. Only 2.5 % of mortality was recorded at test concentrations: 1.5, 4.7 and 8.4 mg test item/kg soil dw.

No statistically significant different values for the growth relative to the control were observed at test all concentrations.

Statistically significant different values for the number of juveniles relative to the control were observed at the test concentrations of 4.7 and 8.4 mg test item/kg soil dw.

The results from the second test run on mortality and changes in body weight of the adults after an exposure period of 28 days and the number of offspring per test vessel after 56 days are shown in the table below:

Table B.9.7.1.1.1-2: Effects on mortality and reproduction of *Eisenia fetida* in a sub-chronic test in the second test run.

		Eisenia fetida				
		mg Diflufenican + Flufenacet SC 600 G /kg soil dw				
2 nd test run						
Exposure	Control	0.8	1.5	2.6	4.7	8.4
Mortality of adult earthworms [%] after 28 days	0	0	2.5	0	2.5	2.5
Mean change of body weight of the adults from day 0 to day 28 [%] (Standard deviation)	+63.1 (± 15.4)	+64 (± 1.4)	+64.5 (±7.1)	+62.8 (±3.1)	+62.8 (± 8.8)	+61.5 (±22.5)
Mean number of offspring per test vessel after 56 days (Standard deviation)	116.8 (± 17.2)	111 (± 13.4)	113.8 (± 18.7)	104.3 (±11.5)	83** (± 10.7)	68** (± 13.2)
% reproduction compared to control	100	95.0	97.4	89.3	71.1**	58.2**

** Mean value statistically significant different compared to the control (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$, $p < 0.05$)

Toxic standards:

The results of positive control (carbendazim) used in the first test run , tested in separately report, indicated that the number of juveniles was reduced by 58.1 and approximately 100% at concentration of 2.5 and 5.0 mg carbendazim./kg dry weight soil (mean number of juveniles=95.3 and 0.8) after 56 days of test duration, when compared to the control (mean number of juveniles =227.4).

The results of positive control (Nutzdazim 50 FLOW, 51.5% carbendazim) used in the second test run, tested in separately report, indicated that the number of juveniles was reduced by 65 and 92% at concentration of 5 and 10 mg product/kg soil dw (mean number of juveniles =50.5 and 11) after 56 days of test duration when compared to the control (mean number of juveniles =143.3).

Conclusions:

NOEC_{reproduction}=2.6 mg test item/kg soil dw

NOEC_{growth}= 48 mg test item/kg soil dw

NOEC_{adult mortality}= 48 mg test item/ kg soil dw

LOEC = 4.7 mg test item/kg soil dw

EC₅₀>48 mg test item /kg soil dw

Comment RMS:

The earthworm reproduction study was conducted according to the OECD test guideline 222 (2004).
 Based on the validity criteria stated in the test guideline OECD 222 (2004) the study is considered acceptable.
 The mortality of adults in the control was below 10% (being: 0%).
 The number of juveniles per control replicate was greater than 30 (being 80-121 juveniles per replicate in the 1st test run and 98-149 juveniles per replicate in the 2nd test run).
 The coefficient of variation of reproduction in the control was $\leq 30\%$ (being 14.9 and 14.7% in the first test run and the second test run, respectively).

Agreed endpoints:

NOEC_{reproduction} = 2.6 mg test item/kg soil dw

NOEC_{growth} = 48 mg test item/kg soil dw

NOEC_{adult mortality} = 48 mg test item/ kg soil dw

LOEC = 4.7 mg test item/kg soil dw

EC₅₀ > 48 mg test item /kg soil dw

Field studies**DFF+FFA SC 200+400 G**

B.9.7.1.1.1.2. DFF+FFA SC 200+400 G – A field study to investigate effects on the earthworm fauna in Southern Germany.

Reference: DFF+FFA SC 200+400 G – A field study to investigate effects on the earthworm fauna in Southern Germany.

Author(s), year: Hamberger, A.; 2014

Report/Doc. number: Study No: M-478092-01-1

Guideline(s): BBA, Part VI, 2 - 3 (January 1994), ISO Guideline 11268-3, 1999;
 ISO Guideline 23611-1, 2006;
 KULA et al., 2006
 SANCO/3029/99 rev.4
 Regulation (EC) No 1107/2009 (EC, 2009)
 Guideline 7029/VI/95 rev. 5 to Directive 91/414/EEC and Regulations (EU) 283/2013 and 284/2013 implementing Regulation (EC) 1107/2009
 US EPA OCSPP Guideline No. 860.1500

GLP: Yes

Material and methods:

Test substance: DFF + FFA SC 600 (content of Diflufenican (analysed): 209.5 g/L; Flufenacet. (analysed): 410.0 g/L, Batch-No.: 2011-005209.

Test site:

The study was conducted in Southern Germany near Bodelshausen/Baden-Württemberg under field conditions after one autumn application of Diflufenican SC 500A on bare soil at a rate of 243.75 g diflufenican/ha (application 1) on followed by once application of DFF+ FFA SC 200+400 G (diflufenican+flufenacet, application 2): at different rates (0.6 L product/ha, 1.2 L product/ha and 1.8 L product/ha. The control plots were sprayed once with tap water, the toxic reference item plots were treated with Twist WP® at rate 17152.66 g product/ha (equivalent to 10000 g a.s. carbendazim/ha).

Field story:

The field history is shown in Table B.9.7.1.1.1.2-1 below.

Table B.9.7.1.1.1.2-1: Field story.

Pesticide usage		
Crop season 2009	May 2009	Clio Star 0,8 L/ha (50g/L topramezone;160g/L dicamba)
		Motivell 0,8L/ha (40g/L nicosulfuron)
		Cetrol B 0,3L/ha (235 g/L bromoxynil)
Crop season 2010	March 2010	Attribut 100g/ha (70% propoxycarbazonenatrim)
		Artus 40g/ha (10% metsulfuron-methyl, 40% carfentrazone-ethyl)
		Cycocel 720, 0,8L/ha (chlormequat-chlorid, 720 g/L
Crop season 2011	April 2011	Axial 50, 100g/ha (50g/L pinoxaden)
		Biathlon 0,75L/ha bixafen; (714g/L tritosulfuron)
	Juni 2011	Fandango 0,65 L/ha (100g/L prothioconazole; 100g/L fluoxastrobin)
		Aviator 0,65 L/ha (75g/L bixafen; 150g/L prothioconazole)
		Biscaya 0,2L/ha (240g/L thiacloprid)
Crop season 2012	April 2012	Attribut 100g/ha (70% propoxycarbazonenatrium)
		Artus 40g/ha (10% metsulfuron-methyl, 40% carfentrazone-ethyl)
Crop season 2013	-	no pesticide usage

During the study period, the following agricultural practice was conducted: after application of Diflufenican SC 500 on bare soil winter wheat was drilled, no pesticides and fertilizers were applied during the trial, and after harvest of winter cereals, grass-clover mixture was planted.

Soil type/substrate:

The application rates were verified by soil core samplings in the test item and control plots directly after each application. Soil cores were collected using a soil corer with acetate liners (5 cm diameter, 10 cm depth). Samples were deep frozen within a maximum of 6 hours after sampling and were stored at $\leq -18^{\circ}\text{C}$. Samples were shipped deep frozen to the analytical PI for residue analysis to verify the application rates.

In addition a soil sample was prepared from 10 soil cores (each 50 mm wide by 20 cm deep) taken across the field site and the following characteristics were determined for the sample: soil type, particle size distribution (USDA), pH (CaCl_2), cation exchange capacity, total carbon, total organic carbon and maximum water holding capacity.

Table B.9.7.1.1.2-2: Soil parameters

Test parameter	Results
pH	7.15
Water holding capacity	37.3
CEC [meq/100 g dry weight]	16.7
%Corg	1.9
% sand	8.2
% silt	60.8%
% clay	31%
Classification USDA	Silty clay loam

Climatic condition:

The following environmental parameters were recorded throughout the test: mean, maximum and minimum air temperature, daily mean air humidity, daily mean wind speed, daily mean soil temperatures at depths of approximately 5 and 20 cm, soil moisture at depths of approximately 5 and 20 cm and daily rainfall. Weather data recording by the data-logging weather station cannot be considered full GLP, but was carried out to a standard commensurate with GLP.

Long term precipitation data and air temperature data were taken from the weather station of the Deutscher Wetter

Dienst(DEUTSCHERWETTERDIENST, 2012) in Hechingen (approx. 6.5 km air-line distance to the field site) covering the period of 1961-1990.

Table B.9.7.1.1.2-3: Monthly values of air temperature, soil temperature, precipitation, soil humidity and wind speed at the field site at Bodelshausen, Germany.

Month	mean air temp.	max. air temp.	min. Air temp.	Soil tem. 5cm	Soil tem. 20cm	Precipi- tation	Sol Humidity 5cm	Sol Humidity 20cm	Wind Speed
	°C	°C	°C	°C	°C	mm	Volume%	Volume%	m/s
Oct. 2012	8.6	22.1	-3.9	9.4	9.9	60.2	41.4	42.2	1.9
Nov.2012	5.3	17.0	-5.7	5.4	6.0	127.8	43.0	44.3	2.5
Dec.2012	1.6	17.5	-15.7	1.8	2.3	86.0	43.4	44.4	5.4
Jan.2013	2.2*	14.9*	-7.4*	1.7*	2.4*	69.4*	43.0*	44.7*	4.9*
Feb.2013	-1.7	8.3	-14.8	0.4	0.8	57.6	43.8	45.2	4.0
Mar.2013	1.5	16.7	-9.7	1.7	2.6	34.6	43.9	45.2	3.4
Apr.2013	8.5	24.9	-4.3	6.8	8.2	70.4	42.7	43.3	4.8
May 2013	10.6	22.7	-0.6	10.5	11.7	130.0	42.2	43.5	3.2
Jun. 2013	15.7	33.7	5.0	14.2	16.1	79.4	36.9	35.5	1.9
Jul. 2013	19.6	36.1	6.1	18.5	20.6	111.8	34.3	26.6	1.8
Aug. 2013	17.6	34.7	6.3	17.5	19.0	111.0	39.8	34.9	0.1
Sep. 2013	13.9	29.0	5.0	14.1	15.1	109.2	41.1	39.4	0.8
Oct. 2013	10.9	22.4	-1.9	11.1	11.6	97.6	41.6	42.6	2.5

* Data from DEUTSCHER WETTERDIENST (2012). weather station Hechingen.
covering the period of 1961-1990

** Data missing from 16-18.01.2013 and 20-21.01.2013

Table B.9.7.1.1.2-4: Monthly values of measured mean air temperature, long term mean air temperature and their deviation.

Month	Mean air temp. Measured	Long term air temperature	Deviation
	°C	°C	°C
Oct. 2012	8.6	9.2	-0.6
Nov.2012	5.3	3.7	1.6
Dec.2012	1.6	0.4	1.2
Jan.2013	2.2**	-0.5	2.7
Feb.2013	-1.7	0.7	-2.4
Mar.2013	1.5	3.9	-2.4
Apr.2013	8.5	7.5	1.0
May 2013	10.6	11.9	-1.3
Jun. 2013	15.7	15.0	0.7
Jul. 2013	19.6	17.3	2.3
Aug. 2013	17.6	16.7	0.9

Month	Mean air temp. Measured	Long term air temperature	Deviation
	°C	°C	°C
Sep. 2013	13.9	13.5	0.4
Oct. 2013	10.9	9.2	1.7
Mean	8.8	8.3	+0.5

* Data from Deutscher Wetterdienst, weather station Hechingen, covering the period of 1961-1990

** Data missing from 16-18.01.2013 and 20-21.01.2013

Table B.9.7.1.1.2.-5: Monthly values of measured and long term precipitation

Month	Monthly precipitation measured	Monthly precipitation measured +irrigation	Long term monthly precipitation	Deviation	Deviation +irrigation
	mm	Mm	mm	Mm	mm
Oct. 2012	60.2	85.2	54.4	5.8	30.8
Nov.2012	127.8	127.8	60.6	67.2	67.2
Dec.2012	86.0	86.0	52.9	33.1	33.1
Jan.2013	69.4**	69.4	48.0	21.4	21.4
Feb.2013	57.6	57.6	49.7	7.9	7.9
Mar.2013	34.6	34.6	54.0	-19.4	-19.4
Apr.2013	70.4	70.4	74.5	-4.1	-4.1
May 2013	130.0	130.0	95.2	34.8	34.8
Jun. 2013	79.4	79.4	107.7	-28.3	-28.3
Jul.2013	111.8	111.8	87.9	23.9	23.9
Aug.2013	111.0	111.0	93.0	18.0	18.0
Sep.2013	109.2	109.2	58.2	51.0	51.0
Oct.2013	97.6	97.6	54.5	43.1	43.1
Sum	1145.0	1170.0	890.6	+254.4	+279.4

Application:

On the test site, twenty 12 x 12 m plots were arranged in 4x5 formation, each plot surrounded by least a 5 m quard row between the plots.

Four replicates to each treatment group of DFA+FFA SC, the water treated control and the reference were made.

Application was made in 300 L water/ha using calibrated boom sprayer with 6 m width.

Diflufenican SC 500A G was applied once on 08 October 2012 at a rate of 487.5 mL product/ha corresponding to 243.75 g diflufenican/ha (0.325 mg diflufenican/kg soil, plateau application A 1) to all treatment groups. After application, Diflufenican SC 500A G was incorporated in the top 5 cm of soil and winter wheat was drilled.

On 17 October the formulation DFF and FFA SC 200+400 G was applied at different rates (application A2). Treatment group 1 (T1) was treated with 0.6 L DFF and FFA SC 200+400 G /ha, treatment group 2 (T2) with 1.2 L DFF and FFA SC 200+400 G /ha and treatment group 3 (T3) with 1.8 L DFF and FFA SC 200+400 G /ha. The control plots were sprayed once with tap water and the toxic reference item plots were treated once with 17152.66 g/ha Twist WP® (nominally equivalent to 10000 g carbendazim/ha) at the second test item application date.

Irrigation was performed up to 5 days after the second application (22 October 2012).

The timing and the amounts of water applied (total 25.0 mm) and natural rainfall resulted in sufficient soil moisture levels such that earthworms were active and thus exposed to the treatments.

The application scenario and the rates are given in Table B.9.7.1.1.1.2-6.

Table B.9.7.1.1.1.2-6: The application scenario.

Application code	Treatment	Timing	Application rate		Spray volume
A1	T1, T2, T3	08.Oct.2012	Diflufenican SC 500A G 487.5 mL product/ha	243.75 g a.s./ha (bare soil)	300 l/ha
A2	C	17 Ot 2012	Water		300 l/ha
	T1		DFF+FFA SC 200+400 G 0.6 L product/ha	120 g DFF/ha 240 g FFA/ha	300 l/ha
	T2		DFF+FFA SC 200+400 G 1.2 L product/ha	240 g DFF/h 480 g FFA/ha	300 l/ha
	T3		DFF+FFA SC 200+400 G 1.8 L product/ha	360 g DFF/ha 720 g FFA/ha	300 l/ha
	Reference Toxic standard		Twist WP® 17152.66 g product/ha	10000 g Carbendazim /ha	300 l/ha

C: control,T: test item treatment, R: toxic reference item treatment

^a based on nominal content of a.s.

Earthworm sampling:

From each plot earthworms were sampled (4 areas of 0.125 m² within the inner 12 x 12 m area of each plot) by hand sorting (top 20 cm) with subsequent formalin sampling extraction for the deeper layers of each sampling hole. The field site was sampled to assess the earthworm

population before the first application and approximately 1, 6 and 12 months after second application.

Earthworm sampling were performed in the Table B.9.7.1.1.1.2-7.

Table B.9.7.1.1.1.2-7: Earthworm sampling.

Assessment	Date of assessment
Sampling 1 (pre-treatment sampling)	0.1 Oct-0.2 October 2012 (7-6 DBA1)
Sampling 2 (1 st post- treatment sampling)	21 Nov-22 Nov 2012 (35-36 DAA2)
Sampling 3 (2 nd post- treatment sampling)	18 April-19 April 2013 (183-184 DAA2)
Sampling 4 (3 rd post-treatment sampling)	16 Oct.-17 Oct. 2013 (364-365 DAA2)

Adult earthworms were determined to species level.

Juveniles of the species *Lumbricus terrestris* were counted separately. Additionally, juveniles of this species were added to the number of adults. If juveniles of particular key species could be determined to species level, they were counted as a separate category, as well as added to the juvenile morphological group counts. For juvenile worms that were difficult to identify, a distinction between tanylobous and epilobous was made.

The following were recorded: abundance and biomass of total earthworms, total adults earthworms, total juvenile earthworms (tanylobous and epilobous), single species (dominant species only), total endogeic, total epigeic and total anecic earthworms.

In addition to the above, surface monitoring for direct mortality effects on earthworms was performed the first three days after application.

Chemical soil analysis:

Overall 5 soil samples were taken per treatment replicate and from untreated control areas on the day of application (22.05.2007) to analytical verify application rate.

Soil samples were analysed for the determination of the residues of Flufenacet in soil by HPLC-MS/MS analysis. LOQ was 4 µg/kg.

Statistical analysis:

The statistical analysis have been applied to both population counts and weights, whether individual species or totals across species. For both, population counts and weights the total count and weight per plot and per square metre, was calculated prior to analysis. Taxa with low abundances (threshold < 5 Ind/m² for at least two sampling occasions in the control plots) and earthworm fragments are not presented within this detailed

analysis but are part of grouped and total earthworm numbers. All statistical analyses were performed using SAS, version 9.3. Graphs were generated with the data outputs from SAS using Excel 2010.

Effect of Test Item:

The normality of the distribution was tested using the Shapiro-Wilk test ($\alpha = 0.05$). Homogeneity of variance was tested with the Levene's test ($\alpha = 0.05$). If the samplings evaluated had the same variance and were distributed normally an ANOVA was calculated. The ANOVA was followed by a Dunnett's t-test. If neither homogeneity of variance nor normality were observed a Kruskal-Wallis test was used on the data set. The test was followed by a Wilcoxon test for pair-wise comparisons of the individual treatments and a correction of p-values according to Bonferroni-Holms procedure (Bonferroni-U-Test). All tests were carried out two-side at the five percent significance level.

All statistical analyses were performed using SAS, version 9.3. Graphs were generated with the data outputs from SAS using Excel 2010.

Statistical analysis was performed only for species >5 ind/m².

Effect of Reference Item:

The normality of the distribution was tested using the Shapiro-Wilk test ($\alpha = 0.05$). Homogeneity of variance was tested with the Levene's test ($\alpha = 0.05$). A Student t-test ($\alpha = 0.05$, two sided) was performed with data that were normally distributed and showed variance homogeneity. With data normally distributed without variance homogeneity a Satterthwaite t-test was performed ($\alpha = 0.05$, two sided). If the data set was non-homogeneous and non-normal distributed the data sets were compared to the control with a non-parametric pair-wise U-Test (Wilcoxon) with the Exact-Statement ($\alpha = 0.05$, two-sided).

Findings:

Soil analysis:

After application of Diflufenican SC 500A G (plateau application) mean residues as percentage of the target rate of 80 % (0.181 mg DFF/kg d.s.), 100 % (0.227 mg DFF/kg d.s.) and 99 % (0.224 mg kg d.s.) were found for treatment groups T1, T2 and T3, respectively.

After application of DFF+FFA SC 200+400 G (application 2) mean residues of DFF of 97 %, (0.326 mg/kg d.s.), 116 % (0.523 mg DFF/kg d.s.) and 122 % (0.686 mg DFF/kg d.s.) as percentage of the target rate were determined in treatment groups T1, T2 and T3, respectively.

Mean residues of FFA of 90 % (0.203 mg FFA/kg d.s.), 98 % (0.443 mg FFA/kg d.s.) and 99 % (0.670 mg kg d.s.) as percentage of the target rate were found in treatment groups T1, T2 and T3. The limit of quantification was 0.001 mg/kg for DFF and 0.004 mg/kg for FFA.

Soil core samples of the control and test item treatments were analysed for residues of diflufenican (DFF) and flufenacet (FFA).

No measurable residues of DFF and FFA were found in soil samples taken after application 1 (plateau application) in the control plots.

In soil samples taken after application 2 mean residues of 0.007 mg DFF/kg dry soil and 0.015 mg FFA/kg dry soil were found in the control plots, representing 1.0 % and 2.2 %, respectively, of the concentrations in highest test item treatment group (0.686 mg DFF/kg and 0.670 mg FFA/kg, respectively).

Thus, since no effects on earthworm populations were observed overall at all test item treatment groups, the impact of this cross contamination can be regarded as negligible.

Meteorology: During the 13 months of the trial (calculated from October 2012 to October 2013) the measured mean air temperature (8.8°C) was 0.5 °C higher than the reported long term mean air temperature (Hechingen, 1961-1990) for this period (8.3°C). Measured mean air temperature values from October 2012 to October 2013 are compared to long term averages in Table 42. During the 13 months of the trial the cumulative measured precipitation including irrigation was higher (1170.0 L/m²) than the reported long term cumulative precipitation (Hechingen, 1961- 1990) for this period (890.6 L/m²) resulting in an excess of 279.4 L/m²_(including irrigation).

Abundances: Twelve different taxa were observed and identified. These taxa will be referred to by their genus and species. The Table B. 9.7.1.1.1.2-8. provides a list of all taxa identified in this study and their ecological grouping in terms of niche occupied. Total juvenile earthworms: comprising all juvenile earthworms (i.e. tanylobous juveniles and epilobous juveniles).

Total adults: comprising all adults (i.e. Allolobophora chlorotica, Aporrectodea caliginosa, Aporrectodea limicola, Aporrectodea longa, Aporrectodea rosea, Lumbricus castaneus, Lumbricus rubellus, Lumbricus terrestris, Murchieona minuscula and Octolasion lacteum).

Total: comprising all taxa (i.e. Allolobophora chlorotica, Aporrectodea caliginosa, Aporrectodea limicola, Aporrectodea longa, Aporrectodea rosea, Lumbricus castaneus, Lumbricus rubellus, Lumbricus terrestris, Murchieona minuscula, Octolasion lacteum, tanylobous juveniles, epilobous juveniles, tanylobous front ends and epilobous front ends).

Additionally, for Lumbricus terrestris total abundances were calculated for the juveniles and adults of this species.

Table B.9.7.1.1.2-8: Taxa indentified during the study.

Genus	Species	Adult/Juvenile	Ecological group
Allolobophora	Chlorotica	adult	endogeic
Aporrectodea	Caliginosa	adult	endogeic
Aporrectodea	Limicola	adult	endogeic
Aporrectodea	Longa	adult	anecic
Aporrectodea	Rosea	adult	endogeic
Lumbricus	Castaneus	adult	epigeic
Lumbricus	Rubellus	adult	epigeic
Lumbricus	Terrestris	adult	anecic
Murchieona	Minuscula	adult	endogeic
Octolasion	Lacteum	adult	endogeic
Tanylobous	spp.	juvenile	-
Epilobous	spp.	juvenile	-
Tanylobous	spp.	front ends	-
Epilobous	spp.	front ends	-

Results:The mean earthworm abundances

The mean earthworm abundances was 382 earthworms/m² across all plots at the start of the trial. The juvenile:adult ratio was 0.7 (equivalent to 41.3 % adults). The initial earthworm population as % of adult earthworms of the field site was characterised by 87.3 % endogeic and 12.6 % anecic earthworms. The dominant endogeic species at trial start was *Aporrectodea rosea* (58 earthworms/m², 15.1 % of total earthworms, 39.0 % of adult earthworms) followed by *Aporrectodea caliginosa* (41 earthworms/m², 10.8 % of total earthworms, 27.9 % of adult earthworms). The dominant anecic earthworm species was *Lumbricus terrestris* (including juveniles: 23 earthworms/m², 6.1 % of total earthworms, 15.6 % of adult earthworms).

The mean earthworm abundance (mean values from control plots only) was 375 earthworms/m² at trial start (pre-sampling was done 6-7 days before application with Diflufenican SC 500A G-(7DBA1) with decreasing to 179 earthworms/m² at 35 DAA2 (35 days after application of DFF+FFA SC 200+400 G), and 183 earthworms/m² at 183 DAA2 (183 days after application of DFF+FFA SC 200+400 G).

At the end of the trial 216 earthworms/m² were found (364 days after after application of DFF+FFA SC 200+400 G). The dominant species in the control plots (7 DBA1) were: *Aporrectodea rosea* (57 earthworms/m²), *Aporrectodea caliginosa* (44/m²) and *Lumbricus terrestris* (19 ind/m² (including juveniles and 18 adults /m²)).

Table B.9.7.1.1.2-9. Set of individual tables characterising abundance and biomass from the field study. Individual tables are marked A-N.

Table A. Statistical analysis on number and biomass of <i>Aporrectodea caliginosa</i> adults.					Table B. Statistical analysis on number and biomass of <i>Aporrectodea rosea</i> .				
Sampling	Abundance				Sampling	Abundance			
	C	T1	T2	T3		C	T1	T2	T3
mean Abundance [n/m ²] ± SD (% of Control)					mean Abundance [n/m ²] ± SD (% of Control)				
7 DBA1	44.0 ± 5.4	38.5 ± 12.2 (87.5 %)	42.0 ± 18.8 (95.5 %)	51.0 ± 22.2 (115.9 %)	7 DBA1	57.0 ± 29.6	57.5 ± 37.1 (100.9 %)	60.0 ± 40.8 (105.3 %)	61.5 ± 12.0 (107.9 %)
35 DAA2	18.0 ± 6.9	16.5 ± 12.2 (91.7 %)	17.5 ± 18.9 (97.2 %)	23.5 ± 7.0 (130.6 %)	35 DAA2	8.0 ± 6.3	6.0 ± 2.8 (75.0 %)	11.5 ± 7.0 (143.8 %)	8.5 ± 5.3 (106.3 %)
183 DAA2	17.5 ± 3.4	16.5 ± 8.4 (94.3 %)	17.0 ± 8.1 (97.1 %)	17.5 ± 6.8 (100.0 %)	183 DAA2	33.5 ± 21.9	31.5 ± 18.7 (94.0 %)	40.0 ± 27.5 (119.4 %)	37.5 ± 19.1 (111.9 %)
364 DAA2	38.0 ± 17.2	40.0 ± 13.8 (105.3 %)	44.0 ± 14.9 (115.8 %)	38.0 ± 13.3 (100.0 %)	364 DAA2	45.0 ± 19.8	40.5 ± 26.4 (90.0 %)	60.0 ± 29.7 (133.3 %)	61.0 ± 28.9 (135.6 %)
Sampling	Biomass				Sampling	Biomass			
	C	T1	T2	T3		C	T1	T2	T3
mean weight [g/m ²] ± SD (% of Control)					mean weight [g/m ²] ± SD (% of Control)				
7 DBA1	6.9 ± 1.0	7.8 ± 2.6 (112.8 %)	7.6 ± 3.5 (110.5 %)	8.0 ± 3.6 (116.2 %)	7 DBA1	6.4 ± 3.0	6.3 ± 3.2 (98.9 %)	6.7 ± 4.3 (104.6 %)	6.7 ± 0.8 (104.7 %)
35 DAA2	2.4 ± 1.2	2.7 ± 1.9 (112.6 %)	2.5 ± 2.6 (103.5 %)	3.6 ± 1.1 (148.8 %)	35 DAA2	3.8 ± 6.1	1.0 ± 0.6 (25.2 %)	1.6 ± 1.1 (42.7 %)	1.2 ± 0.9 (30.4 %)
183 DAA2	2.7 ± 0.5	2.9 ± 1.4 (108.4 %)	3.4 ± 2.2 (126.2 %)	2.9 ± 1.4 (106.7 %)	183 DAA2	4.1 ± 2.3	3.7 ± 1.6 (91.6 %)	5.0 ± 3.3 (121.7 %)	4.2 ± 1.8 (103.4 %)
364 DAA2	8.4 ± 4.4	9.6 ± 3.9 (114.3 %)	11.2 ± 4.6 (133.1 %)	10.4 ± 3.6 (123.4 %)	364 DAA2	6.7 ± 1.9	8.1 ± 4.5 (121.8 %)	7.9 ± 2.7 (117.9 %)	8.0 ± 3.4 (119.4 %)
Table C. Statistical analysis on number and biomass of <i>Allolobophora chlorotica</i> adults.					Table D. Statistical analysis on number and biomass of <i>Lumbricus terrestris</i> adults.				
Sampling	Abundance				Sampling	Abundance			
	C	T1	T2	T3		C	T1	T2	T3
mean Abundance [n/m ²] ± SD (% of Control)					mean Abundance [n/m ²] ± SD (% of Control)				
7 DBA1	9.5 ± 13.2	3.5 ± 3.4 (36.8 %)	8.0 ± 8.2 (84.2 %)	8.0 ± 9.1 (83.3 %)	7 DBA1	18.0 ± 8.3	24.0 ± 8.5 (133.3 %)	17.0 ± 6.6 (94.4 %)	15.0 ± 5.8 (83.3 %)
35 DAA2	10.0 ± 10.7	3.0 ± 3.5 (30.0 %)	3.5 ± 4.4 (35.0 %)	7.5 ± 11.4 (75.0 %)	35 DAA2	12.0 ± 7.1	13.5 ± 3.0 (112.5 %)	18.0 ± 8.6 (150.0 %)	11.5 ± 3.8 (95.8 %)
183 DAA2	6.0 ± 4.3	5.5 ± 6.8 (91.7 %)	4.0 ± 5.7 (66.7 %)	3.5 ± 4.1 (58.3 %)	183 DAA2	8.0 ± 5.9	10.5 ± 3.4 (131.3 %)	9.0 ± 2.6 (112.5 %)	16.5 ± 2.5* (206.3 %)
364 DAA2	7.0 ± 6.6	6.5 ± 5.3 (92.9 %)	4.5 ± 5.3 (64.3 %)	8.0 ± 9.1 (114.3 %)	364 DAA2	12.5 ± 8.1	10.5 ± 6.8 (84.0 %)	8.0 ± 5.2 (64.0 %)	12.0 ± 5.9 (96.0 %)
Sampling	Biomass				Sampling	Biomass			
	C	T1	T2	T3		C	T1	T2	T3
mean weight [g/m ²] ± SD (% of Control)					mean weight [g/m ²] ± SD (% of Control)				
7 DBA1	2.0 ± 2.6	0.8 ± 0.7 (38.7 %)	2.0 ± 1.9 (103.6 %)	1.9 ± 1.9 (95.9 %)	7 DBA1	72.4 ± 33.6	97.9 ± 37.3 (135.2 %)	64.3 ± 35.5 (88.8 %)	60.7 ± 21.3 (83.8 %)
35 DAA2	2.0 ± 2.2	0.7 ± 0.8 (35.3 %)	0.8 ± 0.9 (41.8 %)	1.5 ± 2.4 (76.9 %)	35 DAA2	51.7 ± 32.3	62.5 ± 19.9 (120.9 %)	77.2 ± 31.2 (149.4 %)	47.9 ± 17.3 (92.8 %)
183 DAA2	1.2 ± 0.8	1.0 ± 1.2 (84.0 %)	0.8 ± 1.0 (64.6 %)	0.8 ± 0.9 (61.7 %)	183 DAA2	39.1 ± 33.1	48.4 ± 20.0 (123.7 %)	40.4 ± 13.9 (103.4 %)	74.1 ± 10.1 (189.4 %)
364 DAA2	2.0 ± 1.6	1.7 ± 1.2 (86.1 %)	1.4 ± 1.6 (69.2 %)	2.6 ± 2.7 (130.0 %)	364 DAA2	58.3 ± 38.1	55.5 ± 35.4 (95.2 %)	37.3 ± 25.9 (64.0 %)	56.0 ± 28.7 (96.1 %)
Table E. Statistical analysis on number and biomass of <i>Lumbricus terrestris</i> adults and juveniles.					Table F. Statistical analysis on number and biomass of <i>Octolasion lacteum</i> adults.				
Sampling	Abundance				Sampling	Abundance			
	C	T1	T2	T3		C	T1	T2	T3
mean Abundance [n/m ²] ± SD (% of Control)					mean Abundance [n/m ²] ± SD (% of Control)				
7 DBA1	19.5 ± 9.1	29.5 ± 12.3 (151.3 %)	21.0 ± 7.4 (107.7 %)	23.0 ± 10.5 (118.0 %)	7 DBA1	18.5 ± 8.4	19.5 ± 15.8 (105.4 %)	24.0 ± 14.3 (129.7 %)	15.0 ± 12.3 (81.1 %)
35 DAA2	18.0 ± 9.4	20.0 ± 1.6 (111.1 %)	22.5 ± 8.1 (125.0 %)	18.5 ± 6.0 (102.8 %)	35 DAA2	12.5 ± 10.0	5.5 ± 6.2 (44.0 %)	7.0 ± 5.3 (56.0 %)	4.0 ± 2.8 (32.0 %)
183 DAA2	13.5 ± 8.1	15.5 ± 8.2 (114.8 %)	14.0 ± 5.2 (103.7 %)	22.5 ± 4.1 (166.7 %)	183 DAA2	14.5 ± 13.1	14.0 ± 13.5 (96.6 %)	11.0 ± 10.5 (75.9 %)	6.0 ± 5.7 (41.4 %)
364 DAA2	15.0 ± 8.1	16.5 ± 7.7 (110.0 %)	15.5 ± 7.6 (103.3 %)	15.0 ± 3.8 (100.0 %)	364 DAA2	18.0 ± 19.2	18.5 ± 15.6 (102.8 %)	12.0 ± 9.9 (66.7 %)	14.5 ± 10.0 (80.6 %)
Sampling	Biomass				Sampling	Biomass			
	C	T1	T2	T3		C	T1	T2	T3
mean weight [g/m ²] ± SD (% of Control)					mean weight [g/m ²] ± SD (% of Control)				
7 DBA1	76.2 ± 35.3	106.9 ± 43.3 (140.4 %)	71.1 ± 36.6 (93.3 %)	72.4 ± 29.4 (95.1 %)	7 DBA1	22.0 ± 7.1	18.3 ± 12.5 (83.2 %)	24.1 ± 14.2 (109.9 %)	14.3 ± 10.6 (65.2 %)
35 DAA2	61.6 ± 37.1	78.3 ± 9.1 (127.2 %)	83.5 ± 26.9 (135.6 %)	64.3 ± 22.4 (104.4 %)	35 DAA2	8.4 ± 6.3	3.8 ± 4.1 (45.7 %)	4.9 ± 4.1 (58.1 %)	2.3 ± 1.7 (27.5 %)
183 DAA2	49.3 ± 36.6	62.4 ± 32.5 (126.6 %)	51.0 ± 18.8 (103.4 %)	84.4 ± 10.2 (171.3 %)	183 DAA2	14.0 ± 12.2	12.5 ± 12.3 (89.0 %)	11.0 ± 10.5 (78.9 %)	7.1 ± 7.0 (50.5 %)
364 DAA2	64.6 ± 39.7	66.4 ± 37.6 (102.8 %)	50.0 ± 29.4 (77.4 %)	62.8 ± 22.4 (97.3 %)	364 DAA2	21.3 ± 21.7	18.5 ± 13.1 (86.6 %)	11.2 ± 8.9 (52.6 %)	14.3 ± 8.8 (67.3 %)

Sampling 1= Pre-treatment sampling (01 Oct 2012-02 Oct. 2012, 7-5 DBA1)

Sampling 2= 1st post treatment sampling (21 Nov 2012-22 Nov 2012, 35-36 DAA2)

Sampling 3 = 2nd post treatment sampling (18 Apr 2013-19 Apr 2013, 183-184 DAA2)

Sampling 4 = 3rd post treatment sampling (16 Oct 2013-17 Oct 2013, 364-365 DAA2)

DBA1= days before application 1, DAA2= days after application 2, SD = standard deviation

The results after application 0.6 L, 1.2 and 1.8 L DFF+FFA SC 200+400 G/ha, following application of the the plateau application of diflufenican at a rate of 243.77 g a.s/ha are summarized in the tables Table

B.9.7.1.1.1.2-10 - B.7.1.1.1.2-12.

Table B.9.7.1.1.2-10: Adult and juvenile earthworms, changes in numbers and biomass after application of 0.6 L DFF+FFA SC 200+400 G, following the plateau application of diflufenican at a rate of 243.77 g a.s/ha.

Treatment	DFF+FFA SC 200+400 G 0.6 L product/ha					
	Mean number (Ind/m ²) and change (%)**					
species / group	35 DAA2		183 DAA2		364 DAA2	
<i>Aporrectodea caliginosa</i>	16.5	(-8.3 %)	16.5	(-5.7 %)	40.0	(+5.3 %)
<i>Aporrectodea rosea</i>	6.0	(-25.0 %)	31.5	(-6.0 %)	40.5	(-10.0 %)
<i>Allolobophora chlorotica</i>	3.0	(-70.0 %)	5.5	(-8.3 %)	6.5	(-7.1 %)
<i>Lumbricus terrestris</i>	13.5	(+12.5 %)	10.5	(+31.3 %)	10.5	(-16.0 %)
<i>Lumbricus terrestris</i> adult + juvenile	20.0	(+11.1 %)	15.5	(+14.8 %)	16.5	(+10.0 %)
<i>Octolasion lacteum</i>	5.5	(-56.0 %)	14.0	(-3.4 %)	18.5	(+2.8 %)
<i>Tanylobous</i> juvenile	14.5	(-47.3 %)	16.0	(+88.2 %)	25.0	(+4.2 %)
<i>Epilobous</i> juvenile	84.5	(+9.7 %)	115.0	(+31.4 %)	63.5	(+5.8 %)
Endogeic earthworms	32.5	(-33.0 %)	67.5	(-6.3 %)	108.0	(-1.8 %)
Anecic earthworms	14.5	(+20.8 %)	11.0	(+37.5 %)	11.5	(-8.0 %)
Anecic earthworms adult + juvenile	22.0	(+22.2 %)	16.0	(+18.5 %)	17.5	(+16.7 %)
Total juveniles	99.0	(-5.3 %)	131.0	(+36.5 %)	88.5	(+5.4 %)
Total adults	47.0	(-23.0 %)	78.5	(-1.9 %)	119.5	(-2.4 %)
Total earthworms	164.5	(-7.8 %)	215.5	(+17.4 %)	222.0	(+3.0 %)
	Mean biomass (g/m ²) and change (%)**					
<i>Aporrectodea caliginosa</i>	2.7	(+12.6 %)	2.9	(+8.4 %)	9.6	(+14.3 %)
<i>Aporrectodea rosea</i>	1.0	(-74.8 %)	3.7	(-8.4 %)	8.1	(+21.8 %)
<i>Allolobophora chlorotica</i>	0.7	(-64.7 %)	1.0	(-16.0 %)	1.7	(-13.9 %)
<i>Lumbricus terrestris</i>	62.5	(+20.9 %)	48.4	(+23.7 %)	55.5	(-4.8 %)
<i>Lumbricus terrestris</i> adult + juvenile	78.3	(+27.2 %)	62.4	(+26.6 %)	66.4	(+2.8 %)
<i>Octolasion lacteum</i>	3.8	(-54.3 %)	12.5	(-11.0 %)	18.5	(-13.4 %)
<i>Tanylobous</i> juvenile	20.4	(+51.3 %)	19.9	(+78.8 %)	16.5	(+48.1 %)
<i>Epilobous</i> juvenile	9.3	(+42.5 %)	10.1	(+15.7 %)	7.4	(-2.4 %)
Endogeic earthworms	8.8	(-47.2 %)	20.1	(-8.5 %)	38.8	(-0.2 %)
Anecic earthworms	65.4	(+26.5 %)	48.9	(+25.0 %)	58.1	(-0.3 %)
Anecic earthworms adult + juvenile	82.4	(+33.8 %)	62.9	(+27.7 %)	69.0	(+6.9 %)
Total juveniles	29.6	(+48.5 %)	30.1	(+51.0 %)	23.9	(+27.6 %)
Total adults	74.2	(+8.3 %)	69.0	(+13.0 %)	96.9	(-0.2 %)
Total earthworms	105.7	(+16.9 %)	100.7	(+23.5 %)	123.2	(+5.0 %)

** negative values indicate decrease in earthworm numbers compared to the control

Positive values indicate increase in earthworm numbers compared to the control

DAA2: days after application 2

The following observations on earthworm community after application of 0.6 L DFF+FFA SC 200+400G/ha on sampling dates 35 DDA, 183 DDA and 364 DDA were recorded:

On Day 35 after application DFF+FFA SC 200+400G/ha, *Tanylobous* juveniles were reduced in numbers (-47.3%, not significant) as compared to the control. At the same time, an increase in biomass (+51.3%) was observed. This may indicate that stronger individuals have survived the treatment. The *Tanylobous* juveniles on 183 and 364 days after application increased in numbers (+88.2%) and biomass (+78.8%) as compared to

the control. The increase in Tanylobous juveniles (probably juvenile stadium of *Lumbricidae*) may be related to the increase in adult *Lumbricus terrestris* observed on Day 183 after application of DFF+FFA 200+400 at a rate of 0.6 L/ha. This was probably due to the fact that adults of *L. terrestris* entered the reproduction phase.

Moreover, 35 days after application of DFF+FFA 600 SC 200+400G at the rate 0.6 L/ha, *Allolobophora chlorotica* and *Octolasion lacteum* were found to decrease in numbers (-70% and -56%, respectively, not significant) and biomass (-64.7% and -54.3%, respectively, not significant) as compared to the control.

However, on the following sampling dates, i.e.:183 and 364 days after application, the decrease in biomass for both species was less pronounced (less than 30% as compared to the control).

At the same time, the number of *Octolasion lacteum* increased at the end of the study (+2.8%).

Both the number and biomass of *L. terrestris*, were not affected 35 days after application, as compared to the control. Similar observation was valid for the second most abundant species. i.e. *Aporrectodea caliginosa*. For *A. rosea* the decrease in numbers (-25%, not significant) and biomass (-74.8%, no significant) on Day 35 was observed.

In general, taking into account the category: total earthworms, total adults, total juveniles or ecological groups and single species, not statistically significant reductions in number and biomass occurred after application of the test item at any of the post treatment samplings.

It should be pointed out, that for the category: total adults and total juvenile, despite of the slight reduction in number and biomass (<10%, not significant) on Day 35 after application, an increase in numbers and biomass was noted on the following sampling dates: Day 183 and 364 after application of 0.6 L product/ha.

For the category total adults, a slight decrease in numbers and biomass (<30%, not significant) was observed on Day 35, but this effect was negligible for the following sampling dates: 183 and 364 days after application.

However, it should be noted, that the effect of reduction in numbers for Tanylobous juveniles (-47.35%, not significant) as well as for *Octolasion lacteum* and *Allolobophora chlorotica* in numbers (-56 and -70%, respectively, not significant) and biomass (-54.3% and -64.7%, respectively, not significant), on Day 35 after application of 0.6 L product/ha was apparent, and even if it was not statistically significant, should be taken into account, particularly in the case of *Octolasion lacteum*. *Allolobophora chlorotica*. Reduction in numbers and biomass was noted for the latter species on the sampling date - Day 35 after application of DFF+FFA 600 SC 200+400G also for higher application rates (1.2 l and 1.8 l/ha).

However, since for the next sampling days after application, i.e. 183 and 364, for Tanylobous juveniles the increase in numbers and biomass was noted and for *Octolasion lacteum* and *Allolobophora chlorotica*, these effects were not pronounced (not enhanced, but rather diminished), it can be concluded, that on Day 183 and 364 after application of DFF+FFA SC 200+400 G at a rate of 0.6 L/ha, no observed adverse effects on earthworm community could be recorded for all the tested groups, including individual species.

Table B.9.7.1.1.2-11: Adult and juvenile earthworms, changes in numbers and biomass after application of 1.2 L DFF+FFA SC 200+400 G, following the plateau application of diflufenican at a rate of 243.77 g a.s/ha.

Treatment	DFF+FFA SC 200+400 G 1.2 L product/ha					
	Mean number (Ind/m ²) and change (%)**					
species / group	35 DAA2		183 DAA2		364 DAA2	
<i>Aporrectodea caliginosa</i>	17.5	(-2.8 %)	17.0	(-2.9 %)	44.0	(+15.8 %)
<i>Aporrectodea rosea</i>	11.5	(+43.8 %)	40.0	(+19.4 %)	60.0	(+33.3 %)
<i>Allolobophora chlorotica</i>	3.5	(-65.0 %)	4.0	(-33.3 %)	4.5	(-35.7 %)
<i>Lumbricus terrestris</i>	18.0	(+50.0 %)	9.0	(+12.5 %)	8.0	(-36.0 %)
<i>Lumbricus terrestris</i> adult + juvenile	22.5	(+25.0 %)	14.0	(+3.7 %)	15.5	(+3.3 %)
<i>Octolasion lacteum</i>	7.0	(-44.0 %)	11.0	(-24.1 %)	12.0	(-33.3 %)
<i>Tanylobous</i> juvenile	14.0	(-49.1 %)	14.0	(+64.7 %)	29.5	(+22.9 %)
<i>Epilobous</i> juvenile	78.5	(+2.0 %)	104.0	(+18.9 %)	79.5	(+32.5 %)
Endogeic earthworms	40.5	(-16.5 %)	74.0	(+2.8 %)	128.5	(+16.8 %)
Anecic earthworms	18.5	(+54.2 %)	9.0	(+12.5 %)	10.0	(-20.0 %)
Anecic earthworms adult + juvenile	23.0	(+27.8 %)	14.0	(+3.7 %)	17.5	(+16.7 %)
Total juveniles	92.5	(-11.5 %)	118.0	(+22.9 %)	109.0	(+29.8 %)
Total adults	59.0	(-3.3 %)	83.5	(+4.4 %)	139.0	(+13.5 %)
Total earthworms	164.0	(-8.1 %)	207.5	(+13.4 %)	271.5	(+26.0 %)
	Mean biomass (g/m ²) and change (%)**					
<i>Aporrectodea caliginosa</i>	2.5	(+3.5 %)	3.4	(+26.2 %)	11.2	(+33.1 %)
<i>Aporrectodea rosea</i>	1.6	(-57.3 %)	5.0	(+21.7 %)	7.9	(+17.9 %)
<i>Allolobophora chlorotica</i>	0.8	(-58.2 %)	0.8	(-35.4 %)	1.4	(-30.8 %)
<i>Lumbricus terrestris</i>	77.2	(+49.4 %)	40.4	(+3.4 %)	37.3	(-36.0 %)
<i>Lumbricus terrestris</i> adult + juvenile	83.5	(+35.6 %)	51.0	(+3.4 %)	50.0	(-22.6 %)
<i>Octolasion lacteum</i>	4.9	(-41.9 %)	11.0	(-21.1 %)	11.2	(-47.4 %)
<i>Tanylobous</i> juvenile	9.8	(-27.4 %)	15.1	(+35.2 %)	19.0	(+71.1 %)
<i>Epilobous</i> juvenile	6.6	(+1.3 %)	11.0	(+25.2 %)	12.2	(+60.6 %)
Endogeic earthworms	10.1	(-39.5 %)	20.3	(-7.7 %)	32.3	(-17.0 %)
Anecic earthworms	78.4	(+51.7 %)	40.4	(+3.4 %)	41.7	(-28.4 %)
Anecic earthworms adult + juvenile	84.7	(+37.6 %)	51.0	(+3.4 %)	54.4	(-15.7 %)
Total juveniles	16.4	(-18.0 %)	26.0	(+30.8 %)	31.2	(+66.8 %)
Total adults	88.5	(+29.2 %)	60.9	(-0.3 %)	74.1	(-23.8 %)
Total earthworms	105.9	(+17.1 %)	87.5	(+7.3 %)	109.6	(-6.6 %)

** negative values indicate decrease in earthworm numbers compared to the control positive values indicate increase in earthworm numbers compared to the control DAA2: days after application 2 (DFF+FFA SC 200+400G)

The following observations on earthworm community after application of 1.2 L DFF+FFA SC 200+400G/ha on sampling dates 35 DDA, 183 DDA and 364 DDA were recorded:

On Day 35 after application of DFF+FFA SC 200+400G/ha, the *Tanylobous* juveniles were reduced in numbers (-49.1%, not significant), as compared to the control. At the same time, a decrease in biomass was observed even though below 30% (being -27.4%, not significant). For the next sampling dates, i.e.: 183 and 364 days after application these effects were not observed. To the contrary, increases in numbers (+64.7%) and biomass (+35.2%) were recorded for this group.

For endogeic species: *Allolobophora chlorotica* and *Octolasion lacteum*, a reduction in numbers was observed after application of DFF+FFA SC 200+400G/ha for all the sampling dates, i.e.: 35 (-65% and -44%, respectively, not significant), 184 and 363 days.

It should be pointed out, that at the end of the study, a decreasing trend of reduction in numbers for both species was clearly seen (about -30%), as compared to the control.

However, at the end of the study, a clear effect of biomass reduction for *O. lacteum* was noticed (-47%, not significant), as compared to the control.

For *Aporrectodea rosea*, the increase in numbers (+ 43.8%) was recorded after 35 days following the treatment. This species was dominant throughout all the experiment.

Even though *Lumbricus terrestris* was observed to increase in numbers and biomass after 35 and 183 days following the application, a reduction in numbers (-36%, not significant) was recorded at the end of the experiment. The latter effect may probably be related to ageing of *L. terrestris* population. At the same time, in view of the fact that there were recorded both increases in numbers (+64.7%) and biomass (+35.2%) for Tanylobous juveniles, and increases in numbers (+3.3.) for *L. terrestris* + juveniles, it may be safely assumed that the total population of *Lumbricus terrestris* would be maintained.

In general, taking into account the category: total earthworms, total adults, total juveniles or ecological groups and single species, no statistically significant reductions in biomass and numbers occurred at any of the post treatment samplings, after application of the test item.

It should be noted, that for category: total adults, total juvenile and total earthworms, despite a slight, not significant, reduction in numbers and biomass on Day 35 after application, the increase in numbers as compared to the control was noted on the following sampling dates i.e.:183 and 364 days after application.

However, the effect of reduction in numbers for Tanylobous juvenile (-49.1%, not significant) and biomass (-27%, not statistically significant) and for the species *Allolobophora chlorotica* and *Octolasion lacteum* in numbers (-65% and -44%, respectively , not significant) and biomass (-58.2% and - 41.9%) on Day 35 after application of 1.2 l product/ha was apparent (in case of numbers), and even if it was not statistically significant, should be taken into account, particularly in the case of *Octolasion lacteum*.

For this species the reduction in numbers (not significant) and in biomass (not significant) was noted for all the tested rates of DFF+FFA SC 200+400 G. For *Octolasion lacteum* and *Allolobophora chlorotica* species these effects reduction in numbers and in biomass seen were on Day 183 and 364 after application of DFF+FFA SC 200+400 G at a rate of 1.2 L/ha.

However, since for the next sampling days after application, i.e. 183 and 364, for Tanylobous juveniles the increase in numbers and biomaas was noted on Day 183 and 364 after application of DFF+FFA SC 200+400 G at a rate of 1.2 L/ha.

Table B.9.7.1.1.2-12: Adult and juvenile earthworms, changes in numbers and biomass after application of 1.8 L DFF+FFA SC 200+400 G, following the plateau application of diflufenican at a rate of 243.77 g a.s/ha.

Treatment	DFF+FFA SC 200+400 G 1.8 L product/ha					
	Mean number (Ind/m ²) and change (%)**					
species / group	35 DAA2		183 DAA2		364 DAA2	
<i>Aporrectodea caliginosa</i>	23.5	(+30.6 %)	17.5	(±0.0 %)	38.0	(±0.0 %)
<i>Aporrectodea rosea</i>	8.5	(+6.3 %)	37.5	(+11.9 %)	61.0	(+35.6 %)
<i>Allolobophora chlorotica</i>	7.5	(-25.0 %)	3.5	(-41.7 %)	8.0	(+14.3 %)
<i>Lumbricus terrestris</i>	11.5	(-4.2 %)	16.5 *	(+106.3 %)	12.0	(-4.0 %)
<i>Lumbricus terrestris</i> adult + juvenile	18.5	(+2.8 %)	22.5	(+66.7 %)	15.0	(±0 %)
<i>Octolasion lacteum</i>	4.0	(-68.0 %)	6.0	(-58.6 %)	14.5	(-19.4 %)
Tanylobous juvenile	19.5	(-29.1 %)	12.0	(+41.2 %)	35.5	(+47.9 %)
Epilobous juvenile	68.5	(-11.0 %)	127.5	(+45.7 %)	81.0	(+35.0 %)
Endogeic earthworms	45.0	(-7.2 %)	66.0	(-8.3 %)	129.0	(+17.3 %)
Anecic earthworms	12.0	(±0.0 %)	17.5 *	(+118.8 %)	14.5	(+16.0 %)
Anecic earthworms adult + juvenile	19.0	(+5.6 %)	23.5	(+74.1 %)	18.0	(+20.0 %)
Total juveniles	88.0	(-15.8 %)	139.5	(+45.3 %)	116.5	(+38.7 %)
Total adults	57.0	(-6.6 %)	84.0	(+5.0 %)	143.5	(+17.1 %)
Total earthworms	157.0	(-12.0 %)	228.0	(+24.6 %)	273.5	(+26.9 %)
	Mean biomass (g/m ²) and change (%)**					
<i>Aporrectodea caliginosa</i>	3.6	(+48.8 %)	2.9	(+6.7 %)	10.4	(+23.4 %)
<i>Aporrectodea rosea</i>	1.2	(-69.6 %)	4.2	(+3.4 %)	8.0	(+19.4 %)
<i>Allolobophora chlorotica</i>	1.5	(-23.1 %)	0.8	(-38.3 %)	2.6	(+30.0 %)
<i>Lumbricus terrestris</i>	47.9	(-7.2 %)	74.1	(+89.4 %)	56.0	(-3.9 %)
<i>Lumbricus terrestris</i> adult + juvenile	64.3	(+4.4 %)	84.4	(+71.3 %)	62.8	(-2.7 %)
<i>Octolasion lacteum</i>	2.3	(-72.5 %)	7.1	(-49.5 %)	14.3	(-32.7 %)
Tanylobous juvenile	23.1	(+71.5 %)	12.6	(+12.8 %)	14.0	(+26.2 %)
Epilobous juvenile	5.2	(-19.7 %)	9.9	(+13.2 %)	11.1	(+45.7 %)
Endogeic earthworms	9.2	(-44.9 %)	14.9	(-31.9 %)	37.0	(-4.8 %)
Anecic earthworms	48.0	(-7.0 %)	75.5	(+93.0 %)	60.5	(+3.8 %)
Anecic earthworms adult + juvenile	64.4	(+4.5 %)	85.8	(+74.1 %)	67.7	(+4.9 %)
Total juveniles	28.3	(+41.8 %)	22.5	(+13.0 %)	25.1	(+34.1 %)
Total adults	57.2	(-16.4 %)	90.6	(+48.4 %)	97.5	(+0.4 %)
Total earthworms	86.4	(-4.5 %)	113.6	(+39.2 %)	124.4	(+6.0 %)

* significantly different from control ($p \leq 0.05$)

** negative values indicate decrease in earthworm numbers compared to the control positive values indicate increase in earthworm numbers compared to the control

The following observations on earthworm community after application of 1.8 L DFF+FFA SC 200+400G/ha on sampling dates 35 DDA, 183 DDA and 364 DDA were recorded:

On Day 35 after application of 1.8 L DFF+FFA SC 200+400G/ha, the Tanylobous and *Epibolus* juveniles slightly decreased in numbers (-29.1% and -11%, not significant, respectively), as compared to the control. It should be emphasized, that the reduction in numbers of Tanylobolus juveniles are lower in comparison to these effects observed for application at the rates of 0.6 l/ha and 1.2 l/h a on Day 35.

For the next sampling dates, i.e. 183 and 364 days after application of DFF+FFA SC 200+400G/ha, at a rate of 1.8 l/ha, both juvenile groups increased in numbers (+41.2% and +45.7%, respectively) and biomass (+12.8% and +13.2%, respectively).

The statistically significant increase in numbers (+106%) and biomass (+89%) was observed for *L. terrestris* on Day 183 after application with an abrupt reduction on Day 364 in both number (-4%) and biomass (-3.9%), as compared to the control. This effect may be due to ageing of earthworm population, and may be explained by the fact that Tanylobous juveniles increased simultaneously in numbers (+47.9%) and biomass (+26.2 %, respectively).

On the other hand, the population of *Aporrectodea caliginosa* remained constant throughout the time of the experiment.

For *Aporrectodea rosea*, the increase in numbers was recorded in all sample dates. However, the reduction in biomass (-69.6%, not significant) on day 35 after application was observed.

This species was dominant throughout all the experiment.

For *Allolobophora chlorotica*, on the sampling date 183 days after application of 1.8 L product/ha, a decrease in numbers (-41.7%, not significant) was more clear than on the previous sampling Day 35 (-25%, not significant).

However, it should be pointed out that at the end of the study (on Day 364), an increase in numbers (+14.3%) and biomass (+30%) was observed for this species, as compared to control.

For *Octolasion lacteum*, on all the sampling dates, i.e. 35, 183 and 364 days after application of 1.8 L DFF+FFA SC 200+400G/ha, a decrease in numbers (-68%, -58.5 and -19 %, respectively, not significant) and biomass (-72%, -49% and -32%, respectively, not significant) was observed. It should be noted, that at the end of the study this effect was below 30%, as compared to the control.

In general, taking into account the category: total earthworms, total adults, total juveniles or ecological groups and single species, no statistically significant reductions in biomass and in numbers occurred at any of the post treatment samplings, after application of the test item.

It should be pointed out, that for the category: total adults, total juvenile and total earthworms, despite a slight, insignificant, reduction in numbers and biomass on Day 35 after application, the increase in numbers as compared to the control was noted on the following sampling dates, i.e.: 183 and 364 days after application.

The biomass of earthworms for these categories also increased as compared to control.

However, the effect of reduction in numbers for endogeic species *Octolasion lacteum* in numbers (-68% %, not significant) and biomass (-72.2%, not significant) on Day 35 and on Day 183 after application of DFF+FFA SC 200+400 G at a rate 1.8 L/ha, even though not statistically significant, should be taken into account, because it may be related to the activity of the item.

However, it should be noted that biological significant effects, -19% (reduction in number), -33% (reduction in biomass) could still be observed on the population *Octolasion lacteum* after 364 d at rates of 1.2. and 1.8 L/ha.

Conclusion:

Not statistically significant reduction in numbers and in biomass of total earthworms, total juveniles, total adults and single species occurred at any post treatment samplings after application of the test item at rates of 0.6, 1.2 and 1.8 L/ha, following the plateau application of diflufenican at a rate of 243.77 g a.s/ha.

However, it should be noted that biological significant effects (19-33%) could still be observed on the population *Octolasion lacteum* after 364 d at rates of 1.2. and 1.8 L/ha.

At rate of 0.6 L/ha biological significant but transient effects for this species were observed.

Therefore, NOAER of 0.6 L product/ha (leading to 0.203 mg flufenacet/kg soil dw) was estimated by RMS from the study.

Comments RMS:

The study was performed according to the guidelines BBA, Part VI, 2 - 3 (January 1994), ISO Guideline CD 11268-3 (E), (1999).

To verify the validity criteria of this study, RMS took into consideration the recommendations given in the test Dutch Guidance (Kula et al. 2006).

According to the recommendations given in that last guideline, the following criteria should be considered:

- Earthworm abundance (average) should be > 60 earthworms/m² at test initiation for all treatment groups.

The mean earthworm abundances was 382 earthworms/m² across all plots at the start of the trial.

- Species composition: representative, regional specific earthworm species of different ecological life forms (i.e. endogeics and anecics) have to be present in sufficiently high numbers of at least 10 individuals per m² or at least 10 % of total adult earthworm abundance.

In the study under consideration, several ecological groups of earthworms were observed.

Control plots: - study period - 7 DBA1

The dominant endogeic species at trial start was *Aporrectodea rosea* (58 earthworms/m², 15.1 % of total earthworms, 39.0 % of adult earthworms) followed by *Aporrectodea caliginosa* (41 earthworms/m², 10.8 % of total earthworms, 27.9 % of adult earthworms). The dominant anecic earthworm species was *Lumbricus terrestris* (including juveniles): 23 earthworms/m², 6.1 % of total earthworms, 15.6 % of adult earthworms.

Control plots – study period -35 DDA2

The dominant endogeic species was *Aporrectodea caliginosa* (18 earthworms/m², 10% of total earthworms, 29% of adult earthworms) followed by *Allophobora chlorotica* (10 earthworms/m², 5.6 % of total earthworms, 16.4 % of adult earthworms). The dominant anecic earthworm species was *Lumbricus terrestris* (including juveniles): (18 earthworms/m², 10.1 % of total earthworms, 29.5 % of adult earthworms).

- Toxic reference effect:

Reduction of the earthworm abundance by the toxic reference item: > 50 % in comparison to the control plots for at least one post-treat. The treatment with the reference substance carbendazim at 10,000 g/ha showed strong effects on the earthworm community in comparison to the control, and decreased the abundance of earthworms by 71.1 % at 35 DAA2, 69.4 % at 183 DAA2 and 45.2 % at 364 DAA2, thus confirming the validity of the test system.

The following deviations from the study protocol were noted:

- In soil samples taken after application DFF+FFA 200+400 G , mean residues of 0.007 mg DFF/kg dry soil and 0.015 mg FFA/kg dry soil, were found in the control plots, representing 1.0 % and 2.2 %, respectively, of the concentrations in highest test item treatment group (0.686 mg DFF/kg and 0.670 mg FFA/kg, respectively).

-In addition, it should be noted, that the statistical power of the study effects seem to be quite low and only allow the detection of >100% effects as statistically significant.

In opinion of RMS this deviation has no significant impact of the validity of the study

Thus, the study is considered acceptable.

Agreed endpoint:

NOAER of 0.6 Lproduct/ha (leading to 0.203 mg flufenacet/kg soil dw).

This value is considered appropriate to use in the risk assessment.

B.9.7.2. Effects on non-target soil meso-and macrofauna (other than earthworms)**B.9.7.2.1. Toxicity data**

New studies on other soil macro-organisms (*Hypoaspis aculeifer*, *Folsomia candida*) were submitted with the active substance – flufenacet and with representative formulation DFF+FFA SC 600. Studies with the soil metabolites such as: FOE oxalate, FOE sulfonic acid-Na-salt, FOE methylsulfone, TFA, FOE trifluoroethane sulfonic acid and FOE-Thiadone were conducted addressing the risk soil organisms. Therefore, laboratory studies with the other than earthworms soil macro-organisms *Hypoaspis aculeifer* and *Folsomia candida* were performed. The study summaries for studies with the active substance flufenacet and the soil metabolites are provided in the RAR, Volume 3, B.9 (CA). The study summaries for the studies with representative formulation DFF+FFA SC 600 are given in the Table B.9.7.2.1-1 below.

Table B.9.7.2.1-1: Ecotoxicological endpoints for other soil meso-and macrofauna exposed to flufenacet and its metabolites.

Test substance	Test species	Endpoint	Reference
Flufenacet	<i>Folsomia candida</i>	NOEC=31.5* mg a.s./kg dws	Frommholz (2010) M-394712-01-1
	<i>Hypoaspis aculeifer</i>	NOEC=281* mg a.s./kg dws	Kratz (2013) M-455214-01-1
FOE oxalae	<i>Folsomia candida</i>	NOEC ≥ 100 mg p.m./kg dws	Frommholz (2010) M-394712-01-1
	<i>Hypoaspis aculeifer</i>	NOEC ≥ 100 mg p.m./kg dws	Kratz (2010) M-393634-01-1
FOE sulfonic acid-Na-salt	<i>Folsomia candida</i>	NOEC ≥ 100 mg p.m./kg dws	Frommholz (2010) M-396039-01-1
	<i>Hypoaspis aculeifer</i>	NOEC ≥ 100 mg p.m./kg dws	Kratz (2013) M-455654-01-1
FOE methylsulfone	<i>Folsomia candida</i>	NOEC ≥ 100 mg p.m./kg dws	Frommholz (2010) M-392345-01-1
	<i>Hypoaspis aculeifer</i>	NOEC= 500 mg p.m./kg dws	Kratz (2009) M-357707-01-1
TFA	<i>Folsomia candida</i>	NOEC ≥ 100 mg p.m./kg dws	Frommholz (2012) M-436127-01-1
	<i>Hypoaspis aculeifer</i>	NOEC ≥ 100 mg p.m./kg dws	Kratz (2012) M-436326-01-1
FOE 5043-trifluoroethane sulfonic acid	<i>Folsomia candida</i>	NOEC ≥ 100 mg p.m./kg dws	Frommholz (2012) M-436128-01-1
	<i>Hypoaspis aculeifer</i>	NOEC ≥ 100 mg p.m./kg dws	Kratz (2012) M-436315-01-1
FOE-Thiadone	<i>Folsomia candida</i>	NOEC=1.8 mg p.m./kg dws	Frommholz (2012) M-440372-01-1
	<i>Hypoaspis aculeifer</i>	NOEC=32 mg p.m./kg dws EC ₁₀ =28 mg p.m./kg dws	Kratz (2012) M-442897-01-1

* Endpoints corrected to allow for log P_{ow} > 2
pm - pure metabolite

Table B.9.7.2.1-2: Ecotoxicological endpoints for other soil meso-and macrofauna exposed to DFF + FFA SC 600.

Test substance	Test species	Endpoint	Reference
DFF + FFA SC 600	<i>Folsomia candida</i>	NOEC _{corr} =89 mg product/kg* dws	Frommholz (2011) M-415903-01-1
	<i>Hypoaspis aculeifer</i>	NOEC ≥65.3 mg prod/kg dws ¹	Feije (2002) M-061660-04-1

* Endpoints corrected to allow for log P_{ow} > 2

¹⁾ The factor of 2 for the test of natural soil was not considered relevant as the LUFA soils contains only 2% organic matter, which is considered to be more respective of natural soil condition.

9.7.2.1.1. Diflufenican + flufenacet SC 600 (200+400) G: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.

Reference:	Diflufenican + flufenacet SC 600 (200+400) G: Influence on the reproduction of the collembolan species <i>Folsomia candida</i> tested in artificial soil.
Author(s), year:	Frommholz U., 2011
Report/Doc. number:	Study No: E 314 4124-9, Report No: FRM-Coll-125/11, Reference BCS N: M-415903-01-1
Guideline(s):	OECD 232 adopted, September 07, 2009.
GLP:	Yes

<u>Test substance</u>	Diflufenican + Flufenacet SC 600 (200+400) G, Batch No: EV56002670, 16.4 % w/w diflufenican (AE F088657) equivalent to 203.8 g/L 32.7 % w/w flufenacet (FOE 5043) equivalent to 407.5 g/L (analyzed).
Density:	1.246 g/mL
Test species:	<i>Collembola Folsomia candida</i>
Number of organism:	8 replicates per control group and 4 replicates per treatment group, each with 10 individuals. Additional 2 replicates per treatment and control to check the pH and water content of the test substrate after 28 days.
Life stage, age:	Juveniles/Adult, 9-12 days old
Type of test, duration	Laboratory sub-lethal limit test, 28 days
<u>Applied concentrations:</u>	
Nominal:	0 (control), 100, 178, 316, 562, 1000 mg test substance/kg soil dw
Toxic standard	Boric acid tested at concentrations of 44, 67, 100, 150 and 225 mg Boric acid /kg soil dw.
Test substrate	Artificial soil, 5 % sphagnum peat, air dried and finely ground, 20 % kaolin clay, 74.8 % fine quartz sand , 0.2% calcium carbonate (for the adjustment to pH to 6 ±0.5).

Substrate/Test vessel	<p>Test containers were glass vessels (volume 140 ml, diameter 5 cm at the bottom, height 7 cm).</p> <p>Each test vessel contained 30 g wet weight artificial soil.</p> <p>The test vessels were covered with glass lids to prevent the collembolans from escaping but allowing aeration during the test period.</p>
Temperature	20±2 °C
Light regime:	16 hours light/8 hours dark. Light intensity: 573-727 Lux
Water content:	<p>Test start: 19.52-20.02% (equivalent to 47.88-49.40 % of WHC)</p> <p>Test end: 19.37-20.19% (equivalent to 47.42-49.91% of WHC)</p>
pH:	<p>Test start: 5.64 -5.68</p> <p>Test end: 5.62-5.72</p>
Feeding:	<p>Directly after the addition of the collembolans, they were fed with granulated dry yeast. Feeding was also done 14 days after test start.</p> <p>Approximately 2 mg (one spatula tip) per test vessel was added per feeding date.</p>
Test parameters:	<p>pH and water content were determined at test start and test end.</p> <p>Water content maintenance was checked two weeks after application.</p> <p>Mortality of adults, behavioral effects and number of juvenile Collembola were assessed after 28 days.</p>
Statistic:	<p>Data of reproduction were tested for normal distribution and homogeneity of variance using Kolmogorov - Smirnov-Test and Cochran's-Test ($\alpha = 0.05$) respectively. Data of reproduction were normally distributed and homogeneity of variances was given.</p> <p>Therefore William's-t test (one-sided-smaller, $\alpha = 0.05$) was used to determine NOEC and LOEC values. Mortality was calculated by Probit analysis. The software used to perform the statistical analysis was ToxRat Professional 2.10.</p>

Findings:

Effects on mortality and reproduction of *Folsomia candida* in sub-chronic test are presented in the Table B 9.7.2.1.1-1 below.

Table B 9.7.2.1.1-1: Effects on mortality and reproduction of Folsomia candida in a sub-chronic test.

Exposure	Diflufenican + Flufenacet SC 600 (mg test item/ kg soil dw)					
	Control	100	178	316	562	1000
Mortality of adult [%] after 14 days	5	7.5	7.5	30	27.5	42.5
Mean number of offspring per test vessel after 14 days (±SD)	1539.3 (±117.0)	1566.0 (±110.1)	1490.0 (±123.3)	1228.0 (±160.7)	335.3 (±87.6)	155 (±59.3)
% reproduction compared to control		101.7	96.8	79.8*	21.8*	10.1*
EC ₁₀ , EC ₂₀ , EC ₅₀ (mg test item/kg soil dw)		Adult mortality (mg test item/kg soil dw)				Reproduction mg test item/kg dws
EC ₁₀		222				261
EC ₂₀		411				309
EC ₅₀		1338				428

*Statistically significant compared to the control (William's T-test, one-sided-smaller, $\alpha=0.05$)

Toxic standard

The most recent test with the reference item Boric acid showed an EC₅₀ of 91 mg test item/kg soil dw for reproduction. The NOEC_{reproduction} was calculated to be 44 mg Boric acid/kg soil dw and the LOEC_{reproduction} was 7 mg Boric acid/kg soil dw.

Conclusions:

NOEC_{reproduction}: 178 mg test item/kg soil dw

LOEC_{reproduction}: 316 mg test item/kg soil dw

LC₁₀ (adult mortality): 222 mg test item/kg soil dw (95 % CI: 20 – 310 mg test item/kg soil dw)

LC₂₀ (adult mortality): 411 mg test item/kg soil dw (95 % CI: 290 – 566 mg test item/kg soil dw)

LC₅₀ (adult mortality): 1338 mg test item/kg soil dw (95 % CI: 881 – 3211 mg test item/kg soil dw)

EC₁₀ reproduction: 261 mg test item/kg soil dw (95 % CI: 148 – 322 mg test item/kg soil dw)

EC₂₀ reproduction: 309 mg test item/kg soil dw (95 % CI: 206 – 367 mg test item/kg soil dw)

EC₅₀ reproduction: 428 mg test item/kg soil dw (95 % CI: 357 – 511 mg test item/kg soil dw)

Comments RMS:

The Collembola reproduction study was conducted according to the OECD test guideline 232 (2009).

Based on the validity criteria stated in OECD test guideline 232 (2009) the study was considered acceptable.

The mean mortality of the adults in the control was below 20 % (being 5%).

The mean number of juveniles per control replicate was greater than 100 (being 1539.3 per replicate).

The coefficient of variation of reproduction in the control was < 30 (being 7.6%).

The agreed endpoints:

NOEC_{reproduction} = 178 mg test item/kg soil dw

LOEC_{reproduction} = 316 mg test item/kg soil dw

LC₁₀ (adult mortality) = 222 mg test item/kg soil dw (95 % CI: 20 – 310 mg test item/kg soil dw)

LC₂₀ (adult mortality) = 411 mg test item/kg soil dw (95 % CI: 290 – 566 mg test item/kg soil dw)

LC₅₀ (adult mortality) = 1338 mg test item/kg soil dw (95 % CI: 881 – 3211 mg test item/kg l soil dw)

EC₁₀ reproduction = 261 mg test item/kg soil dw

EC₂₀ reproduction = 309 mg test item/kg soil dw (95 % CI: 206 – 367 mg test item/kg soil dw)

EC₅₀reproduction = 428 mg test item/kg soil dw (95 % CI: 357 – 511 mg test item/kg soil dw)

B. 9.7.2.1.2. Flufenacet & Diflufenican SC 600: The effects on survival and reproduction of the predaceous mite *Hypoaspis aculeifer* Canestrini (Acari: Laelapidae) in standard soil (LUFA 2.1).

Reference:	Flufenacet & Diflufenican SC 600: The effects on survival and reproduction of the predaceous mite <i>Hypoaspis aculeifer</i> Canestrini(Acari: Laelapidae) in standard soil (LUFA 2.1).
Author(s), year:	Feije, R 2002
Report/Doc. number:	Study No: B094HAE, Reference BCS No: M-061660-01-1
Guideline(s):	SECOFASE, Final Report. Development, improvement and standardization of test systems for assessing sub-lethal effects of chemicals on fauna in the soil ecosystem (Løkke & van Gestel 1996). Guidance document on regulatory testing procedures for pesticides with non-target arthropods (Barrett et al. 1994)
GLP:	Yes

Test substance:	Flufenacet & Diflufenican SC 600, Batch No.: 07205/0024 0006, active substances: FOE 5043 and Diflufenican, 32.6% and 16.5 % respectively: Total active substances: 612.28 g /L. Density=1.247 g/mL
Test species:	Predatory mites, <i>Hypoaspis aculeifer</i>
Number of organism:	4 replicates for each concentrations, 5 for control and 3 for toxic reference, each with 10 individuals
Life stage, age:	Protonymphs (maximum 2 days old)
Type of test, duration:	Laboratory survival and reproduction test, 28-29 days
Applied concentrations:	0 (control, water) 0.0435, 0.0760, 0.135, 0.245, 0.435 g product/L test solution equivalent to 0 (control) 3.2, 5.6, 10, 18 and 32 mg active substances (DFF+FFA)/kg soil dw, based on test item containing 612.28 g s.a./L
	Toxic standard: Dimethoate tested at rate 4.50 mg a.s./kg soil dw.

Test substrate:	Standard soil: LUFA 2.1 Corg: 0.9% pH =5.2 CEC : 6 mval/100 g WHC: 30 g/100g Soil texture According to USDA: clay: 3.8%, silt: 9.9% and 87.2% sand Soil type: sand
Bioassay procedure:	<p>The bioassay was initiated within 1 hour after application by confining 20 protonymphs of <i>Hypoaspis aculeifer</i> per mortality unit (inert glass material).</p> <p>Fourteen days after initiation mortality was assessed by counting the live males/females/juveniles and corpses, and the occurrence of offspring (eggs and/or new juveniles). Reproductive success was determined for mites of the deionised water control and the 2 highest test rates below the expected LR_{50} (viz. 18 and 32 mg a.s./kg dry soil). Hereto all surviving mites of these treatments were transferred to untreated mating units (keeping replicate groups together). After a 7-day mating period 20 females, of the 18 and 32 mg a.s./kg dry soil-treatment and the water treatment, were transferred to reproduction units (1 mite/unit) to determine egg production. After 3 days all females were transferred to a second series of identical reproduction units and 4 days later the females were removed. In this way there were two oviposition assessments in a 7-day period.</p> <p>Reproduction units were kept for egg hatch determination for an additional 4-5 days.</p>
Feeding:	<p>Feeding was done before test and, 4, 8, and 10 days after test start (mortality phase) and in the following occasions: before mating phase and four days after and during reproduction phase (two oviposition period).</p> <p>The predatory mites were fed cheese mites (<i>Tyrophagus putrescentiae</i>).</p>
<u>Test condition</u>	
Temperature:	$25 \pm 2^{\circ}\text{C}$
Relative humidity:	$65 \pm 15\%$ in the dark Light intensity: 0.02 Lux measured at the start of the mortality phase
Test parameters:	<p>Mortality was assessed after 14 day exposure.</p> <p>Reproduction over 7 day period for surviving females in the demonized water control and two highest test item rates was recorded.</p> <p>For both series of oviposition units, juveniles and eggs were counted 4-5 days after removal of the females.</p>
Statistic:	Mortality data were analysed with Fisher's Exact test. The relationship between (corrected) mortality and application rate, as well as the calculation of LR_{50} -values was established through Probit analysis.

The mean numbers of fertile eggs (cumulative totals per female, per 7 days) were compared among treatment groups. The hypothesis that treatment means are not different from the control was tested using ANOVA and multiple comparison procedures. ANOVA assumptions concerning normality of residuals and homogeneity of variances were tested using Lilliefors1 test and Bartlett's test, respectively.

Findings:

The mortality of all test rates of the Flufenacet & Diflufenican SC 600 treatment, 3.2, 5.6, 10, 18 and 32 mg s.a./kg dry soil, were not statistically significantly different from the mortality of control. Reproduction was assessed in Flufenacet & Diflufenican SC 600 treatment groups 18 and 32 mg s.a./kg dry soil. Reproduction in these groups was 24.0 and 24.4 fertile eggs per female, respectively. Relative to the control this was 99.1% and 100.6%, respectively. This was not statistically significantly different from the control performance.

Effects on mortality and the number of offspring per test vessel are shown in the table below.

Table B 9.7.2.1.2-1: Effects on mortality and reproduction of *Hypoaspis aculeifer* in a sub-chronic test.

Test item	Flufenacet & Diflufenican SC 600			
Exposure (mg Flufenacet + mg Diflufenican/ kg soil dw)	Mortality after 14 days (%)		Reproduction (fertile eggs/female/7 days)	
Control (water) (±SD)	10		24.2 (±3.5)	
	Corrected mortality after 14 days		Reproduction after 7 days (% reduction compared to control)	
3.2 mg a.s./kg soil dw (2.12 mg flufenacet/kg soil dw)	-3 %	P= 0.608	Not assessed	
5.6 mg a.s./kg soil dw (3.72 mg flufenacet/kg soil dw)	3 %	P= 0.639	Not assessed	
10 mg a.s./kg soil dw (6.64 mg flufenacet/kg soil dw)	1 %	P= 0.811	Not assessed	
18 mg a.s./kg soil dw (11.95 mg flufenacet/kg soil dw)	10 %	P= 0.128	24.0 (99.1 %)	P= 0.843
32 mg a.s./kg soil dw (21.24 mg flufenacet/kg dw soil)	1 %	P= 0.811	24.4 (100.6 %)	P= 0.898
Toxic reference	100 %	P <0.001*	Not assessed	
	LR ₅₀ > 32 mg a.s./kg soil dw (21.24 mg flufenacet/kg soil dw)		NOEC ≥32 mg a.s./kg soil dw (21.24 mg flufenacet/kg sdw)	

*Statistically significant compared to control
(Fisher's Exact test for mortality, ANOVA/Fisher's LSD test for reproduction).
a.s-total active substances

In most recent test with the reference item Perfection (a.s. dimethoate) tested in separately report, applied at concentrations of 4.50 mg Dimethoate/kg soil dw, dimethoate showed a 100% of mortality of adult *Hypoaspis aculifer*.

Conclusion:

LR₅₀ > 32 mg s.a./kg dry soil (equivalent to 21.24 mg flufenacet/kg soil dw)

NOEC ≥ 32 mg s.a./kg dry soil (equivalent to 21.24 mg flufenacet/kg soil dw)

Comments RMS:

Predatory mite production study was conducted according to the SECOFASE, Final Report And G, Barret at 1994). Taking into consideration the validity criteria stated in that guideline the study is considered acceptable.

The control mortality is below 25% (being 10%),

The mean reproduction control was ≥ 10 eggs/female/7 days (being 24.4 fertile eggs/female/7 days)

The mean corrected mortality in the toxic reference was 100%.

Taking into account, the current valid OECD test guideline 232 (July 2006) the following validity criteria are given:

The mean mortality of the adults in the control were below 20 % (being 10% per replicate)

The mean number of juveniles per control replicate was greater than 100.

The coefficient of variation of reproduction in the control were < 30 (being 14%).

However, the test design differs from the methodology recommended for *Hypoaspis aculifer* in OECD 232, test guideline at the following areas:

- The test lasts 7 day instead of 14 days
- The soil test used is different than recommended by the test guideline OECD 232.

However, in opinion of RMS, since there were no effects at the top dose (the reproduction 100.6% of control and mortality only 1%) it is not expected significant effects to appear if the study will extend over another 7 days. In addition, it should be pointed out that that the NOEC wasn't just no statistically significant effect but no effect at all.

Therefore, the study is considered acceptable.

Agreed endpoints:

LR₅₀ > 32 mg s.a./kg dry soil (equivalent to 21.24 mg flufenacet/kg soil dw), corresponding to > 65.30 mg product/ha

NOEC ≥ 32 mg s.a./kg dry soil (equivalent to 21.24 mg flufenacet/kg soil dw), corresponding to 65.30 mg product/ha

B.9.8. RISK ASSESSMENT FOR NON-TARGET SOIL MESO-AND MACROFAUNA

B.9.8.1. Risk assessment for earthworms

The risk assessment for effects on earthworms is conducted according to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002 rev. 2).

Exposure:

The exposure of soil organisms to flufenacet and its metabolites was estimated by calculating the maximum Predicted Environmental Concentrations in soil (PEC_{SOIL}) (please refer to Volume 3 (CA), B.8.).

The maximum PEC_{SOIL} of the formulation DFF+FFA SC 600, flufenacet and its metabolites in soil have been assessed using the FOCUS groundwater crop interception values (FOCUS 2011) and the maximum DT₅₀ values.

Since DT₅₀ values for three metabolites of flufenacet: FOE sulfonic acid-Na-salt, FOE-methylsulfone and TFA, are >100 days, potential for accumulation in soil was also taken into account by calculation of plateau concentrations. Plateau PEC_{soil} values were summed up with maximum initial PEC_{soil} values in order to obtain PEC_{soil,accu}, recommended for long-term risk assessment.

Based on the recommended uses rate of 1 x 240 g s.a./ha, 1 x 160 g a.s./ha and 1 x 120 g a.s./ha, an as spray application in winter cereals, the max PEC_{SOIL} values are presented in Table B.9.8.1-1.

Table B.9.8.1-1: Maximum Predicted Environmental Concentration of the compounds in soil after application of the DFF + FFA SC 600 formulation to cereals.

Compound	Winter cereals 1x240 g a.s./ha BBCH 10-13, CI= 0%		Winter cereals 1x160 g a.s./ha BBCH 11-13, 0% CI=0%		Winter cereals 1x120 g a.s./ha BBCH 00-22, 0% CI=0%	
	PECs ¹ (mg/kg)	PEC _{acc.} ² (mg/kg)	PECs ¹ (mg/kg)	PEC _{acc.} ² (mg/kg)	PECs ¹ (mg/kg)	PEC _{acc.} ² (mg/kg)
Flufenacet	0.3200	0.3210	0.2133	0.2140	0.1600	0.1605
FOE oxalate	0.0186	0.0187	0.0124	0.0125	0.0093	0.0094
FOE sulfonic acid-Na-salt	0.0452	0.0574	0.0302	0.0383	0.0226	0.0287
FOE methylsulfone	0.0134	0.0150	0.0089	0.010	0.0067	0.0075
TFA	0.0888	0.6184	0.0592	0.4122	0.0444	0.3092
FOE 5043-trifluoroethane sulfonic acid	0.0160	0.0160	0.0107	0.0107	0.0080	0.0080
FOE-Thiadone	0.0236	0.0237	0.0157	0.0157	0.0118	0.0119
DFF + FFA SC 600*	1		0.667		0.500	

¹ PECs

² PEC_{acc}

In bold

*PEC

PEC_{soil} actual

Accumulated PEC_{soil}

PECs used in the risk assessment

formulation, mg product/kg dws, density=1.251 g/mL

For substances with log Pow >2, the endpoints (LC₅₀ and the NOEC/EC₁₀) should be divided by a factor of 2 irrespective of the peat content in the study according to recommendations given during the EFSA expert meeting (PRAS 91, April 2012) and these corrected endpoints should be used in the risk assessments. Log Pow values of flufenacet and its soil metabolites are presented in the Table B.9.8.1-2 below:

Table B.9.8.1-2: Log Pow values for flufenacet and its metabolites.

Substance	log P _{ow}
Flufenacet	3.5
FOE oxalate (M01)	0.80
	pH-dependent
	-2.0 (pH 5)
	-2.2 (pH 7)
FOE sulfonic acid (M02)	-2.4 (pH 9)
	Not pH-dependent
FOE methylsulfide (M05)	-2.72
	2.6 (pH 5)
	2.6 (pH 7)
	2.6 (pH 9)
FOE methylsulfone (M07)	1.7 (pH 5)
	1.7 (pH 7)
	1.7 (pH 9)
FOE-thiadone (M09)	pH-dependent
	1.92 (pH 4.3)
	0.62 (pH 7)
	-0.90 (pH 9.4)
FOE 5043-trifluoroethanesulfonic acid (M44)	pH-dependent
	-3.0 (pH 5)
	-2.95 (pH 7)
	-3.16 (pH 9)
trifluoroacetic acid (TFA) (M45)	pH-dependent
	-2.5 (pH 5)
	-2.6 (pH 7)
	-2.8 (pH 9)

*Section 2.7 “Partition coefficient n-octanol/water” in the CA

Toxicity-exposure ratio

The long-term toxicity exposure ratios (TER_{LT}) were calculated according to the following equation:

$$TER_{LT} = \frac{NOEC}{Maximum\ PEC_{soil}}$$

Since the log P_{OW} of a.s.-flufenacet exceeds the trigger value of 2 (a log P_{OW} value of 3.5), the toxicity endpoints were corrected by a factor 2.

Calculation of TER values

In Tables B.9.8.1-3 to B.9.8.3.1-5 maximum PEC_s values for flufenacet, representative formulation DDF + FFA SC 600 and flufenacet soil metabolites are compared to the chronic toxicity data to derive TERs.

Table B.9.8.1-3: Chronic risk (TER_{LT}) to earthworms for flufenacet and metabolites, formulation based on max PEC_{SOIL} values, for the intended uses in cereals.

Species	Test substance	Endpoint	max PEC _{SOIL} (mg/kg)	TER _{LT}	Trigger
Winter cereals, 1 x 120 ga.s./ha					
Eisenia fetida	DFF+FFA SC 600	NOEC 1.3 * mg form./kg sdw	0.500 mg product/kg dws	2.6	≥5
	Flufenacet (DFF+FFA SC 600)	NOAER 0.203 mg a.s./kg s dw (measured value)	0.1600	1.26	≥1
	FOE oxalate	NOEC ≥100 mg p.m./kg sdw	0.0093	10752.68	≥5
	FOE sulfonic acid-Na-salt	NOEC 500 mg p.m./ kg sdw	0.0287 ¹	17421.60	≥5
	FOE methylsulfone	NOEC 125 mg p.m./kg sdw	0.0075 ¹	16666.66	≥5
	TFA	NOEC 320 mg p.m./kg sdw	0.3092 ¹	1034.92	≥5
	FOE 5043-trifluoroethane sulfonic acid	NOEC 100 mg p.m./kg sdw	0.0080	12500	≥5
	FOE-Thiadone	NOEC 3.2 mg p.m./kg sdw	0.0118	271.18	≥5

* Endpoints corrected to allow for log Pow > 2

¹ Accumulated PEC_{soil}

pm-pure metabolite

a.s.-active substance -flufenacet

in bold value not achieved the trigger

Table B.9.8.1-4: Chronic risk (TER_{LT}) to earthworms for flufenacet and metabolites, formulation based on max PEC_{SOIL} values, for the intended uses in cereals.

Species	Test substance	Endpoint	max PEC _{SOIL} (mg/kg)	TER _{LT}	Trigger
Winter cereals, 1 x 160 g a.s./ha					
Eisenia fetida	DFF+FFA SC 600	NOEC 1.3* mg form./kg s dw	0.667 mg product/kg s dw	1.94	≥5
	Flufenacet (DFF+FFA SC 600)	NOAER 0.203 mg a.s./kg s dw	0.2133	0.95	≥1
	FOE oxalate	NOEC ≥100 mg p.m./kg sdw	0.0124	8064.51	≥5
	FOE sulfonic acid-Na-salt	NOEC 500 mg p.m./kg sdw	0.0383 ¹	13054.83	≥5
	FOE methylsulfone	NOEC 125 mg p.m./kg sdw	0.010 ¹	12500	≥5
	TFA	NOEC 320 mg p.m./kg sdw	0.4122 ¹	776.32	
	FOE 5043-trifluoroethane sulfonic acid	NOEC 100 mg p.m./kg s dw	0.0107	9345.79	≥5
	FOE-Thiadone	NOEC 3.2 mg p.m./ kg sdw	0.0157	203.82	≥5

* Endpoints corrected to allow for log Pow > 2

¹ Accumulated PEC_{soil}

pm-pure metabolite

a.s.-active substance -flufenacet

in bold value not achieved the trigger

Table B.9.8.1-5: Chronic risk (TER_{LT}) to earthworms for flufenacet and metabolites, formulation based on max PEC_{SOIL} values, for the intended uses in cereals.

Species	Test substance	Endpoint	max PEC _{SOIL} (mg/kg)-	TER _{LT}	Trigger
Winter cereals, 1 x 240 ga.s./ha					
Eisenia fetida	DFF+FFA SC 600	NOEC 1.3* mg form./kg s dw	1.000	1.3	≥5
	Flufenacet (DFF+FFA SC 600)	NOAER 0.203 mg a.s./kg s dw	0.320	0.63	≥1
	FOE oxalate	NOEC ≥100 mg p.m./kg sdw	0.0186	5376.34	≥5
	FOE sulfonic acid-Na-salt	NOEC 500 mg p.m./ kg sdw	0.0574 ¹	8710.80	≥5
	FOE-methylsulfone	NOEC 125 mg p.m./ kg sdw	0.0150 ¹	8333.33	≥5
	TFA	NOEC 320 mg p.m./ kg sdw	0.6184 ¹	517.46	≥5
	FOE 5043-trifluoroethane sulfonic acid	NOEC 100 mg p.m./ kg sdw	0.0160	6250	≥5
	FOE-Thiadone	NOEC 3.2 mg p.m./ kg sdw	0.0236	135.59	≥5

* Endpoints corrected to allow for log Pow > 2

¹ Accumulated PEC_{soil}

pm-pure metabolite

a.s.-active substance -flufenacet

in bold value not achieved the trigger

For all metabolites of flufenacet the TER_{LT} values exceed the critical trigger value of 5 indicating low risk for earthworm. The critical trigger value of 5 is not passed for all proposed uses of DFF+FFA SC 600 indicating a potential risk of the mixture for earthworm populations.

Due to the fact that the endpoint for flufenacet obtained from laboratory study was not considered valid (see Kratz 1997, Vol. 3, B9 CA) the risk assessment for that compound was based on the NOAER = 0.203 mg Flufenacet/kg soil dw (measured value), determined in the field study for the representative formulation DFF+FFA SC 600, applied in amount 0.6 L/ha.

A one-year earthworm field study with the representative formulation DFF+FFA SC 600 was conducted in Southern under field conditions after one autumn application of Diflufenican SC 500A on bare soil at a rate of 243.75 g diflufenican/ha (application 1) on followed by once application of DFF+ FFA SC 200+400 G (diflufenican+flufenacet, application 2): at different rates (0.6 L product/ha, 1.2 L product/ha and 1.8 L product/ha. The control plots were sprayed once with tap water, the toxic reference item plots were treated with Twist WP® at rate 17152.66 g product/ha (equivalent to 10000 g a.s. carbendazim/ha).

Not statistically significant reduction in numbers and in biomass of total earthworms, total juveniles, total adults and single species occurred at any post treatment samplings after application of the test item at rates of 0.6, 1.2 and 1.8 L/ha following the plateau application of diflufenican at a rate of 243.77 g a.s./ha.

However, biological significant effects (19-33%) could still be observed on the population *Octolasion lacteum* after 364 d at rates of 1.2 and 1.8 L product/ha. At rate of 0.6 L product/ha biological significant but transient effects for this species were observed.

Based on all the study results it was concluded that there was no long term adverse effects from maximum application of 0.6 L DFF+FFA SC 600 /ha (leading 0.203 mg flufenacet/kg soil dw) for population of earthworms. Therefore, for the application rates resulting in concentrations higher than 0.203 mg flufenacet/kg dw, corresponding to the field application rate of the representative formulation of 0.6L/ha, the long-term risk for earthworms cannot be considered acceptable.

B. 9.8.2. Risk assessment for non-target soil meso- and macrofauna (other than earthworms)

As for earthworms, the risk assessment for effects on non-target soil meso- and macrofauna is conducted according to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002 rev. 2).

Exposure

The exposure of soil organisms to flufenacet and its metabolites was estimated by calculating the maximum Predicted Environmental Concentrations in soil (PEC_{SOIL}) (see Volume 3 (CA), Section B.8).

The maximum PEC_{SOIL} of the formulation DFF+FFA SC 600, flufenacet and its metabolites in soil (initial PEC_{SOIL}) have been assessed using the FOCUS groundwater crop interception values (FOCUS 2011) and the maximum DT₅₀ values. Since DT₅₀ values for three metabolites - FOE sulfonic acid-Na-salt, FOE-methylsulfone and TFA, are >100 days, potential for accumulation in soil was also taken into account by calculation of plateau concentrations. Plateau PEC_{soil} values were summed up with maximum initial PEC_{soil} values in order to obtain PEC_{soil,accu}, recommended for long-term risk assessment.

Based on the recommended uses rate of 1 x 240 g a.s. /ha, 1 x 160 g a.s./ha and 1 x 120 g a.s./ha, an as spray application in winter cereals, the max PEC_{SOIL} values are presented in Table B.9.8.2-1.

Table B. 9.8.2-1: Maximum Predicted Environmental Concentration of the compounds in soil after application of the DFF + FFA SC 600 formulation to cereals.

Compound	Winter cereals 1x240 g a.s./ha BBCH 10-13, CI= 0%		Winter cereals 1x160 g a.s./ha BBCH 11-13, 0% CI=0%		Winter cereals 1x120 g a.s./ha BBCH 00-22, CI=0%	
	PECs ¹ (mg/kg)	PEC _{acc.} ² (mg/kg)	PECs ¹ (mg/kg)	PEC _{acc.} ² (mg/kg)	PECs ¹ (mg/kg)	PEC _{acc.} ² (mg/kg)
Flufenacet	0.3200	0.3210	0.2133	0.2140	0.1600	0.1605
FOE oxalate	0.0186	0.0187	0.0124	0.0125	0.0093	0.0094
FOE sulfonic acid-Na-salt	0.0452	0.0574	0.0302	0.0383	0.0226	0.0287
FOE methylsulfone	0.0134	0.0150	0.0089	0.010	0.0067	0.0075
TFA	0.0888	0.6184	0.0592	0.4122	0.0444	0.3092
FOE 5043 trifluoroethane sulfonic acid	0.0160	0.0160	0.0107	0.0107	0.0080	0.0080
FOE-Thiadone	0.0236	0.0237	0.0157	0.0157	0.0118	0.0119
DFF + FFA SC 600*	1.00		0.667		0.500	

¹ PECsPEC_{soil} actual² PEC_{acc.}Accumulated PEC_{soil}*PECs formulation, mg product/kg soil, density: 1.251 g/mL
In bold PECs used in the risk assessment

For substances with log Pow >2, the endpoints (LC₅₀ and the NOEC/EC₁₀) should be divided by a factor of 2 irrespective of the peat content in the study according to recommendations given during the EFSA expert meeting (PRAS 91, April 2012) and these corrected endpoints should be used in the risk assessments. Log Pow values of flufenacet and its soil metabolites are presented in the Table B.9.8.2-2 below:

Table B.9.8.2-2: Log Pow values for flufenacet and its metabolites.

Substance	log P _{ow}
Flufenacet	3.5
FOE oxalate (M01)	0.80
	pH-dependent
	-2.0 (pH 5)
	-2.2 (pH 7)
FOE sulfonic acid (M02)	-2.4 (pH 9)
	Not pH-dependent
FOE methylsulfide (M05)	- 2.72
	2.6 (pH 5)
	2.6 (pH 7)
	2.6 (pH 9)
FOE methylsulfone (M07)	1.7 (pH 5)
	1.7 (pH 7)
	1.7 (pH 9)
FOE-thiadone (M09)	pH-dependent
	1.92 (pH 4.3)
	0.62 (pH 7)
	- 0.90 (pH 9.4)
FOE 5043-trifluoroethanesulfonic acid (M44)	pH-dependent
	-3.0 (pH 5)
	-2.95 (pH 7)
	-3.16 (pH 9)

trifluoroacetic acid (TFA) (M45)	pH-dependent -2.5 (pH 5) -2.6 (pH 7) -2.8 (pH 9)
----------------------------------	---

*Section 2.7 "Partition coefficient n-octanol/water" in the CA

Toxicity-exposure ratio

As only chronic toxicity endpoints for non-target soil meso- and macrofauna are available, only long-term toxicity exposure ratios (TER_{LT}) were calculated according to the following equation:

$$TER_{LT} = \frac{NOEC}{Maximum\ PEC_{soil}}$$

In Tables B.9.8.2-3 to B.9.8.2-5 maximum PEC_{soil} values for flufenacet, DDF + FFA SC 600 and flufenacet soil metabolites are compared to the chronic toxicity data to derive TERs.

Table B.9.8.2-3: Chronic risk (TER_{LT}) to non-target soil macro-organisms for representative formulation DFF+FFA SC 600, flufenacet and its metabolites based on max PEC_{soil} values, for the intended uses in cereals.

Test substance	Test organism	Endpoint	max PEC _{soil} (mg/kg) ¹	TER _{LT}	Trigger
Winter cereals, 1 x 120 ga.s./ha					
DFF+FFA SC 600	Folsomia candida	NOEC 89 mg form./kg dws*	0.500	178.0	5
	Hypoaspis aculeifer	NOEC ≥65.30 mg form./kg dws **	0.500	130.60	
Flufenacet	Folsomia candida	NOEC 31.5 mg a.s./kg dws*	0.160	196.87	
	Hypoaspis aculeifer	NOEC 281 mg a.s./kg dws*		1756.25	
FOE oxalate	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0093	10752.68	
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws		10752.68	
FOE sulfonic acid-Na-salt	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0287 ¹	3484.32	
	Hypoaspis aculeifer	NOEC ≥100 mg p.m./kg dws		3484.32	
FOE methylsulfone	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0075 ¹	13333.33	
	Hypoaspis aculeifer	NOEC 500 mg p.m./kg dws		66666.66	
TFA	Folsomia candida	NOEC ≥ 100 mg p.m./kg dws	0.3092 ¹	323.41	
	Hypoaspis aculeifer	NOEC ≥100 mg p.m./kg dws		323.41	
FOE 5043-trifluoroethane sulfonic acid	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0080	12500	
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws		12500	
FOE-Thiadone	Folsomia candida	NOEC 1.8 mg p.m./kg dws	0.0118	152.54	
	Hypoaspis aculeifer	EC ₁₀ 28 mg p.m./kg dws		237.28	

* Endpoints corrected to allow for log P_{ow} > 2

** The factor of 2 for the test of natural soil was not considered relevant as the LUFA soils contains only 2% organic matter, which is considered to be more representative of natural soil condition.

¹ Accumulated PEC_{soil}

Table B.9.8.2-4: Chronic risk (TER_{LT}) to non-target soil macro-organisms for representative formulation DFF+FFA SC 600, flufenacet and its metabolites based on max PEC_{SOIL} values, for the intended uses in cereals.

Test substance	Test organism	Endpoint	max PEC _{SOIL} (mg/kg)	TER _{LT}	Trigger
Winter cereals, 1 x 160 ga.s./ha					
DFF+FFA SC 600	Folsomia candida	NOEC 89 mg form. /kg dws*	0.667	133.43	5
	Hypoaspis aculeifer	NOEC ≥ 65.30 mg form./kg dws**	0.667	97.90	
Flufenacet	Folsomia candida	NOEC 31.5 mg a.s./kg dws*	0.2133	147.67	
	Hypoaspis aculeifer	NOEC 281 mg a.s./kg dws*		1317.39	
FOE-oxalate	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0124	8064.51	
	Hypoaspis aculeifer	NOEC ≥100 mg p.m./kg dws		8064.51	
FOE sulfonic acid-Na-salt	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0383 ¹	2610.96	
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws		2610.96	
FOE methylsulfone	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.010 ¹	10 000	
	Hypoaspis aculeifer	NOEC 500 mg p.m./kg dws		50 000	
TFA	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.4122 ¹	242.60	
	Hypoaspis aculeifer	NOEC ≥100 mg p.m./kg dws		242.60	
FOE 5043-trifluoroethane sulfonic acid	Folsomia candida	NOEC ≥ 100 mg p.m./kg dws	0.0107	9345.79	
	Hypoaspis aculeifer	NOEC ≥100 mg p.m./kg dws		9345.79	
FOE-Thiadone	Folsomia candida	NOEC 1.8 mg p.m./kg dws	0.0157	114.65	
	Hypoaspis aculeifer	EC ₁₀ 28 mg p.m./kg dws		1783.43	

* Endpoints corrected to allow for log P_{ow} > 2

** The factor of 2 for the test of natural soil was not considered relevant as the LUFA soils contains only 2% organic matter, which is considered to be more representative of natural soil condition.

¹ Accumulated PEC_{soil}

Table B.9.8.2-5: Chronic risk (TER_{LT}) to non-target soil macro-organisms for representative formulation DFF+FFA SC 600, flufenacet and its metabolites based on max PEC_{SOIL} values, for the intended uses in cereals.

Test substance	Test organism	Endpoint	max PEC _{SOIL} (mg/kg)	TER _{LT}	Trigger
Winter cereals, 1 x 240 ga.s./ha					
DFF+FFA SC 600	Folsomia candida	NOEC 89 mg form./kg dws*	1.000	89	>5
	Hypoaspis aculeifer	NOEC ≥65.30 mg form./kg dws**	1.000	65.30	
Flufenacet	Folsomia candida	NOEC 31.5 mg a.s./kg dws*	0.320	98.43	
	Hypoaspis aculeifer	NOEC 281 mg a.s./kg dws*		878.12	
FOE-oxalate	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0186	5376.34	
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws		5376.34	
FOE sulfonic acid-Na-salt	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0574 ¹	1742.16	
	Hypoaspis aculeifer	NOEC ≥100 mg p.m./kg dws		1742.16	
FOE methylsulfone	Folsomia candida	NOEC ≥ 100 mg p.m./kg dws	0.0150 ¹	6666.66	
	Hypoaspis aculeifer	NOEC 500 mg p.m./kg dws		33333.33	
TFA	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.6184 ¹	161.70	
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws		161.70	
FOE 5043-trifluoroethane sulfonic acid	Folsomia candida	NOEC ≥100 mg p.m./kg dws	0.0160	6250	
	Hypoaspis aculeifer	NOEC ≥100 mg p.m./kg dws		6250	
FOE-Thiadone	Folsomia candida	NOEC 1.8 mg p.m./kg dws	0.0236	76.27	
	Hypoaspis aculeifer	EC ₁₀ 28 mg p.m./kg dws		1186.44	

* Endpoints corrected to allow for log P_{ow} > 2

**The factor of 2 for the test of natural soil was not considered relevant as the LUFA soils contains only 2% organic matter, which is considered to be more respective of natural soil condition.

¹ Accumulated PEC_{soil}

Based on the laboratory toxicity endpoints and maximum PEC_{SOIL} values the long-term TER values for Hypoaspis aculeifer and Folsomia candida are higher than the annex VI trigger value of 5, indicating an acceptable risk.

B 9.9. EFFECTS ON SOIL NITROGEN TRANSFORMATION

In the first EU approval of the active substance flufenacet nitrogen and carbon mineralization studies were submitted addressing the risk to soil micro-flora. According to the EU data requirements for active substances (Regulation 283/2013) and plant protection products (Regulation 284/2013) the impact on soil microbial activity should be evaluated, in terms of nitrogen transformation. Hence, the available studies on micro-flora respiration (carbon transformation) are given as additional information only. Please refer to Volume 3, B.9 (CA). Studies with the soil metabolites such as: FOE oxalate, FOE sulfonic acid-Na-salt, FOE methylsulfone, TFA, FOE 5043-trifluoroethane sulfonic acid and FOE-Thiadone were conducted by Applicant addressing the risk to soil micro-flora and were evaluated in RAR, Volume 3, B.9 (CA). In addition to the studies with the active substance and its metabolites a new nitrogen mineralization study with the representative formulation DFF+FFA SC 600 was submitted and summarised under Table 9.9.-1

Table 9.9-1: Effects of flufenacet, relevant soil metabolites and formulation DFF+FFA SC 600 on soil nitrogen transformation.

Test item	Test design	Max tested concentration	% effect on soil nitrogen transformation rate at 28 days after treatment compared to control	Reference
Flufenacet a.s.	silty sand soils, 28 d	0.83 mg s.a./kg dws	+3.20	Anderson, (1994) M-003871-01-2
		4.13 mg s.a./kg dws	-0.60	
Formulation DFF+FFA SC 600	1 soil, 28 d	0.6 L form./ha (equiv to 0.983 mg form./kg dws)	-3	Frommholz, (2009) M-357934-01-1
		3.0 L product/ha (equiv.to 4.916 mg form./kg soil dw)	-8	
FOE oxalate	1 soil, 28 d	2.48 mg p.m./kg dws	+8	Lechelt-Kunze, (2005) M-250511-01-1
FOE sulfonic acid-Na-salt	1 soil, 28 d	3.27 mg p.m./kg dws	-8	Lechelt-Kunze, (2005) M-250265-01-1
FOE methylsulfone	1 soil, 28 d	0.60 mg p.m./kg dws	+4	Frommholz (2010) M-398568-01-1
		6.01 mg p.m./kg dws	-5	
TFA	1 soil, 28 d	0.32 mg p m /kg dws	+3.1	Schulz (2013) M-444423-01-1
		1.60 mg p.m./kg dws 1.61	+24.4	
FOE 5043-trifluoroethane sulfonic acid	1 soil, 28 d	0.164 mg p.m./kg dws	-2.3	Schulz (2013) M-457331-01-1
		0.820 mg p.m./kg dws	+15.4	
FOE-Thiadone	1 soil, 28 d	0.149 mg p.m./kg dws	+19.3	Schulz (2013) M-457326-01-1
		0.749 mg p.m./kg dws	-3.2	

B.9.9.1. Diflufenican + flufenacet SC 600 (200+400) G: Determination of effects on nitrogen transformation in soil

Reference:	Diflufenican + flufenacet SC 600 (200+400) G: Determination of effects on nitrogen transformation in soil.
Author(s),	Frommholz U., 2009
Report/Doc. number:	Study No: E 337 3702-5, Reference BCS No: M-357934-01-1.
Guideline(s):	OECD Guideline 216, Adopted January 21, 2000
GLP:	Yes

<u>Test substance</u>	Diflufenican + Flufenacet SC 600 (200+400) G: diflufenican, 191.4 g/L, flufenacet, 394.5 g/L; (analysed). Batch No.: EV56001418, TOX-No.: FAR 01403-00), Density: 1.229 g/mL
Test species:	Soil microflora
Type of test, duration:	Nitrogen transformation test 28 days
Applied concentrations:	0 (control), 0.8 and 4.0 µL test item/kg dw soil 3 replicates per control and treatment groups. The test item was applied as requested at an application rate equivalent to 0.6 L test item/ha and a fivefold application rate of 3 L test item/ha. Lucerne-grass-green meal was added to soils. (5 g/kg soil dw) to stimulate nitrogen transformation.
Toxic standard	Sodium chloride was used as a reference standard in the tests. In tests (non-GLP) with the agricultural soil described above, 16 g NaCl/kg soil dw had a distinct and long-term (> 28 days) influence on microbial mineralization of nitrogen
Test substrate	Agriculturally soil removed to a depth of 20 cm, from a field located in Germany, Laacher Hof. No application of and plant protection chemicals since 2000. The plot has been under grass and has not been treated with fertilizers since 2000. C _{ORG} 1.57% pH: 5.90 Carbon content of microbial biomass: 488 mg microbial C/kg soil dw (corresponding to % of C _{ORG} 3.0% Total nitrogen content: 0.01% (CEC): 6.63 meq/100 g dry weight soil WHC=42.65 g /100 g dws Texture according to DIN 11277: 10.4 % clay, 17.4 % silt, 72.2% sand 0.5% (i.e. 1.0 g/200 g soil dw) lucerne grass meal

Soil class: sandy loam

Incubation:	20±2°C, darkness
Water content	The start :19.18-19.19 g /100g dw (corresponding to 45% of WHC) Test end: 18.46 – 18.91g/100 g soil dw (corresponding to 43-44% of WHC)
pH	Test start: 5.93-5.94 Test end: 6.13-6.14
Test parameters:	The nitrogen transformation was determined on day 0 and at intervals of 14 and 28 days after application. Samples (10 g soil dw) were extracted with 50 mL 1M KCl by agitating for 60 minutes on a horizontal shaker at approximately 150 strokes/min. Extracts, which could not be analyzed immediately, were stored frozen. For the quantitative determination of the mineralized part of nitrogen the Autoanalyzer was used.
Statistic:	Cochran's Test, $\alpha = 0.05$ normality and homogeneity of variance. Student t-test, two sided, $\alpha = 0.05$: test for significant differences between the treatment groups and the control group. The statistical calculations were carried out using ToxRatPro 2.09
Findings:	No adverse effects of on nitrogen transformation in soil could be observed at both test concentrations (0.8 µL item /kg dry soil and 4.0 µL item mg/kg dry soil) during the 28 day experiment. Differences from the control of +3% (test concentration 0.8 µL/kg soil dw /kg dry soil) and +8 % (test concentration 4.0 µL/kg dws were measured at the end of the 28-day incubation period (time interval 14-28).

Table B. 9.9.1-1: Nitrate formation -Nitrogen mean values and CV in the control.

Days	NO3-Nitrogen (mg/ kg soil dry weight) Mean values	
	Control	
	Nitrate-N Content (±SD)	Replicate Variation ¹
Day 0	15.40±0.3	2
Day 7	2.41±0.57	24
Day 14	10.51±1.67	16
Day 28	36.18±3.53	10

¹ % variation within control replicates (coeff. of variation, calculated as standard deviation / mean value * 100)

Table B. 9.9.1-2: Effects of Diflufenican + Flufenacet SC 600 (200+400) G on nitrate formation-Nitrogen formation rates.

Time Interval (days)	NO ₃ -Nitrogen- Nitrate formation Rate (mg/kg soil dry weight/time interval/d) ¹				
	Control	Diflufenican + flufenacet SC 600 (200+400) G			
		0.8 µL/kg soil dw equivalent to 0.6 L test item/ha		4.0 µL/kg soil dw equivalent to 3 L test item/ha	
	Nitrate-N ¹ (±SD)	Nitrate-N ¹ (±SD)	% difference to control ²	Nitrate-N ¹ (±SD)	% difference to control ²
0-7	-1.86	-1.93	+4 ^{n.s.}	-1.80	-3 ^{n.s.}
7-14	1.16	1.13	-2 ^{n.s.}	1.03	-11 ^{n.s.}
14-28	1.83	1.79	-3 ^{n.s.}	1.68	-8 ^{n.s.}

¹ Rate: Nitrate-N in mg/kg soil dw/time interval/day, mean of 3 replicates

² % deviation to control; + = stimulating effect; - = inhibitory effect

n.s. No statistically significant difference to the control (Student-t Test, two-sided, $\alpha = 0.05$).

Conclusions:

Diflufenican + Flufenacet SC 600 (200+400) caused no adverse effects (difference to control < 25 % on the soil nitrogen transformation (expressed as NO₃-N production) at the end of the 28-day incubation period up and including the highest test concentration of dry soil 4.0 µL test item /kg soil dw corresponding to 3 l test item/L and 1.6 mg flufenacet/kg soil dw.

Comments RMS:

The nitrogen transformation test was conducted to the OECD test guideline 216 (2000).

According to the test guideline, the study is considered valid if the coefficient variation in the control for NO₃-N is ≤ 15%.

In this study, the highest coefficient of variation (CV) between nitrate-N concentrations in replicate control samples was 10%.

The following deviation from the recommendation given in the test guideline was noted:

-The organic carbon content of soil was 1.57 % (should be <1.5 %).

Indicated deviations is however are considered as having no impact on the study results, since all validity criteria were met. The study is considered acceptable.

Agreed endpoints:

Effects on soil nitrogen transformation on day 28:

0.8 µL test item/kg soil dws equivalent to 0.6 L product/ha and 0.983 mg form./kg dws

4 µL test item/kg soil dws equivalent to 3 L product/ha and 4.916 mg form./kg soil dw

B 9.10. RISK ASSESSMENT FOR SOIL NITROGEN TRANSFORMATION

The risk assessment for effects on soil organisms is conducted according to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SAN+CO/10329/2002 rev. 2).

Exposure

The exposure of soil organisms to flufenacet and its metabolites and formulation was estimated by calculating the maximum Predicted Environmental Concentrations in soil (PEC_{SOIL}) (see Volume 3 (CA), Section B.8.2). The maximum PEC_{SOIL} of the formulation DFF+FFA SC 600, flufenacet and its metabolites in soil have been assessed using the FOCUS groundwater crop interception values (FOCUS 2011) and the maximum DT₅₀ values. Since DT₅₀ values for metabolites - FOE sulfonic acid-Na-salt, FOE methylsulfone and TFA, are >100 days, potential for accumulation in soil was also taken into account by calculation of plateau concentrations. Plateau PEC_{soil} values were summed up with maximum initial PEC_{soil} values in order to obtain PEC_{soil,accu}, recommended for long-term risk assessment.

Based on the recommended uses rate of 1 x 240 g a.s. /ha, 1 x 160 g a.s./ha and 1 x 120 g a.s./ha, an as spray application in cereals, the summary of max PEC_{SOIL} values are presented in Table B.9.10-1.

Table B.9.10-1: Maximum Predicted Environmental Concentration of the compounds in soil after application of the DFF + FFA SC 600 formulation to cereals.

Compound	Winter cereals 1x240 g a.s./ha BBCH 10-13, CI= 0%		Winter cereals 1x160 g a.s./ha BBCH 11-13, 0% CI=0%		Winter cereals 1x120 g a.s./ha BBCH 00-22, 0% CI=0%	
	PECs ¹ (mg/kg)	PEC _{acc} ² (mg/kg)	PECs ¹ (mg/kg)	PEC _{acc} ² (mg/kg)	PECs ¹ (mg/kg)	PEC _{acc} ² (mg/kg)
Flufenacet	0.3200	0.3210	0.2133	0.2140	0.1600	0.1605
FOE oxalate	0.0186	0.0187	0.0124	0.0125	0.0093	0.0094
FOE sulfonic acid-Na-salt	0.0452	0.0574	0.0302	0.0383	0.0226	0.0287
FOE methylsulfone	0.0134	0.0150	0.0089	0.010	0.0067	0.0075
TFA	0.0888	0.6184	0.0592	0.4122	0.0444	0.3092
FOE 5043-trifluoroethane sulfonic acid	0.0160	0.0160	0.0107	0.0107	0.0080	0.0080
FOE-Thiadone	0.0236	0.0237	0.0157	0.0157	0.0118	0.0119
DFF + FFA SC 600*	1.0		0.667		0.500	

¹ PECs PEC_{soil} actual

² PEC_{acc} Accumulated PEC_{soil}

In bold PECs used in the risk assessment

Risk assessment:

The risk to soil nitrogen transformation of active substance and its metabolites and representative formulation DFF+ FFA SC 600 is based on comparison of PEC_{SOIL} values to the test concentrations at which < 25% effects were observed in the soil nitrogen transformation tests.

The maximum soil exposure for application to winter cereals at rate 1 x 0.6 L DFF + FFA SC 600/ha, corresponding to 240 g flufenacet/ha was used as the worst case exposure scenario, covers all other intended uses.

Table B.9.10-2: Summary of the soil nitrogen transformation endpoints for the active substance and its metabolites, representative formulation DFF+FFA SC 600 and risk assessment based on max PEC_{SOIL} values, for the intended uses in winter cereals.

Test item	PEC _{soil} / [mg/kg dw soil]	Max tested concentration with less than 25% effects	% effect on soil nitrogen transformation rate at 28 days after treatment compared to control	Margin of safety
Flufenacet a.s.	0.320	4.13 mg s.a./kg dws	-0.60	12.9
Formulation DFF+FFA SC 600	1 mg form/kg dws	equiv. to 4.916 mg form./kg soil dw	-8	4.91
FOE oxalate	0.0186	2.48 mg p.m./kg dws	+8	133.33
FOE sulfonic acid-Na-salt	0.0574	3.27 mg p.m./kg dws	-8	56.96
FOE methylsulfone	0.0150	6.01 mg p.m./kg dws	-5	400.67
TFA	0.6184	1.60 mg p.m./kg dws	+24.4	2.58
FOE 5043-trifluoroethane sulfonic acid	0.0160	0.820 mg p.m./kg dws	+15.4	51.25
FOE-Thiadone	0.0236	0.749 mg p.m./kg dws	-3.2	31.73

*density of the formulation=1.229 g/mL
form.- formulation

According to the current regulatory requirements the risk is considered acceptable if the effect on nitrogen transformation at the recommended application rate of a compound/product is ≤ 25% after 100 days. In none of the above presented studies the deviations from the control exceed 25% 28 days after application of the recommended application rate. Therefore, the risk from the representative formulation DFF + FFA SC 600, flufenacet and its degradation products in soil can be considered to be low.

B.9.11. EFFECTS ON TERRESTRIAL NON-TARGET HIGHER PLANTS**B.9.11.1. Summary of screening data**

No screening studies using the formulated product have been submitted.

B.9.11.2. Testing on non-target plants

For the purpose of the current evaluation, the new representative formulation DFF+FFA SC 600 was submitted. That formulation was also declared a representative formulation for the EU-evaluation of the active substance diflufenican performed in 2007 by the UK acting as the RMS for that compound. In addition, the study for formulation Flufenacet SC 500 was evaluated by RMS to show the effects for flufenacet singularly. Summary of obtained results is presented in Table 9.11.2-1 below.

Table B.9.11.2-1: Tier 2 tests: Effects on seedling emergence and vegetative vigour of representative formulation DFF+FFA SC 600.

Formulation D1: TFA SC 66%				
Species	Family	Shoot fresh weight ER ₅₀ (g of sum a.s./ha)		Reference
		Vegetative vigour study	Seedling emergence study	
MONOCOTYLEDONS				
Allium cepa (onion)	Liliaceae	> 332.3	100.00	Kalsch. W. 2002 M-071692-01-1 (Vegetative vigour test)
Avena sativa (oat)	Poaceae	227.54	207.88	
DICOTYLEDONS				
Brassica napus (oilseed rape)	Brassicaceae	92.07	214.22	Kalsch. W. 2002 M-072308-01-1 (Seedling emergence test)
Cucumis sativa (cucumber)	Cucurbitaceae	27.75	218.41	
Glycine max (soybean)	Fabaceae	55.14	>332.3	
Lycopersicon esculentum (tomato)	Solanaceae	n.d.	>332.3	

Note: The single lowest endpoint for seedling emergence and vegetative vigour is indicated in bold.

Note: The endpoints are expressed in terms of active substances (Flufenacet +Diflufenican).

Flufenacet SC 500**Table B.9.11.2-2: Tier 2 tests: Effects on seedling emergence and vegetative vigour of Flufenacet SC 500 formulation.**

Species	Family	Shoot fresh weight EC ₅₀ (g a.s./ha)*		Reference
		Vegetative vigour study	Seedling emergence study	
MONOCOTYLEDONS				
Zea mays (Corn)	Gramineae	>600	477.9	Friedrich S., (2005) M-248250-01-1 (Vegetative vigor test) Friedrich S., (2005) M-248250-01-1
Avena sativa (Oats)	Gramineae	196	80.9	
Sorghum bicolor (Sweet sorghum)	Gramineae	43	10.5	
Lolium perenne (Perennial ryegrass)	Gramineae	17	11.5	
Alium cepa (Onion)	Liliaceae	132	53.3	
DICOTCOTYLEDONS				(Seedlings emergence test)
Brassica rapa (Turnip)	Brassicaceae	167	282.7	
Beta vulgaris (Sugar beet)	Chenopodiaceae	525	275.4	
Cucumis sativa (Cucumber)	Cucurbitaceae	102	101.1	
Lycopersion esculentum (Tomato)	SolanaceaFabacea	>600	93.6	
Soybean (Glycine max)	Fabaceae	168	>600	
		HC5:19.1 g a.s./ha Lolium perenne (Rye grass)	HC5: 8.34 g a.s./ha Sorghum bicolor (Sorghum)	

Note: The single lowest endpoint for seedling emergence and vegetative vigour is indicated in bold.

Note: a.s.-flufenacet only

B. 9.11.2.1. Flufenacet & Diflufenican SC 600: Vegetative Vigour Test on terrestrial non-target plants of 6 families (2 Monocotyledoneae, 4 Dicotyledoneae)

Reference:	Flufenacet & Diflufenican SC 600: Vegetative Vigour Test on terrestrial non-target plants of 6 families (2 Monocotyledoneae, 4 Dicotyledoneae).
Author(s), year:	Kalsch, W., 2002
Report/Doc.number:	Stud No: P3PB, Reference Bayer No: M-071692-01-1
Guideline (s):	Plant Test 208 B: Vegetative Vigour Test",
GLP:	Yes

Material and Methods

Test substance	Flufenacet & Diflufenican SC 600, content of a.s.: Diflufenican 205.00 g/L (analyzed) Flufenacet 406.25 g/L (analysed), sum of active substance 611.25g/l, Batch No: 07205/0024(0006), density: 1.25 g/mL (valid from Feb. 6, 2002).
Type of test	Vegetative vigour test
Test duration	21 days
Test species	4 dicotyledonous (Brassica napus, Cucumis sativus, Glycine max, Lycopersicon esculentum) and 2 monocotyledonous (Allium cepa, Avena sativa) plant species.
Test soil	<p>A standard soil LUFA Sp 2.2 (loamy sand) collected from a hay meadow at Hanhofen, Germany. The side did not received organic carbon fertilizers or plant protection product since 1996.</p> <p>Two batches of LUFA Sp2.2 were used in this study.</p> <p>The batch no Sp 223501 for testing (Avena sativa) and the batch no: Sp 220302 for remaining species.</p> <p>The properties of soil used in the test:</p> <p><u>The batch no: Sp 223501</u></p> <p>% of organic carbon: 2.28 ± 0.16</p> <p>pH: 5.8 ± 0.3,</p> <p>WHC: $51 \pm 4\%$</p> <p><u>The batch no: Sp 220302</u></p> <p>% of organic carbon 2.3 ± 0.2,</p> <p>pH: 5.6 ± 0.4,</p> <p>WHC: $50 \pm 5\%$.</p> <p>Since LUFA Sp2.2 is relatively poor in nutrients, a nutrient solution (the Steinberg nutrient solution according to ISO Working Draft 20079) was</p>

used for watering to ensure the development of the plants throughout the test period.

Applied concentration:

Control: Deionizer water

Test item: 3.2, 10.0, 32.15, 103.4 and 332.3 g active substances/ha.

Replicates:

The number of treatment replicates varied from 5 to 15 depending on the number of plants per test container. The minimum number of plants per treatment was 30. The number of control replicates was at least 6.

Table B. 9.11.2.1-1: The number of replicates used in the vegetative vigor test.

	Allium cepa Avena saliva	Brassica napus Lycopersicon esculentum	Glycine max	Cucumis sativus
no. of plants per test container*	6	4	3	2
no. of replicates of treatments (controls)	5 (6)	8 (8)	10 (10)	15 (15)
total no. of containers	31	48	60	90
no. of plants per treatment (control)	30 (36)	32 (32)	30 (30)	30 (30)

*The test container is defined as the replicate and not the individual plant

Exposure route

Seeds of monocotyledoneous species and dicotyledoneous species were planted in a standard soil LUFA Sp 2.2 (loamy sand) and were allowed to emerge and grow until the two-leaf stage was reached. Plants were grown in container (polystyrene vessels Bellaplast 590:18 x 13.5x6.5x6.5 cm) containing 800 ± 10 g soil. A hole (10 mm) was punched in the bottom of the test container and a glass fibre wick was inserted. During the test each test container was placed above a polystyrene beaker serving as water reservoir in a way that the wicks reached the water reservoirs filled with deionised water or nutrient solution. Application was carried out, when the plants had reached the two-leaf stage. Applications were made using a plot sprayer equipped with one flat spraying nozzle.

Test condition:

The test plants were cultivated in two rooms equipped with artificial lighting 13000 ± 2000 lx with a photoperiod of 16 h/d.

The temperature in the test rooms was kept at 22 ± 3 °C.

Humidity: not reported:

Test parameter: At day 7, 14 and 21 a visible inspection of the plants was made. In addition, the plants were harvested at day 21 and their length and biomass were determined.

Analytical parameters:

Test concentrations were confirmed by analytical verification of the highest test solution.

At each time, when the test substance was applied, a sample of the respective test solution was taken and deep-frozen immediately after the preparation of the test solution.

Statistic:NOEC/LOEC:

Shoot length and biomass at day 21 were evaluated for homogeneity of variances by Cochran's test. Subsequently, ANOVA was used to detect differences between biological data of the different concentration levels. Dunnett's Test was used to compare the data of the treated replicates to the control replicates in order to determine significant differences from the control vessels. The highest concentration level showing no statistically significant difference from the controls was defined as NOEC.

The lowest concentration level showing a statistically significant difference from the controls was defined as LOEC. The statistical software used was SPSS for Windows, version 7.5.

EC₅₀ values were derived from logistic regression analysis. The statistical software used was SPSS for Windows, version 7.5

Findings:Analytical findings:

All calculations were based on nominal concentrations. Analytical verification of the highest test solution resulted in recoveries of 96.0 – 99.5 % (sum of active substances).

Biological findings:

Summary of results considering shoot length and fresh weight of the plants at day 21 are presented at the Table B.9.11.2.1-2 below:

Table B. 9.11.2.1-2: Shoot length and shoot fresh weight of the plants at day 21.

Flufenacet & Diflufenican SC 600 (g of total a.s./ha in 300 L/ha)	Plant species					
	Monocotyledoneae		Dicotyledoneae			
	<i>Allium cepa</i> (onion)	<i>Avena sativa</i> (oats)	<i>Brassica napus</i> (turnip)	<i>Cucumis sativus</i> (cucumber)	<i>Glycine max</i> (Soybean)	<i>Lycopersicon esculentum</i> (tomato)
Fresh weight (g), ±SD						
Control	0.894 ±0.287	2.791 ±0.16	3.964 ±0.52	11.443 ±2.09	7.913 ±1.06	3.549 ±1.52
3.2	0.629 ±0.316	2.716 ±0.30	4.417 ±0.98	9.972 ±2.16	7.172 ±1.10	1.739 ±1.79
10.0	0.658 ±0.134	2.505 ±0.20	4.629 ±0.59	6.991 ±1.59	6.348 ±0.96	2.328 ±1.82
32.1	0.788	2.636	3.139	5.884	4.385	2.185

Plant species						
Flufenacet & Diflufenican SC 600 (g of total a.s./ha in 300 L/ha)	Monocotyledoneae		Dicotyledoneae			
	<i>Allium cepa</i> (onion)	<i>Avena sativa</i> (oats)	<i>Brassica napus</i> (turnip)	<i>Cucumis sativus</i> (cucumber)	<i>Glycine max</i> (Soybean)	<i>Lycopersicon esculentum</i> (tomato)
	±0.14	±0.16	±0.65	±2.26	±1.07	±1.82
103.4	0.728 ±0.32	2.336 ±0.18	2.238 ±0.58	4.033 ±1.38	3.273 ±0.97	1.099 ±0.98
332.3	0.665 ±0.15	0.787 ±0.15	0.356 ±0.19	1.767 ±0.88	2.165 ±0.30	0.583 ±0.35
Shoot length, ±SD (mm)						
Control	231.9 ±18.64	509.4 ±11.99	185.1 ±7.03	243.4 ±38.14	1008.0 ±150.73	225.2 ±27.34
3.2	207.3 ±27.53	502.3 ±32.12	192.4 ±18.00	238.5 ±42.14	944.7 ±96.70	174.0 ±34.58
10.0	206.5 ±21.08	487.3 ±18.33	187.0 ±8.56	212.0 ±43.12	881.3 ±57.34	216.3 ±52.68
32.1	226.5 ±23.06	501 ±20.77	173.1 ±13.66	198.4 ±38.99	691.7 ±95.95	171.2 ±59.41
103.4	211.2 ±34.92	427.5 ±23.37	160.1 ±12.75	165.0 ±26.40	498.2 ±92.24	150.8 ±41.67
332.3	200.3 ±0.32	305.2 ±12.09	115.5 ±10.76	113.4 ±29.29	330.9 ±39.00	104.3 ±20.80

SD standard deviation

Table B. 9.11.2.1-3: Shoot fresh weight (percentage of untreated control).

Test item (g a.s./ha)	Plant species					
	Monocotyledoneae		Dicotyledoneae			
	<i>Alium cepa</i> (onion)	<i>Avena sativa</i> (oats)	<i>Brassica Napus</i> (turnip)	<i>Cucumis Sativus</i> (cucumber)	<i>Glycine Max</i> (soybean)	<i>Lycopersicon Esculentum</i> (tomato)
Control	-	-	-	-	-	-
3.2	70.36	97.31	111.43	87.14	90.64	49.00
10	73.60	89.75	116.78	61.09	80.22	65.60
32.1	88.14	94.45	79.19	51.42	55.42	61.57
103.4	81.43	83.70	56.46	35.24	41.36	30.97
332.3	74.38	28.20	8.98	15.44	27.36	16.43

Table B. 9.11.2.1-4: Shoot length (percentage of untreated control).

Test item (g a.s./ha)	Plant species					
	Monocotyledoneae		Dicotyledoneae			
	<i>Alium cepa</i> (onion)	<i>Avena sativa</i> (oats)	<i>Brassica napus</i> (turnip)	<i>Cucumis Sativus</i> (cucumber)	<i>Glycine max</i> (soybean)	<i>Lycopersicon Esculentum</i> (tomato)
Control	-	-	-	-	-	-
3.2	89.39	98.61	103.94	97.99	93.72	77.26
10	89.05	95.66	101.03	87.10	87.43	96.05
32.1	97.67	98.35	93.52	81.51	68.62	76.02
103.4	91.07	83.92	86.49	67.79	49.42	66.96
332.3	86.37	59.91	62.40	46.59	32.83	46.31

Table B. 9.11.2.1-5: Visual observations.

Study period	Test item (g a.s./ha)	Plant species					
		Monocotyledoneae		Dicotyledoneae			
		Alium cepa	Avena sativa	Brassica napus	Cucumis sativus	Glycine max	Lycopersicon esculentun
Chlorosis or discoloration							
7 days after application of test item	Control	0	0	0	13.3	0	0
	3.2	3.8	0	65.5	86.7	76.9	32.3
	10	3.8	6.9	89.7	93.3	100.0	87.5
	32.1	0	36.7	100.0	77.8	100.0	96.3
	103.4	0	69.0	100.0	46.7	100.0	96.8
	332.3	0	90.0	100.0	60.7	96.4	96.7
	Deformations, wilting or developmental abnormalities %						
	Control	0	0	3.4	40.0	0	0
	3.2	0	0	6.9	83.3	3.8	9.7
	10	0	0	7.1	90.0	17.9	9.4
	32.1	3.7	0	6.9	96.3	28.6	7.4
	103.4	6.9	0	58.6	100.0	63.0	0
	332.3	0	6.7	53.6	96.4	96.4	33.3
14 days after application of test item	Chlorosis or discoloration						
	Control	0	0	0	6.7	0	0
	3.2	0	0	13.8	90.0	30.8	71.0
	10	0	0	51.7	90.0	85.7	100.0
	32.1	0	13.3	100.0	96.3	89.3	92.6
	103.4	0	51.7	100.0	83.3	29.6	80.6
	332.3	20.0	93.3	75.0	75.0	0	53.1
	Deformations, wilting or developmental abnormalities %						
	Control	0	0	0	0	3.4	0
	3.2	42.3	0	0	16.7	30.8	25.8
	10	23.1	0	0	56.7	85.7	31.3
	32.1	14.8	0	6.9	88.9	100.0	74.1
	103.4	10.3	0	62.1	76.7	100.0	100.0
	332.3	16.0	6.7	100.0	89.3	100.0	100.0
21 days after application of test item	Chlorosis or discoloration						
	Control	0	0	0	0	6.9	3.1
	3.2	0	0	3.4	100.0	84.6	41.9
	10	0	0	50.0	100.0	100.0	84.4
	32.1	14.8	0	100.0	96.2	60.7	100.0
	103.4	3.4	10.3	100.0	90.0	22.2	100.0
	332.3	30.0	100.0	42.9	69.2	13.8	100.0
	Deformations, wilting or developmental abnormalities %						
	Control	2.9	0	0	0	0	3.1
	3.2	42.3	0	0	93.3	0	64.5
	10	57.7	0	0	100.0	3.6	71.9
	32.1	59.3	0	57.1	100.0	57.2	100.0
	103.4	51.7	6.9	89.7	100.0	100.0	100.0
	332.3	60.0	16.7	100.0	100.0	100.0	100.0
21 d visual NOEL	-	< 3.2 ²	32.1 ²	< 3.2 ¹	< 3.2 ¹	<3.2 ¹	< 3.2 ²

¹ Effects 21 days after application such as chlorosis or discoloration at all rates, but diminished as rates increased. Deformation increase with the higher rates.

² Effects 21 days after application such as chlorosis or discoloration at all rates increase with higher rates.

Visual observations:

Control

The following phytotoxic effects were observed during the study:

Day 7

For *Cucumis sativus*, chlorosis/discolouration affected 13.3% of the test plants and deformations/wilting affected 40% of the plants. For *Brassica napus*, deformations/wilting affected 3.4% of the test plants.

Day 14

For *Cucumis sativus*, chlorosis/discolouration affected 6.7% of the tested plants even as these effects were less pronounced as compared to those on Day 7. No deformations/wilting were observed for these plants, although 40% of the plants were affected on Day 7.

In addition, deformations/wilting affected 3.4% of *Glycine max* plants.

Day 21

On Day 21, 2.9% of control plants of *Allium cepa* were partly wilted, whilst 3.1% of *Lycopersicon esculentum* plants had a few brownish speckled leaves, and chlorosis/discolouration affected 3.1% of these plants. In addition, 6.9% of *Glycine max* plants had chlorotic spots.

Treatment

Phytotoxic effects in the exposed plants were observed soon after application.

On Day 7 following application, the dicotyledonous species were more affected than the monocotyledonous ones. Chlorosis was the most frequently observed effect.

Brassica napus and *Glycine max*, the two dicot species, were affected by chlorosis/discolouration even at the lowest treatment rate of 3.2 g a.s./ha, that is 65.5 and 86.7% of the test plants, respectively. An increase of up to 100% of affected plants was noted at higher application rates.

For *Cucumis sativus*, the third dicot species, even at the lower application doses i.e.: 3.2; 10.1 and 32.1 g a.s./ha, chlorosis/discolouration affected 86.7, 93.3 and 77.8% of the test plants, respectively. However, that effect was less pronounced at the two highest doses: 46.7% for the application rate of 103.4 g a.s./ha and 60.7% for the application rate of 332.3 g a.s./ha.

Chlorosis/discolouration in *Lycopersicon esculentum* plants, except for the lowest rate i.e. 3.2 g a.s./ha, was identified to be at the range of from 87.5 to 96.7% of the plants tested.

From among the two monocot species, *Avena sativa* turned to be more affected by chlorosis, and at the two highest rates of 103.4 and 332.4 g a.s./ha, 69 and 90% of the plants, respectively, were affected.

Leaf deformations/wilting or other developmental abnormalities were most pronounced in *Cucumis sativus*. For this species, the effect was observed in 83.3% of the test plants and at the lowest application rate of 3.2 g a.s./ha. It rose up to 96.4- 100% of the test plants affected for the remaining application rates. *Brassica napus* and *Glycine max* were strongly affected as well, mainly at the two highest rates, i.e.: 103.4 and 332.4 g a.s./ha.

Only slight deformations were noted for the two monocot species: *Allium cepa* and *Avena sativa*.

On Day 14, deformations/ wilting or both were frequently observed in all the treated plants whereas chlorosis in monocot plants was more frequent with *Avena sativa*. For the two dicot species: *Cucumis sativus* and *L. esculentum*, even at the lowest application rate of 3.2. g a.s./ha, the chlorosis symptom was observed in 90 and

71 % of these plants, respectively. However, decreasing of this effect at the higher rate of 332.3 g a.s./ha was observed as well as in all dicot plants. No dose response pattern could be determined. *Glycine max*, was the species affected by deformation or wilting from among the dicot plants, whereas *Allium cepa* from among the monocot plants.

.At 21 day deformations/wilting and chlorosis were observed for all plants.

The most sensitive species was a dicot plant-*Cucumis sativus* for which 100% of the plants were affected by chlorosis/discoloration and 93.3 % of plants indicated deformations/wilting effects at the lowest treatment rate of 3.2 g a.s./ha. *Lycopersicon esculentum* was also sensitive plant, particularly at rate the rate of 3.2 g a.s./ha and higher where 100% of plants indicated chlorosis/discoloration and deformations or wilting.

Monocot plants were usually affected by chlorosis at the higher treatment rate of 332.3 g a.s./ha, however, *Avena sativa* was a more sensitive species.

At the rate of 32 g a.s./ha or higher ,most plants of the dicotyledonous species were affected by wilting or deformations of leaves.

From among monocot species, *Allium cepa* more most affected than *Allium sativa*.

Summary of endpoints for phytotoxic effects empirically estimated by RMS are presented in

Table B.9.11.2.1-6 below.

Table B. 9.11.2.1-6: Effects of Flufenacet + Diflufenican SC 600 on six plant species: early post-mergence treatment.

Plant species						
Monocotyledoneae			Dicotyledoneae			
21 days after 50 % emergence of controls	<i>Allium cepa</i>	<i>Avena sativa</i>	<i>Brassica napus</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>	<i>Lycopersicon esculentum</i>
Flufenacet & Diflufenican SC 600 (g a.s./ha in 300 L/ha)						
Visual EC ₅₀ * based on signs of chlorosis/discoloration	> 332.3 ¹⁾	103.4-332.3 g a.s./ha	nd ² 100% of plants affected at 32.1 g a.s./ha but decrease at higher rates was noted	nd ² 100% of plants affected at 3.2 g a.s./ha but decrease at higher rates was noted	nd ² >50% of plants affected at 3.2 g a.s./ha, but decrease at higher rates was noted	3.2-10 g a.s./ha
Visual EC ₅₀ * based on deformations	3.2-10 g a.s./ha	> 332.3 ¹⁾	10-32 g a.s./ha	< 3.2 >50% of plants affected at 3.2 g a.s./ha and this effect increased with higher rates	10-32 g a.s./ha	<3.2 g a.s./ha 50% of plants effects at rate 3.2 g a.s./ha and increasing with higher rates

* 21 d visual EC₅₀ - empirically predicted by RMS

¹⁾ EC₅₀ could not be predicted because of less than 50 % effects were recorded. Therefore estimated to be > 332.3 g a.s./ha.

nd² It could be not predicted due to irregular dose response patterns. The effects are >50% at lowest rate 3.2. g a.s./ha but diminished as rates increased.

Summary of endpoints considering shoot length and fresh weight are presented in the Table B.9.11.2.1-7. below:

Table B.9.11.2.1-7: Effects of Flufenacet + Diflufenican SC 600 on six plant species: early post-emergence treatment.

Plant species						
Monocotyledoneae			Dicotyledoneae			
21 days after 50 % emergence of controls	<i>Allium cepa</i>	<i>Avena sativa</i>	<i>Brassica napus</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>	<i>Lycopersicon esculentum</i>
Flufenacet & Diflufenican SC 600 (g a.s./ha in 300 L/ha)						
Shoot length						
EC ₅₀	> 332.3 ¹⁾	> 332.3 ¹⁾	> 332.3 ¹⁾	279.30	102.44	> 332.3 ¹⁾
NOEL	≥ 332.3 ²⁾	32.1	32.1	10.0	≥ 332.3 ²⁾	32.1
LOEL	n.d.	103.4	103.4	32.1	n.d.	103.4
Fresh weight						
EC ₅₀	> 332.3 ¹⁾	227.54	92.07	27.75	55.14	nd ³
NOEL	≥ 332.3 ²⁾	32.1	32.1	3.2	3.2	32.1
LOEL	n.d.	103.4	103.4	10.0	10.0	103.4
Visual effects 21 d NOEL	< 3.2	32.1	< 3.2	< 3.2 ^{4,5}	<3.2 ^{4,5}	< 3.2 ⁶

¹⁾ EC₅₀ could not be calculated because of less than 50 % effect. Therefore estimated to be > 332.3 g a.s./ha.

²⁾ Not significant effect within the range tested

n.d. Not determined

n.d.³ Not determined due to irregular dose response patterns and high variance

⁴ Effects at all rates but diminished as rates increased (chlorosis/discoloration)

⁵ Effects at all rates and increased at higher rates (deformations, wilting)

⁶ Effects at all rates and increased at higher rates (deformations, wilting and chlorosis/discoloration)

The quantitative parameters

The monocotyledonous species were less sensitive than the dicotyledonous species with respect to fresh weight. The EC₅₀ for fresh weight was 227.54 and > 332.3 g a.s./ha for *A. sativa* and *A. cepa*, respectively.

The lowest EC₅₀ value was determined to be 27.75 g a.s./ha for the monocotyledonous species *C. sativus*.

However, for the dicot species – *L. esculentum*, the EC₅₀ value was not determined, due to irregular dose response patterns and high variance during the study.

Effects on the fresh weight were more pronounced than on the shoot length.

An EC₅₀ for the fresh weight could not be determined for *Allium cepa* because inhibition was less than 50%. It could be determined for all other species with *Cucumis sativus* being the most sensitive one (27.75g a.s./ha).

The lowest NOEC and LOEC were observed for *Cucumis sativus* and *Glycine max* (3.2 and 10.0 g a.s./ha).

Lycopersicon esculentum was strong affected.

Conclusion:

Taking into account the quantitative parameters such as fresh shoot length and fresh weight, the most sensitive parameter was the fresh weight followed by shoot length.

The most sensitive species was *Cucumis sativus* with EC₅₀ of 27.75 g a.s./ha – fresh weight.

It is worth noting that for the vegetative vigour study the phytotoxic effects were widespread in all dicot species including also the most sensitive species tested - *Cucumis sativus* with the lowest toxicity endpoint $EC_{50}=27.75$ g a.s./ha (based on foliar fresh weight), and could have resulted in a lower endpoint- than that calculated based on foliar fresh weight (21 d visual $EC_{50} < 3.2$ g sum of a.s./ha - endpoints empirically estimated by RMS).

In addition, the phytotoxicity data in this study are not adequate to assess at what level there would be no effects for this particular formulation for the most sensitive species *Cucumis sativus* (visual 21 d NOEL < 3.2 g a.s./ha, endpoints empirically estimated by RMS).

Taking into consideration that the representative formulation consists of two active substances – flufenacet and diflufenican, the level of the caused by solo a.s.-flufenacet, could not be possibly to determined based on phytotoxicity effects.

However, since the measurement of phytotoxicity effects is rather subjective; the biomass reduction measurement is used in preference as a more quantitative assessment of the toxicological effect on non-target plant species.

In opinion of RMS, the MSs should note that the risk assessment based on the quantitative parameter EC_{50} of 27.75 g a.s./ha (fresh weight) will not cover the phytotoxic effects from this particular formulation.

RMS comments:

The vegetative vigor test was conducted according to the OECD test guideline 208 B (July 2000), draft .

In order for the test to be considered valid, following performance criteria must be met in the control:

- The mean seedling growth doesn't exhibit visible phytotoxic effect
- The plant survival in vegetative vigour test is at least 90% at the end of the study

Taking into account the current valid OECD test guideline 227 (July 2006) the following validity criteria are given:

- The seedling emergence is at least 70% (control and treatment groups).

In this study the seedling emergence ranged between 88 and 98 % in the treatment group and 88-100% in the control group

- In the control groups the plants do not exhibit visible phytotoxic effects

(e.g. Chlorosis, necrosis, and wilting. leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species.

During the study the following phytotoxic effects for control plants were observed:

Visual observations:-

Control

The following phytotoxic effects were observed during the study:

Day 7

For *Cucumis sativus*, chlorosis/discolouration affected 13.3% of the test plants and deformations/wilting affected 40% of the plants. For *Brassica napus*, deformations/wilting affected 3.4% of the test plants.

Day 14

For *Cucumis sativus*, chlorosis/discolouration affected 6.7% of the tested plants even as these effects were less

pronounced as compared to those on Day 7. No deformations/wilting were observed for these plants, although 40% of the plants were affected on Day 7.

In addition, deformations/wilting affected 3.4% of Glycine max plants.

Day 21

On Day 21, 2.9% of control plants of *Allium cepa* were partly wilted, whilst 3.1% of *Lycopersicon esculentum* plants had a few brownish speckled leaves, and chlorosis/dicolouration affected 3.1% of these plants. In addition, 6.9% of Glycine max plants had chlorotic spots.

- In the control groups the mean plant survival is at least 90% for the duration of the study.

No mortality in the control group was observed for the duration of the study

- In the control groups environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix support media or substrate from the same source.

In this study the standard soil LUFA Sp2.2 (loamy sand) was used and environmental conditions were similar in study two experimental rooms (temperature and photoperiod).

- According to the OECD 227 (2006) test guideline the temperature and the humidity has to be in a range of $22\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ and $70\% \pm 25\%$, respectively.

The temperature in both experimental rooms was in a range of $20 - 24.5^{\circ}\text{C}$

- Minimum 16 h photoperiod was recommended and light intensity – $350\text{ }\mu\text{E}/\text{m}^2/\text{s}$.

Additional lighting may be necessary if intensity decreases below $200\text{ }\mu\text{E}/\text{ms}$, wavelength 400 - 700 nm except for certain species whose light requirements are less.

The lighting of $13000 \pm 2000\text{ lx}$ (16 hours) was maintained during the study.

The following deviations were recorded from the guidelines:

- Steinberg nutrient solution was used instead of Hoagland solution.
- The organic carbon content of the soil was higher (being 2.8%) than the recommended value given in OECD test guideline (should be 1.5%).
- No data of humidity is reported in the study protocol.
- EC_{50} value was determined only for 5 species (six species are recommend by Sanco/10329/2002 rev. 2 final 17 October, guidance document). However, in OECD 208 and 227 test guidelines the number of tested plants is not specified.

Taking into account the validity criteria given in the OECD 227 (2006) test guideline the validity criteria were met except the requirement of the lack of phytotoxic effects in control plants.

For *Cucumis sativus* the most sensitive species with the lowest toxicity endpoint $\text{EC}_{50} = 27.75\text{ g a.s./ha}$ (based on fresh weight) the phytotoxic effects in the control group were observed on Day 7 (13.3% chlorosis, 40% deformations) and a slight chlorosis was held until Day 14 after application (6.7% of plants).

No phytotoxic effects were seen for this species on Day 21.

Taking into consideration that the effects were $< 10\%$ by 14 days and absent by 21 days showing that whatever problem caused the effect in the control this was only in the start of the study and the plants seemed to have recovered from it and a clear difference between control and treatment could be seen.

At the same time a high survival should be stressed of the control groups. Since it can be concluded that survival was not affected in the control.

In addition, should be stressed, that the phytotoxic effects in this vegetative vigour study were widespread and for all dicot species including also the most sensitive species tested (*Cucumis sativus*) could have resulted in a lower endpoint - 21 d visual $EC_{50} < 3.2$ g sum of a.s./ha, than that calculated with regard to the foliar fresh weight - $EC_{50} = 27.75$ g a.s./ha. In addition, the phytotoxicity data are not adequate to assess at what level there would be no effects for this particular formulation for the most sensitive species *Cucumis sativus* (visual 21 d NOEL < 3.2 g a.s./ha).

Taking into consideration that the representative formulation consists of two active substances – flufenacet and diflufenican, the level effects caused by solo a.s.-flufenacet, could not be not possibly determined based on phytotoxicity effects observed in this study.

For this reason, RMS evaluated also the solo formulation of Flufenacet 500 SC (500 g flufenacet/L) to show the effects for flufenacet singularly.

Agreed endpoints:

$EC_{50} > 332.3$ g total active substances/ha, based on fresh weight (*Alium cepa*)

$EC_{50} = 227.54$ g total active substances/ha, based on fresh weight (*Avena sativa*)

$EC_{50} = 92.07$ g total active substances/ha, based on fresh weight (*Brassica napa*)

$EC_{50} = 55.14$ g total active substances/ha, based on fresh weight (*Glycine max*)

$EC_{50} = 27.75$ g total active substances/ha, based on fresh weight (*Cucumis sativus*)

The most sensitive species:

$EC_{50} = 27.75$ g total active substances, based on fresh weight (*Cucumis sativa*)

For L. esculentum RMS proposes to re-calculate by Applicant EC_{50} value for this species taking into account the lowest value as outlier.

When that value becomes available, the Applicant is kindly requested to calculate the HC_5 for all six test species listed above.

B 9.11.2.1.2. Flufenacet & Diflufenican SC 600: Seedling Emergence and Seedling Growth

Test on terrestrial non-target plants of 6 families (2 Monocotyledoneae, 4 Dicotyledoneae).

Reference:	Flufenacet & Diflufenican SC 600: Seedling Emergence and Seedling Growth Test on terrestrial non-target plants of 6 families (2 Monocotyledoneae, 4 Dicotyledoneae).
Author(s). year:	Kalsch. W. 2002
Report/Doc.	Study No: P2PA, Reference BCS No: M-072308-01-1
Guideline (s):	OECD 208 A
GLP:	Yes

Test substance	Flufenacet & Diflufenican SC 600. content of a.s.: Diflufenican 205.00 g/L. Flufenacet 406.25 g/L (analysed). sum of active substance 611.25 g/L, batch no: 07205/0024(0006), density: 1.25 g/mL (valid from Feb. 6. 2002).
----------------	---

Type of test	Seedling emergence test
Test duration	21 days
Test species	4 dicotyledonous (<i>Brassica napus</i> , <i>Cucumis sativus</i> , <i>Glycine max</i> , <i>Lycopersicon esculentum</i>) plant species. 2 monocotyledonous (<i>Allium cepa</i> , <i>Avena sativa</i>) plant species.
<u>Test soil</u>	A standard soil LUFA Sp2.2 (loamy sand) collected from a hay meadow at Hanhofen/Germany. The side did not received organic carbon fertilizers or plant protection product since 1996. Two batches of LUFA Sp2.2 soil were used in this study. The batch no: Sp 223501 for testing (<i>Avena sativa</i>) and The batch no: Sp 220302 for remaining species. The properties of soils used in the test: <u>The batch no: Sp 223501</u> % of organic carbon: 2.28 ± 0.16 . pH: 5.8 ± 0.3 . WHC: $51 \pm 4\%$ <u>The batch no: Sp 220302</u> % of organic carbon : 2.3 ± 0.2 . pH: 5.6 ± 0.4 . WHC: $50 \pm 5\%$. Since LUFA Sp2.2 was relatively poor in nutrients a nutrient solution (the Steinberg nutrient solution according to ISO Working Draft 20079) was used for watering to ensure the development of the plants throughout the test period.
Applied concentration:	Control: Deionizer water Test item: 3.2, 10.0, 32.15, 103.4 and 332.3 g total active substances (diflufenican + flufenacet)/ha.
Replicates:	The number of treatment replicates varied from 5 to 15 depending on the number of plants per test container. The minimum number of plants per treatment was 30. The number of control replicates was at least 6.

Table B 9.11.2.1.2-1: The number of replicates in seedling emergence test.

	Allium cepa Avena saliva	Brassica napus Lycopersicon Esculentum	Glycine max	Cucumis sativus
no. of seeds per test container*	6	4	3	2
no. of replicates of treatments (controls)	5 (6)	8 (8)	10 (10)	15 (15)
total no. of containers	31	48	60	90
no. of seeds per treatment (control)	30 (36)	32 (32)	30 (30)	30 (30)

* the test container is defined as the replicate and not the individual plant

Exposure route	<p>Seeds of monocotyledoneous species and dicotyledoneous species were planted in a standard soil LUFA Sp2.2 (loamy sand). Immediately after sowing Flufenacet & Diflufenican SC 600 was sprayed at concentrations corresponding to 3.2 - 10.0 - 32.15 - 103.4 - 332.3 g a.s./ha and a water application rate of 300 L/ha on the soil surface. Following application of the test item the plants were allowed to emerge and grow for 21 days following 50 % emergence of the control plants under laboratory conditions.</p> <p>Plants were grown in container (polystyrene vessels Bellaplast 590 (18 x 13.5 x 6.5 x 6.5 cm) containing 800 ± 10 g soil. A hole (10 mm) was punched in the bottom of the test container and a glass fibre wick was inserted. During the each test container was placed above a polystyrene beaker serving as water reservoir in a way that the wicks reached the water reservoirs filled with deionised water or nutrient solution. Applications were made using a plot sprayer equipped with one flat spraying nozzle.</p>
Test condition:	<p>The test plants were cultivated in two rooms equipped with artificial lighting 13000 ± 2000 lux with a photoperiod of 16 h/d .</p> <p>The temperature in the test rooms was kept at 22 ± 3 °C</p>
Humidity:	not reported:
<u>Test parameter</u>	<p>Test concentrations were confirmed by analytical verification of the highest test solution. At each time when the test substance was applied, a sample of the respective test solution was taken and deep-frozen immediately after the preparation of the test solution.</p>
Statistic	<p>Shoot length and biomass at day 21 were evaluated for homogeneity of variances by Cochran's test.</p> <p>Subsequently ANOVA was used to detect differences between biological data of the different concentration levels.</p> <p>Dunnett's test was used to compare the data of the treated replicates to the control replicates in order to determine significant differences from the control containers.</p> <p>The statistical software used was SPSS for Windows, version 7.5. For the number of survived plants a non-parametric analysis of variance (Kruskal-Wallis-Test) was performed.</p> <p>In addition, Bonferroni U-Test was performed to detect differences between the treatment Statistical significant differences between survival in</p>

treatments compared to the control were difficult to detect because the number of plants per test vessel was rather low and the control to determine NOEC and LOEC values.

Findings:

Analytical findings:

All calculations were based on nominal concentrations.

Analytical verification of the highest test solution resulted in recoveries of 92.8 – 97.4 % (sum of active ingredients).

Biological results:

Table B 9.11.2.1.2-2: Survival of live plants on day 21 (percentage of total seeds sown).

Test item (g a.s./ha)	Plant species					
	Monocotyledoneae		Dicotyledoneae			
	<i>Alium cepa</i>	<i>Avena sativa</i>	<i>Brassica napus</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>	<i>Lycopersicon esculentum</i>
Control	80.6	100	90.0	95	86.7	97.5
3.2	86.7	100	90	90	76.7	97.5
10	73.3	100	97.5	95	86.7	90
32.1	83.3	100	85.0	85	76.7	87.5
103.4	83.3	100	87.5	87.5	76.7	95
332.3	40	76.7	97.5	97.5	70	100

Table B 9.11.2.1.2-3: Shoot fresh weight (percentage of untreated control).

Test item (g a.s./ha)	Plant species					
	Monocotyledoneae		Dicotyledoneae			
	<i>Alium cepa</i>	<i>Avena sativa</i>	<i>Brassica napus</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>	<i>Lycopersicon esculentum</i>
Control	-	-	-	-	-	-
3.2	78.91	94.26	87.90	104.75	92.70	82.29
10	82.55	89.76	76.66	94.58	96.51	86.23
32.1	73.45	96.73	67.64	87.65	94.72	89.47
103.4	58.91	75.85	51.66	83.30	95.78	97.03
332.3	31.27	28.65	51.14	28.79	89.84	77.02

B 9.11.2.1.2-4: Shoot length (percentage of untreated control).

Test item (g a.s./ha)	Plant species					
	Monocotyledoneae		Dicotyledoneae			
	<i>Alium cepa</i>	<i>Avena sativa</i>	<i>Brassica napus</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>	<i>Lycopersicon esculentum</i>
Control	-	-	-	-	-	-
3.2	94.13	99.05	94.31	100.59	90.14	94.45
10	92.32	99.55	96.87	96.51	95.51	101.58
32.1	94.19	102.19	93.77	99.67	110.26	97.53
103.4	79.37	75.35	93.77	96.82	92.95	105.68
332.3	45.64	33.01	91.34	67.40	101.72	79.83

Table B 9.11.2.1.2-5: Visual observations.

Study period	Plant species						
	Monocotyledoneae			Dicotyledoneae			
	Test item (ga.s.)	Alium cepa	Avena sativa	Brassica napus	Cucumis sativus	Glycine max	Lycopersicon esculentum
Chlorosis or discolouration							
7 days after 50% emergence of controls	Control	0	0	0	0	0	0
	3.2	0	0	0	0	4.3	0
	10	0	0	3.2	0	0	0
	32.1	4	0	22.2	3.3	8.7	7.1
	103.4	34.6	0	25.0	3.4	8.0	6.7
	332.3	59.3	3.3	58.1	33.3	13.6	25.0
	Deformations, wilting or developmental abnormalities %						
	Control	0	0	0	0	0	0
	3.2	0	0	3.4	0	0	0
	10	0	0	0	0	11.5	0
	32.1	0	0	0	0	8.7	7.1
	103.4	0	0	0	6.9	28.0	10
	332.3	59.3	76.7	0	14.8	18.2	9.4
14 days after 50% emergence of controls	Chlorosis or discolouration						
	Control	0	0	0	0	0	0
	3.2	0	0	0	0	0	0
	10	0	3	6.5	0	0	3.4
	32.1	12	0	25.9	3.3	0	3.6
	103.4	46.2	0	28.6	17.2	0	10.0
	332.3	55.6	96.7	65.5	92.9	4.5	59.4
	Deformations, wilting or developmental abnormalities %						
	Control	0	0	0	0	15.4	3.2
	3.2	3.8	0	0	0	43.5	0
	10	0	0	0	0	26.9	0
	32.1	0	0	0	3.3	30.4	3.6
	103.4	0	20	0	3.4	32	10
	332.3	18.5	6.7	3.2	0	22.7	62.5
21 days after 50% emergence of controls	Chlorosis or discolouration						
	Control	0	0	0	0	0	12.9
	3.2	0	0	0	0	0	9.7
	10	0	0	0	0	0	20.7
	32.1	0	3.3	0	6.7	0	28.6
	103.4	26.9	3.3	0	17.2	0	36.7
	332.3	33.3	36.7	0	96.4	0	31.3
	Deformations, wilting or developmental abnormalities %						
	Control	0	0	0	0	0	3.2
	3.2	0	0	0	0	4.3	3.2
	10	0	0	3.2	0	7.4	10.3
	32.1	0	0	11.1	6.7	0	0
	103.4	7.7	23.3	25.0	3.4	8	3.3
	332.3	11.1	60	67.7	96.4	4.5	53.1

Visual observations:

Control

At day 7 after application, no phytotoxicity effects were observed in control plants.

At the 14th day after application in the control deformation, wilting or developmental abnormalities for *Glycine max* attained 15.5% of plants and for *Lycopersicon esculentum* this effect was observed in 3.2% of tested plants.

At day 21, 3.2% of *Lycopersicon esculentum* control plant had deformed leaves. Four plants (12,6%) had somewhat pale green leaves and were thus classified as "damaged". However, such light green leaves can occasionally be observed at untreated tomato plants without an effect on biomass production.

Treatment

The test item had no significant effect on the emergence of the seedlings. At day 7 some effects were observed. *Avena sativa* was the least sensitive species: only at the highest application rate some chlorotic and abnormal plants were found. *Allium cepa* showed chlorotic leaves even at 103.4 g a.s./ha. The dicotyledoneous species showed symptoms at 32.1 g a.s./ha and above (typically chlorosis of cotyledons or first leaves).

At day 14 effects on *Avena sativa* were observed at 103.4 g a.s./ha and above (chlorosis and abnormalities) and very few plants had chlorotic leaves even at 10.0 g a.s./ha. *Allium cepa* showed effects at 32.1 g a.s./ha and above (mainly chlorosis). The dicotyledoneous plants showed effects mainly at 10.0 g a.s./ha and above. The typical symptom was chlorosis except for *Glycine max* which in contrast showed wilted or deformed leaves.

At day 21, the observed pattern was similar to day 14 except that some plants of *Glycine max* had recovered and chlorosis of *Lycopersicon esculentum* was now observed more often.

Table B 9.11.2.1.2-6: Effects of Flufenacet + Diflufenican SC 600 on six plant species: early post-emergence treatment.

21 days after 50 % emergence of controls	Plant species					
	Monocotyledoneae		Dicotyledoneae			
	<i>Allium cepa</i>	<i>Avena sativa</i>	<i>Brassica napus</i>	<i>Cucumis sativus</i>	<i>Glycine max</i>	<i>Lycopersicon esculentum</i>
Flufenacet & Diflufenican SC 600 (g a.s./ha in 300 L/ha)						
survival ¹⁾						
ER ₅₀	331.52	> 332.3 ²⁾	> 332.3 ²⁾	> 332.3 ²⁾	> 332.3 ²⁾	> 332.3 ²⁾
NOEC	103.4	≥ 332.3 ³⁾	≥ 332.3 ³⁾	≥ 332.3 ³⁾	≥ 332.3 ³⁾	≥ 332.3 ³⁾
LOEC	332.3	n.d.	n.d.	n.d.	n.d.	n.d.
Shoot length						
ER ₅₀	308.96	210.99	> 332.3	> 332.3 ²⁾	> 332.3 ²⁾	> 332.3 ²⁾
NOEC	32.1	32.1	3.2	32.1	≥ 332.3 ³⁾	103.4
LOEC	103.4	103.4	10.0	103.4	n.d.	332.2
Fresh weight						
ER ₅₀	190.43	207.88	100 ⁴⁾	218.41	> 332.3 ²⁾	> 332.3 ²⁾
NOEC	32.1	32.1	3.2	32.1	≥ 332.3 ³⁾	≥ 332.3 ³⁾
LOEC	103.4	103.4	10.0	103.4	n.d.	n.d.
Visual effects	32.1	10	3.2	10.0	<3.2	3.2
21 d NOEL						

1) no. of surviving plants.

2) ER₅₀ could not be calculated because of less than 50 % effect, therefore estimated to be > 332.3 g a.s./ha

3) estimated value, no significant effect within the range tested.

n.d. not determined, no significant effect within the range tested.

Since ~50% effects are observed at 103.4 and 332.3 g a.s./ha, EC₅₀ of 214.22 g a.s./ha estimation of EC₅₀ is not reliable. Therefore, EC₅₀ of 100 g a.s./ha value for this species.

was proposed by RMS

The monocotyledonous species were slightly more sensitive than the dicotyledonous species with respect to fresh weight, which was the most sensitive parameter for which EC_{50} could be derived. The EC_{50} for fresh weight for A cepa was 190.43 g a.s./ha. Among the dicotyledonous species Brassica napus was the most sensitive with an EC_{50} for fresh weight EC_{50} for the fresh weight 100 g a.s./ha determined by RMS since about 50% effects are observed at 103.4 and 332.3 g a.s./ha.

Effects of the test item on the shoot fresh weight were more pronounced than on the shoot length.

An EC_{50} could not be derived for all species and endpoints when effects were less than 50%.

The NOEC ranged from 3.2 to 332.2 g a.s./ha- the highest concentration tested and the lowest LOEC was 10.0 g a.s./ha.

Taking into account the visual 21 d value of EC_{50} (ranged between 103.4-332.3 g.a.s./ha), it should be noted that it seems to be covered by $EC_{50}=100$ g a.s./ha (based on fresh weight).

The 21d visual NOEL is estimated to be < 3.2 g.a./ha.

Conclusion:

The most sensitive parameter was the fresh weight followed by shoot length then survival.

The most sensitive species was Brassica napus with an EC_{50} of 100 g a.s./ha (fresh weight) followed by Avena sativa (EC_{50} of 210.99 g a.s./ha). The 21d visual NOEL is estimated to be < 3.2 g.a./ha.

Comments RMS:

The seedling emergence test was conducted according to the OECD test guideline 208 A (July 2000, Draft).

In order for the test to be considered valid the following performance criteria must be met in the control:

- a seedling emergence of at least 65% has to be observed
- the mean seedling growth does not exhibit visible phytotoxic effects.

Taking into account the current valid OECD test guideline 208 (July 2006) the following validity criteria are given:

- The seedling emergence in the controls is at least 70%

In this study the seedling emergence in the control ranged between 80-100%

- In the controls the plants do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis and wilting leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species.

In this study the following effects in the control plants were observed:

On day 7 after application, no phytotoxicity effects were observed in control plants.

On the 14th day after application, in the control, deformation, wilting or developmental abnormalities for *Glycine max* affected 15.5% of plants, whereas for *Lycopersicon esculentum* this effect was observed in 3.2% of tested plants.

On day 21, 3.2% of *Lycopersicon esculentum* control plant had deformed leaves. Four plants (12.6%) had somewhat pale green leaves and were thus classified as "damaged". However, such light green leaves can occasionally be observed at untreated tomato plants without any effect on biomass production.

- In the controls the mean plant survival is at least 90% for the duration of the study.

No mortality of control plants was observed for the duration of the study

- In the controls environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix. support media or substrate from the same source. In this study the standard soil LUFA Sp2.2 (loamy sand) was used and environmental conditions were similar in study two experimental rooms (temperature and photoperiod).

- According to the OECD 208 (2006) test guideline the temperature and the humidity has to be in a range of $22\text{ }^{\circ}\text{C} \pm 10\text{ }^{\circ}\text{C}$ and $70\% \pm 25\%$, respectively.

The temperature in both experimental rooms was in a range of $22 \pm 3^{\circ}\text{C}$. Humidity was not reported in the study protocol

- Minimum 16 h photoperiod was recommended and light intensity – $350\text{ }\mu\text{E}/\text{m}^2/\text{s}$. Additional lighting may be necessary if intensity decreases below $200\text{ }\mu\text{E}/\text{ms}$, wavelength 400 - 700 nm except for certain species whose light requirements are less.

The lighting of $13000 \pm 2000\text{ lx}$ (16 hours) was maintained during the study.

The following deviations were noted from the 208 (2006) test guideline:

- Steinberg nutrient solution was used instead of Hoagland solution
- The organic carbon content of the soil was higher (2.8%) than the recommended value (1.5%).
- No data of humidity was recorded during the study

Taking into account the validity criteria given in the OECD 208 A (2000) and OECD 208 (2006) test guidelines, the validity criteria was met, except the requirements of lack of effects in the control plants - for Glycine max and Lycopersicon esculentum species.

However, it should be note that, for this two species no significant effects on survival, shoot length or fresh weight at the highest rate of $332.3\text{ g sum a.s/ha}$ were noted.

No phytotoxic symptom have been reported for the control of onion – the most sensitive plants.

Agreed endpoints:

$\text{EC}_{50} = 190.43\text{ g total active substances/ha}$, based on fresh weight (*Alium cepa*)

$\text{EC}_{50} = 207.88\text{ g total active substances/ha}$, based on fresh weight (*Avena sativa*)

$\text{EC}_{50} = 100\text{ g total active substances/ha}$, based on fresh weight (*Brassica napus*)

$\text{EC}_{50} = 218.41\text{ g total active substances/ha}$, based on fresh weight (*Cucumis sativus*), based on based on fresh weight $\text{EC}_{50} > 332.3\text{ g total active substances/ha}$, based on fresh weight (*Glycine maxx* and *L. esculentum*), based on based on fresh

The most sensitive species:

$\text{EC}_{50} = 100\text{ g total active substances/ha}$, based on fresh weight (*Brassica napa*)

The Applicant is kindly requested to calculate the HC_5 for all six test species listed above.

B 9.11.2.1.3. Flufenacet SC 500: Vegetative vigour test on non target terrestrial plants

Reference:	Flufenacet SC 500: Vegetative vigour test on non target terrestrial plants
Author(s), year:	Friedrich S., 2005
Report/Doc.number:	Report No: 041048105, Reference BCS No: M-248251-01-1
Guideline (s):	Plant Test 208 B: Vegetative Vigour Test
GLP:	Yes

<u>Test substance:</u>	Flufenacet 500 SC, Product code: AE F133402 00 SC42 A102, containing s.a.- flufenacet (FOE 5043), batch no: EFKFT000175, purity: 42.3% w/w . density of formulation: 1.194 g/mL
Type of test:	Vegetative vigour test
Test duration:	21 days
Test species:	10 species tested: five monocotyledoneae and 5 Dicotyledoneae, belong to seven families:

Table B 9.11.2.1.3-1: Plants tested.

Family	Class	Species
Gramineae	Monocotyledoneae	Zea mays (corn)
Gramineae	Monocotyledoneae	Avena sativa (oats)
Gramineae	Monocotyledoneae	Lolium perene (perennial ryegrass)
Gramineae	Monocotyledoneae	Sorghum biclor (sweet sorgum)
Liliaceae	Monocotyledoneae	Alium cepa (onion)
Brassicacea	Dicotyledoneae	Brasica rapa (turnip)
Chenopodiaceae	Dicotyledoneae	Beta vulgaris (sugar beet)
Cucurbitaceae	Dicotyledoneae	Cucumis sativa (cucumber)
SolanaceaFabacea	Dicotyledoneae	Lycopersicon escu.(tomato)
Fabaceae	Dicotyledoneae	Glycine max (soybean)

Test soil:	A agricultural soil collected from fallow land from site Gerichshain, Germany. The side did not received pesticides or fertilizers for least 5 years.
------------	---

The properties of the soil used in the test:

C_{org}: 0.9%

Humus content: 1.5%

pH: 6.0

WHC: 36.5 g/100 g dry weight

Particle size: <0.002 mm (clay): 12.4%

0.002-0.063 mm (silt): 48.8%

0.063-2.00 mm (sand): 38.9%

Soil type:

- loam soil, according to USDA

- sandy loam soil, according to BBA 1994

Applied concentration:Nominal:

Control: (Deionizer water), 18.75, 37.5, 75, 150, 300, 600 g a.s./ha

(corn, oats, turnip, sugar beet, cucumber, tomato, soybean)

Control: (Deionizer water), 4.7, 9.4, 18.75, 37.5, 75 and 150 g a.s./ha

(onion, perennial ryegrass)

Control: (Deionizer water), 2.4, 4.7, 9.4, 18.75, 37.5 and 75 g a.s./ha

(sweet sorghum)

Test pot:

Plastic flower pot (Ø15 cm)

(Capacity 1.6 kg fresh soil; amount soil/pot: 1.4 kg)

Replicates:

The number of replicates/treatment varied from 7 to 16 depending on the number of plants per test pot. The minimum number of plants per treatment was 32.

Table B 9.11.2.1.3-2: The number of replicate used in the vegetative vigor test.

	Corn, cucumber, tomato, soybean	Sweet sorghum, turnip, sugar beet	Oats, onion, perennial ryegrass
no. of plants /pot	2	4	5
no. of replicates=number of pots/ treatments (controls)	16 (16)	8 (8)	7 (7)
total no. of plants/treatment	32	32	35

Exposure route

Seeds of monocotyledonous species and dicotyledonous species were planted in a soil and were allowed to emerge and grow until the two-leaf stage was reached. Plants were grown in a plastic flower pot (Ø 15 cm) containing 1.4 kg soil/pot.

Fertilizing was done once, 7 days after germination (100 mL/pot)

Irrigation was conducted daily with 40 mL tap water (pH: 7.3, conductivity 580 µS/cm).

Application was carried out, when the plants had reached the two-leaf stage, three to five weeks after emerged.

Test solutions were sprayed onto the plants and the soil substrate in an automatic application cabin at a spray volume equivalent to 400 L/ha of water.

Test condition:

The test plants were cultivated in greenhouse equipped with artificial lighting with a photoperiod of 16 h/d (including 6 h HQ-light)

The temperature:

11 ± 31 °C

Humidity:

35-82%

Test parameter:	<p>The plants were observed weekly during 21 days after application (after 7, 14 and 21 days) for visual phytotoxicity and plant survival/mortality.</p> <p>A plant was defined as dead, when it was photosynthetically inactive, i.e. when there was 100 % necrosis and no chlorophyll.</p> <p>Severity of phytotoxic symptoms: e.g. chlorosis, necrosis, wilting was expressed as the %/plant.</p> <p>At the end of the test, final shoot fresh weight (per pot) and observations of phytotoxicity were taken from surviving plants.</p> <p>The assessments included all variations, either inhibitory or stimulatory, between treated and untreated plants and also the stage of development.</p>
Analytical parameters:	Test concentrations were confirmed by analytical verification of the highest test solution.
Statistic:	<p>Calculation of the dose-response relationships and EC₅₀/LC₅₀ values, using the software ToxRat Professional 2.07 (RATTE 2002).</p> <ul style="list-style-type: none"> - Statistical analysis of data obtained for fresh weight for significant differences between treated and untreated plants (ToxRat Professional 2.07 (RATTE 2002)). - Determination of NOEC and LOEC (using a <i>multiple</i> test method, i.e. multiple sequentially rejective U-test after BONFERRONI-HOLM and DUNNETT) - For all tests a significance level of $\alpha = 0.05$ was used
Findings:	
Analytical findings:	The highest dosed test solutions at the start of the test were analysed for Flufenacet and resulted in recoveries between 87.6% and 103.9% of nominal.
Survival:	<p>The 100 % survival of the control was recorded at the study termination.</p> <p>The foliar application of Flufenacet SC 500 had no toxic effect on survival of tested plants. The 100 % of survival was identified in the tested plants with one exception. The eleven percentage of mortality was identified for Lolium perenne at the highest application rate of 150 g a.s./ha.</p>

TableB 9.11.2.1.3-3: The seedling survival at 21 days after application of Flufenacet 500 SC.

21 days after app.	Zea mays	Avena sativa	Lolium perenne	Sorgum bicolor	Allium cepa	Brassica napra	Beta vulgaris	L. escu.	C. sativa	Glycine max
	Monocotyledoneae					Dicotyledoneae				
LC ₅₀ (ga.s./ha)	>600	>600	>150	>150	>75	>600	>600	>600	>600	>600

Summary of results considering shoot fresh weight of the plants at day 21 are presented at the Table B.9.11.2.1.3-4 and Table B.11.2.1.3-5 below:

Shoot fresh weight – Monocotyledoneae

Table B 9.11.2.1.3-4: Shoot fresh weight of monocot plants 21 days after application of Flufenacet 500 SC.

Type of plant	Plant species	Application rate (g a. s./ha)	Shoot fresh weight (g)	% reduction ^a	Shoot fresh weight	
					EC ₅₀	NOEC
					g a.s./ha	
Monocotyledonous plants	<i>Zea mays</i> (Maize/corn)	Control (0)	15.06±1.75		>600	300
		18.75	15.34±1.35	-2		
		37.5	14.86±1.53	4		
		75.0	14.21±1.68	1		
		150	14.21±1.68	6		
		300	13.72±1.64	9		
		600	12.34±1.35	18*		
	<i>Avena sativa</i> (Oats)	Control (0)	16.42±0.87		196	37.5
		18.75	16.12±0.73	2		
		37.5	15.29±1.31	7		
		75.0	13.63±1.16	17*		
		150	10.33±1.60	37*		
		300	4.69±0.63	71*		
		600	2.92±0.37	83*		
	<i>Sweet sorghum</i> (<i>Sorghum bicolor</i>)	Control (0)	4.50±0.42	-	43	9.4
		2.4	4.41±0.44	2		
		4.7	4.57±0.68	-2		
		9.4	4.08±0.60	9		
		18.75	3.70±0.36	18*		
		37.5	2.71±0.49	40*		
		75.0	1.07±0.18	76*		
	<i>Lolium perenne</i> (Perennial ryegrass)	Control (0)	11.61±1.29	-	17	4.7
		4.7	10.80±1.47	7		
		9.4	9.05±1.08	22		
		18.75	3.70±0.36	59*		
		37.5	1.93±0.34	83*		
		75.0	0.83±0.18	93*		
		150	0.34±0.10	97*		
	<i>Allium cepa</i> (Onion)	Control (0)	2.48±0.39	-	132	9.4
		4.7	2.52±0.37	-1		
		9.4	2.46±0.41	1		
		18.75	1.98±0.26	20*		
		37.5	1.71±0.16	31*		
		75.0	1.62±0.32	35*		
		150	1.19±0.25	52*		

± Standard deviation (SD)

a (%): reduction of fresh weight compared to control

* Statistically significant compared to control (Dunnett-test, Bonefroni-Holm-U-test, $p \leq 0.05$)

Taking into account the quantitative parameters, such as shoots fresh weight, the most sensitive monocotyledonous species was *Lolium perenne* with EC₅₀ of 17 g a.s./ha, followed by *Sorghum bicolor* with EC₅₀ of 43 g a.s./ha, *Allium cepa* with EC₅₀ of 132 g a.s./ha, *Avena sativa* with EC₅₀ of 196 g a.s./ha and *Avena sativa* EC₅₀ >600 g a.s./ha.

Shoot fresh weight – Dicotyledoneae**Table B 9.11.2.1.3-5: Shoot fresh weight of dicot plants 21 days after application of Flufenacet 500 SC.**

Type of plant	Plant species	Application rate (nominal) of the test item (g a. s./ha)	Shoot fresh weight (g)	% reduction ^a	Shoot fresh weight	
					EC ₅₀	NOEC
					(g a.s./ha)	
Dicotyledonous plants	Brassica rapa (Turnip)	Control (0)	20.39±1.96	-	167	37.5
		18.75	20.80±1.86	-2		
		37.5	18.19±1.80	11		
		75.0	15.89±1.43	22*		
		150	10.95±2.72	46*		
		300	6.32±1.82	69*		
		600	2.08±0.53	90*		
	Beta vulgaris (Sugar beet)	Control (0)	36.03±3.17	-	525	150
		18.75	35.60±2.45	1		
		37.5	34.84±1.87	3		
		75.0	34.06±3.49	5		
		150	32.48±2.34	10		
		300	27.52±2.21	24*		
		600	15.33±2.97	57*		
	Cucumis sativa (Cucumber)	Control (0)	35.28±2.30	-	102	<18.75
		18.75	28.89±2.70	18*		
		37.5	26.40±2.41	25*		
		75.0	17.90±2.10	46*		
		150	13.38±2.32	62*		
		300	10.56±2.42	70*		
		600	8.28±1.26	77*		
	Lycopersicon esculentum (Tomato)	Control (0)	34.79±2.87	0	>600	18.75
		18.75	32.60±2.50	6		
		37.5	30.84±3.70	11*		
		75.0	28.07±2.81	19*		
		150	26.06±2.98	25*		
		300	21.42±2.09	38*		
		600	19.12±2.21	45*		
	Glycine max (Soybean)	Control (0)	13.47±1.56	-	168	18.75
		18.75	12.97±1.11	6		
		37.5	11.60±1.44	16*		
		75.0	10.63±1.89	23*		
		150	6.55±1.25	52*		
		300	4.49±1.04	67*		
		600	3.08±0.69	78*		

± Standard deviation (SD)

a (%): reduction of fresh weight compared to control

* Statistically significant compared to control (Dunnett-test, Bonefroni-Holm-U-test, p≤p0.05)

Cucuma sativa was the most sensitive dicotylodenous species with EC₅₀ of 102 g s.a./ha, followed by Brassica napa with EC₅₀ of =167 g a.s./ha, Glycine max with EC₅₀ of 168 g s.a./ha , Beta vulgaris with EC₅₀ of 525 g a.s./ha, and Lycopersicon ecs. with EC₅₀ >600 g a.s./ha.

The visual observation during the study were presented in the Tables below:

Table B 9.11.2.1.3-6: Phytotoxicity and growth inhibitory effects observed 7 days after application of Flufenacet 500 SC.

7 days after application (g a.s./ha)	Zea mays (Corn)	Avena sativa (Oats)	Lolium perenne (ryegrass)	Sorghum bicolor (sweet sorghum)	Allium cepa (Onion)	Brassica rapa	Beta vulgaris	Lycopersicon escu. (tomato)	Cucumis sativa (cucumber)	Glycine max (soybean)
Monocotyledoneae						Dicotyledoneae				
Necrosis (%)						Necrosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	2	3
75	0	0	1	0	0	4	0	0	5	5
150	0	0	3	-	1	8	1	2	7	9
300	0	0	-	-	-	9	2	4	8	13
600	0	0	-	-	-	10	5	8	10	18
Chlorosis (%)						Chlorosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	2	3
75	0	0	0	0	0	0	0	2	5	7
150	0	0	0	-	0	2	2	4	6	8
300	0	0	-	-	-	4	4	9	7	11
600	0	0	-	-	-	8	11	12	8	12
Deformation (%)						Deformation (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0
150	0	0	0	-	0	0	0	0	0	0
300	0	0	-	-	-	1	0	0	0	0
600	0	0	-	-	-	3	0	0	0	0
Growth inhibition (%)						Growth inhibition (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	4	0
37.5	0	0	4	8	0	0	0	0	7	4
75	0	0	11	20	4	7	0	8	12	11
150	0	0	24	-	9	16	7	14	23	23
300	0	8	-	-	-	34	16	20	39	31
600	2	21	-	-	-	45	38	25	45	51
BBCH Growth stage						BBCH Growth stage				
Control	13	13	14	13	13	14	13	14	13	13-14
2.4	-	-	-	13	-	-	-	-	-	-
4.7	-	-	14	13	13	-	-	-	-	-
9.4	-	-	14	13	13	-	-	-	-	-
18.75	13	13	14	13	13	14	13	14	13	13-14
37.5	13	13	13	13	13	14	13	14	12	13
75	13	13	12	12	13	14	13	13	12	13
150	13	13	12	-	12	13	13	12	12	12
300	13	12	-	-	-	12	12	12	12	12
600	13	12	-	-	-	12	12	12	12	12

- not tested rate

Table B 9.11.2.1.3-7: Phytotoxicity and growth inhibitory effects observed 14 days after application of Flufenacet 500 SC.

7 days after application (g a.s./ha)	Zea mays (Corn)	Avena sativa (Oats)	Lolium perenne (perennial ryegrass)	Sorghum bicolor (sweet sorghum)	Allium cepa (Onion)	Brassica rapa	Beta vulgaris	Lycopersicon esculentum (tomato)	Cucumis sativus (cucumber)	Glycine max (soybean)
Monocotyledoneae						Dicotyledoneae				
Necrosis (%)						Necrosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	1	1
37.5	0	0	1	0	0	4	0	0	6	4
75	0	0	3	0	3	6	0	3	7	6
150	0	0	5	-	2	13	2	4	8	16
300	0	0	-	-	-	14	4	5	9	20
600	0	0	-	-	-	11	6	9	11	18
Chlorosis (%)						Chlorosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	1	1
37.5	0	0	0	0	0	0	0	2	4	4
75	0	0	1	1	1	1	0	4	6	9
150	0	0	3	-	4	4	1	9	8	9
300	0	0	-	-	-	6	3	13	9	8
600	0	0	-	-	-	11	8	15	10	6
Deformation (%)						Deformation (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0
150	0	0	0	-	0	2	0	0	0	0
300	0	0	-	-	-	3	0	0	0	0
600	0	0	-	-	-	4	0	0	0	0
Growth inhibition (%)						Growth inhibition (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	2	0	0	-	-	-	-	-
18.75	0	0	29	8	5	0	0	0	7	1
37.5	0	6	57	21	9	4	0	0	8	7
75	0	38	67	58	23	11	0	12	26	15
150	0	75	86	-	36	21	11	20	42	29
300	3	88	-	-	-	46	22	24	48	39
600	8	98	-	-	-	55	48	29	56	43
BBCH Growth stage						BBCH Growth stage				
Control	14	12	21	15	14	16	14	16	14	14-15
2.4	-	-	-	15	-	-	-	-	-	-
4.7	-	-	21	15	14	-	-	-	-	-
9.4	-	-	21	14-15	14	-	-	-	-	-
18.75	14	12	21	14-15	14	16	14	16	14	14-15
37.5	14	12	15	13	14	16	14	16	13	14
75	14	11-12	12	12-13	13-14	15	14	15	13	13-14
150	14	10-11	12	-	12	13-14	14	14	12	12-13
300	14	10	-	-	-	12-13	13-14	13-14	12	12
600	13-14	10	-	-	-	12	12-13	12-13	12	12

- not tested rate

Table B. 9.11.2.1.3-8: Phytotoxicity and growth inhibitory effects observed 21 days after application of Flufenacet 500 SC.

21 days after application (g a.s./ha)	Zea mays (Corn)	Avena sativa (Oats)	Lolium perenne (perennial ryegrass)	Sorghum bicolor (sweet sorghum)	Allium cepa (Onion)	Brassica napá	Beta vulgaris	Lycopersicon escu. (tomato)	Cucumis sativa (cucumber)	Glycine max (soybean)
Monocotyledoneae						Dicotyledoneae				
Necrosis (%)						Necrosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	3	2
37.5	0	0	3	0	0	3	0	2	7	5
75	0	0	4	0	5	6	0	4	10	7
150	0	0	4	2	8	11	2	6	11	17
300	0	0	-	-	-	14	5	8	12	22
600	0	0	-	-	-	20	6	12	14	20
Chlorosis (%)						Chlorosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	2	2
37.5	0	0	2	0	2	0	0	0	5	5
75	0	0	4	0	5	2	0	2	6	8
150	0	0	2	0	10	5	2	5	9	10
300	0	0	-	-	-	5	5	10	7	8
600	0	0	-	-	-	6	9	8	6	5
Deformation (%)						Deformation (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	0	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	2	0	0	0	0
300	0	0	-	-	-	5	0	0	0	0
600	0	0	-	-	-	2	0	0	0	0
Growth inhibition (%)						Growth inhibition (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	0	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	10	0	0	-	-	-	-	-
18.75	0	0	54	5	12	0	0	0	10	0
37.5	0	2	77	10	20	6	0	10	14	11
75	0	16	85	40	30	13	2	18	35	20
150	0	35	92	70	40	31	11	24	47	38
300	5	64	-	-	-	55	25	28	56	44
600	11	77	-	-	-	69	54	33	62	53
BBCH Growth stage						BBCH Growth stage				
Control	15	21	23	21	15	19	15-16	55	61	21
2.4	-	-	-	uz	-	-	-	-	-	-
4.7	-	-	23	21	15	-	-	-	-	-
9.4	-	-	23	21	15	-	-	-	-	-
18.75	15	21	22-23	15	15	19	15-16	55	61	21
37.5	15	21	21	15	14-15	19	15-16	55	61	21
75	15	21	12-13	14	13-14	18	15-16	54	60	14
150	15	21	12	13	12	14	15-16	51	60	13
300	15	13-14	-	-	-	13	14-15	15-16	55	12-13
600	14-15	13	-	-	-	12	12-14	13-14	12	12

- not tested rate

Visual observations:

No phytotoxic effects in control plants have been observed over the entire study period (at 7, 14 and 21 days) after application.

In order to determine the 21 visual NOEL and 21 visual EC₅₀, RMS describe the effects observable at 21 days after treatment.

For the monocotyledoneae plants the growth inhibition effects (stunting) was the most sensitive visual parameter. The growth inhibition effects attained 54% *Lolium perenne* plants and 70% *Sorghum bicolor* plants at rates 18.75 g a.s./ha and 150 g a.s./ha, respectively.

For *Avena sativa* plants, these effects were recorded in 64 and 77% tested plants at two highest rates of 300 and 600 g a.s./ha, respectively.

The monocot plants were slightly affected by chlorosis or necrosis. These effects were mainly observed for *Allium cepa* (8% necrosis and 10% chlorosis) at the highest tested rate of 150 g a.s./ha wherein, for *Zea mays* and *Avena sativa* plants the lack of these effects were recorded during the study. No deformation or stimulation effects were observed for all treatment plants. For dicot plants the growth inhibition effects were also the most sensitive visual parameter. The most growth inhibition effects were observed for *Cucumis sativa*-62%, followed by *Glycine max*-53% at maximum application rate - 600 g a.s./ha. Necrosis was observed for all dicot plants but these effects were more pronounced at two highest application rates 600 g a.s./ha and ranged between 8 and 22%. Slight chlorosis was recorded for all dicot plants but for *Cucumis sativa* and *Glycine max* these effects were observed for all application rates.

The chlorosis did not attain more than 10% of tested plants even in the highest tested rate of 600 g a.s./ha.

No deformation or stimulation effects were observed for all treatment plants.

The summary of endpoints obtained from the study including the values of 21 visual NOEL and 21 d visual EC₅₀ are presented in the Table B. 9.11.2.1.3-9 below:

Table B 9.11.2.1.3-9: Survival, shoot fresh weight and phytotoxic effects.

Plant species	Plant survival LC ₅₀ g s.a./ha	Shoot fresh weight			Visual effects			BBCH
		EC ₅₀	NOEC	LOEC	Application rate g a.s/ha	% effects*	21 NOEL visual** g a.s./ha	
		g s.a./ha						
Monocotyledonea								
Zea mays	>600	>600	300	600	18.75	0	150	14-15
					37.5	0		
					75	0		
					150	0		
					300	5		
					600	11		
Avena sativa	>600	196	37.5	75	18.75	0	18.75	13-21
					37.5	2		
					75	16		
					150	35		
					300	64		
					600	77		
Allium cepa	>150	132	9.4	18.75	4.7	0	9.4	12-15
					9.4	0		
					18.75	12		
					37.5	20		
					75	30		
					150	40		
Lolium perenne	>150	17	4.7	9.4	4.7	0	4.7	12-23
					9.4	10		
					18.75	54		
					37.5	77		
					75	85		
					150	92		
Sorghum bicolor	>75	43	9.4	18.75	4.7	0	9.4	13-21
					9.4	0		
					18.75	5		
					75	40		
					150	70		
Dicotyledoneae								
Brassica rapa	>600	167	37.5	75	18.75	0	18.75	12-19
					37.5	6		
					75	13		
					150	31		
					300	55		
					600	69		
Beta vulgaris	>600	525	150	300	18.75	0	37.5	12-16
					37.5	0		
					75	2		
					150	11		
					300	25		
					600	54		
Cucumis sativa	>600	102	< 18.75	18.75	18.75	10	<18.75	12-61
					37.5	14		
					75	35		
					150	47		
					300	56		
					600	62		
Lycopersicon esculentum	>600	>600	18.75	37.5	18.75	0	18.75	13-55
					37.5	10		
					75	18		
					150	24		
					300	28		
					600	33		
Glycine max	>600	168	18.75	37.5	18.75	0	<18.75	12-21
					37.5	11		
					75	20		
					150	38		
					300	44		
					600	53		

* % effects based on the most sensitive visual parameter - % inhibition growth effects (stunting)

** 21 d No observed effect level (empirically estimated by RMS)

Note: In bold, the visual effects - more than 50% affected plants

Conclusion:

Taking into account the quantitative parameters, such as shoots fresh weight, the most sensitive monocotyledonous species was *Lolium perenne*, with EC₅₀ of 17 g a.s./ha. The most sensitive dicotyledonous species was *Cucumis sativus* with EC₅₀ of 102 g s.a./ha.

The growth inhibition effects (stunting) were most pronounced visual parameter for both tested groups of plants. It can be concluded that empirically predicted visual values of EC₅₀ (54% stunting at rate 18.75 g a.s./ha, Table B 9.11.2.1.3-9) will be close to the values of EC₅₀ based on most sensitive quantitative parameter - shoot fresh weight.

The lowest visual value of 21 d NOEL for phytotoxic effects - 4.7 g a.s./ha, were recorded for the most sensitive monocot plants. For the dicot plants the lowest recorded value of 21 d visual NOEL, based on phytotoxicity, was < 18.78 g a.s./ha.

RMS comments:

The vegetative vigor test was conducted according to the OECD test guideline 208 B (July 2000).

In order for the test to be considered valid, the following performance criteria must be met in the control:

- The mean seedling growth does not exhibit visible phytotoxic effects and the plant survival in the vegetative vigor test is at least 90% at the end of the test.

The test is considered acceptable given the validity criteria regarding phytotoxic effects (No phytotoxic effects were observed in the control groups) and survival of the plants (100 % survival at the end of the test).

Therefore, the study is considered acceptable according to the OECD guideline 208 B (July 2000).

Taking into account the current valid OECD test guideline 227 (July 2006) the following validity criteria are given:

- The seedling emergence is at least 70% (control and treatment groups).

No information of the seedling emergence was not given in the study protocol.

• In the control groups the plants do not exhibit visible phytotoxic effects (e.g. Chlorosis, necrosis, and wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species.

No phytotoxic effects were recorded in the control group during the study.

- In the control groups, the mean plant survival is at least 90% for the duration of the study.

The mean plant survival was 100% for the duration of the study.

- In the control groups, environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix support media or substrate from the same source.

According to the OECD 227 (2006) test guideline the following conditions are recommended:

- The temperature: 22 °C ± 10 °C
- The humidity: 70 % ± 25%, respectively
- The light intensity: 350±μE/m²/s. Additional lighting may be necessary if decreases below 200 μE/m²/s, wavelength 400-700 nm except for certain species whose light requirements are less.

The following deviations were noted from OECD 227 guideline:

- There is no information in the study protocol address possible effects on the test plants due to the low humidity – 35% in the greenhouse. However, under consideration of the observed effects on plant survival and plant phytotoxicity the influence of the humidity on the test plants is considered to be low.
- Four seeds of sugar beet /pot were sown instead of one to two seeds/pot as recommended in the test guideline. However, there was no mortality or phytotoxicity observed in control, therefore it is considered that the growth conditions were sufficient for tested plants and they did not suffer with crowding.
- No information of the seedling emergence was given in the study protocol.
- No information about light intensity is given

Even though no information on the seedling emergence in the control and treatment groups are given a sufficient emergence of seedlings can be considered taking into account the available data on plant survival (100%) and phytotoxic effects.

Agreed endpoints:

EC₅₀ = 17 g a.s./ha, *Lolium perene*

EC₅₀ = 43 g a.s./ha, *Sorgum biclor*

EC₅₀ = 132 g s.a./ha, *Allium cepa*

EC₅₀ = 196 g s.a./ha, *Avena sativa*

EC₅₀ > 600 g a.s./ha, *Zea mays*

EC₅₀ = 102 g s.a./ha, *Cucuma sativa*

EC₅₀ = 167 g a.s./ha, *Brassica rapa*

EC₅₀ = 525 g a.s./ha, *Beta vulgaris*

EC₅₀ > 600 g a.s./ha, *Lycopersicon*

EC₅₀ = 168 g a.s./ha, *Glycine max*

The most sensitive plant species:

Lolium perene with EC₅₀ = 17 g a.s./ha, based on shoot fresh weight

B 9.11.2.1.4.: Flufenacet 500 S: Seedling emergence and seedling growth test on terrestrial non-target plants.

Reference:	Flufenacet 500 S: Seedling emergence and seedling growth test on terrestrial non-target plants.
Author(s). year:	Friedrich S., 2005
Report/Doc.	Study Number: 04 10 48 104, Reference BCS No: M-248251-01-1
Guideline (s):	OECD 208 A
GLP:	Yes

Test substance:	Flufenacet 500 SC, Product code: AE F133402 00 SC42 A102, containing s.a.- flufenacet (FOE 5043), batch no: EFKFT000175, purity: 42.3% w/w . density of formulation: 1.194 g/mL
Type of test:	Seedling emergence test
Test duration:	21 days after 50% seedling emergence
Test species:	10 species tested: 5 monocotyledoneae and 5 Dicotyledoneae, belong to seven families:

B 9.11.2.1.4-1: Plants tested.

Family	Class	Species
Gramineae	Monocotyledoneae	Zea mays (corn)
Gramineae	Monocotyledoneae	Avena sativa(oats)
Gramineae	Monocotyledoneae	Lolium perene (perennial ryegrass)
Gramineae	Monocotyledoneae	Sorghum biclor (sweet sorgum)
Liliaceae	Monocotyledoneae	Alium cepa(onion)
Brassicacea	Dicotyledoneae	Brasica rapa (turnip)
Chenopodiaceae	Dicotyledoneae	Beta vulgaris (sugar beet)
Cucurbitaceae	Dicotyledoneae	Cucumis sativa (cucumber)
SolanaceaFabacea	Dicotyledoneae	Lycopersicon escu.(tomato)
Fabaceae	Dicotyledoneae	Glycine max (soybean)

Test soil:	The agricultural soil collected from fallow land from site Gerichshain, Germany. The side did not received pesticides or fertilizers for least 5 years. The properties of the soil used in the test: C _{org} : 0.9% Humus content: 1.5% pH: 6.0 WHC: 36.5 g/100 g dry weight Particle size: <0.002 mm (clay): 12.4%
------------	--

0.002-0.063 mm (silt): 48.8%

0.063-2.00 mm (sand): 38.9%

Soil type: loam soil, according to USDA; sandy loam soil, according to BBA

Applied concentration:

Nominal:

Control: (Deionizer water), 18.75, 37.5, 75, 150, 300, 600 g a.s./ha
(corn, oats, turnip, sugar beet, cucumber, tomato, soybean)

Control: (Deionizer water), 4.7, 9.4, 18.75, 37.5, 75 and 150 g a.s./ha
(onion, perennial ryegrass)

Control: (Deionizer water), 2.4, 4.7, 9.4, 18.75, 37.5 and 75 g a.s./ha
(sweet sorghum)

Test pot:

Plastic flower pot (Ø 15 cm)

(capacity 1.6 kg fresh soil; amount soil/pot: 1.4 kg)

Replicates:

The number of replicates/treatment varied from 7 to 16 depending on the number of plants per test pot. The minimum number of plants per treatment was 32.

Table B 9.11.2.1.4-2: The number of replicates used in the seedling test.

	Corn, cucumber, tomato, soybean	Sweet sorghum, turnip, sugar beet	Oats, onion, perennial ryegrass
no. of plants /pot	2	4	5
no. of replicates=number of pots/ treatments (controls)	16 (16)	8 (8)	7 (7)
total no. of plants/treatment	32	32	35

Germination rate:

The minimum seed germination rates were reached for all used seeds of all tested species and attained level for 98% for *Zea mays*, 96% for *Avena sativa*, 88% of *Allium cepa*, 94% of *Lolium perenne*, 86% of *Sorghum bicolor*, 96% for *Brassica napa*, 94% for *Beta vulgaris*, 98% for *Cucumis sativus*, 96% *Lycopersicon escul.* and 88% for *Glycine max*.

Exposure route:

Seeds of monocotyledonous species and dicotyledonous species were planted in a loamy soil (according to USDA).

The control and test item treatments were applied once onto the soil immediately after sowing. Test solutions were sprayed onto the soil substrate in an automatic application cabin at a spray volume equivalent to 400 L/ha of water.

Test condition:

The test plants were cultivated in greenhouse equipped with artificial lighting with a photoperiod of 16 h/d (including 6 h HQ-light)

The temperature: 11 ± 31 °C

Humidity: 35-82%

The emergence rate was determined 7 days after 50 % of the plants had emerged in the control group. Thereafter plants were observed weekly during 21 days (after 7, 14 and 21 days after 50 % emergence) for seedling emergence, visual phytotoxicity and plant survival/mortality. A plant was

defined as dead, when it was photo synthetically inactive, i.e. when there was 100 % necrosis, no emergence and no chlorophyll.

At the end of the test, emergence/survival of the seedlings, final shoot fresh weight (per pot) and observations of phytotoxicity were taken from surviving plants. The assessments included all variations, either inhibitory or stimulatory, between treated and untreated plants and also the stage of development. The recorded effects were used to calculate the EC_{50}/LC_{50} values, LOEC and NOEC, where possible.

Statistic:

Calculation of the dose-response relationships and EC_{50}/LC_{50} values by software ToxRat Professional 2.07 (RATTE 2002).

- Statistical analysis of data obtained for fresh weight for significant differences between treated and untreated plants (ToxRat Professional 2.07 (RATTE 2002).

- Determination of NOEC and LOEC where possible

(using a *multiple* test method, i.e. multiple sequentially rejective U-test after BONFERRONI-HOLM and DUNNETT)

-For all tests a significance level of $\alpha = 0.05$ was used

Findings:

Analytical findings:

The recovery of the analysed highest dosed test solutions ranged from 87.6 - 103.9 %.

Germination, emergency of seedlings and survival of seedlings after application of Flufenacet 500 SC are preasented in the Table B. 9.11.2.1.4-3.

Table B 9.11.2.1.4-3: Germination, Emergency of seedlings and survival of seedlings after application of Flufenacet 500 SC in monocot plants.

Type of plant	Plant species	Application rate of the test item [g a. s./ha]	Germination – number of seeds:		Emergence of seedlings, 7 days after application of the test item, expressed as:		Survival of seedlings, 21 days after application of the test item		LC ₅₀ (g a. s./ha) for	
			Sown	Germinated	No. of seedlings	% of germinated	No. of seedlings	% survived	Emergence of seedlings	Plant survival after emergence
Monocotyledonous plants	Zea mays (Ccorn)	Control (0)	32	31	30	97	30	100	> 600	> 600
		18.75	32	31	30	97	31	100		
		37.5	32	31	30	97	30	100		
		75.0	32	31	28	90	29	100		
		150	32	31	30	97	30	100		
		300	32	31	30	97	30	100		
		600	32	31	28	90	29	100		
	Avena sativa (Oats)	Control (0)	35	34	33	97	33	100	472.3	418.0
		18.75	35	34	34	100	34	100		
		37.5	35	34	34	100	34	100		
		75.0	35	34	31	91	31	100		
		150	35	34	33	97	33	100		
		300	35	34	26	76	25	96		
		600	35	34	11	42	0	0		
	Sorghum bicolor (Sweet sorghum)	Control (0)	32	27	27	100	27	100	34.7	36.6
		2.4	32	27	26	96	27	100		
		4.7	32	27	26	96	27	100		
		9.4	32	27	25	93	26	100		
		18.75	32	27	18	67	20	100		

		37.5	32	27	15	56	7	47		
		75.0	32	27	5	19	0	0		
	Lolium perenne (Perennial ryegrass)	Control (0)	35	33	33	100	33	100	47.2	18.0
		4.7	35	33	32	97	32	100		
		9.4	35	33	33	100	33	100		
		18.75	35	33	32	97	13	41		
		37.5	35	33	18	55	0	0		
		75.0	35	33	12	36	0	0		
		150	35	33	0	0	0	----		
	Allium cepa (Onion)	Control (0)	35	31	30	97	31	100	> 150	> 150
		4.7	35	31	29	94	31	100		
		9.4	35	31	28	90	30	100		
		18.75	35	31	29	94	30	100		
		37.5	35	31	26	84	29	100		
		75.0	35	31	27	87	29	100		
		150	35	31	24	67	15	100		

The most sensitive monocot plants was *Sorghum bicolor* with the lowest endpoints of LC₅₀ of 34 g a.s./L (based on emergence of seedling) and LC₅₀ of 36 mg a.s./L (based on plant survival after emergence).

Table B 9.11.2.1.4-4: Germination, Emergency of seedlings and survival of seedlings after application of Flufenacet 500 SC in dicot plants.

Type of plant	Plant species	Application rate (nominal) of the test item [g a.s./ha]	Germination – number of seeds:		Emergence of seedlings, 7 days after application of the test item, expressed as:		Survival of seedlings, 21 days after application of the test item		LC ₅₀ (g a. s./ha) for	
			Sown	Germinated	No. of seedlings	% of germinated	No. of seedlings	% survived	Emergence of seedlings	Survival
Dicotyledonous plants	<i>Brassica rapa</i> (Turnip)	Control (0)	32	31	30	97	30	100	> 600	> 600
		18.75	32	31	31	100	31	100		
		37.5	32	31	30	97	30	100		
		75.0	32	31	30	97	30	100		
		150	32	31	28	90	38	100		
		300	32	31	29	94	29	100		
		600	32	31	26	84	26	100		
	<i>Beta vulgaris</i> (Sugar beet)	Control (0)	32	30	29	97	29	100	> 600	> 600
		18.75	32	30	30	100	30	100		
		37.5	32	30	29	97	30	100		
		75.0	32	30	30	100	30	100		
		150	32	30	28	93	29	100		
		300	32	30	30	100	30	100		
		600	32	30	28	93	29	100		
	<i>Cucumis sativa</i> (Cucumber)	Control (0)	32	31	29	94	30	100	> 600	> 600
		18.75	32	31	28	90	29	100		
		37.5	32	31	30	97	30	100		
		75.0	32	31	29	94	30	100		
		150	32	31	29	94	30	100		
		300	32	31	29	94	30	100		
		600	32	31	16	84	28	100		
	<i>Lycopersicon esculentum</i> (Tomato)	Control (0)	32	31	28	90	28	100	> 600	365.1
		18.75	32	31	29	94	29	100		
		37.5	32	31	28	90	28	100		
		75.0	32	31	29	94	29	100		
		150	32	31	27	87	27	100		
		300	32	31	24	77	20	83		
		600	32	31	24	77	0	0		
	<i>Soybean</i> (<i>Glycine max</i>)	Control (0)	32	28	27	96	28	100	> 600	> 600
		18.75	32	28	27	96	28	100		
		37.5	32	28	28	100	28	100		
		75.0	32	28	27	96	28	100		
		150	32	28	27	96	28	100		
		300	32	28	25	89	28	100		
		600	32	28	26	93	27	100		

The foliar application of Flufenacet SC 500 had no toxic effect on emergence of seedling for all tested dicot plants. The survival of seedling 21 days after application was also not affected, except Lycopersion esc. plants with the lowest value of LC₅₀ of 365.1 g a.s./ha.

Table B 9.11.2.1.4-5: Phytotoxicity and growth inhibitory effects observed 7 days after application of Flufenacet 500 SC.

7 days after application (g a.s./ha)	Zea mays (Corn)	Avena sativa (Oats)	Lolium perenne (perennial ryegrass)	Sorghum bicolor (sweet sorghum)	Allium cepa (Onion)	Brassica rapa	Beta vulgaris	Lycopersicon esculentum (tomato)	Cucumis sativus (cucumber)	Glycine max (soybean)
Monocotyledoneae						Dicotyledoneae				
Necrosis (%)						Necrosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0
150	0	0	0	-	0	0	0	0	0	0
300	0	0	-	-	-	0	0	0	0	0
600	1	0	-	-	-	0	0	0	0	0
Chlorosis (%)						Chlorosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0
150	0	0	0	-	0	0	0	1	0	0
300	0	0	-	-	-	0	0	4	0	0
600	1	0	-	-	-	0	0	9	0	0
Deformation (%)						Deformation (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0
150	0	0	0	-	0	0	0	0	0	0
300	0	0	-	-	-	0	0	0	0	0
600	0	0	-	-	-	0	0	0	0	0
Growth inhibition (%)						Growth inhibition (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	8	12	0	-	-	-	-	-
18.75	0	0	73	86	0	0	0	0	0	0
37.5	0	6	86	92	8	0	0	0	0	0
75	0	38	92	99	28	0	0	0	0	0
150	4	75	100	-	56	6	19	6	7	0
300	9	88	-	-	-	11	39	14	12	4
600	30	98	-	-	-	26	67	26	22	13
BBCH Growth stage						BBCH Growth stage				
Control	11	12	11	12	11	10-11	10	10	10-11	10
2.4	-	-	-	12	-	-	-	-	-	-
4.7	-	-	11	12	11	-	-	-	-	-
9.4	-	-	11	11-12	11	-	-	-	-	-
18.75	11	12	10-11	10-11	11	10-11	10	10	10-11	10
37.5	11	12	10-11	10	11	10-11	10	10	10-11	10
75	11	11-12	10	10	10-11	10-11	10	10	10-11	10
150	11	10-11	n.d.	-	10	10-11	10	10	10-11	10
300	11	10	-	-	-	10-11	10	10	10	10
600	10-11	10	-	-	-	10-11	10	10	10	10

- not tested rate

Table B 9.11.2.1.4-6: Phytotoxicity and growth inhibitory effects observed 14 days after application of Flufenacet 500 SC.

7 days after application (g a.s./ha)	Zea mays (Corn)	Avena sativa (Oats)	Lolium perenne (perennial ryegrass)	Sorghum bicolor (sweet sorghum)	Allium cepa (Onion)	Brassica rapa	Beta vulgaris	Lycopersicon esculentum (tomato)	Cucumis sativus (cucumber)	Glycine max (soybean)
	Monocotyledoneae					Dicotyledoneae				
	Necrosis (%)					Necrosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	1	2	0	0	0	0	0	0
37.5	0	0	4	3	0	0	0	0	0	0
75	0	1	2	1	3	0	0	0	0	0
150	0	4	0	-	5	0	0	3	0	0
300	0	2	-	-	-	5	1	4	0	0
600	2	1	-	-	-	2	3	8	3	0
	Chlorosis (%)					Chlorosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	1	0	0	0	0	0
75	0	0	0	0	4	0	0	0	0	0
150	0	0	0	-	8	0	0	3	0	0
300	0	0	-	-	-	2	2	6	1	0
600	2	0	-	-	-	2	3	1	3	0
	Deformation (%)					Deformation (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	0	0	0	0	0	0	0	0
37.5	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0
150	0	0	0	-	0	2	1	0	0	0
300	0	0	-	-	-	2	3	0	0	0
600	0	0	-	-	-	5	4	0	0	0
	Growth inhibition (%)					Growth inhibition (%)				
Control	0	0	0	0	0	0	0	0	0	0
2.4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	13	22	0	-	-	-	-	-
18.75	0	0	81	89	4	0	0	0	0	0
37.5	0	8	91	95	17	0	0	6	5	0
75	0	43	98	99	46	5	3	14	27	0
150	11	79	100	-	76	21	22	43	56	0
300	24	90	-	-	-	28	45	79	74	8*
600	54	98	-	-	-	53	75	90	80	19*
	BBCH Growth stage					BBCH Growth stage				
Control	12	13-14	13	13	12	14	12	11	11-12	12
2.4	-	-	-	13	-	-	-	-	-	-
4.7	-	-	13	13	12	-	-	-	-	-
9.4	-	-	13	12	12	-	-	-	-	-
18.75	12	13-14	10-11	10-11	12	14	12	11	11-12	12
37.5	12	13-14	10	10	12	14	12	11	11-12	12
75	12	13	n.d.	10	11	13	12	11	11-12	12
150	12	12	n.d.	-	10	13	10-12	10-11	11	12
300	11-12	10-11	-	-	-	12-13	10-12	10	10-11	12
600	11	10	-	-	-	10-11	10	10	10	11-12

- not tested rate

Table B 9.11.2.1.4-7: Phytotoxic effects observed 21 days after application of Flufenacet 500 SC.

On day 21 after 50% emergence	Zea mays (Corn)	Avena sativa (Oats)	Lolium perene (perennial ryegrass)	Sorghum biclor (sweet sorghum)	<u>Alium</u> <u>cepa</u> (onion)	Brassica napa	Beta vulgaris	Lycopersicon escu. (tomato)	Cucumis sativa (cucumber)	Glycine max (soybean)
Monocotyledoneae						Dicotyledoneae				
Necrosis (%)						Necrosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2,4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
18.75	0	0	3	0	2	0	0	0	0	0
37.5	0	0	6	4	4	0	0	2	0	0
75	0	5	2	4	5	0	0	4	0	0
150	0	5	0	1	7	0	0	3	2	0
300	0	2	-	-	-	5	3	9	4	0
600	2	2	-	-	-	2	3	9	4	0
Chlorosis (%)						Chlorosis (%)				
Control	0	0	0	0	0	0	0	0	0	0
2,4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	2	-	-	-	-	-
18.75	0	0	0	0	5	0	0	0	0	0
37.5	0	0	0	0	5	0	0	0	0	0
75	0	0	0	0	5	0	0	0	2	0
150	0	0	0	0	4	0	0	9	5	0
300	0	0	-	-	-	2	2	6	4	0
600	2	0	-	-	-	2	5	0	2	0
Deformation (%)						Deformation (%)				
Control	0	0	0	0	0	0	0	0	0	0
2,4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	0	0	0	-	-	-	-	-
0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
75	0	0	0	0	0	0	0	0	0	0
150	0	0	0	0	0	3	2	0	0	0
300	0	0	-	-	-	5	5	0	0	0
600	0	0	-	-	-	11	5	0	0	0
Growth inhibition (%)						Growth inhibition (%)				
Control	0	0	0	0	0	0	0	0	0	0
2,4	-	-	-	0	-	-	-	-	-	-
4.7	-	-	0	0	0	-	-	-	-	-
9.4	-	-	21	0	0	-	-	-	-	-
18.75	0	0	85	33	10	0	0	0	0	0
37.5	0	11	94	89	25	0	0	17	7	0
75	0	46	98	95	56	6	5	27	31	0
150	13	81	100	99	84	34	26	63	64	0
300	30	91	-	-	-	51	51	84	82	12*
600	52	98	-	-	-	76	80	91	91	27*
BBCH Growth stage						BBCH Growth stage				
Control	13	15	21	15	13	16	12-13	12	12-13	14
2,4	--	--	--	0	-	-	-	-	-	-
4.7	nt	nt	21	15	13	-	-	-	-	-
9.4	nt	nt	21	15	13	-	-	-	-	-
18.75	13	15	10-12	14-15	12-13	16	12-13	12	12-13	14
37.5	13	15	nd	10-11	12-13	16	12-13	12	12-13	14
75	13	14-15	nd	10	11-12	14-15	12-13	12	12-13	14
150	13	13	nd	n.d	10	14-15	12	11-12	12	14
300	12-13	10-11	-	-	-	13-14	12	10	10-11	14
600	12	10	-	-	-	11-12	10-12	n.t.	10-11	13-14

n.d. not determinable

* plants weakened in the habit

- rate not tested

Visual observations:

No phytotoxic effects in control plants have been observed over the entire study period (at 7, 14 and 21 days) after application.

In order to determine the 21 visual NOEL and 21 visual EC₅₀, RMS describe the effects observable at 21 days after treatment.

Growth inhibition effects (stunting) were the most sensitive visual parameter for the monocotyledon plants.

The effects were attained in above 50% of tested plants for *Lolium perenne* (85%) and for *Sorghum bicolor* (89%) at the rates of 18.75 and 37.5 g a.s./ha, respectively.

The plants were slightly affected by chlorosis or necrosis. Both effects were mainly observed for *Allium cepa* plants, yet they were not pronounced (maximum 7% of plants were affected by necroses at the max application rate of 75 g a.s./ha, and 4-5% of plants by chloroses at the rates between 18.75 and 150 g a.s./ha).

For *Zea mays* plants, chloroses and necroses were observed at the level of 2%, at the highest tested rate of 600 g a.s./ha. No deformation nor any stimulation effects were observed for all the treated plants.

The growth inhibition effects were also the most sensitive visual parameter for the dicotyledon plants. The most pronounced stunting was observed for *Cucumis sativus* in 64% of plants, and for *Lycopersicon esculentum* in 63% of plants at the rate of 150 g a.s./ha, while 91% of the tested plants indicated these effects at the highest rate of 600 g a.s./ha. For the remaining dicotyledons, i.e. *Brassica napa* and *Beta vulgaris*, the growth inhibition effects were observed in more than 50% of plants at the higher rates – 300 and 600 g a.s./ha, respectively.

For three dicotyledon species only slight necrosis was observed, including *Brassica napa* (5-2% of tested plants), *Beta vulgaris* (3% of tested plants) and *Cucumis sativus* (4% of tested plants), mainly at the rates 300 and 600 g a.s./ha. Among all the plants tested, *Cucumis sativus* turned to be most affected by necrosis. However, maximum 9% of the plants were affected at max application rates of 300 and 600 g a.s./ha. Chlorosis was observed for most dicotyledons under study. On the other hand, no chlorosis nor necrosis were observed in *Glycine max* at any application rate. Slight deformation was observed only for *Beta vulgaris* and *Brassica napa* plants at the rate of 150 g a.s./ha. No stimulation effects were observed for all the plants under treatment.

The shoot fresh weight of monocot and dicot plants was presented in the Tables: B.9.11.2.1.4-8 and B.9.11.2.1.4-9.

Shoot fresh weight Monocotyledoneae**Table B 9.11.2.1.4-8: Shoot fresh weight of monocot plants on day 21 after 50% emergence.**

Type of plant	Plant species	Application rate [g a. s./ha]	Shoot fresh weight (g)	% reduction	Shoot fresh weight	
					EC ₅₀	NOEC
					g a.s./ha	
Monocotyledonous plants	Zea mays (Corn)	Control (0)	10.47±1.37	-	477.9	75
		18.75	10.81±1.31	-3		
		37.5	10.11±1.26	3		
		75.0	9.86±1.79	6		
		150	8.98±1.10	14*		
		300	7.04±1.59	33*		
		600	4.31±1.00	59*		
	Avena sativa (Oats)	Control (0)	9.42±0.99	-	80.9	37.5
		18.75	9.84±1.38	-4		
		37.5	8.25±1.04	12		
		75.0	5.04±2.27	47*		
		150	1.67±0.49	82*		
		300	0.74±0.34	92*		
		600	-	100*		
	Sorghum bicolor; (Sweet sorghum)	Control (0)	1.97±0.28	-	10.5	4.7
		2.4	1.81±0.33	8		
		4.7	1.83±0.42	7		
		9.4	1.21±0.21	39*		
		18.75	0.18±0.08	91*		
		37.5	0.02±0.01	99*		
		75.0	-	100*		
	Lolium perenne (Perennial ryegrass)	Control (0)	4.61	-	11.5	4.7
		4.7	4.31±0.27	7		
		9.4	3.49±0.66	24*		
		18.75	0.15±0.12	97*		
		37.5	-	100*		
		75.0	-	100*		
		150	-	100*		
	Allium cepa (Onion)	Control (0)	0.78±0.10	-	53.3	18.75
		4.7	0.81±0.12	-4		
		9.4	0.78±0.17	0		
		18.75	0.67±0.06	15		
		37.5	0.51±0.14	35*		
		75.0	0.51±0.14	63*		
		150	0.51±0.14	88*		

± Standard deviation (SD)

%: reduction of fresh weight compared to control

* Statistically significant compared to control (Dunnett-test, Bonferroni-Holm-U-test, $p \leq 0.05$)

Taking into account the quantitative parameters such as shoots fresh weight, the most sensitive monocotyledonous species was Sorghum perenne, with EC₅₀ of 10.5 g a.s./ha, followed by Lolium perenne with EC₅₀ of 11.5 g a.s./ha, Allium cepa with EC₅₀ of 53.3 g a.s./ha, Avena sativa with EC₅₀ of 80.9 g a.s./ha and Zea mays with EC₅₀ of 477.9 g a.s./ha.

Shoot fresh weight - Dicotyledoneae

Table B 9.11.2.1.4-9: Shoot fresh weight of dicot plants on day 21 after 50% emergence.

Type of plant	Plant species	Application rate (nominal) [g a. s./ha]	Shoot fresh weight (g)	% reduction	Shoot fresh weight	
					EC ₅₀	NOEC
					g a.s./ha	
Dicotyledonous plants	Brassica rapa (Turnip)	Control (0)	21.19±2.01	-	282.7	75
		18.75	21.46±1.19	-1		
		37.5	21.27±2.06	0		
		75.0	19.28±2.07	9		
		150	15.12±4.65	29*		
		300	10.54±3.28	50*		
		600	4.62±1.5	78*		
	Beta vulgaris (Sugar beet)	Control (0)	15.64±1.53	-	275.4	75
		18.75	15.84±1.14	-1		
		37.5	15.54±1.21	1		
		75.0	15.18±1.46	3		
		150	11.70±1.94	25*		
		300	6.76±1.48	57*		
		600	3.43±0.50	78*		
	Cucumis sativa (Cucumber)	Control (0)	13.19±1.73	-	101.1	18.75
		18.75	12.89±2.09	2		
		37.5	11.33±1.79	14*		
		75.0	8.74±1.19	34*		
		150	3.76±0.86	71*		
		300	1.65±0.45	87*		
		600	0.95±0.25	93*		
	Lycopersion esculentum (Tomato)	Control (0)	6.83±1.15	-	93.6	18.75
		18.75	6.18±1.40	10		
		37.5	5.18±0.90	24*		
		75.0	4.57±1.37*	33*		
		150	2.31±0.98	66*		
		300	0.25±0.27	96*		
		600	1	100*		
	Soybean (Glycine max)	Control (0)	8.02±1.23	-	>600	150
		18.75	8.37±1.24	-4		
		37.5	7.88±1.29	2		
		75.0	7.85±1.41	2		
		150	7.0±0.88	12		
		300	5.31±1.03	34*		
		600	4.30±0.97	46*		

± Standard deviation (SD)

Response (%): reduction of fresh weight compared to control

* Statistically significant compared to control (Dunnett-test, Bonefroni-Holm-U-test, $p \leq 0.05$)

Lycopersion esculentum was the most sensitive dicotyledonous species with EC₅₀ of 93.6 g s.a./ha, followed by Cucumis sativus with EC₅₀ of 101.1 g a.s./ha, Beta vulgaris with EC₅₀ of 275.4 g a.s./ha, Brassica napa with EC₅₀ of 282.7 g a.s./ha and Glycine max with EC₅₀>600 g a.s./ha.

The summary of endpoints obtained from the study including 21 visual NOEL and 21 d visual EC₅₀ are presented in the Table B.9.11.2.4-1-10 below:

Table B 9.11.2.1.4-10: Seedling emergence, survival, shoot fresh weight and visual effects after application of Flufenacet 500 SC.

Plant species	Seedling emergence LC ₅₀ g s.a./ha	Plant survival after emergence LC ₅₀ g s.a./ha	Shoot fresh weight			Phytotoxic* effects			BBC H
			EC ₅₀	NOEC	LOEC	Application rate g a.s./ha.	% effects 21 d	21 visual NOEL	
			g s.a./ha						
Monocotyledonea									
Zea mays	>600	>600	477.9	75	150	18.75	0	75	12-13
						37.5	0		
						75	0		
						150	13		
						300	30		
						600	52		
Avena sativa	472.3	418.0	80.9	37.5	75	18.75	0	18.75	10-15
						37.5	11		
						75	46		
						150	81		
						300	91		
						600	98		
Allium cepa	>150	>150	53.3	18.75	37.5	4.7	0	4.7	10-13
						9.4	21		
						18.75	85		
						37.5	94		
						75	98		
						150	100		
Lolium perenne	47.2	18	11.5	4.7	9.4	4.7	0	4.7	10-21
						9.4	21		
						18.75	85		
						37.5	94		
						75	98		
						150	100		
Sorghum bicolor	34.7	36.6	10.5	4.7	9.4	4.7	0	9.4	10-15
						9.4	0		
						18.75	33		
						37.5	89		
						75	95		
						150	99		
Dicotyledoneae									
Brassica rapa	>600	>600	282.7	75	150	18.75	0	37.5	
						37.5	0		
						75	6		
						150	34		
						300	51		
						600	76		
Beta vulgaris	>600	>600	275.4	75	150	18.75	0	37.5	10-13
						37.5	0		
						75	5		
						150	26		
						300	51		
						600	80		
Cucumis sativa	>600	>600	101.1	18.75	37.5	18.75	0	18.75	10-13
						37.5	7		
						75	31		
						150	64		
						300	82		
						600	91		
Lycopersicon esculentum	>600	365.1	93.6	18.75	37.5	18.75	0	18.75	10-12
						37.5	17		
						75	27		
						150	63		
						300	84		
						600	91		
Glycine max	>600	>600	>600	150	300	18.75	0	150	13-14
						37.5	0		
						75	0		
						150	0		

Plant species	Seedling emergence LC ₅₀ g s.a./ha	Plant survival after emergence LC ₅₀ g s.a./ha	Shoot fresh weight			Phytotoxic* effects			BBC H
			EC ₅₀	NOEC	LOEC	Application rate g a.s./ha.	% effects 21 d	21 visual NOEL	
			g s.a./ha						
						300	12 ¹		
						600	12 ¹		

¹ plants weakened in the habit

* % effects based on the most sensitive visual parameter - % inhibition growth effects (stunting),** no observed effect level (empirically estimated by RMS)

In bold , the visual effects - more than 50% affected plants

Conclusion:

Taking into account the quantitative parameters such as shoots fresh weight, the most sensitive monocotyledonous species was *Sorghum bicolor* with EC₅₀ of 10.5 g a.s./ha and most sensitive dicot species was *Lycopersicon esculentum* with EC₅₀ of 93.6 g s.a./ha. The growth inhibition effects (stunting) are most pronounced visual parameter for both tested groups of plants.

Taken into account the most sensitive visual parameter-growth inhibition effects, it can be concluded that empirically predicted visual values of EC₅₀ (between 18.75-37.5 g a.s./ha, Table B. 9.11.2.1.4-10) will be close to predicted value of EC₅₀ based on most sensitive quantitative parameter - shoot fresh weight.

The lowest visual 21 d NOEL for phytotoxic effects- 4.7 g a.s./ha was recorded for *Lolium perenne*.

The lowest value of 21 d NOEL for dicot plants, based on phytotoxicity, was estimated to be 18.78 g a.s./ha for *Cucumis sativa* and *Lycopersicon esculentum*.

Comment RMS:

The seedling emergence test was conducted according to the OECD test guideline 208 A (July 2000).

In order for the test to be considered valid the following performance criteria must be met in the control:

- The seedling emergence of at least 65% has to be observed.
- The mean seedling growth does not exhibit visible phytotoxic effects.

Taking into account the current valid OECD test guideline 208 (July 2006) the following validity criteria are given:

- The seedling emergence in the controls is at least 70%
- The seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis and wilting leaf and stem deformations) and plants exhibit only normal variation in growth and morphology for that particular species)
- The mean survival of emerged control seedlings is at least 90% for the duration of the study
- In the controls, environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix support media or substrate from the same source.

According to the OECD 208 (2006) test guideline the following conditions are recommended:

- The temperature: 22 °C ± 10 °C
- The humidity: 70 % ± 25%, respectively
- The light intensity: 350 ± μE/m²/s. Additional lighting may be necessary if decreases below 200, μE/m²/s, wavelength 400-700 nm except for certain species whose light requirements are less.

Taking into account the validity criteria given in the OECD 208 (2000) and OECD 208 (2006) test guidelines

all validity criteria were met.

- The seedling emergence in the controls was above 90 %
- The seedlings do not exhibit visible phytotoxic effects for the duration of the study
- The mean survival of emerged control seedlings was 100% for the duration of the study
- The temperature was in a required range and was 11-31 °C and 16 hours photoperiod.

The following deviations were noted from the study guideline:

- There is no information in the study protocol address possible effects on the test plants due to the low humidity – 35% in the greenhouse. However, under consideration of the observed effects on plant survival and plant phytotoxicity the influence of the humidity on the test plants is considered to be low.
- Four seeds of sugar beet /pot were sown instead of one to two seeds/pot as recommended in the test guideline. However, there was no mortality or phytotoxicity observed in control, therefore it is considered that the growth conditions were sufficient for tested plants and they did not suffer with crowding.

Agreed endpoints:

EC₅₀ = 11.5 g a.s./ha, *Lolium perenne*

EC₅₀ = 10.5 g a.s./ha, *Sorghum bicolor*

EC₅₀ = 53.3 g s.a./ha, *Allium cepa*

EC₅₀ = 80.9 g s.a./ha, *Avena sativa*

EC₅₀ = 477.9 g s.a./ha, *Zea mays*

EC₅₀ > 600 g s.a./ha, *Cucumis sativus*

EC₅₀ = 282.7 g a.s./ha, *Brassica rapa*

EC₅₀ = 275.4 g a.s./ha, *Beta vulgaris*

EC₅₀ > 365.1g a.s./ha, *Lycopersicon esc.*

EC₅₀ > 600 g a.s./ha, *Glycine max*

The most sensitive plant species : *Sorghum bicolor* with EC₅₀ =10.5 g a.s./ha, based on shoot fresh weight

B.9.11.3. Extended laboratory studies on non-target plants

No extended laboratory studies on non-target plants using the formulated product have been submitted.

B.9.11.4. Semi-field and field tests on non-target plants

No semi-field and field tests on non-target plants using the formulated product have been submitted.

B.9.12. RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

The risk assessment for effects on terrestrial non-target higher plants is conducted according to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002 rev. 2).

Exposure**Representative formulation: DFF + FFA SC 600**

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to the spray drift. The amount of spray drift reaching off-crop habitats was calculated using estimates derived by the BBA (2000) from the spray-drift predictions of Ganzelmeier & Rautmann (2000).

Off-field exposure was calculated according to following formula:

$$PER_{\text{off-field}} = \text{Application rate} \times \text{Drift factor}$$

where:

Drift factor % of active substance drifted outside the field during application, depending on number of applications and target crop

The representative formulation DFF+FFA SC 600 is intend to applied as an herbicide in winter cereals in the following application rates:

Table B.9.12-1: Intended uses of the representative formulation DFF+FFA SC 600.

Crop	Timing of application	No of app.	Maximum application rate (L prod./ha)	Maximum appl. rate g a.s./ha		g of sum a.s. (DFF+FFA)
				FFA*	DFF**	
Cereals	10-13	1	0.6	240	120	360
Cereals	11-13	1	0.4	160	80	240
Cereals	0-22	1	0.3	120	60	180

* a.s.: Flufenacet

** a.s.: Diflufenican

The corresponding off-field predicted environmental rates (PER) for three different use patterns are presented in the Table B. 9.12-2. below:

Table B. 9.12-2: Predicted environmental rates (PER) at 1 m distance from edge of field.

Crop	Timing of application	No of app.	Maximum application rate g of sum a.s./ha*	DRIFT ¹	MAF	PER off-field (at 1 m distance) g of sum a.s./ha
Cereals	Post emergence 10-13	1	360	2.77%	1	9.972
Cereals	Post emergence 11-13	1	240	2.77%	1	6.648
Cereals	Pre-emergence	1	180	2.77%	1	4.986

¹ BBA drift values (1 application, field crops), see Terr. Guidance Doc. SANCO/10329/2002 rev 2 final

Deterministic risk assessment

Representative formulation - DFF + FFA SC 600

According to the Terrestrial Guidance Document the risk to non-target plants is evaluated by comparing the lowest ER₅₀ observed in the laboratory studies with the drift rates (PER_{off-field}) as reported in table B.9.12.2.

TER value was calculated according to following equation:

$$\text{TER} = \frac{\text{ER}_{50} \text{ (g a.s./ha)}}{\text{PER (g a.s./ha)}}$$

TER values below the trigger value of 5 indicate an unacceptable risk to NTPs in the off-field environment.

Calculated TER values for the single proposed applications of DFF+FFA SC 600 are listed in Table B.9.12-3.

Table 9.12-3: Deterministic risk assessment for DFF+FFA SC 600 based on vegetative vigor for test for application rate 1 x 360 g sum of a.s./ha (0.6 L product/ha).

Distance (m)	Drift rate ¹ (%)	PER off-field (g sum of a.s./ha)	Toxicity (g of sum a.s./ha)	TER			
				No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	9.972	ER₅₀=27.75 (Cucumis sativus)	2.78	5.56	11.13	27.82
5	0.57	2.052		13.52	27.05	54.09	135.23
10	0.29	1.044		26.58	53.16	106.32	265.80

¹ BBA drift values (1 application, field crops), see Terr. Guidance Doc. SANCO/10329/2002 rev 2 final

Note: In bold the TER values above trigger value of 5.

Table 9.12-4: Deterministic risk assessment for DFF+FFA SC 600 based on vegetative vigor test for application rate 1 x 240 g sum of a.s./ha(0.4 L/ha).

Distance (m)	Drift rate ¹ (%)	PER off-field (g sum of a.s./ha)	Toxicity (g of sum a.s./ha)	TER			
				No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	6.648	ER₅₀=27.75 (Cucumis sativus)	4.17	8.34	16.69	41.36
5	0.57	1.368		20.28	40.57	81.14	202.85
10	0.29	0.696		39.87	79.74	159.48	398.70

¹ BBA drift values (1 application, field crops), see Terr. Guidance Doc. SANCO/10329/2002 rev 2 final

Note: In bold the TER values above trigger value of 5.

Table 9.12-5: Deterministic risk assessment for DFF+FFA SC 600 based on vegetative vigor test for application rate 1 x 180 g sum of a.s./ha (0.3 L/ha).

Distance (m)	Drift rate ¹ (%)	PER off-field (g sum of a.s./ha)	Toxicity (g of sum a.s./ha)	TER			
		No drift reduction		No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	4.986	ER₅₀=27.75 (Cucumis sativus)	5.56	11.13	22.26	55.61
5	0.57	1.026		27.04	54.03	108.18	270.46
10	0.29	0.522		53.16	106.32	212.64	531.60

¹ BBA drift values (1 application, field crops), see Terr. Guidance Doc. SANCO/10329/2002 rev 2 final

Note: In bold the TER values above trigger value of 5.

Conclusion of RMS

Representative formulation: DFF + FFA SC 600

According to EU requirements the risk for non-target terrestrial plants is considered acceptable, if a 5m buffer zone is kept without drift reduction or no buffer zone and a 50% drift reducing spray equipment, if 600 mL product /ha (360 g sum of a.s./ha) is applied.

At application rates, 400 mL product/ha, (corresponding to 240 g sum of a.s./ha) a 5 m buffer zone without drift reduction or no buffer zone and 50% drift reducing spray equipment is sufficient in order to protect the non-target flora on field margins.

At application rates, 300 mL product/ha, (corresponding to 180 g of sum of a.s./ha) no risk mitigation is required.

It is worth noting, that for the vegetative vigour study, the phytotoxic effects were widespread and for all dicot species including the most sensitive species tested (Cucumis sativus) could have resulted in a lower endpoint than that calculated with regards to the most sensitive quantitative parameters - foliar fresh weight (visual ER₅₀ <3.2 g sum of a.s./ha for most tested plants). In the seedling emergence study (Kalsch W, 2012b) , the 21 d NOEL was also estimated to be <3.2 g total active substances/ha.

In addition, the phytotoxicity data for DFF+ FFA 600 SC are not adequate to assess at what level there would be no phytotoxic effects for this particular formulation (visual 21 d NOEL < 3.2 g a.s./ha for most tested plants).

Taken into consideration that the representative formulation DFF+FFA SC 600 is consisted of two active substances – flufenacet and diflufenican, the level of the effects caused by solo a.s.-flufenacet, could not be determined based on visual phytotoxicity effects observed in this study.

In respect to representative formulation DFF+FFA SC 600, the MSs should noting that the risk assessment based on the lowest value of ER₅₀=27.75 g total active substances/ha (based on fresh weight) for most sensitive plants Cucumis sativa will not cover the phytotoxic visual effects caused by representative formulation.

For the probabilistic risk assessment for representative formulation not sufficient data are available.

Therefore, RMS took into account two additional studies conducted with straight formulation Flufenacet 500 SC containing only flufenacet (500 g a.s./L) to evaluated the intrinsic toxicity of the active substance – flufenacet.

Therefore, the probabilistic risk assessment based on the data with Flufenacet 500 SC can be used as surrogate in a kind of bridging, since lower endpoints were obtained with this formulation.

For clarity of evaluation the risk assessment for Flufenacet 500 SC was presented separately under endpoint B.9.12.1 below.

B.9.12.1.The risk assessment performed for Flufenacet 500 SC (500 g a.s./L)

The risk assessment for effects on terrestrial non-target higher plants is conducted according to the Guidance Document on Terrestrial Ecotoxicology under Council Directive 91/414/EEC (SANCO/10329/2002 rev. 2). Flufenacet 500 SC – singular formulation .

Exposure:

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to the spray drift. The amount of spray drift reaching off-crop habitats was calculated using estimates derived by the BBA (2000) from the spray-drift predictions of Ganzelmeier & Rautmann (2000).

Off-field exposure was calculated according to following formula:

$$PER_{\text{off-field}} = \text{Application rate} \times \text{Drift factor}$$

where:

Drift factor % of active substance drifted outside the field during application, depending on number of applications and target crop

Flufenacet 500 SC is intend to applied as an herbicide in winter cereals in the following application rates :

Table B.9.12.1-1: Intended uses of Flufenacet

Crop	Timing of application	Number of application	Maximum application rate g a.s./ha *
Cereals	10-13	1	240
Cereals	11-13	1	160
Cereals	0-22	1	120

*a.s.: Flufenacet

The corresponding off-field predicted environmental rates (PER) for three different use patterns are presented in the table below:

Table B. 9.12.1-2: Predicted environmental rates (PER) at 1 m distance from edge of field.

Crop	Timing of application	Number of application	Maximum application rate (g a.s./ha)*	% DRIFT ¹	MAF	PER off-field (at 1 m distance) g a.s./ha
Cereals	Post emergence 10-13	1	240	2.77%	1	6.648
Cereals	Post emergence 11-13	1	160	2.77%	1	4.432
Cereals	Pre-emergence 0-22 BBCH	1	120	2.77%	1	3.324

*a.s.: Flufenacet

¹ BBA drift values (1 application, field crops), see Terr. Guidance Doc. SANCO/10329/2002 rev 2 final**Probabilistic Risk assessment**

Terrestrial Guidance Document recommends the use of the HC5 (the concentration below which less than 5% of the species will be harmed above the EC₅₀ level) which can be calculated from the data sets of ER₅₀ growth inhibition levels. The EU guidance document for terrestrial ecotoxicology states that *if the ED₅₀ for less than 5 % of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable*. Thus, the HC5 itself (TER =1) can be regarded to be protective.

Table B.9.12.1-3: The vegetative vigour and seedling emergence EC₅₀ values for 10 species exposed to Flufenacet 500 SC.

Species	M/D	Seedling test EC ₅₀ [g as/ha], 21 days	Vegetative test [g as/ha], 21 EC ₅₀
		Shoot fresh weight	Shoot fresh weight
Zea mays	M	477.9	>600
Avena sativa	M	80.9	196
Allium cepa	M	53.3	132
Lolium perenne	M	11.5	17
Sorghum bicolor	M	10.5	43
Brassica rapa	D	282.7	167
Beta vulgaris	D	275.4	525
Cucumis sativa	D	101.1	102
Lycopersicon esculentum	D	93.6	>600
Glycine max	D	>600	168

M = Monocotyledoneous, D = Dicotyledoneous

The HC5 was calculated according to $HC5 = 10 \exp^{(avg-ks*std)}$

With:

avg=mean of log10 transformed EC₅₀ values

std=standard deviation of log10 transformed EC₅₀ values

ks = extrapolation factor

HC₅ calculation

The respective HC₅ calculation have been conducted using the computer program ETX .20. by rivm (RIV, 2004). In the first step, the toxicity data (EC₅₀) 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 accepted. The analysis of the EC₅₀ values show normal distribution of the data. The results of the ETX^{2.0} calculation based on the EC₅₀ values and the respective graphs of terrestrial plants sensitivity distribution are presented below.

Vegetative vigour test

Analysis of normality for terrestrial plants

Table B.9.12.1-4: Parameters of the log-normal distribution for data set of terrestrial plants (vegetative vigour study)

Name	Value	Description
mean	2.057	mean of the log toxicity values
s.d.	0.4504	sample standard deviation
n	8	sample size*

*The EC₅₀ value of >600 g a.s./ha was not used in the calculation.

Figure B.9.12.-1: Species sensitivity distribution for non-target terrestrial plants.

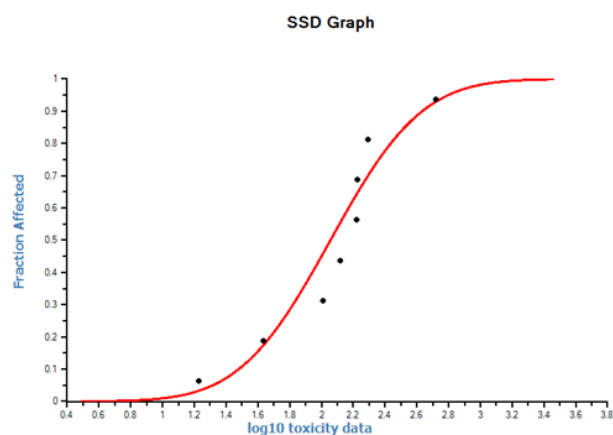


Table B.9.12.1-5: Goodness of fit tests - results for non-target terrestrial plants.

Sign. level	Tests for normality n = 8					
	Anderson-Darling		Kolmogorov-Smirnov		Cramer von Mises	
0.1	0.631	Accepted	0.819	Accepted	0.10	Accepted
0.05	0.752	Accepted	0.895	Accepted	0.126	Accepted
0.025	0.873	Accepted	0.995	Accepted	0.148	Accepted
0.01	1.035	Accepted	1.035	Accepted	0.179	Accepted
Statistic	0.40750064		0.64712377		0.06094718	

Table B.9.12.1-6: HC₅ results [g flufenacet/L] for non-target terrestrial plants.

Name	Value	Log10 (Value)	Description
LLHC ₅	4.180358665	0.621213545	lower estimate of the HC ₅
HC ₅	19.17002552	1.28262269	median estimate of the HC ₅
UL HC ₅	42.19079646	1.625217724	upper estimate of the HC ₅
Spr HC ₅	10.09262598	1.004004179	spread of the HC ₅ estimate

*The EC₅₀ value of >600 g a.s./ha was not used in the calculation.

Table B.9.12.1-7: HC₅-figures for vegetative vigour with Flufenacet 500 SC.

HC ₅	Vegetative vigour [g as/ha],
HC ₅ based on endpoint from all species	19.17

Seedling emergence test

Analysis of normality for terrestrial plants

Parameters of the log-normal distribution for data set of terrestrial plants

Table B.9.12.1-8: Parameters of the log-normal distribution for data set of terrestrial plants

Name	Value	Description
mean	1.918	mean of the log toxicity values
s.d.	0.5834	sample standard deviation
n	9	sample size*

*The EC₅₀ value of >600 g a.s./ha was not used in the calculation.

Figure B.9.12.1-1: Species sensitivity distribution for non-target terrestrial plants.

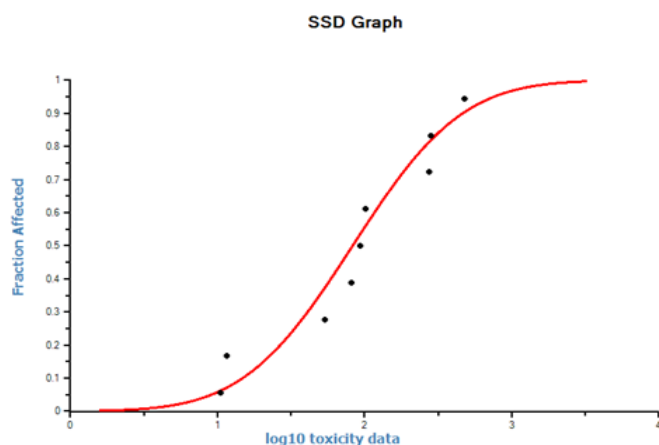


Table B.9.12.1-9: Goodness of fit tests - results for non-target terrestrial plants

Sign. level	Tests for normality n = 9					
	Anderson-Darling		Kolmogorov-Smirnov		Cramer von Mises	
0.1	0.631	Accepted	0.819	Accepted	0.10	Accepted
0.05	0.752	Accepted	0.895	Accepted	0.126	Accepted
0.025	0.873	Accepted	0.995	Accepted	0.148	Accepted
0.01	1.035	Accepted	1.035	Accepted	0.179	Accepted
Statistic	0.40101352		0.52275498		0.04560763	

Table B.9.12.1-10: HC₅ results [g flufenacet/L] for non-target terrestrial plants.

Name	Value	Log10 (Value)	Description
LLHC5	1.41164148	0.14972441	lower estimate of the HC5
HC5	8.33829033	0.92107701	median estimate of the HC5
UL HC5	21.9102137	1.34064661	upper estimate of the HC5
Spr HC5	15.5210895	1.1909222	spread of the HC5 estimate

Table B.9.12.1-11: HC₅-figures for vegetative vigour with Flufenacet 500 SC.

HC ₅	Seedling emergence [g as/ha]
HC ₅ based on endpoint from all species	8.34

Table B.9.12.1-12: Probabilistic off risk assessment for non target terrestrial plants based on effects on seedling emergence test for formulation Flufenacet SC 500.

Cereals, 1 x 240 g a.s./ha; HC ₅ = 8.34 g a.s/ha						
Distance	Drift ¹	PER	TER			
[m]	(%)	no drift reduction [g a.s./ha]	No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	6.648	1.25	2.51	5.02	12.55
5	0.57	1.368	6.10	12.19	24.39	60.96
10	0.29	0.696	11.98	23.97	47.93	119.83

¹ BBA drift values (1 application, field crops), see Terr. Guidance Doc. SANCO/10329/2002 rev 2 final

Note: In bold the TER values above trigger value of 1.

Table B.9.12.1-13: Probabilistic off risk assessment for non target terrestrial plants based on effects on seedling emergence test for formulation Flufenacet SC 500.

Cereals, 1 x 160 g a.s./ha; HC ₅ = 8.34 g a.s/ha						
Distance	Drift ¹	PER	TER			
[m]	(%)	no drift reduction [g a.s./ha]	No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	4.432	1.88	3.76	7.52	18.81
5	0.57	0.912	9.14	18.28	36.57	91.44
10	0.29	0.464	17.98	35.95	71.89	179.74

¹ BBA drift values (1 application, field crops), see Terr. Guidance Doc. SANCO/10329/2002 rev 2 final

Note: In bold the TER values above trigger value of 1.

Table B.9.12.1-14: Probabilistic off risk assessment for non target terrestrial plants based on effects on seedling emergence test for formulation Flufenacet SC 500.

Cereals, 1 x 120 g a.s./ha; HC ₅ = 8.34 g a.s/ha						
Distance	Drift ¹	PER	TER			
[m]	(%)	no drift reduction [g a.s./ha]	No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	3.324	2.57	5.01	10.03	25.09
5	0.57	0.684	12.19	24.38	48.77	121.92
10	0.29	0.348	23.96	47.93	95.86	239.65

¹ BBA drift values (1 application, field crops), see Terr. Guidance Doc. SANCO/10329/2002 rev 2 final ufenacet

Note: In bold the TER values above trigger value of 1.

RMS conclusion:

Since Flufenacet SC 500 has stronger effects on seedling emergence than on the vegetative vigor of young plants seedling emergence data determine the risk assessment.

Based on the probabilistic risk assessment for solo formulation Flufenacet 500 SC (containing 42.3% a.s.-flufenacet), the risk for non-target terrestrial plants is considered acceptable with no buffer zone or drift reducing spraying equipment.

Consideration of metabolites

Bioefficacy tests performed with flufenacet soil metabolites (please refer to Volume 3 (CA), B.9) demonstrated that metabolites are considerably less biologically active than the parent compound. For this reason it is not expected that any of metabolites would be more toxic to non-target terrestrial plants than flufenacet. On this basis it is concluded that performed evaluation is sufficiently protective and metabolites were not considered in risk assessment for non-target terrestrial plants.

According to the results of the propabilistic approach involving the most sensitive endpoint in the seedling emergence study the risk to non-target plants is considered acceptable at distance of 1 m. No risk mitigation measures are required to refine the risk to terrestrial plants in the off-field.

B.9.13. EFFECTS ON OTHER TERRESTRIAL ORGANISMS (FLORA AND FAUNA)

-

B.9.14. RISK ASSESSMENT FOR OTHER TERRESTRIAL ORGANISM (FLORA AND FAUNA)

-

B.9.15. REFERENCES RELIED ON

Annex pointn / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCP 10.2.1 /01	Dorgerloh, M.; Sommer, H.	2001	FOE 5043 & diflufenican SC 600 - Influence on the growth of the green alga, Selenastrum capricornutum Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: DOM 20073, Edition Number: <u>M-073137-01-1</u> Date: 2001-09-18 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.2.1 /02	Dorgerloh, M.; Sommer, H.	2001	FOE 5043 & diflufenican SC 600 - Toxicity (7 days) to Lemna gibba G3 in a static test Bayer AG, Leverkusen, Germany Bayer CropScience, Report No.: DOM 20074, Edition Number: <u>M-073160-01-1</u> Date: 2001-09-18 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.3.1.1.1 /01	Schmitzer, S.; Sekine, T.	2009	Effects of diflufenican + flufenacet SC 600 (200+400) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 47501035, Edition Number: <u>M-356881-01-1</u> Date: 2009-10-05 GLP/GEP: yes, unpublished ...also filed: KCP 10.3.1.1.2 /01	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.3.1.1.2 /01	Schmitzer, S.; Sekine, T.	2009	Effects of diflufenican + flufenacet SC 600 (200+400) G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 47501035, Edition Number: <u>M-356881-01-1</u> Date: 2009-10-05 GLP/GEP: yes, unpublished ...also filed: KCP 10.3.1.1.1 /01	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal

Annex pointn / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCP 10.3.2.1 /01	Gossmann, A.	2001	Effects of flufenacet & diflufenican SC 600 on the predatory mite Typhlodromus pyri Scheuten (Acari, Phytoseiidae) in the laboratory - dose response design - IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 9352063, Edition Number: <u>M-058604-01-1</u> Date: 2001-07-04 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.3.2.1 /02	Moll, M.; Buetzler, R.	2001	Effects of flufenacet & diflufenican SC 600 on the parasitoid Aphidius rhopalosiphii in the laboratory - limit test - IBACON GmbH, Rossdorf, Germany Bayer CropScience, Report No.: 9351001, Edition Number: <u>M-058618-01-1</u> Date: 2001-07-04 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.3.2.2 /01	Chauzat, M. P.	2002	The effects of flufenacet & diflufenican SC 600 on Typhlodromus pyri (Acari: Phytoseiidae) on natural substrate in laboratory (extended laboratory test) Promo-Vert S.A., Serres Castet, France Bayer CropScience, Report No.: 01TYBYL12, Edition Number: <u>M-034242-01-1</u> Date: 2002-01-14 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.3.2.2 /02	Waibel, J.	2009	Toxicity to the green lacewing Chrysoperla carnea STEPH. (Neuroptera, Chrysopidae) using an extended laboratory test on Zea mays; Flufenacet + Diflufenican SC 400 + 200 g/L Bayer CropScience, Report No.: CW09/010, Edition Number: <u>M-352372-01-1</u> Date: 2009-07-28 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.

Annex pointn / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evalutio
KCP 10.3.2.2 /03	Roehlig, U.	2009	Chronic toxicity (ER50) of Diflufenican + Flufenacet SC 600 g/L to the rove beetle <i>Aleochara bilineata</i> GYLL. under extended laboratory conditions BioChem agrar GmbH, Gerichshain, Germany Bayer CropScience, Report No.: 09 10 48 027 A, Edition Number: <u>M-353760-01-1</u> Date: 2009-07-28 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submmited for the renewal of a.s.
KCP 10.3.2.2 /04	Jans, D.	2009	Toxicity to the predatory mite <i>Typhlodromus pyri</i> SCHEUTEN (Acari, Phytoseiidae) using an extended laboratory test (under semi-field conditions aged residues on <i>Zea mays</i>), Flufenacet + Diflufenican SC 400 + 200 g/L Bayer CropScience, Report No.: CW09/026, Edition Number: <u>M-355238-01-1</u> Date: 2009-09-08 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submmited for the renewal of a.s.
KCP 10.4.1.1 /01	Leicher, T.	2010	Diflufenican + Flufenacet SC 600 G: Effects on survival, growth and reproduction on the earthworm <i>Eisenia fetida</i> tested in artificial soil with 5 % peat Bayer CropScience, Report No.: LRT-RG-R-70/10, Edition Number: <u>M-362809-01-1</u> Date: 2010-02-08 GLP/GEP: yes, unpublished	N	Y	New data requirement	Bayer Crop Science	New study submmited for the renewal of a.s.
KCP 10.4.1.2 /01	Hamberger, A.	2014	DFF+FFA SC 200+400 G - A field study to investigate effects on the earthworm fauna in southern Germany eurofins-GAB GmbH, Niefern-Oeschelbronn, Germany Bayer CropScience, Report No.: S12-03897/EBFON089, Edition Number: <u>M-478092-01-1</u> Date: 2014-02-24 GLP/GEP: yes, unpublished	N	Y	Earthworm field study, needed for refinement	Bayer Crop Science	New study submmited for the renewal of a.s.

Annex pointn / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCP 10.4.2.1 /01	Feije, R.	2002	Flufenacet & diflufenican SC 600: The effects on survival and reproduction of the predaceous mite Hypoaspis aculeifer Canestrini (Acari: Laelapidae) in standard soil (LUFA 2.1) MITOX Stichting Bevoordering Duurzame Plaagbestrijding, Amsterdam, Netherlands Bayer CropScience, Report No.: B094HAE, Edition Number: M-061660-01-1 Date: 2002-04-26 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.4.2.1 /02	Frommholz, U.	2011	Diflufenican + flufenacet SC 600 (200+400) G: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil. Bayer CropScience, Report No.: FRM-Coll-125/11, Edition Number: M-415903-01-1 Date: 2011-10-11 GLP/GEP: yes, unpublished	N	Y	New data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.5 /01	Frommholz, U.	2009	Diflufenican + flufenacet SC 600 (200+400) G: determination of effects on nitrogen transformation in soil Bayer CropScience, Report No.: FRM-N-121/09, Edition Number: M-357934-01-1 Date: 2009-10-27 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
KCP 10.6.2 /01	Kalsch, W.	2002	Flufenacet & diflufenican SC 600: Vegetative vigour test on terrestrial non-target plants of 6 families (2 Monocotyledoneae, 4 Dicotyledoneae) ECT Oekotoxikologie GmbH, Floersheim, Germany Bayer CropScience, Report No.: P3PB, Edition Number: M-071692-01-1 Date: 2002-07-12 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.

Annex pointn / reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner	Previous evaluation
KCP 10.6.2 /02	Kalsch, W.	2002	Flufenacet & diflufenican SC 600: Seedling emergence and seedling growth test on terrestrial non-target plants of 6 families (2 Monocotyledoneae, 4 Dicotyledoneae) ECT Oekotoxikologie GmbH, Floersheim, Germany Bayer CropScience, Report No.: P2PA, Edition Number: <u>M-072308-01-1</u> Date: 2002-07-12 GLP/GEP: yes, unpublished	N	Y	Data requirement	Bayer Crop Science	New study submitted for the renewal of a.s.
-	Friedrich, S.	2005	Flufenacet SC 500: Vegetative vigour test on non target terrestrial plants. Bayer Crop Science Report/Doc.number: Report No: 041048105, Edition Number: <u>M-248251-01-1</u> Date: Data:2005-03-25 GLP: yes, unpublished	N	Y		Bayer Crop Science	New study submitted for the renewal of a.s.
-	Friedrich, S.	2005	Flufenacet 500 S: Seedling emergence and seedling growth test on terrestrial non-target plants. Study Number: 04 10 48 104 Edition Number: <u>M-248251-01-1</u> Data: 2005-03-25 GLP/GEP: yes, unpublished	N	Y		Bayer Crop Science	New study submitted for the renewal of a.s.

The additional publications/articles:

- Maynard et al. "Weeds in the treated field – a realistic scenario for pollinator risk assessment?"
Hazards of pesticides to bees - 12th International Symposium of the ICP-PR Bee Protection Group, Ghent (Belgium), September 15-17, 2014, 56 Julius-Kühn-Archiv, 450 (2015),
- Seitz, B.-J. 1989. *Beziehungen zwischen Vogelwelt und Vegetation im Kulturland - Untersuchungen im südwestdeutschen Hügelland. Beihefte zu den Veröffentlichungen für Naturschutz und Landschaftspflege in Baden-Württemberg 54: 1-236 (cf Appendix 1)*
- Scheffer/Schachtschnabel (1998) *Lehrbuch der Bodenkunde. Stuttgart, Enke Verlag.*
- Edwards CA & Lofty JR (1972) *The biology of earthworms. London, Chapman and Hall.*

Position Paper :

- *The risk assessment for Bees for flufenacet Annex I Renewal (11th May, 2016), authors: M.Almanza, U.Koelzer*