Bayer CropScience

Document Title

Summary of the ecotoxicological studies Diflufenican+Flufenacet SC600 (200+400)G

Data Requirements

EU Regulation 1107/2009 & EU Regulation 284/2013

Document MCP

Section 10: Ecotoxicological studies

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Introduction

The representative formulation FFA WG 60 submitted in the first Annex I listing process is no longer considered to be the representative formulation. The new representative formulation used for the submission of the renewal of the Annex I listing of flufenacet is a mixture formulation of flufenacet and diflufenican, Flufenacet + Diflufenican SC 600 (DFF+FFA SC600, Herold SC 600). The respective summaries will be presented in this Supplemental Dossier.

The risk assessment for Non-Target Organisms is presented for flufenacet using the formulation DFF + FFA SC 600, for the use as herbicide in winter cereals. Ecotoxicological endpoints used in the following risk assessment were derived from studies with the formulated product, the active substance flufenacet and the metabolites listed in the residue definition for risk assessment. In some cases where due to the study design the use of a technical substance is not possible, a solo formulation of flufenacet is used to address the intrinsic toxicity of flufenacet.

For the second active substance in the representative formulation, diflufenican, reference is made to the EU agreed endpoints according to the EFSA Scientific Report (2007) 122. For the Annex I listing process of diflufenican also the formulation Flufenacet + Diflufenican SC 600 (DFF+FFA SC600, 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. This evaluation was done under the Council Directive 91/414/EEC and all respective data requirements were addressed. With the present dossier 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.

In this Supplemental Dossier only endpoints used for the risk assessment are presented. For an overview of all available endpoints for flufenacet and its metabolites please refer to the respective section of the MCA document. In order to facilitate discrimination between new and information submitted during the first Annex I inclusion process, the old information is written in grey letters.

According to the guidance of EFSA on the "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (EFSA Journal 2011, 9 (2), 2092), literature for the active substance and it`s metabolites need to be presented, covering the last 10 years prior to the submission of this Annex I renewal dossier. In case where reliable and adequate literature is found for flufenacet and its metabolites during this literature search, summaries are integrated in the respective sections of this document.

In addition literature older than 10 years is included for the common and ubiquitous in the environment occurring metabolite trifluoroacetic acid (TFA). However these articles were not evaluated according to the above mentioned EFSA Guidance. Summaries are presented in the respective sections in the MCA document. Ecotoxicological endpoints extracted from these articles will be used in the risk assessment for the metabolite trifluoroacetic acid (TFA) and presented in this supplemental dossier.

Use pattern considered in this risk assessment

Table10- 1: Intended application pattern

Crop	Timing of application (range)	Number of applications	Application interval [days]	Maximum label rate (range) [L/ha]	Maximum application rate, individual treatment (ranges) [g/ha]	
					Diflufenican	Flufenacet
Cereals	10-13	1	-	0.6	120	240
Cereals	11-13	1		0.4	80	160
Cereals	00-22	1	-	0.3	60	120

Product density according to Section 2, MCP 2.6: 1.251 g/mL at 20 °C

Definition of the residue for risk assessment for flufenacet

Due to changes in triggers for metabolites to be further assessed as well as due to new studies on the route of degradation in various environmental compartments, additional metabolites are proposed to be included in the residue definition for the risk assessment (see Table 8-1). Accordingly, studies have been prepared to describe the ecotoxicological profile of these metabolites in the relevant environmental compartment.

Table 10- 2: Definition of the residue for risk assessment*

Compartment	Residue Definition for Risk Assessment
Soil	Flufenacet, FOE oxalate, FOE sulfonic acid, FOE methylsulfone, FOE-thiadone, FOE 5043-trifluoroethanesulfonic acid and trifluoroacetic acid
Groundwater	Same as for soil
Surface water	Same as for soil plus FOE methylsulfone
Sediment	flufenacet
Air	flufenacet

*Justification for the residue definition for risk assessment is provided in MCA Sec.7, Point 7.4.1 and MCA Sec. 6, Point 6.7.1.

In addition, a list of metabolites, which contains the structures, the synonyms and code numbers attributed to the compound flufenacet is presented in Document N3 of this dossier.

CP 10.1 Effects on birds and other terrestrial vertebrates

The risk assessment has been performed according to "European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438).

In addition to the parent compound flufenacet, a risk assessment (screening level only) is performed also for the metabolite trifluoroacetic acid (TFA). TFA has been identified as an environmental metabolite of different chemicals, including pesticide active substances as e.g. flufenacet. TFA has a pKa values < 2, therefore it occurs only in its deprotonated form under environmental conditions. As residues of TFA may occur in plant food items of birds and wild mammals, it was considered necessary to establish appropriate ecotoxicological endpoints to be used for risk assessment purposes. However, toxicity endpoints are only available for mammals. As birds are not expected to be more susceptible to TFA than mammals, these endpoints were also used for the bird screening assessment.

CP 10.1.1 Effects on birds

The summary of the toxicity profile of the active substances flufenacet and diflufenican to birds is provided in the following tables. For diflufenican reference is made to the EU agreed endpoints according to the EFSA Scientific Report (2007) 122.

Only endpoints used for the risk assessment are presented here. For an overview of all available endpoints on flufenacet please refer to the respective section of the MCA document.

It should be noted that the long-term risk assessment for flufenacet is based on a reproductive endpoint established in Mallard ducks. The product DFF+FFA SC 200+400, however, is applied to winter cereals in autumn at a time of the year when European birds do not reproduce. Flufenacet is quickly metabolized and excreted. Therefore it does not accumulate in birds' bodies and in addition irreversible or persistent adverse effects on the reproductive performance are not known for this compound. From this it is obvious that using a reproductive endpoint for the bird long-term risk assessment reflects a real worst case scenario for autumn uses.

Table 10.1.1- 1 Endpoints used in risk assessment

Test substance		species/origin	Endpoint		Reference
Flufenacet	Acute risk assessment	Lowest LD_{50} from Canary	LD_{50}	**434** mg as/kg bw	[redacted] 2013 M-468210-01-1 KCA 8.1.1.1/01
	Long-term risk assessment	Mallard duck	NOAEL	**9.87** mg as/kg bw/d	[redacted] & [redacted] (1994) M-003858-01-1

Table 10.1.1- 2 Endpoints of mixing partner diflufenican

Test substance	Test species	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84	
Diflufenican	'Bird' acute, oral	LD_{50}	>2150 mg as/kg bw 5537 mg as/kg bw [2]
	Bobwhite quail, reproduction	NOAEL	91.84 mg as/kg bw/d

[1] geometric mean of extrapolated LD_{50} values according to EFSA GD 2009

Toxicity of the formulation

No study was performed with the formulation on birds due to animal welfare reasons. A comparison of the acute endpoint of the formulation (LD_{50}) derived from a study on rats with a calculated value (calculated according to Finney's formula (IFAP, 1990)) is shown in Table 10.1.1- 3.

Table 10.1.1- 3: Comparison of acute toxicity: active ingredients vs. formulation

Species	Diflufenican 16.4%+ Flufenacet 32.5%	DFF + FFA SC 600
	Calculated [mg product/kg]*	Study results [mg product/kg]
Bird (Bobwhite quail)	3314	not available
Mammal (Rat)	1682 [2]	$500 < LD_{50} < 2000$

[1] based on: diflufenican – LD_{50} > 2150 mg/kg bw; flufenacet – LD_{50} 1608 mg/kg bw

[2] based on: diflufenican – LD_{50} > 5000 mg/kg bw; flufenacet – LD_{50} 589 mg/kg bw

* Based on a formulation density of 1.251 g/cm³ (Section 1)

Assessment: The comparison of available toxicity data from an experimental study with results from Finney calculations shows that the preparation is not more toxic than expected on basis of its content of active ingredients.

Table 10.1.1- 4 Relevant generic avian focal species for screening risk assessment

Crop	Indicator species	Shortcut value	
		For acute RA based on RUD_{90}	For long-term RA based on RUD_m
Bare soil	Small granivorous bird	24.7	11.4
Cereals	Small omnivorous bird	158.8	64.8

Table 10.1.1- 5 Relevant generic avian focal species for Tier 1 risk assessment

Crop	Growth stage (BBCH)	Generic focal species	Representative species	Shortcut value	
				Long-term RA based on RUD_m	acute RA based on RUD_{90}
Bare soil[1)]	BBCH <10	Small granivorous bird "finch"	Linnet (*Carduelis cannabina*)	11.4	24.7
		Small omnivorous bird "lark"	Woodlark (*Lullula arborea*)	8.2	17.4
		Small insectivorous bird "wagtail"	Yellow wagtail (*Motacilla flava*)	5.9	10.9
Cereals	BBCH 10-29	Small omnivorous bird "lark"	Woodlark (*Lullula arborea*)	10.9	24.0
	Early (shoots) autumn –winter BBCH 10-29	Large herbivorous bird "goose"	Pink-footed goose (*Anser brachyrhynchos*)	16.2	30.5

BOLD: Species considered in risk assessment (only worst case for each species)
[1)] scenario only representative for lowest application rate of 0.5 L DFF+FFA SC600, equivalent to 120 g FFA/ha

Risk assessment for birds

ACUTE DIETARY RISK ASSESSMENT FOR FLUFENACET

Table 10.1.1- 6 Tier 1 acute DDD and TER calculation for birds

Crop	Generic focal species	DDD			DDD	LD_{50} [mg/kg bw]	TER_A	Trigger
		Appl. rate [kg/ha]	SV_{90}	MAF_{90}				
Flufenacet – 0.6 L/ha								
Cereals	Small omnivorous bird "lark" <woodlark>	0.240	24.0	1	5.76	434	75	10
	Large herbivorous bird "goose" <pink-footed goose>		30.5		7.32		59	
Flufenacet – 0.4 L/ha								
Cereals	Small omnivorous bird "lark" <woodlark>	0.160	24.0	1	3.84	434	113	10
	Large herbivorous bird "goose" <pink-footed goose>		30.5		4.88		89	
Flufenacet – 0.3 L/ha								
Bare soil	Small granivorous bird "finch" <linnet>	0.120	24.7	1	2.96	434	147	10
	Small omnivorous bird "lark" <woodlark>		17.4		2.09		208	
	Small insectivorous bird "wagtail" <yellow wagtail>		10.2		1.31		331	
Cereals	Small omnivorous bird "lark" <woodlark>	0.120	24.0	1	2.88	434	151	10
	Large herbivorous bird "goose" <pink-footed goose>		30.5		3.66		119	

Assessment: The acute risk scenario results in TER values far above the trigger of 10 indicating that DFF+FFA SC 200+400 is safe for birds.

Acute risk assessment for birds drinking contaminated water from pools in leaf whorls

An assessment of the risk potentially posed by consumption of contaminated water is required according to the EFSA Guidance Document for Birds and Mammals (2009).

Leafy scenario

According to EFSA (2009) the potential exposure of birds via drinking water from pools on leaves or formed in leaf axils after the application should be addressed for acute risk assessment for birds. This scenario is only relevant for leafy vegetables forming heads at growth stage 4 (BBCH 41-49). This is not the case for cereals at early BBCH stages.

Puddle scenario

The acute risk from water in 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 is covered by the long-term risk assessment presented below.

LONG-TERM REPRODUCTIVE RISK ASSESSMENT FOR FLUFENACET

Table 10.1.1- 7 Tier 1 long-term DDD and TER calculation for birds

Compound / Crop	Generic focal species BBCH	DDD				DDD	NOAEL mg kg/bw/d	TER_{LT}	Trigger
		Appl. rate [kg/ha]	SV_m	MAF_m	TWA				
Flufenacet – 0.6 L/ha									
Cereals	Small omnivorous bird "lark" <woodlark>	0.240	10.9	1.0	0.53	1.39	9.87	7.1	5
	Large herbivorous bird "goose" <pink-footed goose>		16.2			2.06		4.8	
Flufenacet – 0.4 L/ha									
Cereals	Small omnivorous bird "lark" <woodlark>	0.160	10.9	1.0	0.53	0.92	9.87	10.7	5
	Large herbivorous bird "goose" <pink-footed goose>		16.2			1.37		7.2	
Flufenacet – 0.3 L/ha									
Bare soil	Small granivorous bird "finch" <linnet>	0.120	12.4	1.0	0.53	0.73	9.87	13.5	5
	Small omnivorous bird "lark" <woodlark>		8.2			0.52		19.0	
	Small insectivorous bird "wagtail" <yellow wagtail>		5.9			0.38		26.0	
Cereals	Small omnivorous bird "lark" <woodlark>	0.120	10.9	1.0	0.53	0.70	9.87	14.1	5
	Large herbivorous bird "goose" <pink-footed goose>		16.2			1.03		9.6	

Assessment: For use rates of 0.3 and 0.4 kg/ha the long-term risk scenario results in TER values greater than the trigger of 5 indicating that DFF+FFA SC 200+400 is safe for birds. Only for the large herbivorous bird the TER is marginally below the trigger at a use rate of 0.6 kg/ha; a refined assessment for this scenario is presented below.

Refined Risk Assessment

For the refined risk assessment addressing large herbivorous birds flufenacet-specific residue decline data established in cereals (wheat and barley) is taken into account ([redacted], 1995; M-004928-01 -1; [redacted] & [redacted], 2012, M-443138-01-1; [redacted] & [redacted], 2013, M-451178-01-1).

Refinement of f_{twa}

On basis of measured residue data from winter wheat a DT_{50} of ca. 3 days was determined for flufenacet ([redacted], 1995; M-004928-01 -1). This value has been confirmed in a new study where a DT_{50}

of 2.97 days was found for cereals ([redacted] & [redacted], 2012; M-443138-01-1; [redacted] & [redacted], 2013, M-451178-01-1). From this a f_{twa} value of **0.2025** results.

Table 10.1.1- 8 Refined long-term DDD and TER calculation for large herbivorous birds

Compound / Crop	Generic focal species BBCH	DDD				DDD	NOAEL mg kg/bw/d	TER_{LT}	Trigger
		Appl. rate [kg/ha]	SV_m	MAF_m	TWA				
Flufenacet 0.6 L/ha									
Cereals	Large herbivorous bird "goose" <pink-footed goose>	0.240	16.2	1.0	0.2025	0.787	9.87	12.5	5

Assessment: The refined long-term risk assessment based on flufenacet-specific residue decline data results in an acceptable TER value also for large herbivorous birds.

Long-term risk assessment for birds drinking contaminated water in puddles

Table 10.1.1- 9 Evaluation of potential concern for exposure of birds drinking water (escape clause)

Crop	Koc [L/kg]	Application rate * MAF [g as/ha]	NOAEL [mg as/ kg bw/d]	Ratio (Application rate * MAF) / NOAEL	"Escape clause" No concern if ratio	Conclusion
Flufenacet						
Cereals	215	240 x 1.0	9.87	24.3	**≤ 50**	No concern

Assessment: The "escape clause" calculation shows that DFF+FFA SC 200+400 would not result in unacceptable risk for birds drinking contaminated water.

SCREENING ASSESSMENT FOR TFA

The risk assessment on screening level has been performed for bare soil for an application rate of 0.3 L product/ha and for cereals for 0.6 L/ha, corresponding to 120 g flufenacet/ha and 240 g flufenacet/ha, respectively. As a worst case assumption, a formation of 100% TFA from flufenacet was used. The application rate of TFA was then estimated correcting the application rate of the parent for the difference in molecular mass between flufenacet (363.33 g/mol) and TFA (114.04 g/mol). This results in maximum application rates for TFA of 37.7 g/ha (0.3 L/ha DFF+FFA SC 600) and 75.4g/ha (0.6 L/ha DFF+FFA SC 600).

Table 10.1.1- 10 Screening step acute DDD and TER calculation for birds - TFA

Crop	Indicator species	LD_{50} [mg/kg bw]	DDD			DDD	TER_A	Trigger
			Appl. rate [kg/ha]	SV_{90}	MAF_{90}			
TFA								
Bare soils	Small granivorous bird	>2000	0.0377*	24.7	1	0.931	2148	10
Cereals	Small omnivorous bird	>2000	0.0754*	158.8	1	11.97	167	10

* 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.

Table 10.1.1- 11 Screening step long-term DDD and TER calculation for birds - TFA

Crop	Indicator species	NOAEL [mg/kg bw/d]	DDD				DDD	TER_{LT}	Trigger
			Appl. rate [kg/ha]	SV_m	MAF_m	f_{twa}			
TFA									
Bare soils	Small granivorous bird	98	0.0377*	11.4	1	0.53	0.228	430	5
Cereals	Small omnivorous bird	98	0.0754*	64.8	1	0.53	2.59	38	5

* * 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.

Assessment: All TER values are above the requested trigger of 10 for acute exposure and 5 for long-term exposure. Accordingly, no risk is discernible for birds from residues of TFA following uses of DFF+FFA SC 200+400.

RISK ASSESSMENT OF SECONDARY POISONING

Table 10.1.1- 12 Log Pow values (for details please refer to section 2.7 "Partition coefficient n-octanol/water" in the MCA)

Substance	log P_{ow}
Flufenacet	3.2
	3.5
FOE oxalate (M01)	0.80
	pH-dependent -2.0 (pH 5) -2.2 (pH 7) - 2.4 (pH 9)
FOE sulfonic acid (M02)	No 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 4.6) 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)

Table 10.1.1- 13 Avian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic avian indicator species	Body weight [g]	Example	FIR/bw
Earthworm eater	100	Blackbird	1.05
Fish eater	1000	Heron	0.159

Long-term DDD and TER calculation for earthworm-eating birds

Table 10.1.1- 14 Tier 1 long-term DDD and TER calculation for earthworm-eating birds

	Cereals	
Flufenacet	**240 g a.s./ha**	**160 g a.s./ha* 120 g a.s./ha***
P_{OW}	3200	3200
K_{OC} [mL/g]	215	215
f_{OC}	0.02	0.02
BCF_{worm}	9.13	9.13
$PEC_{soil\ (twa,\ 21\ d)}$[mg/kg]	0.203	0.135
PEC_{worm} [mg/kg]	1.853	1.232
FIR/bw	1.05	1.05
DDD [mg/kg bw/d]	1.946	1.294
NO(A)EL [mg/kg bw/d]	9.87	9.87
TER_{LT}	5.1	7.6
Trigger	5	5

*see MCP, section 9, Efate – same PEC soil for 160/120 g a.s./ha due to different interception

The TER value is above the trigger of 5 for all application rates indicating that DFF+FFA SC 200+400 is safe for earthworm eating birds.

Long-term DDD and TER calculation for fish-eating birds

Table 10.1.1- 15 Tier 1 long-term DDD and TER calculation for fish-eating birds

	Cereals		
Flufenacet	**240 g a.s./ha**	**160 g a.s./ha**	**120 g a.s./ha**
BCF_{fish}	71.4	71.4	71.4
$PEC_{SW\ (twa,\ 21\ d)}$[mg/L]	0.0193	0.0129	0.0126
PEC_{fish} [mg/kg]	1.378	0.921	0.899
FIR/bw	0.159	0.159	0.159
DDD [mg/kg bw/d]	0.22	0.15	0.14
NO(A)EL [mg/kg bw/d]	9.87	9.87	9.87
TER_{LT}	45	67	69
Trigger	5	5	5

The TER value is above the trigger of 5 for all application rates: Hence the risk to fish-eating birds form the use of the product in cereals is considered acceptable.

CP 10.1.1.1 Acute oral toxicity

One new acute oral toxicity study with flufenacet on canary birds was performed. For details on this study, please refer to the MCA section 8.1.1.1.

CP 10.1.1.2 Higher tier data on birds

No additional studies were considered necessary. For details on studies to determine residues of flufenacet on insects and plants please refer to the MCA section 8.1.1.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

The summary of relevant toxicity endpoints of the active substances flufenacet and the metabolite TFA in mammals is provided in the following tables. For diflufenican references is made to the EU agreed endpoints according to the EFSA Scientific Report (2007) 122.

Only endpoints used for the risk assessment are presented here. For an overview of all available endpoints on flufenacet please refer to the respective section of the MCA document.

Table 10.1.2- 1 Endpoints used in risk assessment

Test substance	Scenario	species / origin	Endpoint		Reference
Flufenacet	Acute risk assessment	Rat	LD_{50}	**589** mg as/kg bw	[redacted] & [redacted] (1993) M-004865-02-1
	Long-term risk assessment	Rat	$NOAEL_{parental}$ $NOAEL_{reproduction}$	500 ppm **35.4** mg as/kg bw/d	[redacted] & [redacted] (1995) M-004984-03-1
			NOAEL	**37.4** mg as/kg bw/d	Endpoint evaluation: [redacted] (2014) M-476600-01-1 KCA 8.1.2.2/01
TFA	Acute risk assessment	Rat	LD_{50}	>2000 mg as/kg bw	[redacted]-[redacted] (2013) M-444479-01-1 KCA 5.8.1/24
	Long-term risk assessment	Rat	NOAEL	1000 ppm 98 mg as/kg bw/d	[redacted]-[redacted] (2007) M-283994-01-1 KCA 5.8.1/27
			NOAEL	**98** mg as/kg bw/d	Endpoint evaluation: [redacted] (2014) M-477154-01-1 KCA 8.1.2.2/02

Table 10.1.2- 2 Endpoints of mixing partner diflufenican

Test substance	Test species	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84	
Diflufenican	Rat acute, oral	LD_{50}	> 5000 mg as/kg bw
	Rat reproduction	NOAEL	35.5 mg as/kg bw/d

Table 10.1.2- 3 Relevant generic focal species for Tier 1 risk assessment

Crop group*	Scenario	Generic focal species	Representative species	Shortcut value	
				Long-term RA based on RUD_m	acute RA based on RUD_{90}
Bare soil[1)]	< 10	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvaticus*)	**5.7**	**14.3**
Cereals	10 - 19	Small insectivorous mammal "shrew"	Common shrew (*Sorex araneus*)	**4.2**	**7.6**
	≥ 20			1.9	5.4
	Early (shoots)	Large herbivorous mammal "lagomorph"	Rabbit (*Oryctolagos cuniculus*)	**22.3**	**42.1**
	10-29	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvaticus*)	**7.8**	**17.2**

BOLD: Species considered in risk assessment (only worst case for each species)
[1)] scenario only representative for lowest application rate of 0.3 L DFF+FFA SC600, equivalent to 120 g FFA/ha

Risk assessment for other terrestrial vertebrates

The risk assessment on screening level has been performed for bare soil for an application rate of 0.3 L product/ha and for cereals for 0.6 L/ha, corresponding to 120 g flufenacet/ha and 240 g flufenacet/ha, respectively. As a worst case assumption, a formation of 100% TFA from flufenacet was used. The application rate of TFA was then estimated correcting the application rate of the parent for the difference in molecular mass between flufenacet (363.33 g/mol) and TFA (114.04 g/mol). This results in maximum application rates for TFA of 37.7 g/ha (0.3 L/ha DFF+FFA SC 600) and 75.4g/ha (0.6 L/ha DFF+FFA SC 600).

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.2- 4 Tier 1 acute DDD and TER calculation for mammals

Crop	Generic focal species	DDD			DDD	LD_{50} [mg/kg bw]	TER	Trigger
		Appl. rate [kg/ha]	SV_{90}	MAF_{90}				
Flufenacet – 0.6 L/ha								
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.240	7.6	1	1.82	589	324	10
	Large herbivorous mammal "lagomorph" <Rabbit>		42.1		10.1		58	
	Small omnivorous mammal "mouse" <Woodmouse>		17.2		4.13		143	
TFA								
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.0754*	7.6	1	0.57	>2000	>3508	10
	Large herbivorous mammal "lagomorph" <Rabbit>		42.1		3.17		>630	
	Small omnivorous mammal "mouse" <Woodmouse>		17.2		1.30		>1538	
Flufenacet – 0.4 L/ha								
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.160	7.6	1	1.22	589	483	10
	Large herbivorous mammal "lagomorph" <Rabbit>		42.1		6.74		87	
	Small omnivorous mammal "mouse" <Woodmouse>		17.2		2.75		214	
TFA								
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.0502*	7.6	1	0.38	>2000	>5263	10
	Large herbivorous mammal "lagomorph" <Rabbit>		42.1		2.11		>947	
	Small omnivorous mammal "mouse" <Woodmouse>		17.2		0.86		>2325	
Flufenacet – 0.3 L/ha								
Bare soil	Small omnivorous mammal "mouse" <Woodmouse>	0.120	14.3	1	1.72	589	342	10
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.120	7.6	1	0.91		647	10
	Large herbivorous mammal "lagomorph" <Rabbit>		42.1		5.05		117	
	Small omnivorous mammal "mouse" <Woodmouse>		17.2		2.06		286	
TFA								
Bare soil	Small omnivorous mammal "mouse" <Woodmouse>	0.0377*	14.3	1	0.54	>2000	>3703	10
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.0377*	7.6	1	0.29		>6896	10
	Large herbivorous mammal "lagomorph" <Rabbit>		42.1		1.59		>1257	
	Small omnivorous mammal "mouse" <Woodmouse>		17.2		0.65		>3076	

*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.

LONG-TERM REPRODUCTIVE ASSESSMENT

Table 10.1.2- 5 Tier 1 long-term DDD and TER calculation for mammals

Compound / Crop	Generic focal species BBCH	DDD				DDD	NOAEL mg kg/bw/d	TER_{LT}	Trigger
		Appl. rate [kg/ha]	SV_m	MAF_m	TWA				
Flufenacet – 0.6 L/ha									
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.240	4.2	1.0	0.53	0.53	37.4	71	5
	Large herbivorous mammal "lagomorph" <Rabbit>		22.3			2.84		13.2	
	Small omnivorous mammal "mouse" <Woodmouse>		7.8			0.99		38	
TFA									
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.0754*	4.2	1.0	0.53	0.17	98	576	5
	Large herbivorous mammal "lagomorph" <Rabbit>		22.3			0.89		110	
	Small omnivorous mammal "mouse" <Woodmouse>		7.8			0.31		316	
Flufenacet – 0.4 L/ha									
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.160	4.2	1.0	0.53	0.67	37.4	56	5
	Large herbivorous mammal "lagomorph" <Rabbit>		22.3			1.89		20	
	Small omnivorous mammal "mouse" <Woodmouse>		7.8			0.66		57	
TFA									
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.0502*	4.2	1.0	0.53	0.11	98	891	5
	Large herbivorous mammal "lagomorph" <Rabbit>		22.3			0.59		166	
	Small omnivorous mammal "mouse" <Woodmouse>		7.8			0.21		467	

Table 10.1.2- 5 (cont.) Tier 1 long-term DDD and TER calculation for mammals

Compound / Crop	Generic focal species BBCH	DDD				DDD	NOAEL mg kg/bw/d	TER_{LT}	Trigger
		Appl. rate [kg/ha]	SV_m	MAF_m	TWA				
Flufenacet – 0.3 L/ha									
Bare soil	Small omnivorous mammal "mouse" <Woodmouse>	0.120	5.7	1.0	0.53	0.36	37.4	104	5
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.120	4.2	1.0	0.53	0.27	37.4	139	5
	Large herbivorous mammal "lagomorph" <Rabbit>		22.3			1.42		26	
	Small omnivorous mammal "mouse" <Woodmouse>		7.8			0.50		75	
TFA									
Bare soil	Small omnivorous mammal "mouse" <Woodmouse>	0.0377*	5.7	1.0	0.53	0.11	98	891	5
Cereals	Small insectivorous mammal "shrew" <Common shrew>	0.0377*	4.2	1.0	0.53	0.09	98	1089	5
	Large herbivorous mammal "lagomorph" <Rabbit>		22.3			0.45		218	
	Small omnivorous mammal "mouse" <Woodmouse>		7.8			0.16		613	

*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.

Assessment: The acute and long-term risk assessment addressing flufenacet and the metabolite TFA results in acceptable TER values for all use rates indicating that DFF+FFA SC 200+400 is safe for mammals.

LONG-TERM RISK ASSESSMENT FOR MAMMALS DRINKING CONTAMINATED WATER

The puddle scenario is relevant for the long-term risk assessment.

Table 10.1.2- 6 Evaluation of potential concern for exposure of mammals drinking water

Crop	Koc [L/kg]	Application rate * MAF [g as/ha]	NO(A)EL [mg as/ kg bw/d]	Ratio (Application rate * MAF) / NOAEL	"Escape clause" No concern if ratio	Conclusion
Flufenacet						
Cereals	215	240 x 1.0	37.4	6.4	**≤ 50**	No concern

Assessment: According to the evaluation for flufenacet, the risk to mammals drinking water from puddles on soil following the use of DFF+FFA SC 600 on bare soil and on cereals is acceptable.

RISK ASSESSMENT OF SECONDARY POISONING

As outlined in Point 10.1.1 a risk assessment of secondary poisoning has to be performed for the following compounds: flufenacet.

Table 10.1.2- 7 Mammalian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic focal species	Body weight [g]	Example	FIR/bw
Earthworm eater	10	Common shrew	1.28
Fish eater	3000	Otter	0.142

Long-term DDD and TER calculation for earthworm-eating mammals

Table 10.1.2- 8 Tier 1 long-term DDD and TER calculation for earthworm eating mammals

	Cereals	
Flufenacet	**240 g a.s./ha**	**160 g a.s./ha* 120 g a.s./ha***
PEC_{worm} [mg/kg]	1.853	1.232
FIR/bw	1.28	1.28
DDD [mg/kg bw/d]	2.37	1.58
NOAEL [mg/kg bw/d]	37.4	37.4
TER_{LT}	15.8	23.7
Trigger	5	5

*see MCP, section 9, Efate – same PECsoil for 160/120 g a.s./ha due to different interception rates

Assessment: No risk to earthworm-eating mammals is discernible from the use of DFF+FFA SC 200+400 in cereals.

Long-term toxicity exposure ratio for fish-eating mammals

Table 10.1.2- 9 Tier 1 long-term DDD and TER calculation for fish eating mammals

	Cereals		
Flufenacet	**240 g a.s./ha**	**160 g a.s./ha**	**120 g a.s./ha**
PEC_{fish} [mg/kg]	1.378	0.921	0.899
FIR/bw	0.142	0.142	0.142
DDD [mg/kg bw/d]	0.20	0.13	0.13
NOAEL [mg/kg bw/d]	37.4	37.4	37.4
TER_{LT}	187	286	293
Trigger	5	5	5

Assessment: No risk to fish-eating mammals is discernible form the use of DFF+FFA SC 200+400 in cereals.

CP 10.1.2.1 Acute oral toxicity to mammals

Table 10.1.2.1- 1 Endpoints for the representative formulation

Test species	Test design	Ecotoxicological endpoint [mg product/kg bw]	Reference
Rat	acute, oral	$500 < LD_{50} < 2000$	[redacted], 2002, M-055334-01-1 KCP 7.1.1 /01

Toxicity of the formulation

A comparison of the acute endpoint of the formulation derived from a study on rats with calculated theoretical endpoints (calculated according to Finney's formula GIFAP, 1990) is shown in Table 10.1.1- 3.

Table 10.1.1- 16: Comparison of acute toxicity: active ingredients vs. formulation

Species	Diflufenican 16.4%+ Flufenacet 32.5%	DFF + FFA SC 600
	Calculated [mg product/kg bw]*	Study results [mg product/kg bw]
Mammal (Rat)	1682[1]	$500 < LD_{50} < 2000$

[1] based on: Diflufenican – LD_{50} > 5000 mg/kg bw; Flufenacet – LD_{50} 589 mg/kg bw

* Based on a formulation density of 1.251 g/cm³ (Section 1)

Assessment: A comparison of available toxicity data from an experimental study with results from the Finney calculation shows that the preparation is not more toxic than expected on basis of its content of active ingredients.

CP 10.1.2.2 Higher tier data on mammals

No additional studies were considered necessary. For details on studies to determine residues of flufenacet on insects and plants please refer to the MCA section 8.1.1.

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

No studies were conducted on reptiles or amphibians with the formulation. An acute toxicity study on the African clawed frog (*Xenopus laevis*) using flufenacet technical was performed ([redacted]n et al. 2013, M-471899-01-1; KCA 8.2.8/03). The 48 h NOEC based on mortality and sublethal effects is 10 mg a.s./L equivalent to the highest dose rate tested.

CP 10.2 Effects on aquatic organisms

The risk assessment is based on the current Guidance Document on Aquatic Ecotoxicology, SANCO/3268/2001, rev 4 final, 17 October 2002. Some implications of the new Aquatic Guidance Document (EFSA Journal 2013, 11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290), which is not yet notified, have been taken into consideration as well.

In the first Annex I listing process data on aquatic species for a different formulation of flufenacet were submitted and evaluated. The formulation FFA WG60 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 will be presented with this dossier. For the Annex I listing process of diflufenican also the formulation Flufenacet + Diflufenican SC 600 (DFF+FFA SC600, 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.

The summary of the toxicity profile of the active substances flufenacet and diflufenican to aquatic organisms is provided in the following tables. For diflufenican reference is made to the EU agreed endpoints according to the EFSA Scientific Report (2007) 122.

Only endpoints used for the risk assessment are presented here. For an overview of all available endpoints on flufenacet and its metabolites please refer to the respective section of the MCA document.

Ecotoxicological endpoints used in risk assessment

Table 10.2- 1 Endpoints for the representative formulation used in risk assessment

Test substance	Test species	Endpoint	Reference
DFF+FFA SC 600	*Selenastrum capricornutum* 72h, static	E_rC_{50} 0.00663 mg as/L	[redacted] & [redacted] (2001) M-073137-01-1 KCP 10.2.1/01
	Lemna gibba 7d, static	E_rC_{50} 0.307 mg as/L	[redacted] & [redacted] (2001), M-073160-01-1 KCP 10.2.1/02

Table 10.2- 2 Endpoints for flufenacet and its metabolites used in risk assessment

Test substance	Test species	Endpoints used in risk assessment	Reference
Flufenacet	Fish acute *Lepomis macrochirus*	LC_{50} 2.13 mg a.s./L	[redacted] (1995) M-002378-01-1
	Fish, chronic, ELS *Oncorhynchus mykiss*	NOEC 0.334 mg a.s./L (1)	[redacted] (1995) M-002357-01-1
	Fish, chronic, FFLC *Pimephales promelas*	NOEC **0.138** mg a.s./L (3)	[redacted] & [redacted] (2002) M-082934-01-1 KCA 8.2.2.2/01

Test substance	Test species	Endpoints used in risk assessment		Reference
	Invertebrate, acute *Daphnia magna*	EC_{50}	30.9 mg a.s./L	[redacted] (1994) M-003805-01-1
	Invertebrate, chronic *Daphnia magna*	NOEC	3.26 mg a.s./L	[redacted] & [redacted] (1994) M-003795-01-1
	Sediment dweller, chronic *Chironomus riparius* (spiked water)	NOEC	**5.0** mg a.s./L	[redacted] (2010) M-372857-01-1 KCA 8.2.3.3/01
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	**0.0144** mg a.s./L	Geometric mean of the three endpoints listed (see MCA 8.2)
	Aquatic plant *Lemna gibba*	E_rC_{50}	**0.0139** mg a.s./L[4]	[redacted] (2013) M-451198-01-1 KCA 8.2.7/11
	Lemna gibba (Duckweed)	Justification to use the new E_rC_{50} ([redacted], 2013) for risk assessment purposes		[redacted] (2014) M-478762-01-1 KCA 8.2.7/13
	Lemna gibba (Duckweed)	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_rC_{50} >0.126 mg/L	[redacted] (2013) M-452567-01-1 KCA 8.2.7/12
Flufenacet - Saltwater organisms	Fish, acute *Cyprinodon variegatus*	LC_{50}	3.31 mg a.s./L	[redacted] & [redacted] (1994) M-002422-01-1 KCA 8.2.1/05
	Fish, chronic, ELS *Cyprinodon variegatus*	NOEC	**0.049** mg a.s./L	[redacted], [redacted] & [redacted] (2013) M-464909-01-1 KCA 8.2.2.1/02
	Invertebrate, acute *Mysidopsis bahia*	LC_{50}	**5.6** mg a.s./L	[redacted], M.B. et al. (2013) M-452205-01-1 KCA 8.2.4.2/03
	Invertebrate, chronic *Mysidopsis bahia*	NOEC	**0.221** mg a.s./L	[redacted], M.B. et al. (2013) M-452207-01-1 KCA 8.2.5.2/01
	Algae *Skeletonema costatum*	4d-E_rC_{50}	**0.00949** mg a.s./L	[redacted] (1995) M-002353-02-1 recalculated: [redacted] (1998) M-086470-01-1 KCA 8.2.6.2/07
FOE oxalate	Algae *Pseudokirchneriella subcapitata*	E_bC_{50} E_rC_{50}	> 100 mg p.m./L [5] **> 100** mg p.m./L [5]	[redacted] (2009) M-358823-01-1 KCA 8.2.6.1/08
	Aquatic plant *Lemna gibba*	E_rC_{50}	**> 100** mg p.m./L[5]	[redacted] (2009) M-359515-02-1 KCA 8.2.7/05

Test substance	Test species	Endpoints used in risk assessment		Reference
FOE Sulfonic acid	Fish, acute *Oncorhynchus mykiss*	LC_{50}	> **86.7** mg p.m./L	[redacted] (1995) M-004932-01-1
	Invertebrate, acute *Daphnia magna*	EC_{50}	> **87.3** mg p.m./L	[redacted] (1995) M-004930-01-1
	Algae *Desmodesmus subspicatus*	E_rC_{50}	> **86.7** mg p.m./L	[redacted] (1995) M-004931-01-1
	Aquatic plant *Lemna gibba*	EC_{50}	> **75.9** mg p.m./L	[redacted] (1995) M-004929-01-1
FOE Methylsulfide	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	**83.8** mg p.m./L	[redacted] (1998) M-002341-01-1
	Aquatic plant *Lemna gibba*	E_rC_{50}	**106** mg p.m./L	[redacted] (2010) M-393709-01-1 KCA 8.2.7/07
FOE Methylsulfone	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	> **10.0** mg p.m./L	[redacted] (2010) M-364591-01-1 KCA 8.2.6.1/10
	Aquatic plant *Lemna gibba*	EC_{50}	> **100** mg p.m./L	[redacted] (2010) M-369703-01-1 KCA 8.2.7/06
TFA	Fish, acute *Brachydanio rerio*	LC_{50}	> **1200** mg p.m./L	[redacted] et al., (1992) M-247889-01-1 KCA 8.2.1/10
	Brachydanio rerio (Zebra fish)	NOEC	**300** mg p.m./L	[redacted] et al. 2013; M-462660-01-1 KCA 8.2.2.1/01
	Invertebrate, acute *Daphnia magna*	EC_{50}	> **1200** mg p.m./L	[redacted] et al. (1992) M-247890-01-1 KCA 8.2.4.1/04
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	**160** mg p.m./L	[redacted] et al. (1992) M-247820-01-1 KCA 8.2.6.1/12
	Aquatic plant *Lemna gibba*	EC_{50}	**618.3** mg p.m./L	[redacted] & [redacted] (2004) M-455787-01-1 KCA 8.2.7/14
	Aquatic plant *Myriophyllum spicatum*	EC_{50}	**312.9** mg p.m./L	
FOE 5043-trifluoroethane sulfonic acid	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	> **100** mg p.m./L	[redacted] (2012) M-444217-01-1 KCA 8.2.6.1/15
	Aquatic plant *Lemna gibba*	EC_{50}	> **10** mg p.m./L	[redacted] (2013) M-445884-01-1 KCA 8.2.7/10
FOE-Thiadone	fish, acute *Oncorhynchus mykiss*	LC_{50}	**9.1** mg p.m./L	[redacted] & [redacted] (1998) M-005388-01-1 KCA 8.2.1/06
	Invertebrate, acute *Daphnia magna*	EC_{50}	**31.7** mg p.m./L	[redacted] & [redacted] (1998) M-005390-01-1 KCA 8.2.4.1/03

Test substance	Test species	Endpoints used in risk assessment	Reference
	Algae *Pseudokirchneriella subcapitata*	E_bC_{50} **4.1** mg p.m./L	[redacted] & [redacted] (1999) M-009214-01-1 KCA 8.2.6.1/06
	Aquatic plant *Lemna gibba*	E_rC_{50} **18.3** mg p.m./L (5)	[redacted] (2010) M-393718-01-1 KCA 8.2.7/08
FOE-Thiadone – Saltwater organisms	Fish, acute *Cyprinodon variegatus*	LC_{50} **15.3** mg p.m./L	[redacted] & [redacted] (1999) M-009684-01-1 KCA 8.2.1/07
	Invertebrate, acute *Mysidopsis bahia*	EC_{50} **>15.1** mg p.m./L	[redacted] & [redacted] (1998) M-005110-01-1 KCA 8.2.4.2/02
Flufenacet WG 60	Macrophytes & periphyton indoor microcosm	NOEC **0.012 mg a.s./l** EAC **0.022 mg a.s./l** (2)	[redacted] & [redacted] (1999) M-023412-01-1 [redacted] (2009) M-329959-01-1 KCA 8.2.8/03

(1) The fish-ELS NOEC-value reported in the dossier is 0.334 mg/L. The endpoint fixed by the EC is 0.2 mg/L ("value where a significant reduction of growth was measured" at post-hatch day 33). The choice of this value is not supported by BCS. Justification: Growth, measured as fish length, was statistically different from controls on post-hatch day 33 (study-day 66). This proved to be biologically not relevant on post-hatch day 62 (study-day 97), where no effects were observed for length. The biological significance of this transient effect is questionable. Measurements of length at this study time are based on picture analysis, which is a doubtful method and not required in OECD 210 (US-specific; see also comment of study-author on page 19 of study report). The NOEC for growth (as length) at the end of the study is given as 0.8 mg/l (measured 0.735 mg/L). Therefore, the NOEC for the whole study should be based on the parameters "percent swim-up" and "97d-dry weight": 0.4 mg/L (measured 0.334 mg/L).

(2) The microcosm study has been further evaluated by an expert statement confirming the EAC as relevant endpoint ([redacted], 2009, M-329959-01-1, see point 10.2.3).

(3) Lower endpoint obtained from a new study.

(4) Former EU agreed endpoint (14-day Lemna study considering only one endpoint (frond counts)) will be replaced by a new 7-day Lemna study ([redacted] 2013, M-451198-01-1) performed according to current valid OECD 221 guideline considering two endpoints (frond number and frond area). The ErC50 from this study will be used in the risk assessment. For details see Statement performed by [redacted] P. 2014 (M-478762-01-1).

(5) No EU agreed endpoint available. Endpoint used for risk assessment obtained from a new study.

(6) Based on mean measured concentrations as proposed in the study report.

Table 10.2-3 Endpoints of mixing partner Diflufenican

Test substance	Test species	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84	
Diflufenican	Fish, acute *Cyprinus carpio*	LC_{50}	> 0.0985 mg as/L
	Fish, chronic *Pimephales promelas*	NOEC	0.015 mg as/L
	Invertebrate, acute *Daphnia magna*	EC_{50}	> 0.240 mg as/L
	Invertebrate, chronic *Daphnia magna*	NOEC	0.052 mg as/L
	Sediment dweller, chronic *Chironomus riparius* (spiked water)	NOEC	0.100 mg as/L
	Sediment dweller, chronic *Chironomus riparius*	NOEC	2.0 mg as/kg

Test substance	Test species	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84	
	(spiked sediment)		
	Algae *Desmodesmus subspicatus*	EC_{50}	0.00025 mg as/L
	Algae *Desmodesmus subspicatus (with recovery)*	Maximum concentration from which recovery is possible[1] overall NOEC[3]	0.0042 mg as/L 0.0001 mg as/L
	Aquatic plant *Lemna gibba*	E_rC_{50}	0.039 mg as/L
AE B107137	Fish, acute *Oncorhynchus mykiss*	LC_{50}	> 17.3 mg/L [2)]
	Invertebrate, acute *Daphnia magna*	EC_{50}	> 20.4* mg/L [2)]
	Algae *Desmodesmus subspicatus*	EC_{50}	> 20.4* mg/L [2)]
AE 0542291	Invertebrate, acute *Daphnia magna*	EC_{50}	> 10 mg/L [2)]
	Algae *Desmodesmus subspicatus*	EC_{50}	36 mg/L

[1)] *EFSA Scientific Report* (2007) 122, 1-84: "In order to cover effects on less sensitive but slower reproducing algal species the safety factor of 10 was maintained in the risk assessment. The exposure pattern of the FOCUS scenarios were analysed and the risk was considered acceptable provided that the peak exposure is below 0.42 µg diflufenican/L and that this exposure does not last longer than 3 days. In order to cover the overall NOEC of 0.1 µg diflufenican/L no other peak exposure should exceed the NOEC of 0.1 µg diflufenican/L.
[2)] above the limit of aqueous solubility
*above the limit of aqueous solubility

Selection of algae endpoints for risk assessment

Processes in ecosystems are dominantly rate driven and therefore, the unit development per time (growth rate) is more suitable to measure effects in algae. Also, growth rates and their inhibition can easily be compared between species, test durations and test conditions, which is not the case for yield or biomass based endpoints. Following current state of science, the test guidelines OECD TG 201, the EU-Method C3, the EC regulation for Classification and Labeling (EC regulation 1272/2008), the PPR Opinion (EFSA Journal 461, 1-44; 2007) and also the EFSA Aquatic Guidance Document (2013, not yet formally noted by SCFCAH), list growth rate as the relevant endpoint of the algae inhibition test. The previous Guidance Document on Aquatic Toxicology (SANCO/3268/2001 rev. 4) still states that "As there is no clear evidence available to indicate which is the most relevant endpoint for the field situation the lower figure should be used in the risk assessment". As this statement is clearly superseded by recent scientific and regulatory developments, toxicity-exposure-ratios in this assessment were based on the E_rC_{50}, when available.

Selection of Lemna endpoints for risk assessment (see also Statement from [redacted] P, 2014, M-478762-01-1, KCA 8.2.7/12)

So far the EU-agreed endpoint for aquatic plants is based on a 14-day Lemna study form 1993 ([redacted] & [redacted]; M-002418-02-1). This study was done according to the FIFRA Guideline 123-2 and the endpoint was based on frond counts solely. In 1998, [redacted] (M-086479-01-1) recalculated a 7-day ErC50 based on frond count out of this study with 31.8 µg/L. This endpoint was early considered by authorities. However, this study by [redacted] & [redacted] (1993; M-002418-02-

1) is considered to be not valid according to current guidelines (OECD 221, 2006) as a second endpoint like frond dry weight or frond area has not been determined.

To address this data requirement with a fully valid study a new 7-day Lemna study ([redacted] 2013; M-451198-01-1) was performed. 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 [redacted] (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 to the 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.

Predicted environmental concentrations used in risk assessment

Table 10.2- 4 Initial max PEC_{sw} values – FOCUS Step 1, 2

Compound	FOCUS Scenario	Winter cereals 1 x 240 g a.s/ha	Winter cereals 1 x 160 g a.s/ha	Winter cereals 1 x 120 g a.s/ha
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
Flufenacet	**STEP 1**	64.38	42.92	32.19
	STEP 2 - North	**21.80**	**14.53**	**14.24**
	STEP 2 - South	17.79	11.86	11.57
FOE sulfonic acid	**STEP 1**	15.73	10.49	7.864
	STEP 2 - North	**5.152**	3.435	3.435
	STEP 2 - South	4.121	2.748	2.748
FOE oxalate	**STEP 1**	12.95	8.634	6.476
	STEP 2 - North	**3.967**	2.645	2.645
	STEP 2 - South	3.174	2.116	2.116
FOE methylsulfone	**STEP 1**	3.615	2.410	1.807
	STEP 2 - North	**1.301**	0.867	0.867
	STEP 2 - South	1.041	0.694	0.694
FOE methylsulfide	**STEP 1**	0.167	0.111	0.084
	STEP 2 - North	**0.167**	0.111	0.084
	STEP 2 - South	0.167	0.111	0.084
FOE-thiadone	**STEP 1**	2.959	1.973	1.480
	STEP 2 - North	**0.975**	0.650	0.510
	STEP 2 - South	0.947	0.631	0.492
FOE 5043-trifluoroethane sulfonic acid	**STEP 1**	2.168	1.445	1.084
	STEP 2 - North	**0.600**	0.400	0.400
	STEP 2 - South	0.480	0.320	0.320
TFA	**STEP 1**	20.46	13.64	10.23
	STEP 2 - North	**7.651**	5.101	5.101
	STEP 2 - South	6.121	4.081	4.081

BOLD – values considered in risk assessment

Table 10.2- 5 Initial max PEC_{sw} values – FOCUS Step 3

Compound	FOCUS Scenario	Winter cereals 1 x 240 g a.s/ha	Winter cereals 1 x 160 g a.s/ha	Winter cereals 1 x 120 g a.s/ha
		$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
Flufenacet	D1 (ditch, 1st)	6.762	4.460	2.767
	D1 (stream, 1st)	4.230	2.782	1.728
	D2 (ditch, 1st)	7.223	4.646	3.750
	D2 (stream, 1st)	4.517	2.905	2.343
	D3 (ditch, 1st)	1.513	1.010	0.758
	D4 (pond, 1st)	1.245	0.812	0.417
	D4 (stream, 1st)	1.892	1.228	0.658
	D5 (pond, 1st)	1.176	0.776	0.575
	D5 (stream, 1st)	1.419	0.946	0.710
	D6 (ditch, 1st)	6.021	3.969	2.950
	R1 (pond, 1st)	0.116	0.077	0.057
	R1 (stream, 1st)	6.341	4.142	3.062
	R3 (stream, 1st)	7.887	5.148	4.173
	R4 (stream, 1st)	5.943	3.936	1.156

Table 10.2- 6 3-day time-weighted average PEC_{sw} values – FOCUS Step 3 + FOCUS Step 4

Compound	FOCUS Scenario	Winter cereals 1 x 240 g a.s/ha	Winter cereals 1 x 160 g a.s/ha	Winter cereals 1 x 120 g a.s/ha
		$PEC_{twa, 3d}$ [µg/L]	$PEC_{twa, 3d}$ [µg/L]	$PEC_{twa, 3d}$ [µg/L]
Step 3				
Flufenacet	D1 (ditch, 1st)	6.634	4.410	2.736
	D1 (stream, 1st)	4.136	2.750	1.706
	D2 (ditch, 1st)	3.759	2.423	2.269
	D2 (stream, 1st)	2.258	1.453	1.326
	D3 (ditch, 1st)	0.403	0.270	0.206
	D4 (pond, 1st)	1.244	0.812	0.417
	D4 (stream, 1st)	1.601	1.030	0.521
	D5 (pond, 1st)	1.172	0.774	0.573
	D5 (stream, 1st)	0.760	0.500	0.373
	D6 (ditch, 1st)	4.246	2.767	2.040
	R1 (pond, 1st)	0.113	0.074	0.055
	R1 (stream, 1st)	0.992	0.649	0.480
	R3 (stream, 1st)	1.536	1.002	1.760
	R4 (stream, 1st)	1.660	1.105	0.318
Step 4, 10m buffer				
Flufenacet	D1 (ditch, 1st)	6.634	4.410	2.736
	D1 (stream, 1st)	4.136	2.750	1.706
	D2 (ditch, 1st)	3.759	2.423	2.269
	D2 (stream, 1st)	2.258	1.453	1.326
	D3 (ditch, 1st)	0.058	0.039	0.029
	D4 (pond, 1st)	1.237	0.807	0.414
	D4 (stream, 1st)	1.601	1.030	0.521
	D5 (pond, 1st)	1.166	0.770	0.570
	D5 (stream, 1st)	0.760	0.500	0.373
	D6 (ditch, 1st)	4.246	2.767	2.040
	R1 (pond, 1st)	0.055	0.036	0.027
	R1 (stream, 1st)	0.444	0.290	0.215
	R3 (stream, 1st)	0.694	0.453	0.809

	R4 (stream, 1st)	0.747	0.498	0.143

Table 10.2- 7 Initial max PEC_{sw} values – FOCUS Step 4 – cereals

Compound	Buffer Width& Type; Drift reduction	FOCUS Scenario	Winter cereals 1 x 240 g a.s./ha single	Winter cereals 1 x 160 g a.s/ha	Winter cereals 1 x 120 g a.s/ha
			$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]	$PEC_{sw, max}$ [µg/L]
Flufenacet	20m SD & RO; 90%	D1, ditch	**6.762**	**4.460**	**2.767**
		D1, stream	**4.230**	**2.782**	**1.728**
		D2, ditch	**7.223**	**4.646**	**3.750**
		D2, stream	**4.517**	**2.905**	**2.343**
		D3, ditch	**0.011**	**0.008**	0.006
		D4, pond	**1.228**	0.801	0.410
		D4, stream	**1.892**	**1.228**	0.613
		D5, pond	**1.161**	0.766	0.567
		D5, stream	**1.347**	0.886	0.656
		D6, ditch, 1st	**6.021**	**3.969**	**2.950**
		R1, pond	0.016	0.011	0.009
		R2, stream	**1.482**	**0.968**	**0.716**
		R3, stream	**1.861**	**1.215**	**1.000**
		R4, stream	**1.402**	**0.928**	**0.272**

BOLD – values considered in risk assessment

Risk assessment for aquatic organisms

The risk assessment is based on the current Guidance Document on Aquatic Ecotoxicology, SANCO/3268/2001, rev 4, final, 17 October 2002. Some implications of the new aquatic guidance document (EFSA Journal 2013;11(7):3290, 268 pp. doi:10.2903/j.efsa.2013.3290), which is not yet noted, have been taken into consideration as well.

Toxicity exposure ratios (TER values) are calculated based on the most sensitive species and worst-case PEC_{SW} values.

The TER-values have been calculated based on the following equations:

$TER_A = LC_{50}$ or EC_{50} / max PEC_{SW}

TER_{LT} = chronic $NOEC_2 (E_rC_{50} / E_bC_{50})$ / PEC_{SW}

The risk is considered acceptable if the TER_A values are ≥ 100, and the TER_{LT} values ≥ 10.

ACUTE RISK ASSESSMENT FOR AQUATIC ORGANISMS

Risk assessment based on formulation endpoints

Endpoints measured for the formulation are compared with the acute mixture toxicity calculated according to the formula of Finney (Finney, GIFAP, 1990):

$$1 / LC_{50\ expected} = \sum ct_{as} / LC_{50}$$

ct_{as} = w/w fraction of active substance in %

Table 10.2- 8: Calculation of the acute mixed toxicity of the formulation according to Finney

	Measured endpoint			Calculated endpoint
	Diflufenican	**Flufenacet**	**DFF+FFA SC 600**	**DFF+FFA SC 600**
Content in the product	17.4 %	32.2 %	-	-
Algae, EC_{50}	0.00025 mg as/L	0.0144 mg/L	0.00663 mg/L	0.0014 mg/L
Aquatic plant, EC_{50}	0.039 mg as/L	0.0139 mg/L	0.307 mg/L	0.0362 mg/L

Based on Finney's formula, the maximum deviation of the expected toxicity of the formulated product from the measured toxicity is 0.0014/0.00663 and as such about a factor of 4.7 from the measured toxicity values. This variation is within the experimental variability of biological systems and below the factor of 10 used in the Aquatic Guidance Document as indication for significant differences. Moreover, the endpoints determined in studies with the formulated product are higher than the predicted values for the considered species. Thus, the risk assessment for the formulated product can be safely based on the data generated on its active substances.

Table 10.2- 9 TER_A calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	TER_A	Trigger
Winter cereals, 1 x 240 g.a.s/ha						
Flufenacet	*L. macrochirus*	LC_{50}	2130	21.80	**98**	100
	C. variegatus	LC_{50}	3310		152	
	D. magna	EC_{50}	3090		142	
	M. bahia	EC_{50}	5600		257	
FOE sulfonic acid	*O. mykiss*	LC_{50}	>86700	5.152	16828	100
	D. magna	EC_{50}	>87300		16945	
TFA	*B. rerio*	LC_{50}	>1 200 000	7.651	156842	100
	D. magna	EC_{50}	>1 200 000		156842	
FOE-thiadone	*O. mykiss*	LC_{50}	9100	0.975	9333	100
	C. variegatus	LC_{50}	15300		15692	
	D. magna	EC_{50}	31700		32513	
	M. bahia	EC_{50}	>15100		15487	

Table 10.2- 10 TER_A calculations based on FOCUS Step 3 – winter cereals

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_A	trigger
Flufenacet, winter cereals 1 x 240 g.a.s/ha					
Fish, acute *L. macrochirus*	LC_{50} 2130	6.762	D1, ditch	315	100
		4.230	D1, stream	504	
		7.223	D2, ditch	295	
		4.517	D2, stream	472	
		1.513	D3, ditch	1408	
		1.245	D4, pond	1711	
		1.892	D4, stream	1126	
		1.176	D5, pond	1811	
		1.419	D5, stream	1501	
		6.021	D6, ditch, 1*	354	
		0.116	R1, pond	18362	
		6.341	R1, stream	336	
		7.887	R3, stream	270	
		5.943	R4, stream	358	

Except for the acute risk to fish all acute TER values for the use in cereals meet the trigger based on the FOCUS Step 2 values. For fish further refinement using FOCUS Step3 values were necessary. The calculations show that for fish all TER values for the use in cereals meet the trigger based on the FOCUS Step 3 values. Therefore, no unacceptable acute risk to aquatic organisms is expected following the application of this product in cereals.

CHRONIC RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 11 TER_{LT} calculations based on FOCUS Step 2

Compound	Species	Endpoint [µg/L]	PECsw,max [µg/L]	TER_{LT}	trigger
Winter cereals, 1 x 240 g.a.s/ha					
Flufenacet	*C. variegatus*	NOEC 49	21.80	**2.2**	10
	D. magna	NOEC 3260		149	
	M. bahia	NOEC 221		10.1	
	C. riparius	NOEC 5000		229	
	S. costatum	4d-E_rC_{50} 9.49		**0.44**	
	L. gibba	E_rC_{50} 13.9		**0.64**	
FOE oxalate	*P. subcapitata*	E_rC_{50} >100000	3.967	25208	10
	L. gibba	E_rC_{50} >100000		25208	
FOE sulfonic acid	*D. subspicatus*	E_rC_{50} > 86700	5.152	16828	10
	L. gibba	EC_{50} >75900		14732	
FOE methylsulfide	*P. subcapitata*	E_rC_{50} 83800	0.167	501796	10
	L. gibba	E_rC_{50} 106000		634731	
FOE methylsulfone	*P. subcapitata*	E_rC_{50} > 10000	1.301	7686	10
	L. gibba	EC_{50} >100000		76804	
TFA	*B. rerio*	NOEC 300000	7.651	39211	10
	P. subcapitata	E_rC_{50} 160000		20912	
	L. gibba	EC_{50} 618300		80813	
	M. spicatum	EC_{50} 312900		40897	
FOE 5043-trifluoroethane sulfonic acid	*P. subcapitata*	E_rC_{50} >100000	0.600	166667	10
	L. gibba	EC_{50} >10000		16667	
Thiadone	*P. subcapitata*	E_bC_{50} 4100	0.975	4205	10
	L. gibba	EC_{50} 18300		18769	
Winter cereals, 1 x 160 g.a.s/ha					
Flufenacet	*C. variegatus*	NOEC 49	14.53	**3.4**	10
	S. costatum	4d-E_rC_{50} 9.49		**0.65**	
	L. gibba	E_rC_{50} 13.9		**0.96**	
Winter cereals, 1 x 120 g.a.s/ha					
Flufenacet	*C. variegatus*	NOEC 49	14.24	**3.4**	10
	S. costatum	4d-E_rC_{50} 9.49		**0.67**	
	L. gibba	E_rC_{50} 13.9		**0.98**	

For flufenacet the TER_{LT} for all use rates in cereals meet the trigger for aquatic invertebrates based on the FOCUS Step 2 values. Therefore, for these species an unacceptable risk is not expected following the application of flufenacet in cereals.
For fish, algae and lemna the triggers were no passed based on FOCUS Step 2 values. Therefore further refinements using FOCUS Step 3 values are necessary.

For the metabolites of flufenacet all TET_{LT} for the highest use rate in cereals meet the trigger based on the FOCUS Step 2 values. Therefore, an unacceptable risk of the metabolites to aquatic organisms is not expected following the use of flufenacet in cereals, even at the highest application rate. Hence, no TER calculations are presented here for the lower application rates.

Table 10.2- 12 TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.6 L/ha

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 240 g.a.s/ha					
C. variegatus	NOEC 49	6.762	D1, ditch	**7.2**	10
		4.230	D1, stream	11.6	
		7.223	D2, ditch	**6.8**	
		4.517	D2, stream	10.8	
		1.513	D3, ditch	32.4	
		1.245	D4, pond	39.4	
		1.892	D4, stream	25.9	
		1.176	D5, pond	41.7	
		1.419	D5, stream	34.5	
		6.021	D6, ditch, 1st	**8.1**	
		0.116	R1, pond	422	
		6.341	R1, stream	**7.7**	
		7.887	R3, stream	**6.2**	
		5.943	R4, stream	**8.2**	
S. costatum	4d-E_rC_{50} 9.49	6.762	D1, ditch	**1.4**	10
		4.230	D1, stream	**2.2**	
		7.223	D2, ditch	**1.3**	
		4.517	D2, stream	**2.1**	
		1.513	D3, ditch	**6.3**	
		1.245	D4, pond	**7.6**	
		1.892	D4, stream	**5.0**	
		1.176	D5, pond	**8.1**	
		1.419	D5, stream	**6.7**	
		6.021	D6, ditch, 1st	**1.6**	
		0.116	R1, pond	82	
		6.341	R1, stream	**1.5**	
		7.887	R3, stream	**1.2**	
		5.943	R4, stream	**1.6**	
L. gibba	E_rC_{50} 13.9	6.762	D1, ditch	**2.1**	10
		4.230	D1, stream	**3.3**	
		7.223	D2, ditch	**1.9**	
		4.517	D2, stream	**3.1**	
		1.513	D3, ditch	**9.2**	
		1.245	D4, pond	11.2	
		1.892	D4, stream	**7.3**	
		1.176	D5, pond	11.8	
		1.419	D5, stream	**9.8**	
		6.021	D6, ditch, 1st	**2.3**	
		0.116	R1, pond	120	
		6.341	R1, stream	**2.2**	
		7.887	R3, stream	**1.8**	
		5.943	R4, stream	**2.3**	

Table 10.2- 13 TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.4 L/ha

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 160 g.a.s/ha					
C. variegatus	NOEC 49	4.460	D1, ditch	11.0	10
		2.782	D1, stream	17.6	
		4.646	D2, ditch	10.5	
		2.905	D2, stream	16.9	
		1.010	D3, ditch	48.5	
		0.812	D4, pond	60.3	
		1.228	D4, stream	39.9	
		0.776	D5, pond	63.1	
		0.946	D5, stream	51.8	
		3.969	D6, ditch, 1st	12.3	
		0.077	R1, pond	636	
		4.142	R1, stream	11.8	
		5.148	R3, stream	**9.5**	
		3.936	R4, stream	12.4	
S. costatum	4d-E_rC_{50} 9.49	4.460	D1, ditch	**2.1**	10
		2.782	D1, stream	**3.4**	
		4.646	D2, ditch	**2.0**	
		2.905	D2, stream	**3.3**	
		1.010	D3, ditch	**9.4**	
		0.812	D4, pond	11.7	
		1.228	D4, stream	**7.7**	
		0.776	D5, pond	12.2	
		0.946	D5, stream	10.0	
		3.969	D6, ditch, 1st	**2.4**	
		0.077	R1, pond	123	
		4.142	R1, stream	**2.3**	
		5.148	R3, stream	**1.8**	
		3.936	R4, stream	**2.4**	
L. gibba	E_rC_{50} 13.9	4.460	D1, ditch	**3.1**	10
		2.782	D1, stream	**5.0**	
		4.646	D2, ditch	**3.0**	
		2.905	D2, stream	**4.8**	
		1.010	D3, ditch	13.8	
		0.812	D4, pond	17.1	
		1.228	D4, stream	11.3	
		0.776	D5, pond	17.9	
		0.946	D5, stream	14.7	
		3.969	D6, ditch, 1st	**3.5**	
		0.077	R1, pond	181	
		4.142	R1, stream	**3.4**	
		5.148	R3, stream	**2.7**	
		3.936	R4, stream	**3.5**	

Table 10.2- 14 TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.3 L/ha

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 120 g a.s/ha					
C. variegatus	NOEC 49	2.767	D1, ditch	17.7	10
		1.728	D1, stream	28.4	
		3.750	D2, ditch	13.1	
		2.343	D2, stream	20.9	
		0.758	D3, ditch	64.6	
		0.417	D4, pond	118	
		0.658	D4, stream	74.5	
		0.575	D5, pond	85.2	
		0.710	D5, stream	69	
		2.950	D6, ditch, 1st	16.6	
		0.057	R1, pond	860	
		3.062	R1, stream	16.0	
		4.173	R3, stream	11.7	
		1.156	R4, stream	42.4	
S. costatum	4d-E_rC_{50} 9.49	2.767	D1, ditch	**3.4**	10
		1.728	D1, stream	**5.5**	
		3.750	D2, ditch	**2.5**	
		2.343	D2, stream	**4.1**	
		0.758	D3, ditch	12.5	
		0.417	D4, pond	22.8	
		0.658	D4, stream	14.4	
		0.575	D5, pond	16.5	
		0.710	D5, stream	13.4	
		2.950	D6, ditch, 1st	**3.2**	
		0.057	R1, pond	166	
		3.062	R1, stream	**3.1**	
		4.173	R3, stream	**2.3**	
		1.156	R4, stream	**8.2**	
L. gibba	E_rC_{50} 13.9	2.767	D1, ditch	**5.0**	10
		1.728	D1, stream	**8.0**	
		3.750	D2, ditch	**3.7**	
		2.343	D2, stream	**5.9**	
		0.758	D3, ditch	18.3	
		0.417	D4, pond	33.3	
		0.658	D4, stream	21.1	
		0.575	D5, pond	24.2	
		0.710	D5, stream	19.6	
		2.950	D6, ditch, 1st	**4.7**	
		0.057	R1, pond	244	
		3.062	R1, stream	**4.5**	
		4.173	R3, stream	**3.3**	
		1.156	R4, stream	12.0	

Refined Risk Assessment

Long-term risk to fish

For the long-term risk to fish, when using the lowest of three available chronic endpoints, the trigger was not passed based on FOCUS Step 3 calculations for the highest application rate of 240 g a.s./ha and the D1, D2 and D6 ditch scenarios and the R1, R3 and R4 stream scenarios. For the lower application rate of 160 g a.s./ha, the D1, D2 and D6 ditch scenarios did not pass the trigger of 10. Therefore, a refined risk assessment based on FOCUS Step 4 calculations is presented below for those scenarios not passing based on FOCUS Step 3 calculations.

Table 10.2- 15 TER_{LT} calculations based on FOCUS Step 4 including mitigation measures– fish

Species	Endpoint [µg/L]	Buffer [m]	Drift reduction	$PEC_{sw,max}$ [µg/L]	TER_{LT}	Trigger
Flufenacet, winter cereals, 1 x 240 g/ha						
D1 ditch						
C. variegatus	NOEC 49	10	0-90%	6.762	**7.2**	10
D2 ditch						
C. variegatus	NOEC 49	10	0-90%	7.223	**6.8**	10
D6 ditch 1st						
C. variegatus	NOEC 49	10	0-90%	6.021	**8.1**	10
R1 stream						
C. variegatus	NOEC 49	10	0-90%	2.845	17.2	10
R3 stream						
C. variegatus	NOEC 49	10	0-90%	3.562	13.8	10
R4 stream						
C. variegatus	NOEC 49	10	0-90%	2.683	18.3	10
Flufenacet, winter cereals, 1 x 160 g/ha						
D1 ditch						
C. variegatus	NOEC 49	10	0-90%	4.460	11.0	10
D2 ditch						
C. variegatus	NOEC 49	10	0-90%	4.646	10.5	10
D6 ditch 1st						
C. variegatus	NOEC 49	10	0-90%	3.969	12.3	10

Flufenacet passes the risk assessment for all FOCUS scenarios with exception of the drainage scenarios D1, D2 and D6, when using the lowest of three available chronic endpoints. For these scenarios no mitigation via buffer zones (FOCUS Step 4) is possible. Thus some drainage scenarios may require refinement or risk mitigation on a national level.

Long-term risk to Algae and aquatic macrophytes

Due to the high sensitivity of green algae and aquatic plants to flufenacet, a microcosm study has been conducted over 84 days involving phytoplankton, zooplankton, periphyton, aquatic macrophytes and macrofauna. The study resulted only in minor adverse trends in the highest test concentration. No statistical significant differences compared to the controls were evaluated for any of the investigated

endpoints. An evaluation of this complete and more relevant study is presented in KCA 8.2.8/04 and defines a NOEC (No Observed Effect Concentration) of 12 µg a.s./L.
The relevance of the results of this microcosm study is supported by an expert statement ([redacted], 2009, M-329959-01-1, see ref: KCA 8.2.8/04). In the statement it was concluded: "No adverse long term effect on the investigated biocoenosis was observed and could be expected in the environment based on the outcome of this microcosm study. Due to the fact that several phytoplanktonic algae species, periphyton and three aquatic macrophytes have been investigated, the study was suitable to investigate potential direct adverse effects on aquatic plants. The testing of a biocoenosis enables the use of this study as well for the determination of indirect effects on zooplankton and/or the macrofauna.
The highest test concentration of 24 µg/L showed only minor, non-significant, differences compared to the control and can be seen as EAC."
This EAC value is to be considered as more relevant and representative to the actual sensitivity of algae and macrophytes to flufenacet. However, as a conservative approach the derived NOEC of 12 µg/L is used for the refined TER calculation. The obtained TER is compared to a trigger value of 5. A refined trigger value is considered to be justified, as the endpoint of the microcosm study is a NOEC and not an E_rC_{50}, and the study as such is higher tier than a standard laboratory study.

Therefore in a first refinement step the NOEC of 12 µg a.s./L from the microcosm study ([redacted] & [redacted] 1999) in combination with an assessment factor of 5 is used for the risk assessment for algae and aquatic plants. For further refinement of peak exposure in stream scenarios, for macrophytes the Lemna peak exposure study is used and for algae a 3d PEC_{twa} is used against the lowest algal endpoint (*S. costatum*, marine diatom). Because only Run-off scenarios showed significant differences between PEC_{max} and PEC_{twa}, only for these scenarios (R1-R4 stream) the PEC_{twa} approach was applied.

Table 10.2- 16: TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.6 L/ha

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 240 g a.s/ha					
algae and aquatic plants	NOEC 12.0	6.762	D1, ditch	**1.8**	5
		4.230	D1, stream	**2.8**	
		7.223	D2, ditch	**1.7**	
		4.517	D2, stream	**2.7**	
		1.513	D3, ditch	7.9	
		1.245	D4, pond	9.6	
		1.892	D4, stream	6.3	
		1.176	D5, pond	10.2	
		1.419	D5, stream	8.5	
		6.021	D6, ditch, 1st	**2.0**	
		0.116	R1, pond	103	
		6.341	R1, stream	**1.9**	
		7.887	R3, stream	**1.5**	
		5.943	R4, stream	**2.0**	

Table 10.2- 17 TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.4 L/ha

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 160 g.a.s/ha					
algae and aquatic plants	NOEC 12.0	4.460	D1, ditch	**2.7**	5
		2.782	D1, stream	**4.3**	
		4.646	D2, ditch	**2.6**	
		2.905	D2, stream	**4.1**	
		1.010	D3, ditch	11.9	
		0.812	D4, pond	14.8	
		1.228	D4, stream	9.8	
		0.776	D5, pond	15.5	
		0.946	D5, stream	12.7	
		3.969	D6, ditch, 1st	**3.0**	
		0.077	R1, pond	156	
		4.142	R1, stream	**2.9**	
		5.148	R3, stream	**2.3**	
		3.936	R4, stream	**3.0**	

Table 10.2- 18 TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.3 L/ha

Species	Endpoint [µg/L]	$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 120 g a.s/ha					
algae and aquatic plants	NOEC 12.0	2.767	D1, ditch	**4.3**	5
		1.728	D1, stream	6.9	
		3.750	D2, ditch	**3.2**	
		2.343	D2, stream	5.1	
		0.758	D3, ditch	15.8	
		0.417	D4, pond	28.8	
		0.658	D4, stream	18.2	
		0.575	D5, pond	20.9	
		0.710	D5, stream	16.9	
		2.950	D6, ditch, 1st	**4.1**	
		0.057	R1, pond	211	
		3.062	R1, stream	**3.9**	
		4.173	R3, stream	**2.9**	
		1.156	R4, stream	10.4	

Refined risk assessment for algae

For further refinement of peak exposure in stream scenarios for algae a 3d PEC_{twa} is used against the lowest algal endpoint (*S. costatum*, marine diatom). This is justified because the algal flow-through experiment and recovery studies have shown FFA to be algistatic (not algicidal) and thus fast recovery is possible and because exposure was maintained in algal toxicity tests.
Because only run-off scenarios showed significant differences between PEC_{max} and PEC_{twa}, only for these scenarios (R1-R4 stream) the PEC_{twa} approach was applied.

Table 10.2- 19 TER_{LT} calculations based on FOCUS Step 3 – cereals

Species	Endpoint [µg/L]	$PEC_{twa, 3d}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 240 g.a.s/ha					
S. costatum	4d-E_rC_{50} 9.49	0.993	R1, stream	**9.6**	10
		1.536	R3, stream	**6.2**	
		1.660	R4, stream	**5.7**	
Flufenacet, winter cereals, 1 x 160 g.a.s/ha					
S. costatum	4d-E_rC_{50} 9.49	0.649	R1, stream	14.6	10
		1.002	R3, stream	**9.5**	
		1.105	R4, stream	**8.6**	
Flufenacet, winter cereals, 1 x 120 g a.s/ha					
S. costatum	4d-E_rC_{50} 9.49	0.480	R1, stream	19.8	10
		1.760	R3, stream	**5.4**	
		0.318	R4, stream	29.8	

For those scenarios that did not pass based on FOCUS Step 3 calculation, a further refined risk assessment based on FOCUS Step 4 calculations is presented below.

Table 10.2- 20 TER_{LT} calculations based on FOCUS Step 4, including a 10m buffer zone – cereals

Species	Endpoint [µg/L]	$PEC_{twa, 3d}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 240 g.a.s/ha					
S. costatum	4d-E_rC_{50} 9.49	0.993	R1, stream	21.4	10
		1.536	R3, stream	13.7	
		1.660	R4, stream	12.7	
Flufenacet, winter cereals, 1 x 160 g.a.s/ha					
S. costatum	4d-E_rC_{50} 9.49	0.453	R3, stream	21.0	10
		0.498	R4, stream	19.0	
Flufenacet, winter cereals, 1 x 120 g a.s/ha					
S. costatum	4d-E_rC_{50} 9.49	0.809	R3, stream	11.7	10

Table 10.2- 21 Overview of the outcome of the chronic risk assessments for algae

	0.6 L/ha (240 g flufenat/ha)				0.4 L/ha (160 g flufenat/ha)				0.3 L/ha (120 g flufenat/ha)			
	tier 1 RA	micro cosm	3d-twa	3d-twa+ 10m buffer	tier 1 RA	micro cosm	3d-twa	3d-twa+ 10m buffer	tier 1 RA	micro cosm	3d-twa	3d-twa+ 10m buffer
D1, ditch												
D1, stream										✔		
D2, ditch												
D2, stream												
D3, ditch		✔				✔			✔	✔		
D4, pond		✔			✔	✔			✔	✔		
D4, stream		✔				✔			✔	✔		
D5, pond		✔			✔	✔			✔	✔		
D5, stream		✔			✔	✔			✔	✔		
D6, ditch, 1st												
R1, pond	✔	✔			✔	✔			✔	✔		
R1, stream				✔			✔	✔			✔	✔
R3, stream				✔				✔				✔
R4, stream				✔				✔	✔	✔	✔	✔

Flufenacet passes the risk assessment for all FOCUS-scenarios with exception of the drainage scenarios D1, D2 and D6. For these scenarios no mitigation via buffer zones (FOCUS Step 4) is possible. Thus some drainage scenarios may require refinement or risk mitigation on a national level.

Refined risk assessment for run-off stream scenarios with short-term peak exposure for macrophytes

No inhibition >50% was observed at any treatment level up to 126 µg a.s./L in the peak exposure study with Lemna ([redacted] (2013), M-452567-01-1). Therefore, a peak EC_{50} of >126 µg a.s./L can be derived from this study. In those cases where the drainage peak in the FOCUS scenario was equal or shorter than the peak exposure considered in the study, the endpoint will be used for refinement.
The reasoning for the use of such studies with variable exposure is based on SETAC Europe workshop ELINK[1] . The study was performed based on the ELINK document. The peak EC_{50} is compared with peak concentrations in combination with standard assessment factor of 10.

[1] [redacted]CM, [redacted]A, [redacted]CD, [redacted]E, [redacted]BFF, [redacted] F, [redacted]CM, [redacted] R and [redacted]M (Eds), 2010a. Linking aquatic exposure and effects: risk assessment of pesticides. SETAC Press & CRC Press, Taylor & Francis Group, Boca Raton, FL, USA, 398 pp

Table 10.2- 22 TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.6 L/ha

Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 240 g.a.s/ha						
Lemna	peak E_rC50	>126	6.341	R1, stream	19.9	10
			7.887	R3, stream	16.0	
			5.943	R4, stream	21.2	

Table 10.2- 23 TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.4 L/ha

Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 160 g.a.s/ha						
Lemna	peak E_rC50	>126	4.142	R1, stream	30.4	10
			5.148	R3, stream	24.5	
			3.936	R4, stream	32.0	

Table 10.2- 24 TER_{LT} calculations based on FOCUS Step 3 – cereals – 0.3 L/ha

Species	Endpoint [µg/L]		$PEC_{sw,max}$ [µg/L]	FOCUS scenario	TER_{LT}	trigger
Flufenacet, winter cereals, 1 x 120 g a.s/ha						
Lemna	peak E_rC50	>126	3.062	R1, stream	41.1	10
			4.133	R3, stream	30.2	
			1.156	R4, stream	109.0	

Table 10.2- 25 Overview of the outcome of the chronic risk assessments for aquatic plants

	0.6 L/ha (240 g flufenat/ha)			0.4 L/ha (160 g flufenat/ha)			0.3 L/ha (120 g flufenat/ha)		
	tier 1 RA	microcosm	peak	tier 1 RA	microcosm	peak	tier 1 RA	microcosm	peak
D1, ditch									
D1, stream								✔	
D2, ditch									
D2, stream									
D3, ditch		✔		✔	✔		✔	✔	
D4, pond	✔	✔		✔	✔		✔	✔	
D4, stream		✔		✔	✔		✔	✔	
D5, pond	✔	✔		✔	✔		✔	✔	
D5, stream		✔		✔	✔		✔	✔	
D6, ditch, 1st									
R1, pond	✔	✔		✔	✔		✔	✔	
R1, stream			✔			✔			✔
R3, stream			✔			✔			✔
R4, stream			✔			✔	✔	✔	✔

Flufenacet passes the risk assessment without mitigations for all FOCUS scenarios with exception of the drainage scenarios D1, D2 and D6. For these scenarios no mitigation via buffer zones (FOCUS Step 4) is possible. Thus some drainage scenarios may require refinement or risk mitigation on a national level.

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Report: **CP 10.2.1/01, [redacted] H, [redacted] H. (2001)**
Title: FOE 5043 & diflufenican SC 600 - Influence on the growth of the green alga, Selenastrum capricornutum
Document N°: M-073137-01-1
Guidelines: Directive 92/69/EEC, C.3 (1992), OECD 201, ISO 8692, ASTM E 1218
GLP: yes (certified laboratory)

Dates of work: Start of experimental work: March 23, 2001
Completion of experimental work: July 11, 2001

Material and methods:
FOE 5043 & Diflufenican SC 600, an SC formulation of Flufenacet (401.5 g/L) and Diflufenican (217.0 g/L), Formulation-No.: 07205/0024(0006), Article-No.: 3000248463, TOX-No.: 5454-00;
Selenastrum capricornutum was exposed under static conditions (shake cultures) for 72 h. Algal growth in the controls was exponential over the entire test period. The following concentrations of nominal: 0.938, 1.88, 3.75, 7.5, 15 and 30 µg test item/L were tested. The

quantities of FOE 5043 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 at the end (day 3) were 62 to 99 % (average 84 %). The calculations are based on nominal concentrations of the test item.
The pH values ranged from 7.81 to 8.13 at test start and 8.10 to 8.71 after 72 h. The incubator was illuminated with 6888 lux. The incubation temperature ranged from 21.5 °C to 23.8 °C measured over the whole period of testing.
Samples were analyzed for the actual concentrations of FOE 5043 only, present in the test medium on day 0 and day 3.

Findings and Observations:

The quantities of FOE 5043 found at the beginning of the test 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. This could have been a handling mistake which did not influence the results, because the E_rC_{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 %).

Effects on algal average growth rate (based on nominal concentrations of the formulation):

Test item	FOE 5043 & Diflufenican SC 600
Test object	*Selenastrum capricornutum*
Exposure	72 h, static
E_rC_{50} (0 - 72 h)	**6.63 µg/L**
LOE_rC (0 - 72 h)	0.938 µg/L
NOE_rC (0 - 72 h)	< 0.938 µg/L

Conclusion:
The E_rC_{50} for the formulation Flufenacet + Diflufenican SC 600 was determined to be 6.63 µg /L.

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Report: **CP 10.2.1/02, [redacted], M., [redacted], H., 2001**
Title: FOE 5043 & Diflufenican SC 600 - Toxicity (7 days) to *Lemna gibba* G3 in a Static Test
Document No.: M-073160-01-1
Guidelines: OECD 221 "Lemna sp. Growth Inhibition Test", Revised Draft Document (October 2000)
GLP: yes (certified laboratory)

Objectives:

The objective of the study was to estimate the toxicity of FOE 5043 & Diflufenican SC 600 to *Lemna gibba* G3 in a 7 day toxicity test under static conditions. The results are expressed as NOEC, LOEC and EC_x for growth rate of the response variables, frond number and total frond area of plants.

Materials and methods:

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), Development-No.: 3000248463, TOX-No.: 5454-01;

Lemna gibba G3 (duckweed), 3 x 12 fronds per test concentration were exposed in a chronic multigeneration test for 7 days under static test conditions to nominal concentrations of 10.0, 20.0, 40.0, 80.0, 160, 320 and 640 µg test item/L in comparison to control.

The pH values ranged from 4.87 to 6.18 and the incubation temperature ranged from 23.6 °C to 26.6 °C measured over the whole period of testing.

Samples were analyzed for the actual concentrations of FOE 5043 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.

Results:

Test conditions met all validity criteria, given by the mentioned guideline.

83 (81-86) fronds were reached after a 7-day cultivation in the controls, corresponding to approximately an 7-fold increase in fronds (biomass) within 7 days (initial frond number: 12) or corresponding to a doubling time (T_d) of 2.5 days, respectively.

Based on analytical findings of FOE 5043 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. This could be due to the adsorption to glass or plants. All results are based on nominal.

The static 7 day growth inhibition test provided the following tabulated effects:

Nominal test levels	Final frond number	Dry weight	% inhibition[1] of average growth rate of	
FOE 5043 & DFF SC 600 [µg/L]	**mean day 7**	**mean day 7 (g)**	**frond numbers**	**Dry weight**
control	83	0.00868	--	
10.0	92	0.0107	-5.1	-28.0
20.0	80	0.0084	2.2	3.2
40.0	72	0.0075	7.6*	14.9
80.0	37	0.0057	41.6*	39.7*
160	28	0.0052	55.8*	46.9*
320	27	0.0043	58.2*	58.2*
640	27	0.0047	28.8*	53.7*

[1]negative values mean growth stimulation
* Results which were significantly different (based on Dunnett´s and Williams α = 0.05) from the control(s)

Observed visual effects:

Test level (µg/L FOE 5043 & DFF SC 600)	Observations
Control	no visual effects observed
10.0	no visual effects observed
20.0	no visual effects observed
40.0	Slight chlorosis on day 5+7
80.0	Slight chlorosis on day 2-7
160	Slight chlorosis on day 2 Middle to strong chlorosis on day 5+7
320	Slight-middle chlorosis on day 2 Middle to strong chlorosis on day 5+7
640	Slight-middle chlorosis on day 2 Middle to strong chlorosis on day 5+7

Results are based on nominal concentrations of FOE 5043 & Diflufenican SC 600

Test item	**FOE 5043 & Diflufenican SC 600**
Test object	*Lemna gibba* G3
Exposure	7 d, static
(0 - 7day)-E_rC_{50} (fronds counts)	**307 µg/L**
(0 - 7day)-LOE_rC (fronds counts)	40.0 µg/L
(0 - 7day)-NOE_rC (fronds counts)	20.0 µg/L

Conclusion: The most sensitive response variable was total frond number of plants resulting in (0-7-day)-E_rC_{50} of 307 µg/L FOE 5043 & Diflufenican SC 600 and a lowest (0-7-day)-NOE_rC of 40.0 µg test item/L.

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

No additional studies were considered necessary with the formulation.

CP 10.2.3 Further testing on aquatic organisms

No additional studies were considered necessary with the formulation.

CP 10.3 Effects on arthropods

Only endpoints used for the risk assessment are presented here. For an overview of all available endpoints available for flufenacet please refer to the respective section of the MCA document.

CP 10.3.1 Effects on bees

The summary of the toxicity profile of the active substances flufenacet and diflufenican and the representative formulation Diflufenican + Flufenacet SC 600 (200+400) G to bees is provided in the

following tables.

For the second active substance in the representative formulation, diflufenican, references is made to the EU agreed endpoints according to the EFSA Scientific Report (2007) 122.

Table 10.3.1- 1 Endpoints of the mixing partner Diflufenican

Test substance	Test species	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84	
Diflufenican	Bee (oral 48 h)	$LD_{50\ (oral)}$	> 112.3 µg as/bee
	Bee (contact 48 h)	$LD_{50\ (contact)}$	> 100 µg as/bee

Table 10.3.1- 2 Honey bee toxicity data generated with technical flufenacet

Test substance	Ecotoxicological endpoint		Reference
Acute oral and contact toxicity (laboratory) in honey bees			
Flufenacet, tech.	LD_{50}-oral, 48 h LD_{50}-contact, 48 h	**> 109.2 µg a.s./bee** **> 100 µg a.s./bee**	[redacted] (2011) M-421687-01-1 KCA 8.3.1.1.1/03
Acute contact toxicity (laboratory) in bumble bees			
Flufenacet, tech.	LD_{50}-contact, 48 h	**> 100 µg a.s./bee**	[redacted] (2014) M-478561-01-1 KCA 8.3.1.1.2/05
Chronic toxicity in adult honey bees (laboratory)			
Flufenacet, tech.	10 d chronic adult feeding study	**LC_{50} > 120 mg a.s./kg** **NOEC ≥ 120 mg a.s./kg**	[redacted] (2014) M-477339-01-1 KCA 8.3.1.2/01

Bold values: Endpoints considered relevant for HQ calculation

Table 10.3.1- 3 Honey bee toxicity data generated with formulated flufenacet

Test substance	Ecotoxicological endpoint		Reference
Acute oral and contact toxicity (laboratory) in honey bees			
Diflufenican + Flufenacet SC 600 (200+400)	48 h-LD_{50}-oral 48 h-LD_{50}-contact	**> 217.87 µg product/bee** **> 200 µg product/bee**	[redacted] & [redacted] (2009) M-356881-01-1 CP 10.3.1.1/01
Bee brood feeding test			
Flufenacet SC 508.8	Honey bee brood feeding (Oomen *et al.*, 1992)	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)	[redacted]-[redacted] (2012) M-456504-01-1 KCA 8.3.1.3/01

Bold values: Endpoints considered relevant for HQ calculation

Risk assessment for bees

Hazard Quotients

An indication of hazard (Hazard Quotient or Q_H) can be derived according to the EPPO risk assessment scheme, by calculating the ratio between the application rate (expressed in g a.s./ha or in g peoduct/ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./bee or in µg product/bee).

Q_H values can be calculated using data from the studies performed with the active substance and with the formulation. Q_H values higher than 50 indicate the need of higher tiered activities to clarify the actual risk to honey bees.

Hazard Quotient, oral: $$Q_{HO} = \frac{\text{maximum application rate}}{LD_{50}\ \text{oral}} = \frac{[\text{g a.s./ha or g product/ha}]}{[\mu\text{g a.s./bee or } \mu\text{g product/bee}]}$$

Hazard Quotient, contact: $$Q_{HC} = \frac{\text{maximum application rate}}{LD_{50}\ \text{contact}} = \frac{[\text{g a.s./ha or g product/ha}]}{[\mu\text{g a.s./bee or } \mu\text{g product/bee}]}$$

The maximum label rate of Diflufenican + Flufenacet SC 600 (200+400) is 0.6 L (600 mL) product/ha in cereals (BBCH 13 - 22). With the content of diflufenican and flufenacet within the formulation being 200 g diflufenican/L and 400 g flufenacet/L, respectively, this accounts to a maximum application rate of 240 g flufenacet a.s./ha. Considering a realistic worst case density of Diflufenican + Flufenacet SC 600 of 1.26 g/mL, 600 mL product/ha corresponds to 760 g product/ha.

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Table 10.3.1- 4 Hazard quotients for bees – oral exposure

Test item	Oral LD_{50} [µg a.s./bee] / *[µg product/bee]*	Max. application rate [g a.s./ha] / *[g product/ha]*	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Max. application rate = 240 g flufenacet a.s. / ha via 0.6 L Diflufenican + Flufenacet SC 600 / ha, which corresponds to 760 g Diflufenican + Flufenacet SC 600 / ha					
Flufenacet, tech.	> 109.2	240	< 2.2	50	yes
Diflufenican + Flufenacet SC 600 (200+400)	>217.87	760	< 3.5	50	yes

The hazard quotient for oral exposure is below the validated trigger value for higher tier testing (i.e. $Q_{HO} < 50$).

Table 10.3.1- 5 Hazard quotients for bees – contact exposure

Test item	Oral LD_{50} [µg a.s./bee] / *[µg product/bee]*	Max. application rate [g a.s./ha] / *[g product/ha]*	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Max. application rate = 240 g flufenacet a.s. / ha via 0.6 L Diflufenican + Flufenacet SC 600 / ha, which corresponds to 760 g Diflufenican + Flufenacet SC 600 / ha					
Flufenacet, tech.	> 100	240	< 2.4	50	yes
Diflufenican + Flufenacet SC 600 (200+400)	>200	760	<3.8	50	yes

The hazard quotient for contact exposure is below the validated trigger value for higher tier testing (i.e. $Q_{HC} < 50$).

Further considerations for the risk assessment

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, flufenacet was subjected to chronic laboratory testing with adult honey bees. This chronic study was designed as a limit test by exposing adult honey bees for 10 consecutive days to a concentration of nominally 120 mg flufenacet a.s./kg in aqueous sugar solution. As flufenacet is only slightly soluble in water (53 - 56 mg/L at 20 °C at pH 4-9), the test was conducted by using technical flufenacet in a combination with 3% acetone in the respective feeding solutions, as flufenacet is highly soluble in acetone and because acetone is of low toxicity to honey bees. The nominal test

concentration as such equals about 2× the water solubility of flufenacet. No adverse lethal-, sub-lethal, behavioural or delayed effects were found by exposing adult honey bees for ten consecutive days exclusively to sugar solution, containing 120 ppm flufenacet (nominal).

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)), which require, amongst other parameters to "*…use formulated products only… products are fed at a concentration recommended for high-volume use…"*. The honey bee brood feeding test is a worst-case screening test, by feeding the honey bees directly in the hive with a treated sugar solution which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration) and by investigating the development of eggs, young and old larvae by employing digital photo imaging technology.

This particular study was conducted by mixing formulated flufenacet via Flufenacet SC 508.8 into 1 litre of aqueous sugar solution, and the tested concentration corresponded to a typical concentration of flufenacet via Diflufenican + Flufenacet SC 600 (200+400) present in the spray tank. The actual test concentration of flufenacet was 1500 mg/L. The administration of 1 litre sugar solution per colony, containing 1500 ppm flufenacet has not resulted in adverse effects. There were neither adverse acute or chronic effects on adult honey bees nor adverse effects on immature honey bee life stages (eggs, young larvae, old larvae, pupae) or on the colony itself. Neither mortality of worker bees and larvae/pupae (as assessed via dead bee traps) nor the termination rate of eggs, young larvae and old larvae (as assessed via digital imaging of individual marked cells) was statistically significantly different from the untreated control.

Conclusion

Flufenacet has a low acute toxicity to honey bees, with LD_{50} (oral and contact) values always above the highest tested dose levels (oral: LD_{50} > 109.2 µg a.s./bee, contact: LD_{50} > 100 µg a.s./bee).
The calculated Hazard Quotients for both, flufenacet and Diflufenican + Flufenacet SC 600 (200+400) are well below the validated trigger value which would indicate the need for a refined risk assessment; no adverse effects on honey bee mortality are to be expected. This conclusion is confirmed by the results of the bee brood feeding study.
The acute laboratory study conducted with bumble bees revealed no sensitivity differences between honey bee and bumble bee foragers.
Regarding potential side effects of flufenacet on immature honey bee life stages as well as on colony development, 1500 ppm flufenacet, a concentration which corresponds to/exceeds a typical concentration of flufenacet via Diflufenican + Flufenacet SC 600 (200+400) present in the spray tank, has not resulted in adverse/statistical significant effects on mortality of worker bees and pupae nor in adverse/statistically significant effects on the termination rate of eggs, young larvae and old larvae (as assessed via digital imaging of individually marked cells) in the bee brood feeding study on colony level. Even at this very high concentration under the worst case conditions of the honey bee brood feeding test, no adverse effects on immature honey bee life stages were found; the findings in this study regarding the absence of chronic/delayed effects on adults honey bees are in line with the absence of adverse chronic effects on adult bees in the chronic 10 day laboratory feeding test with adult honey bees under laboratory conditions (at 120 ppm).

Overall, it can be concluded that flufenacet, when applied at the maximum application rate of 240 g a.s./ha in cereals, even during the flowering period of potentially bee-attractive weeds inside the cropping are, does not pose an unacceptable risk to honey bees and honey bee colonies.

CP 10.3.1.1 Acute toxicity to bees

Report:	**CP 10.3.1.1/01, [redacted], S., [redacted], T., 2009**
Title:	Effects of diflufenican + flufenacet SC 600 (200+400) G (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the Laboratory
Document N°:	M-356881-01-1
Guidelines:	OECD 213: OECD Guideline for the Testing of Chemicals on Honeybee, Acute Oral Toxicity Test, (adopted 21st September 1998) OECD 214: OECD Guideline for the Testing of Chemicals on Honeybee, Acute Contact Toxicity Test, (adopted 21st September 1998)
GLP	yes (certified laboratory)

Objective:

Honey bees (*A. mellifera*) can be affected by pesticide residues as a result of indirect contact on plant surfaces, via oral intake of contaminated food or water, via inhalation of vapour or by direct overspray in the course of an application in the field according to normal agricultural practice. If the proposed use pattern of Diflufenican + Flufenacet SC 600 (200+400) G indicates such a possible exposure of honey bees, acute contact and oral toxicity data is necessary for the registration of the pesticide use in question. This study provides:

- the acute toxicity levels of the test item to honey bees;
- toxicity information comparable to expected residues from standard rates, for assessment of the potential hazard to honey bees;
- information to support precautionary label statements;
- information to indicate the need for further testing e.g. semi-field or field studies.

Material and methods:

Test item: 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 according to certificate of analysis), Specification No,: 102000007948, Batch ID.: EV56001418, density 1.229 g/mL.

Reference item: Dimethoate. Test organism: Honey bee (*Apis mellifera* L.), female worker bees, obtained from a healthy and queen-right colony, bred by IBACON, collected on the morning of use.

Under laboratory conditions *Apis mellifera* (50 worker bees per dose; 10 individuals in 5 replicates per test item dose level, controls and reference item doses) were exposed for 48 hours for topical application (contact) with a single dose of 200.0 µg product per bee and to a single dose of 217.8 µg product per bee for feeding (oral value based on the actual intake of the test item).

<u>Oral toxicity study</u>

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 offered to the bees was 50 %. For the control water and sugar syrup was used at the same ratio (1 + 1). The treated food was offered in syringes,

which were weighed before and after introduction into the cages (duration of uptake was 1.0 hour for the test item treatments). After a maximum of 1.0 hour, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food. The target dose levels (e.g. 200.0 µg product/bee nominal) would have been obtained if 20 mg/bee of the treated food was ingested. In practice, higher dose levels were obtained as the bees had a higher or lower uptake of the test solutions than the nominal 20 mg/bee.
The measured dose level was 217.8 µg product/bee. The test was conducted in darkness, temperature was 25°C and humidity between 42 and 76%. Biological observations including mortality and behavioural changes were recorded at 4, 24 and 48 hours after dosing. Results are based on measured concentrations of the product per bee.

Contact toxicity study
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). A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item. The test was conducted in darkness, temperature was 25°C and humidity between 42 and 76%. Biological observations, including mortality and behavioural changes were recorded at 4, 24 and 48 hours after application. Results are based on nominal concentrations of the product per bee.

Findings:
The results can be considered as valid, as all validity criteria of the test were met: control mortality is 0% in the oral and 0% in the contact test, LD_{50} (24 h) of the toxic standard in the oral test equals 0.10 µg/bee, the LD_{50} (24 h) of the toxic standard in the contact test equals 0.16 µg/bee.
A summary of effects of the test item on mortality and behavioural abnormalities of the bees is given below for both tests:

Mortality and behavioural abnormalities of the bees in the oral toxicity test

consumed dosage	after 4 hours		after 24 hours		after 48 hours	
	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %
test item [µg prod./bee] 217.8	0.0	0.0	0.0	0.0	0.0	0.0
water control	0.0	0.0	0.0	0.0	0.0	0.0
reference item [µg a.s./bee]						
0.33	90.0	10.0	98.0	2.0	100.0	0.0
0.16	24.0	62.0	96.0	0.0	96.0	0.0
0.08	4.0	4.0	48.0	0.0	60.0	0.0
0.06	0.0	0.0	8.0	0.0	8.0	0.0

results are averages from five replicates (ten bees each) per dosage / control

Mortality and behavioural abnormalities of the bees in the contact toxicity test

dosage	after 4 hours		after 24 hours		after 48 hours	
	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %	mortality mean %	behavioural abnormalities mean %
test item [µg prod./bee] 200.0	0.0	0.0	0.0	0.0	2.0	0.0
water control	0.0	0.0	0.0	0.0	0.0	0.0
reference item [µg 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

results are averages from five replicates (ten bees each) per dosage / control

Observations:

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 occurred in the control (water + 0.5 % Adhasit).

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. This dose level led to no mortality after 48 hours. No mortality occurred in the control (50 % sugar solution). No test item induced behavioural effects were observed at any time.

Conclusion:

Toxicity to Honey Bees, laboratory tests

Test Item	Diflufenican + Flufenacet SC 600 (200+400) G	
Test object	*Apis mellifera*	
Application rate (µg product/bee)	217.8	200.0
Exposure	oral (sugar solution)	contact (solution in Adhäsit (0.5 %)/water)
LD_{50} µg product/bee	> 217.8	> 200.0

The toxicity of Diflufenican + Flufenacet SC 600 (200+400) G was tested in both an acute contact and an oral toxicity test on honey bees.

The LD_{50} (48 h) value was > 217.8 µg product/bee in the oral toxicity test.
The LD_{50} (48 h) value was > 200.0 µg product/bee in the contact toxicity test.

CP 10.3.1.1.1 Acute oral toxicity to bees

For details on the study please refer to the MCA Section 10.3.1.1/01.

CP 10.3.1.1.2 Acute contact toxicity to bees

For details on the study please refer to the MCA Section 10.3.1.1/01.

CP 10.3.1.2 Chronic toxicity to bees

A 10 day chronic oral toxicity study was conducted with technical flufenacet, the corresponding summary is filed under KCA, point 8.3.1.2/01.

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A honey bee brood feeding study (Oomen *et al.*) has been conducted with an SC 508.8 straight formulation and is included in the MCA document (see MCA 8.3.1.3/01).

CP 10.3.1.4 Sub-lethal effects

There is no particular study design / test guideline to assess "sub-lethal effects" in honey bees. However, in each laboratory study as well as in any higher-tier study, sub-lethal effects, if occurring, are described and reported.

CP 10.3.1.5 Cage and tunnel tests

Not necessary when considering the outcome of the risk assessment provided above and the results of the lower-tiered studies.

CP 10.3.1.6 Field tests with honeybees

Not necessary when considering the outcome of the risk assessment provided above and the results of the lower-tiered studies.

CP 10.3.2 Effects on non-target arthropods other than bees

The risk assessment was performed according to Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002) and to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, [redacted] et al. 2000[2]).

In the first Annex I listing process non-target arthropod data for a different formulation of flufenacet were submitted and evaluated. The formulation FFA WG60 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 will be presented with this dossier. For the Annex I listing process of diflufenican also the formulation Flufenacet + Diflufenican SC 600 (DFF+FFA SC600, 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.

[2] Candolfi et al.: Guidance document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods; ESCORT 2 workshop (European Standard Characteristics Of Non-Target Arthropod Regulatory Testing), Wageningen, NL, March 21-23, 2000, SETAC Europe; SETAC publication August 2001

Table 10.3.2- 1 Flufenacet + Diflufenican SC 600: Ecotoxicological endpoints for arthropods other than bees

Test species, references	Tested Formulation, study type, exposure	Ecotoxicological endpoint
Typhlodromus pyri M-058604-01-1 Rep.No.: 9352063 [redacted], A.; 2001 KCP 10.3.2.1/01	DFF+FFA SC 600 Laboratory, glass plates 22.5 mL prod./ha 45 mL prod./ha 90 mL prod./ha 180 mL prod./ha 360 mL prod./ha	LR_{50} 81.8 mL prod./ha Corr. Mortality [%] / Effect on Reproduction [%] 1.9 / 1.3 9.2 / -12.5[A] 61.1 / n.a. 92.6 / n.a. 100 / n.a.
Typhlodromus pyri M-034242-01-1 Rep.No.: 01TYBYL12 [redacted], M.P.; 2002 KCP 10.3.2.2/01	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	LR_{50} 110.2 mL prod./ha ER_{50} >83.2 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.
Typhlodromus pyri M-355238-01-1 Rep.Nr.: CW09/026 [redacted], D.; 2009 KCP 10.3.2.2/04	DFF+FFA SC 600 Aged residues, spray deposits on maize plants, 1 appl. of 0.7 L prod./ha Residues aged for 0 days: Residues aged for 14 days: Residues aged for 28 days:	Corr. Mortality [%] / Effect on Reproduction [%] 98.9 / n.a. 87.1 / n.a. 9.5 / 8.4
Aphidius rhopalosiphi M-058618-01-1 Rep.No.: 9351001 [redacted], M.; [redacted], R.; 2001 KCP 10.3.2.1 /02	DFF+FFA SC 600 Laboratory, glass plates 500 mL prod./ha 600 mL prod./ha 700 mL prod./ha	LR_{50} > 700 mL prod./ha ER_{50} > 700 mL prod./ha Corr. Mortality [%] / Effect on Reproduction [%] 0 / 9.0 2.0 / 14.0 2.0 / 3.5
Chrysoperla carnea M-352372-01-1 Rep.No.: CW09/010 [redacted], J.; 2009 KCP 10.3.2.2/02	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	LR_{50} > 600 mL prod./ha No effect on reproduction 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
Aleochara bilineata M-353760-01-1 Rep.No.: 09 10 48 027 A [redacted], U.; 2009 KCP 10.3.2.2/03	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	ER_{50} > 600 mL prod./ha Effect on Reproduction [%] 4.3 -2.3[A] 1.7 5.8 7.9

[A]: A negative value indicates a higher reproduction rate in the treatment than in the control.
n.a.: not assessed

RISK ASSESSMENT FOR OTHER NON-TARGET ARTHROPODS

Potential exposure

The product DFF + FFA SC 600 is intended to be used as a foliar spray (BBCH 10-13) on cereals, with a maximum application rate of 0.6 L product/ha corresponding to 0.12 kg as/ha Diflufenican and 0.24 kg as/ha flufenacet, a maximum of 1 application.

In-field risk assessment for other non-target arthropods

The following equation was used to calculate the hazard quotient (HQ) for the **in-field scenario**.

In field-HQ = max. single application rate * MAF / LR_{50}

The risk is considered acceptable if the calculated HQ is < 2.

The product is intended to be applied once with an application rate of 600 mL/ha. Therefore, the multiple application factor (MAF) was set to 1.

Table 10.3.2- 2 HQ for terrestrial non-target arthropods for the in-field scenario

Crop	Species	Appl. rate [mL/ha]	MAF	LR_{50} [mL/ha]	HQ	Trigger
Cereals	*T. pyri*	600	1	81.8	7.33	2
	A. rhopalosiphi			> 700	0.86	2

The in-field HQ for *A. rhopalosiphi* (HQ = 0.86) indicates an acceptable risk, for *T. pyri* (HQ = 7.33) the HQ indicates the need for a refined in-field risk assessment.

Off-field hazard quotient (HQ) tier 1 risk assessment

The following equation was used to calculate the hazard quotient (Q_H) for the off-field scenario:

Off-field HQ = maximum single application rate * MAF * (drift factor/VDF)*correction factor / LR_{50}

MAF = multiple application factor
Drift factor = i.e 0.0277, 90th percentile for one application (according to Ganzelmeier)
VDF = vegetation distribution factor
Vegetation distribution factor = 10 (to take into account the 3-dimensional structure of the off-field vegetation; only applied in the context of 2D test systems)
Correction factor = 10 (tier 1)

The risk is considered acceptable if the calculated HQ is < 2.

Table 10.3.2- 3 HQ for terrestrial non-target arthropods for the off-field scenario

Crop	Species	Appl. rate [ml/ha]	MAF	Drift [%]	VDF	Correction factor	LR_{50} [ml/ha]	HQ	Trigger
Cereals	*T. pyri*	600	1	2.77	10	10	81.8	0.203	2
	A. rhopalosiphi				10	10	> 700	< 0.024	2

The off-field HQ for *A. rhopalosiphi* (HQ =0.024) and *T. pyri* (HQ = 0.203) indicates an acceptable risk for non-target arthropods.

Refined In-field risk assessment

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 10.3.2- 4 Refined non-target arthropod in-field risk assessment

Crop	Species	Appl. rate [mL/ha]	MAF	$PEC_{in\text{-}filed}$ [mL/ha]	LR_{50}/ER_{50} [mL/ha]	Refinement required?
Cereals	*T. pyri*	600	1	600	>83.2	Yes
	C. carnea	600	1	600	> 600	No
	A. bilineata	600	1	600	> 600	No

The tier 2 in-field risk assessment indicates an acceptable risk on non-target arthropods with sensitive species like *C. carnea*, and *A. bilineata*, whereas the results for *T. pyri* indicate that initial effects cannot be excluded and that the potential for recovery needs to be demonstrated.

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 ([redacted], 2009, M-355238-01-1). 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). Therefore a potential for recovery was shown 28 days after application and no unacceptable adverse effects on non-target arthropods are to be expected from the use of DFF+FFA SC 600 according to the proposed use pattern.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Report:	**KCP 10.3.2.1/01; [redacted], A., 2001**
Title:	Effects of Flufenacet & Diflufenican SC 600 on the Predatory Mite *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) in the Laboratory -Dose Response Design.
Document No:	M-058604-01-1
Guidelines:	Blümel et al., 2000
GLP	Yes

Material and Methods:

Flufenacet & Diflufenican SC 600 (active ingredient: Flufenacet (FOE 5043), Diflufenican (DFF 200) Article No.: 3000248463, formulation No.: 07205/0024 (0006), purity: 401.5 g/L Flufenacet, 217.0 g/L Diflufenican); under laboratory conditions approximately 1 day old protonymphs of *Typhlodromus pyri* (20 individuals per test unit) were exposed to dried spray deposits of 22.5, 45.0, 90.0, 180 and 360 mL/ha (diluted in 200 L deionised water/ha) on glass plates (5 replicates per treatment group).
Deionised water was used as a control treatment and 8 mL Perfekthion EC (417.5 g/L Dimethoate) in 200 L water/ha as a reference treatment. The duration of the mortality part was 7 days. The corrected mortalities at day 7 were used to determine the LR_{50} of the test item. The reproductive performance was examined for another 7 day period in the control and in the test item rates were corrected mortality was < 50 %. The toxic standard treatment caused 100% mortality.

Findings:

Test item	Flufenacet & Diflufenican SC 600						
Test Species	*Typhlodromus pyri*						
Exposure	glass plate						
Test Formulation	Control water	Flufenacet & Diflufenican SC 600					Toxic Stand. Perfekthion
Application (ml/ha)	(200 L/ha)	22.5	45	90	180	360	8
Mortality (%) (1 week after applic.)	10.0	11.7	18.3	65.0	93.3	100	100
Significance (Fisher test, α = 0.05)	-	n.s.	n.s.	*	*	*	*
Corrected Mortality (M)	-	1.9	9.2	61.1	92.6	100	100
LR_{50} (Probit Analysis)	81.8 mL/ha (95% confidence limits: 71.4 - 93.8 mL/ha)						
Reproduction Rate (Mean of Total No. of Eggs per Female)	8.0	7.9	9.0	no reproduction evaluated	no reproduction evaluated	no reproduction evaluated	no reproduction evaluated
Significance (Student-test, α = 0.05)	-	n.s.	n.s.	-	-	-	-
Quotient of treated and untreated Series (R)	-	0.99	1.13	-	-	-	-

* significant compared to the control
n.s. not significant
\- not applicable

Conclusion:
The results of this study do not indicate statistically significant lethal effects on the predatory mite *Typhlodromus pyri* exposed up to 45 mL/ha Flufenacet & Diflufenican SC 600 in 200 L water/ha on a glass plate surface. Significant acute lethal effects were observed at dosages of 90 mL/ha Flufenacet & Diflufenican SC 600/ha and higher (Fisher-exact-test, α = 0.05). The LR_{50} value was determined to be 81.8 mL/ha Flufenacet & Diflufenican SC 600/ha with 95% confidence limits of 71.4 mL/ha to 93.8 mL/ha Flufenacet & Diflufenican SC 600/ha (Probit analysis). The reproduction was statistically not affected at rates up to 45 mL/ha Flufenacet & Diflufenican SC 600/ha (Student-t-test, α = 0.05).

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Report:	**KCP 10.3.2.1/02; [redacted], M. & [redacted], R., 2001**
Title:	Effects of Flufenacet & Diflufenican SC 600 on the Parasitoid *Aphidius rhopalosiphi* in the Laboratory - Limit Test.
Document N°:	M-058618-01-1
Guidelines:	IOBC/WPRS 1988, Mead-Briggs et al. 2000
GLP	Yes

Material and methods:

Effects of Flufenacet & Diflufenican SC 600 (active ingredients: Flufenacet (FOE 5043), Diflufenican (DFF); article-no.: 3000248463, formulation no.: 07205/0024(0006), tox no.: 05454-00, analytical content: Flufenacet 401.5 g/L, Diflufenican 217.0 g/L) on *Aphidius rhopalosiphi* were tested under laboratory conditions. Approximately 48 h old adult *Aphidius rhopalosiphi* (3 males and 7 females per test unit) were exposed to dried spray deposits of 500, 600 and 700 mL product/ha (diluted in 200 L deionised water/ha) on glass plates (5 replicates per treatment group). Deionised water was used as a control treatment and Perfekthion EC (0.3 mL/ha diluted in 200 L deionised water/ha) as a reference treatment. The duration of the mortality part was approximately 48 hours. The reproductive performance of the survivors was examined for another 24 hour period using females from the control and from the test item concentrations where corrected mortality was < 50 %. The toxic standard treatment caused 100% mortality.

Findings:

Test substance	Flufenacet & Diflufenican SC 600	
Test object	*Aphidius rhopalosiphi*	
Exposure	Glass Plates	
Treatment	Mortality after 48 h [%]	Mummies per female
Control	0.0	20.0
Application rate	Corrected mortality after 48 h [%]	Reproductive capacity [%]
500 mL product/ha	0.0	91.0
600 mL product/ha	2.0	86.0
700 mL product/ha	2.0	96.5
LR_{50}	> 700 mL product/ha (the highest rate tested in this experiment). The exact LR_{50} value could not be determined due to the low effects of the test item.	

All validity criteria of the study were met, the control mortality should not exceed 13% (0% in this study), the toxic standard mortality should result in at least 50% mortality (100% in this study) and the control reproduction rate should be > 5 mummies per female (20 in this study) and there should be no more than 2 parasitoids producing zero values (0 in this study).

Conclusion:

The LR_{50} and ER_{50} was estimated to be > 700 mL product/ha.

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CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

This study was already submitted and evaluated for the Annex I listing process of diflufenican. Nevertheless, a full study summary will be presented below.

Report:	**KCP 10.3.2.2/01; [redacted], M.-P., 2002**
Title:	The effects of Flufenacet & Diflufenican SC 600 on *Typhlodromus pyri* (Acari: Phytoseiidae) on natural substrate in laboratory (extended laboratory test).
Document N°:	M-034242-01-1
Guidelines:	IOBC guideline (Blümel et al., 2000)
GLP	Yes

Material and Methods

Flufenacet & Diflufenican SC 600 (Batch No. 07205/0024(0006), Development No. 30-00248463, TOX No. 05803-00, containing 406.52 g/l Flufenacet and 205.76 g/l Diflufenican according to analysis, was diluted in deionised water and applied at rates equivalent to 700 ml product/ha (corresponding to 284.6 g flufenacet/ha + 144.0 g diflufenican/ha), 241.4 ml product/ha (corresponding to 98.1 g flufenacet/ha + 49.7 g diflufenican/ha), 83.2 ml product/ha (corresponding to 33.82 g flufenacet/ha + 17.1 g diflufenican/ha) 28.7 ml product/ha (corresponding to 11.7 g flufenacet/ha + 5.9 g diflufenican/ha) and 9.9 ml product/ha (corresponding to 4.0 g flufenacet/ha + 2.0 g diflufenican/ha). Deionised water was applied as control and the toxic reference Danitol (100 g/l fenpropathrin) was applied at 0.5 l product/ha at 200l/ha. Test units consisted of 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. Plastic boxes were labelled individually with the study number, the treatment, the replicate and the application date. There were 4 replicates for each treatment group. 20 *T. pyri* protonymphs were introduced on each test unit together with 1 spot of walnut-apple (50:50) pollen. Assessments of direct treatment effects on mortality (dead + trapped in the glue barrier + trapped in the water + escapees) were made 1, 3 and 7 days after the application. Assessments of fecundity (number of eggs and juveniles / female) were made 7, 10, 12 and 14 days after the application. The sex-ratio was at least 1 male for 5 females on each fecundity assessment except the last one. Pollen was renewed 1, 3, 5, 7, 10 and 12 days after the application.

Findings:

Treatment	Mortality after 7 days (%)		Fecundity	
	Total	**Corrected**	**Absolute[1]**	**Relative[2]**
Control	12.5	-	4.5	
9.9 mL product/ha (4.0 g flufenacet/ha + 2.0 g diflufenican/ha)	7.5	0.0‡	4.3	95.6
28.7 mL product/ha (11.7 g flufenacet/ha + 5.9 g diflufenican/ha)	10.0	0.0‡	3.9	86.7
83.2 mL product/ha (33.82 g flufenacet/ha + 17.1 g diflufenican/ha)	27.5	17.1	5.3	117.8
241.4 mL product/ha (98.1 g flufenacet/ha + 49.7 g diflufenican/ha)	95.0*	94.3	-	-
700 mL product/ha (284.6 g flufenacet/ha + 144.0 g diflufenican/ha)	100.0*	100	-	-

[1] Mean cumulative number of eggs / female from day 7 to 14
[2] Fecundity relative to the control (%)
‡: Corrected mortality was negative and thus corrected to 0%.
*: Values statistically different from the control
Mortality in the toxic reference treatment was 100% 1 day after the application.

Conclusion:
The LR_{50} (p=0.05) value was 210.2 mL/ha (90.2 < LR_{50} < 402.2). The ER_{50} value was >83.2 mL prod./ha.

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Report: **KCP 10.3.2.2/02; [redacted], J., 2009**
Title: 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.
Document N°: M-352372-01-1
Guidelines: Vogt et al. (2000) modified, Candolfi et al. (2001)
GLP Yes

The aim of the study was to determine the toxicity of freshly dried residues of Flufenacet + Diflufenican SC 400 + 200 g/L applied onto detached maize leaves, to the green lacewing *Chrysoperla carnea*.

Material and methods:
Test item: A suspension concentrate formulation of Flufenacet + Diflufenican SC 400 + 200 g/L was tested, specified by sample description: FAR01403-00; specification no.: 102000007948; batch ID: EV56001418 (analysed content of active ingredient: Diflufenican 15.6% w/w, Flufenacet 32.1% w/w; date of completed analysis: 11 Nov 2008, BCS-D-FT Analysis & Services D-65926 Frankfurt); density: 1.229 g/mL. Test organism: the green lacewing *Chrysoperla carnea*, 2 days old larvae. The experiment was performed in a controlled environment room at a temperature of 23.5 - 25.5°C and a relative humidity of 60 - 80% (with a short decline < 2 hours to 41%). The climatic conditions are continuously recorded with thermohygrographs. The light / dark cycle was 16:8 hours. The light intensity was 1285 - 2830 Lux during the mortality phase and 3080 – 3144 Lux during the reproduction phase (measured once per phase using a Luxmeter). The test item was applied to maize leaves at rates of 30, 63, 134, 284 and 600 mL product/ha and the effects were compared to a toxic reference (as: dimethoate) applied at 53.2 mL product/ha (21 g as/ha), and a water treated control. The

preimaginal mortality was monitored over the duration of the study. The fertility and fecundity of the surviving hatched adults were then evaluated over the period of one week.

Findings:

Test item		Flufenacet + Diflufenican SC 400 + 200 g/L				
Test organism		*Chrysoperla carnea*				
Exposure on		Maize leaves				
		Mortality [%]			Reproduction	
Treatment	mL product/ha	Uncorr.	Corr.	P-value (*)	Eggs per female and day	Fertility [hatching rate in %]
Control	0	2.5			26.4	79.9
Test item	30	2.5	0.0	1.000 n. sign.	24.1	81.4
Test item	63	10.0	7.7	0.718 n. sign.	23.9	80.7
Test item	134	5.0	2.6	1.000 n. sign.	27.5	83.4
Test item	284	10.0	7.7	0.718 n. sign.	28.4	82.5
Test item	600	22.5	20.5	0.036 sign.	27.6	82.7
Reference item	53.2	87.5	87.2		n.d.	n.d.
LR_{50}: > 600 mL product/ha						

* Fisher's Exact test (one-sided), p-values are adjusted according to Bonferroni-Holm
n.d. = not detected
n. sign. = not significant
sign = significant

The results can be considered as valid, as all validity criteria of the test were met. The control mortality was ≤ 20% (2.5% in this study), the corrected mortality in the reference item was > 50% (87.2% in this study), the average number of eggs per female per day in the control group was ≥ 15 (26.4 in this study) and the mean larval hatching rate in the control group ≥ 70% (79.9% in this study). When the preimaginal mortality was corrected for control mortality, the corrected figures for all rates of the test item were below 21%. 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%.

Conclusion:
The dose rates of 30, 63, 134 and 284 mL product/ha had no statistically significant influence on mortality. Only a slight corrected mortality of 20.5% occurred at the highest dose rate of 600 mL product/ha. There were no adverse effects of the test item on the reproductive performance at all rates tested. The LR_{50} was estimated to be > 600 mL product/ha.

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Report:	**KCP 10.3.2.2/03; [redacted], U., 2009**
Title:	Chronic toxicity (ER_{50}) of Diflufenican+Flufenacet SC 600 g/L to the rove beetle *Aleochara bilineata* GYLL. under extended laboratory conditions.
Document N°:	M-353760-01-1
Guidelines:	IOBC Guideline (GRIMM et al. 2000)
GLP	Yes

The purpose of this study was to determine possible effects of the test item (regarding a chronic dose response toxicity) on the reproductive capacity of the rove beetle *Aleochara bilineata* GYLL. in an extended laboratory test. Adult beetles were exposed to dried spray residues of different application rates of the test item applied onto sandy soil (LUFA 2.1). The reproductive capacity was used as test endpoint.

Material and methods:

Test item: 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). Specification No.: 102000007948, Batch ID: EV56001418, density: 1.229 g/cm³, sample description: FAR 01403-00)
Control: The control was treated with deionised water (400 L/ha) only. Reference item: Dimethoate EC 400 (1.5 L product/ha in 400 L water/ha). Test organism: Adults of *Aleochara bilineata* GYLL. (1-7 days old) were exposed in 4 replicates of 20 beetles (per treatment group) to the spray residue of the test item, reference item and control treatments, respectively. During the assessments, the beetles were fed with deep frozen larvae of *Chironomus* spp. Test conditions: Diflufenican + Flufenacet SC 600 g/L was tested under extended laboratory conditions after contact exposure of adults of the rove beetle *Aleochara bilineata* GYLL. to dried spray residues of the test item with rates of 60, 107, 190, 337 and 600 mL product/ha in 400 L deionised water/ha applied on sandy soil (LUFA 2.1). 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.

Findings:

Test item	Diflufenican + Flufenacet SC 600 g/L				
Test organism	*Aleochara bilineata* GYLL.				
Exposure	Dried spray deposits on sandy soil (LUFA 2.1)				
Treatment	Reproductive capacity				
	Total number of hatched beetles of the F_{1}-generation per treatment group	Mean number of hatched beetles of the F1-generation per replicate	Mean number of hatched beetles/host pupa	Parasitisation rate P (%)	Reduction of reproductive capacity (relative to control) R (%)

Control	2644	661	0.441	44.1	-
Application rate [ml product/ha]					
60	2530	633	0.422	42.2	4.3
107	2705	676	0.451	45.1	-2.3
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
ER_{50}	**> 600 mL product/ha**				
Reference item Dimethoate EC 400 1.5 L product /ha	8	2	0.0013	0.13	99.7

No statistically significant differences between the control and the test item treatments were calculated.

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 group was reduced to 0.3 %, compared to the control group. Thus, the test accomplished the validity criteria according to GRIMM et al. (2000) for conducting the extended laboratory test with *Aleochara bilineata* (control group: average number of hatched beetles of the F1-generation > 400, reduction of the reproductive capacity in the reference item treatment group, relative to control > 50 %). The results of the control group indicated that the test organisms were in a good condition (average number of hatched beetles of the F1-generation per replicate: 661). The results of the reference item group indicated that the test system was sensitive to harmful substances (99.7 % reduction of reproductive capacity). Statistical analysis of reproduction (DUNNETT´s multiple t-test, $p \leq 0.05$, 1-sided) revealed no significant difference concerning the reproductive capacity between the control and all test item treatment groups. A calculation of the ER_{50} for reproductive capacity was not possible, because the reduction of reproductive capacity was below 50 % on all test item treatment groups.

Conclusion:
The ER_{50} is empirically estimated to exceed the highest tested application rate, i.e. 600 mL product/ha.

CP 10.3.2.3 Semi-field studies with non-target arthropods

Report:	**KCP 10.3.2.3/01, [redacted], B., 2009**
Title:	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.
Document N°:	M-355238-01-1
Guidelines:	Blümel et al. (2000) modified, Candolfi et al. (2001)
GLP	Yes

The objective of this study was to investigate the lethal and sublethal toxicity of residues of Flufenacet + Diflufenican SC 400 + 200 g/L that were aged under semi-field conditions to the predatory mite *Typhlodromus pyri* when exposed to these residues on treated leaf surfaces.

Material and methods:

Test item: A suspension concentrate formulation of Flufenacet + Diflufenican SC 400 + 200 g/L was tested, specified by sample description: FAR01403-00; specification no.: 102000007948; batch ID: EV56001418 [analysed content of active ingredient: Diflufenican 15.6% w/w, Flufenacet 32.1% w/w; date of completed analysis: 11 Nov 2008, BCS-D-FT Analysis & Services D-65926 Frankfurt]; density: 1.229 g/mL. Test organism: the predatory mite *Typhlodromus pyri*, protonymphs. Control: deionised water only. Toxic reference: Dimethoate was applied at 0.1014 L product/ha (40 g as/ha) in 400 L water/ha on the application day on potted maize plants as well. The test item was applied with 0.7 L product/ha in 400 L water/ha on potted maize plants. For the further exposure dates it was applied directly on the maize leaves (with 0.1014 L/ha in 200 L water/ha). It was included to indicate the relative susceptibility of the test organisms and the test system. Aging of the spray residues of the test item on the potted maize plants took place under natural semi-field conditions with rain protection during the whole study. Mortality of 100 protonymphs 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.

Findings:

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 (p-value < 0.001 significant[b])	87.1 (p-value < 0.001, significant[b])	9.5 (p-value = 0.026, significant[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 (p-value = 0.376, not significant[c]

[a] Days after application
[b] Fisher's Exact test, one-sided, p-values adjusted according
[c] one-way ANOVA, Williams test (one-sided)

In all three bioassays the control mortality was below 20% and the mortality of the toxic reference group was 100%. Furthermore the cumulated number of eggs per female for the reproduction

assessment in the third bioassay was above 4 eggs per female (7.5 after 28 days in this study). Therefore the results of this study can be considered as valid.

Conclusion:
In this extended laboratory test the effects of Flufenacet + Diflufenican SC 400 + 200 g/L residues (aged under semi-field conditions) on the survival of the predatory mite *Typhlodromus pyri* were determined after application of 0.7 L product/ha onto *Zea mays*. 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).

CP 10.3.2.4 Field studies with non-target arthropods

No field studies were deemed necessary.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

No relevant exposure of non-target arthropods is expected by other routes of exposure.

CP 10.4 Effects on non-target soil meso- and macrofauna

Only endpoints used for the risk assessment are presented here. For an overview of all available endpoints for flufenacet and its metabolites please refer to the respective section of the MCA document.

For the second active substance in the representative formulation, diflufenican, references is made to the EU agreed endpoints according to the EFSA Scientific Report (2007) 122.

The risk assessment procedure follows current regulatory requirements and the Guidance Document on Terrestrial Ecotoxicology.

Based on most sensitive endpoints the TER values are calculated using the following equations:

$TER_{LT} = NOEC / PEC_{soil}$

The risk is considered acceptable, if the TER_{LT} is >5.

For lipophilic substances (log P_{ow} > 2) all results from the laboratory studies have to be corrected by a factor 2 when the organic matter is higher or equal to 5% (PRAPER decision, April 2012).

Ecotoxicological endpoints used in risk assessment

Table 10.4- 1 Endpoints for the representative formulation used in risk assessment

Test substance	Test species	Endpoint		Reference
DFF + FFA SC 600	Earthworm, reproduction (5% peat in test soil)	NOEC NOEC	2.6 mg as/kg 1.3* mg as/kg	[redacted] (2010) M-362809-01-1 KCP 10.4.1.1/01
DFF + FFA SC 600	Earthworm field study	NOEAER	1.8 L/ha	[redacted] (2014) M-478092-01-1 KCP 10.4.1.2/01

* endpoints corrected to allow for log P_{ow} > 2

Table 10.4- 2 Endpoints used in risk assessment for flufenacet and its metabolites

Test substance	Test species	Endpoint		Reference
Flufenacet WG 60	Earthworm, reproduction (10% peat in test soil)	NOEC	1.2* mg as/kg	[redacted] (2011) M-004878-01-1 KCA 8.4.1/01
FFA SC 500	Earthworm field study	NOEAER	1.2 L prod/ha 0.6 kg a.s./ha	[redacted] (2008) M-307211-01-1 KCA 8.4.1/11
FOE oxalate	Earthworm, reproduction (10% peat in test soil)	NOEC	≥100 mg p.m./kg	[redacted] (2010) M-398163-01-1 KCA 8.4.1/02
FOE sulfonic acid-Na-salt	Earthworm, reproduction (5% peat in test soil)	NOEC	500 mg p.m./kg	[redacted] (2009) M-358264-01-1 KCA 8.4.1/03
FOE methylsulfone	Earthworm, reproduction (5% peat in test soil)	NOEC	62.5* mg p.m./kg	[redacted] (2010) M-362081-01-1 KCA 8.4.1/04
TFA	Earthworm, reproduction (10% peat in test soil)	NOEC	320 mg p.m./kg	[redacted] (2005) M-251328-01-1 KCA 8.4.1/05
FOE 5043-trifluoroethane sulfonic acid	Earthworm, reproduction (5% peat in test soil)	NOEC	≥100 mg p.m./kg	[redacted] (2012) M-436340-01-1 KCA 8.4.1/06
FOE-Thiadone	Earthworm, reproduction (5% peat in test soil)	NOEC	3.2 mg as/kg	[redacted] (2012) M-442579-01-1 KCA 8.4.1/07

* endpoints corrected to allow for log P_{ow} > 2

Table 10.4- 3 Endpoints of mixing partner diflufenican

Test substance	Test species	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84	
Diflufenican	Earthworm, reproduction (10% peat in test soil)	NOEC	500 mg as/kg dws*

* endpoints corrected to allow for log P_{ow} > 2

Predicted environmental concentrations used in risk assessment

Table 10.4- 4 Initial max PEC_{soil} values

Compound	Winter cereals 1 x 240 g a.s./ha		Winter cereals 1 x 160 g a.s./ha		Winter cereals 1 x 120 g a.s./ha	
	$PEC_{soil, max}$ [mg/kg]	$PEC_{soil\ accu}$ [mg/kg]	$PEC_{soil, max}$ [mg/kg]	$PEC_{soil\ accu}$ [mg/kg]	$PEC_{soil, max}$ [mg/kg]	$PEC_{soil\ accu}$ [mg/kg]
DFF + FFA SC 600	0.748[1)]	--	0.498[2)]	--	0.498[2)]	--
Flufenacet	0.240	--	0.160	--	0.160	--
FOE oxalate	0.039	--	0.026	--	0.026	--
FOE sulfonic acid-Na-salt	--	0.077	--	0.051	--	0.051
FOE methylsulfone	--	0.015	--	0.010	--	0.010
TFA	--	0.275	--	0.183	--	0.183
FOE 5043-trifluoroethane sulfonic acid	0.007	--	0.004	--	0.004	--
FOE-Thiadone	0.007	--	0.004	--	0.004	--

[1)] Calculated product PECsoil, considering the PECsoil for flufenacet (0.240 mg a.s./kg) and a concentration of 32.1 % flufenacet in DFF+FFA SC 600

[2)] Calculated product PECsoil, considering the PECsoil for flufenacet (0.160 mg a.s./kg) and a concentration of 32.1 % flufenacet in DFF+FFA SC 600

CP 10.4.1 Earthworms

Risk assessment for earthworms

The earthworm tier 1 risk assessment for the representative formulation DFF+FFA SC 600, flufenacet, and the relevant metabolites is presented in the table below.

Table 10.4.1- 5 TER calculations for earthworms

Compound	Species	Endpoint [mg/kg]	$PEC_{soil,max/accu}$ [mg/kg]	TER_{LT}	Trigger
Winter Cereals – 240 g a.s./ha					
DFF+FFA SC 600	Earthworm, reproduction	NOEC 1.3*	0.748[1)]	**1.7**	5
Flufenacet	Earthworm, reproduction	NOEC 1.2*	0.240	5	5
FOE oxalate	Earthworm, reproduction	NOEC >100	0.039	3564	5
FOE sulfonic acid-Na-salt	Earthworm, reproduction	NOEC 500	0.077	6494	5
FOE methylsulfone	Earthworm, reproduction	NOEC 62.5*	0.015	4167	5
TFA	Earthworm, reproduction	NOEC 320	0.275	1164	5
FOE 5043-trifluoroethane sulfonic acid	Earthworm, reproduction	NOEC >100	0.007	14286	5
FOE-Thiadone	Earthworm, reproduction	NOEC 3.2	0.007	457	5
Winter Cereals – 160 g a.s./ha / Winter cereals – 120 g a.s./ha					
DFF+FFA SC 600	Earthworm, reproduction	NOEC 1.3*	0.498[2)]	**2.6**	5
Flufenacet	Earthworm, reproduction	NOEC 1.2*	0.160	7.5	5
FOE oxalate	Earthworm, reproduction	NOEC >100	0.026	3846	5
FOE sulfonic acid-Na-salt	Earthworm, reproduction	NOEC 500	0.051	9804	5
FOE methylsulfone	Earthworm, reproduction	NOEC 62.5*	0.010	6250	5
TFA	Earthworm, reproduction	NOEC 320	0.183	1749	5
FOE 5043-trifluoroethane sulfonic acid	Earthworm, reproduction	NOEC >100	0.004	25000	5
FOE-Thiadone	Earthworm, reproduction	NOEC 3.2	0.004	800	5

* endpoints corrected to allow for log P_{ow} > 2

1) Calculated product PECsoil, considering the PECsoil for flufenacet (0.240 mg a.s./kg) and a concentration of 32.1 % flufenacet in DFF+FFA SC 600

2) Calculated product PECsoil, considering the PECsoil for flufenacet (0.160 mg a.s./kg) and a concentration of 32.1 % flufenacet in DFF+FFA SC 600

For flufenacet and the relevant metabolites the TER values exceed the critical trigger value of 5, indicating a low risk to earthworm population if the product is applied up to 0.6 L DFF+FFA SC 600/ha (240 g flufenacet/ha) in winter cereals. For the representative formulation DFF+FFA SC 600 the critical trigger value of 5 is not passed indicating a potential risk of the mixture for earthworm populations. A refined risk assessment is presented below.

Refined Risk Assessment

A one-year earthworm field study is available with the representative formulation DFF+FFA SC 600 ([redacted], 2014; KCP 10.4.1.2/01). The results of this field study give clear evidence that DFF+FFA SC 600 applied on an arable field site at applications rates of up to 1.8 L/ha (720 g flufenacet/ha) has no effects on abundance and biomass of earthworm populations. Thus, a low risk for earthworm population can be considered if the product is applied up to 0.6 L DFF+FFA SC 600/ha (240 g flufenacet/ha) in winter cereals.
Furthermore, a one-year earthworm field study is available with Flufenacet SC500 ([redacted], 2008; KCA 8.4.1/11). This study demonstrates that natural earthworm populations are not affected if Flufenacet SC500 is applied on an arable field up to an application rate of 1.2 L/ha which is equivalent to 600 g Flufenacet/ha. Thus, it can be concluded that earthworms are not at risk if Flufenacet is applied up to 240 g/ha in winter cereals.

CP 10.4.1.1 Earthworms - sub-lethal effects

Report:	**CP 10.4.1.1/01 [redacted], T., 2010**
Title:	Diflufenican + flufenacet SC 600 G: Effects on survival, growth and reproduction on the earthworm *Eisenia fetida* tested in artificial soil with 5% peat
Document N°:	M-362809-01-1
Guidelines:	OECD Guideline No. 222 for the Testing of Chemicals "Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*)" adopted April 13, 2004. International Standard ISO 11268-2 Part 2 (1998) "Soil Quality - Effects of Pollutants on Earthworms (*Eisenia fetida*) - Part 2: Determination of Effects on Reproduction"
GLP	yes (certified laboratory)

Objective:
The purpose of this study was to assess the effect of Diflufenican + Flufenacet SC 600 G, on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure in an artificial soil at 5 different test concentrations.

Materials and Methods:
Test item: Diflufenican + flufenacet SC 600 G, Specification No.: 102000007948, Material No.: 05700094, Batch ID: LV56001418, PAR 01403-00, content of a.s. (analysed): diflufenican: 191.4 g/L (15.6 % w/w); flufenacet: 394.5 g/L (32.1% w/w); density 1.229 g/mL.
Test organism: Adult earthworms (*Eisenia fetida*), approx. 7 months old.
Ten *Eisenia fetida* per replicate (8 for the control group, 4 per test item concentration) were exposed in an artificial soil (with 5% peat content) to the nominal test concentrations of 4.8, 8.5, 15.2, 27.0 and 48.0 mg test item/kg dry weight artificial soil in the 1st test run and 0.8, 1.5, 2.6, 4.7 and 8.4 mg test item/kg dry weight artificial soil in the 2nd test run. The test item was mixed into the soil. After 28 days the number of surviving animals and their weight alteration was determined. They were then removed from the artificial soil. After further 28 days (i.e. after 56 days), the number of offspring was determined.
The 1st test run was conducted at the test facility. A NOEC was not achieved in this test run. Due to capacity constraint the 2nd test run was conducted at the principal investigators facility.

Findings:
The results can be considered as valid, as all validity criteria of the test were met.

Validity criteria	**Recommended**	**Obtained 1st run**	**Obtained 2nd run**
Mortality of the adults in the control	≤ 10 %	0 %	0 %
Mean rate of reproduction of juveniles (Min – Max juveniles per control vessel)	≥ 30	102.4 (80 -121)	116.8 (98 – 149)
Coefficient of variance of reproduction in the control	≤ 30 %	14.9 %	14.7 %

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.

Test object	*Eisenia fetida*					
Test item	Control	Diflufenican + Flufenacet SC 600 G				
1st test run						
Test concentration (mg test item/kg dry weight artificial soil)	---	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 [%]	+ 7.9	+ 14.7	+ 16.6	+ 18.4	+ 16.5	+ 12.6
Statistical comparison to the control*	---	s.	s.	s.	s.	s.
Mean number of offspring per test vessel after 56 days	102.4	84.0	75.3	82.5	81.8	59.5
Standard Deviation	± 15.3	± 15.7	± 7.8	± 18.5	± 14.8	± 14.8
Statistical comparison to the control**	---	s.	s.	s.	s.	s.
2nd test run						
Test concentration (mg test item/kg dry weight artificial soil)	---	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 [%]	+ 63.1	+ 64.0	+ 64.5	+ 62.8	+ 62.8	+ 61.5
Statistical comparison to the control*	---	n. s.	n. s.	n. s.	n. s.	n. s.
Mean number of offspring per test vessel after 56 days	116.8	111.0	113.8	104.3	83.0	68.0
Standard Deviation	± 17.2	± 13.4	± 18.7	± 11.5	± 10.7	± 13.2
Statistical comparison to the control**	---	n. s.	n. s.	n. s.	s.	s.

* Result of a Williams Multiple Sequential t-test, two-sided, $\alpha = 0.05$
** Result of a Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$
n. s. mean value not statistically significant different compared to the control ($p \geq 0.05$)
s. mean value statistically significant different compared to the control ($p < 0.05$)

No mortality of adult earthworms was observed after 28 days of exposure at the control group and all test concentrations of the 1st test run. Just one worm each died in the concentrations 1.5 and 4.7 mg test item/kg dry weight soil of the 2nd test run.

Statistically significant different values for the growth relative to the control were observed at all test concentrations of the 1st test run. Since there is no dose-response relationship these differences are not considered to be treatment related. No statistically significant different values for the growth relative to the control were observed at test all concentrations of the 2nd test run.

Therefore:

NOEC related to growth: ≥ 48.0 mg test item/kg dry weight artificial soil
LOEC related to growth: > 48.0 mg test item/kg dry weight artificial soil

Statistically significant different values for the number of juveniles per test vessel relative to the control were observed at all test concentrations of the 1st test run.
In the 2nd test run statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the test concentrations of 4.7 and 8.4 mg test item/kg dry weight artificial soil.

Therefore, based on statistical significance:

NOEC related to reproduction: 2.6 mg test item/kg dry weight artificial soil
LOEC related to reproduction: 4.7 mg test item/kg dry weight artificial soil

Conclusions:
Overall, based on the biological and statistical significance of the effects observed on reproduction, it is concluded that the NOEC for this study is 2.6 mg test item/kg dry weight artificial soil. The overall LOEC is determined to be 4.7 mg test item/kg dry weight artificial soil.

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CP 10.4.1.2 Earthworms - field studies

Report:	**CP 10.4.1.2/01 [redacted], A.; 2014**
Title:	DFF+FFA SC 200+400 G – A field study to investigate effects on the earthworm fauna in Southern Germany.
Document N°:	M-478092-01-1
Guidelines:	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 (certified laboratory)

Material and methods:
The effects of DFF + FFA SC 600 (content of Diflufenican (analysed): 209.5 g/L; Flufenacet. (analysed): 410.0 g/L, Batch-No.: 2011-005209, TOX-No.: TOX09504-00) on earthworm populations under field conditions were studied. The field study was carried out on an agricultural field in Southern Germany following ISO 11268-3 (ISO 1999) and ISO 23611-1 (ISO 2006). The recommendations by KULA et al. (2006) were considered.
The study consists of three trials: S12-03897-01 (field phase), S12-03897-L1 (analytical phase) and S12-03897-L2 (soil characterisation). The soil of the field site is characterised by the soil type silty clay loam with a silt content of 60.8 %, a clay content of 31.0 % and a sand content of 8.2 % (USDA).
The study included 5 treatment groups with four replicates per treatment group: the tap-water treated control (C), three test item treatment groups (a.s. diflufenican and flufenacet) and the toxic reference treated with Twist WP® (a.s. carbendazim). Diflufenican SC 500A G was applied once at a rate of 243.75 g a.s./ha to reach a target plateau application in soil of 0.325 mg diflufenican/kg soil (application 1). After application 1 diflufenican was incorporated into the top 5 cm of the soil and winter wheat was drilled. DFF+ FFA SC 200+400 G was applied once at different rates (application

2). Treatment group 1 was treated with 0.6 L product/ha, treatment group 2 with 1.2 L product/ha and treatment group 3 with 1.8 L product/ha. The applications were performed in autumn during a period of high earthworm activity.

The control plots were sprayed once with tap water, the toxic reference item plots were treated once with 17152.66 g product/ha Twist WP® (equivalent to 10000 g a.s. carbendazim/ha) at the same time as application 2 in the test item groups was performed. The spray applications were made with a boom sprayer calibrated to apply a spray volume of 300 L/ha on bare soil (applications 1 and 2).

Test organisms were naturally occurring field populations of earthworms in all life stages (juveniles and adults). A pre-treatment sampling was conducted before the first application on 01 October - 02 October 2012 to determine the density, diversity and homogeneity of earthworm distribution at the field site. The field site selected contained representatives of the major earthworm groups and at a number that is recommended in the relevant guidelines.

Earthworm populations were assessed for their abundance and biomass prior to the first application (see above) and approximately 1, 6 and 12 months after the second application (21st/22nd November 2012; 18th/19th April 2013 and 16th/17th October 2013, respectively). Additionally, daily surface-density counts of dead earthworms were performed within the first 3 days after the second application in the control and test item plots. Exposure of the earthworm population to the test item was enhanced through additional irrigation of the field site. The combined natural rainfall and irrigation yielded soil moisture levels that ensured constant earthworm activity and thus exposure to the treatments.

Earthworms were sampled from four 50 cm x 25 cm sampling areas per plot per sampling occasion. Earthworm surface monitoring took place between these sample areas with a minimum distance to the border of the plot of two metres. Additionally, areas for soil residue sampling (soil cores) for analytical verification were located in each plot.

After application of Diflufenican SC 500A G (plateau application) mean residues as percentage of the target rate of 80 %, 100 % and 99 % 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 96 %, 115 % and 121 % as percentage of the target rate were determined in treatment groups T1, T2 and T3, respectively. Mean residues of FFA of 90 %, 98 % and 99 % as percentage of the target rate were determined in treatment group T1, T2 and T3.

Findings and observations:

Earthworm number and diversity in pre-sampling and in the control plots:

The mean earthworm abundance 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 decreasing to 179 earthworms/m2 at 35 DAA2 and 183 earthworms/m2 at 183 DAA2. At the end of the trial 216 earthworms/m2 (364 DAA) were found

Adult and juvenile earthworms, changes in numbers and biomass:

No significant reductions in numbers and biomass of total earthworms, juveniles and individual species occurred during the three post-treatment samplings in all test item treatments.

Treatment	DFF+FFA SC 200+400 G 0.6 L product/ha					
	Mean number (Ind/m²) and change (%)**					
species / group	35 DAA2		183 DAA2		364 DAA2	
Aporrectodea caliginosa	16.5	(-8.3 %)	16.5	(-5.7 %)	40.0	(+5.3 %)
Aporrectodea rosea	6.0	(-25.0 %)	31.5	(-6.0 %)	40.5	(-10.0 %)
Allolobophora chlorotica	3.0	(-70.0 %)	5.5	(-8.3 %)	6.5	(-7.1 %)
Lumbricus terrestris	13.5	(+12.5 %)	10.5	(+31.3 %)	10.5	(-16.0 %)
Lumbricus terrestris adult + juvenile	20.0	(+11.1 %)	15.5	(+14.8 %)	16.5	(+10.0 %)
Octolasion lacteum	5.5	(-56.0 %)	14.0	(-3.4 %)	18.5	(+2.8 %)
Tanylobous juvenile	14.5	(-47.3 %)	16.0	(+88.2 %)	25.0	(+4.2 %)
Epilobous 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/m²) and change (%)**					
Aporrectodea caliginosa	2.7	(+12.6 %)	2.9	(+8.4 %)	9.6	(+14.3 %)
Aporrectodea rosea	1.0	(-74.8 %)	3.7	(-8.4 %)	8.1	(+21.8 %)
Allolobophora chlorotica	0.7	(-64.7 %)	1.0	(-16.0 %)	1.7	(-13.9 %)
Lumbricus terrestris	62.5	(+20.9 %)	48.4	(+23.7 %)	55.5	(-4.8 %)
Lumbricus terrestris adult + juvenile	78.3	(+27.2 %)	62.4	(+26.6 %)	66.4	(+2.8 %)
Octolasion lacteum	3.8	(-54.3 %)	12.5	(-11.0 %)	18.5	(-13.4 %)
Tanylobous juvenile	20.4	(+51.3 %)	19.9	(+78.8 %)	16.5	(+48.1 %)
Epilobous 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 %)
Treatment	**DFF+FFA SC 200+400 G 1.2 L product/ha**					
	Mean number (Ind/m²) and change (%)**					
species / group	**35 DAA2**		**183 DAA2**		**364 DAA2**	
Aporrectodea caliginosa	17.5	(-2.8 %)	17.0	(-2.9 %)	44.0	(+15.8 %)
Aporrectodea rosea	11.5	(+43.8 %)	40.0	(+19.4 %)	60.0	(+33.3 %)
Allolobophora chlorotica	3.5	(-65.0 %)	4.0	(-33.3 %)	4.5	(-35.7 %)
Lumbricus terrestris	18.0	(+50.0 %)	9.0	(+12.5 %)	8.0	(-36.0 %)
Lumbricus terrestris adult + juvenile	22.5	(+25.0 %)	14.0	(+3.7 %)	15.5	(+3.3 %)
Octolasion lacteum	7.0	(-44.0 %)	11.0	(-24.1 %)	12.0	(-33.3 %)
Tanylobous juvenile	14.0	(-49.1 %)	14.0	(+64.7 %)	29.5	(+22.9 %)
Epilobous 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/m²) and change (%)**					
Aporrectodea caliginosa	2.5	(+3.5 %)	3.4	(+26.2 %)	11.2	(+33.1 %)
Aporrectodea rosea	1.6	(-57.3 %)	5.0	(+21.7 %)	7.9	(+17.9 %)
Allolobophora chlorotica	0.8	(-58.2 %)	0.8	(-35.4 %)	1.4	(-30.8 %)
Lumbricus terrestris	77.2	(+49.4 %)	40.4	(+3.4 %)	37.3	(-36.0 %)
Lumbricus terrestris adult + juvenile	83.5	(+35.6 %)	51.0	(+3.4 %)	50.0	(-22.6 %)
Octolasion lacteum	4.9	(-41.9 %)	11.0	(-21.1 %)	11.2	(-47.4 %)
Tanylobous juvenile	9.8	(-27.4 %)	15.1	(+35.2 %)	19.0	(+71.1 %)
Epilobous 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 %)
Treatment	**DFF+FFA SC 200+400 G 1.8 L product/ha**					
	Mean number (Ind/m²) and change (%)**					
species / group	**35 DAA2**		**183 DAA2**		**364 DAA2**	
Aporrectodea caliginosa	23.5	(+30.6 %)	17.5	(±0.0 %)	38.0	(±0.0 %)
Aporrectodea rosea	8.5	(+6.3 %)	37.5	(+11.9 %)	61.0	(+35.6 %)
Allolobophora chlorotica	7.5	(-25.0 %)	3.5	(-41.7 %)	8.0	(+14.3 %)
Lumbricus terrestris	11.5	(-4.2 %)	16.5 *	(+106.3 %)	12.0	(-4.0 %)
Lumbricus terrestris adult + juvenile	18.5	(+2.8 %)	22.5	(+66.7 %)	15.0	(±0 %)
Octolasion lacteum	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/m²) and change (%)**					
Aporrectodea caliginosa	3.6	(+48.8 %)	2.9	(+6.7 %)	10.4	(+23.4 %)
Aporrectodea rosea	1.2	(-69.6 %)	4.2	(+3.4 %)	8.0	(+19.4 %)
Allolobophora chlorotica	1.5	(-23.1 %)	0.8	(-38.3 %)	2.6	(+30.0 %)
Lumbricus terrestris	47.9	(-7.2 %)	74.1	(+89.4 %)	56.0	(-3.9 %)
Lumbricus terrestris adult + juvenile	64.3	(+4.4 %)	84.4	(+71.3 %)	62.8	(-2.7 %)
Octolasion lacteum	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

DAA2: days after application 2

The toxic reference reduced total earthworm abundance significantly 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. Total earthworm biomass in the plots treated with the toxic reference was statistically reduced by 85.1 % at 35 DAA2 and 72.7 % at 183 DAA2.

Treatment	Toxic reference Twist WP® (10 000 g a.s./ha)		
	Mean number (Ind/m²) and change (%)**		
species / group	35 DAA2	183 DAA2	364 DAA2
Aporrectodea caliginosa	2.0 * (-88.9 %)	3.0* (-82.9 %)	19.5 (-48.7 %)
Aporrectodea rosea	4.5 (-43.7 %)	9.0 (-73.1 %)	24.5 (-45.6 %)
Allolobophora chlorotica	0.0 (-100 %)	0.5 (-91.7 %)	1.0 (-85.7 %)
Lumbricus terrestris	1.5* (-87.5 %)	3.0 (-62.5 %)	3.0 (-76.0 %)
Lumbricus terrestris adult + juvenile	1.5* (-91.7 %)	3.0 (-77.8 %)	5.0 (-66.7 %)
Octolasion lacteum	3.5 (-72.0 %)	6.5 (-55.2 %)	19.0 (+5.6 %)
Tanylobous juvenile	5.0 (-81.8 %)	1.5* (-82.4 %)	7.5 * (-68.7 %)
Epilobous juvenile	29.5 * (-61.7 %)	29.0 * (-66.9 %)	26.5 * (-55.8 %)
Endogeic earthworms	10.5* (-78.4 %)	20.0* (-72.2%)	69.5 (-36.8 %)
Anecic earthworms	1.5 * (-87.5 %)	3.0 (-62.5 %)	3.0 (-76.0 %)
Anecic earthworms adult + juvenile	1.5 * (-91.7 %)	3.0 (-77.8 %)	5.0 (-66.7 %)
Total juveniles	34.5 * (-67.0 %)	30.5 * (-68.2 %)	34.0 * (-59.5 %)
Total adults	12.0 * (-80.3 %)	23.0 * (-71.2 %)	74.0 (-39.6 %)
Total earthworms	51.5 * (-71.1 %)	56.0 * (-69.4 %)	118.0 * (-45.2 %)
	Mean biomass (g/m²) and change (%)**		
Aporrectodea caliginosa	0.4 * (-84.4 %)	0.6 * (-76.4 %)	7.1 (-15.6 %)
Aporrectodea rosea	0.7 (-81.9 %)	1.1 (-74.1 %)	3.3 * (-50.0 %)
Allolobophora chlorotica	0.0 (-100.0 %)	0.1 (-88.9 %)	0.4 (-79.9 %)
Lumbricus terrestris	6.1 (-88.1 %)	10.1 (-74.3 %)	11.3 (-80.7 %)
Lumbricus terrestris adult + juvenile	6.1 (-90.0 %)	10.1 (-79.6 %)	14.5 (-77.5 %)
Octolasion lacteum	2.7 (-67.9 %)	5.0 (-64.0 %)	24.6 (+15.5 %)
Tanylobous juvenile	0.5 (-96.3 %)	1.6 * (-85.6 %)	4.9 * (-56.4 %)
Epilobous juvenile	2.6 * (-59.4 %)	3.2 * (-63.2 %)	3.3 * (-56.4 %)
Endogeic earthworms	3.8 * (-77.3 %)	6.9 * (-68.7 %)	36.8 (-5.5 %)

Anecic earthworms	6.1		(-88.1 %)	10.1		(-74.3 %)	11.3	(-80.7 %)
Anecic earthworms adult + juvenile	6.1		(-90.0 %)	10.1		(-79.6 %)	14.5	(-77.5 %)
Total juveniles	3.1	*	(-84.3 %)	4.8	*	(-75.7 %)	8.2	* (-56.3 %)
Total adults	9.9	*	(-85.5 %)	16.9	*	(-72.3 %)	48.8	(-49.8 %)
Total earthworms	13.4	*	(-85.1 %)	22.3	*	(-72.7 %)	59.1	(-49.6 %)

* 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

DAA2: days after application 2

Conclusions:

No statistically significant reductions of total earthworm numbers and biomass nor ecological groups and single species occurred at any of the post treatment samplings after application of the test item. Thus, it can be concluded that after application of the test item DFF+FFA SC 200+400 G at rates of 0.6 L, 1.2 L and 1.8 L product/ha following a plateau application of diflufenican at a rate of 243.75 g a.s./ha no effect on earthworm field populations occurred.

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CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Table 10.4.2- 1 Endpoints for flufenacet and its metabolites used in risk assessment

Test substance	Test species	Endpoint	Reference
DFF + FFA SC 600	*Folsomia candida*	NOEC 178 mg/kg dws **NOEC 89* mg/kg dws**	[redacted] (2011) M-415903-01-1 KCP 10.4.2.1/02
	Hypoaspis aculeifer	NOEC ≥ 65.3 mg prod/kg dws **NOEC ≥ 32.65* mg prod/kg dws**	[redacted] (2002) M-061660-04-1 KCP 10.4.2.1/01
Flufenacet	*Folsomia candida*	NOEC 31.5* mg a.s./kg dws	[redacted] (2010) M-394712-01-1 KCA 8.4.2.1/04
	Hypoaspis aculeifer	NOEC 281* mg a.s./kg dws	[redacted] (2013) M-455214-01-1 KCA 8.4.2.1/12
FOE oxalate	*Folsomia candida*	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2010) M-394712-01-1 KCA 8.4.2.1/04
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2010) M-393634-01-1 KCA 8.4.2.1/03
FOE sulfonic acid-Na-salt	*Folsomia candida*	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2010) M-396039-01-1 KCA 8.4.2.1/05
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2013) M-455654-01-1 KCA 8.4.2.1/13
FOE methylsulfone	*Folsomia candida*	NOEC ≥ 50* mg p.m./kg dws	[redacted] (2010) M-392345-01-1 KCA 8.4.2.1/14
	Hypoaspis aculeifer	NOEC 250* mg p.m./kg dws	[redacted] (2009) M-357707-01-1 KCA 8.4.2.1/01
TFA	*Folsomia candida*	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2012) M-436127-01-1 KCA 8.4.2.1/06
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2012) M-436326-01-1 KCA 8.4.2.1/09
FOE 5043-trifluoroethane sulfonic acid	*Folsomia candida*	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2012) M-436128-01-1 KCA 8.4.2.1/07
	Hypoaspis aculeifer	NOEC ≥ 100 mg p.m./kg dws	[redacted] (2012) M-436315-01-1 KCA 8.4.2.1/08
FOE-Thiadone	*Folsomia candida*	NOEC 1.8 mg p.m./kg dws	[redacted] (2012) M-440372-01-1 KCA 8.4.2.1/10
	Hypoaspis aculeifer	NOEC 32 mg p.m./kg dws	[redacted] (2012) M-442897-01-1 KCA 8.4.2.1/11

* endpoints corrected to allow for log P_{ow} > 2

Table 10.4.2- 2 Endpoints for the mixing partner diflufenican

Test substance	Test species	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84
Diflufenican	*Folsomia candida*	NOEC ≥ 438 mg as/kg dws

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

The tier 1 risk assessment on non-target soil macro-organisms (other than earthworms) for the representative formulation DFF+FFA SC 600, flufenacet, and the relevant metabolites is presented in the table below.

Table 10.4.2- 3 TER calculations for other non-target soil meso- and macrofauna

Compound	Species	Endpoint [mg/kg]	$PEC_{soil,max/accu}$ [mg/kg]	TER_{LT}	Trigger
Winter cereals – 240 g a.s./ha					
DFF+FFA SC 600	*Folsomia candida*	NOEC 89*	0.748[1)]	119	5
	Hypoaspis aculeifer	NOEC 32.65*		44	
Flufenacet	*Folsomia candida*	NOEC 31.5*	0.240	131	5
	Hypoaspis aculeifer	NOEC 281*		1171	
FOE oxalate	*Folsomia candida*	NOEC ≥ 100	0.039	2564	5
	Hypoaspis aculeifer	NOEC ≥ 100		2564	
FOE sulfonic acid-Na-salt	*Folsomia candida*	NOEC ≥ 100	0.077	1299	5
	Hypoaspis aculeifer	NOEC ≥ 100		1299	
FOE methylsulfone	*Folsomia candida*	NOEC ≥ 50*	0.015	3333	5
	Hypoaspis aculeifer	NOEC 250*		16667	
TFA	*Folsomia candida*	NOEC ≥ 100	0.275	364	5
	Hypoaspis aculeifer	NOEC ≥ 100		364	
FOE 5043-trifluoroethane sulfonic acid	*Folsomia candida*	NOEC ≥ 100	0.007	14286	5
	Hypoaspis aculeifer	NOEC ≥ 100		14286	
FOE-Thiadone	*Folsomia candida*	NOEC 1.8	0.007	257	5
	Hypoaspis aculeifer	NOEC 32		4571	
Winter cereals – 160 g a.s./ha / Winter cereals – 120 g a.s./ha					
DFF+FFA SC 600	*Folsomia candida*	NOEC 89*	0.498[2)]	179	5
	Hypoaspis aculeifer	NOEC 32.65*		66	
Flufenacet	*Folsomia candida*	NOEC 31.5*	0.160	197	5
	Hypoaspis aculeifer	NOEC 281*		1756	
FOE oxalate	*Folsomia candida*	NOEC ≥ 100	0.026	3846	5
	Hypoaspis aculeifer	NOEC ≥ 100		3846	
FOE sulfonic acid-Na-salt	*Folsomia candida*	NOEC ≥ 100	0.051	1961	5
	Hypoaspis aculeifer	NOEC ≥ 100		1961	
FOE methylsulfone	*Folsomia candida*	NOEC ≥ 50*	0.010	5000	5
	Hypoaspis aculeifer	NOEC 250*		25000	
TFA	*Folsomia candida*	NOEC ≥ 100	0.183	546	5
	Hypoaspis aculeifer	NOEC ≥ 100		546	
FOE 5043-trifluoroethane sulfonic acid	*Folsomia candida*	NOEC ≥ 100	0.004	25000	5
	Hypoaspis aculeifer	NOEC ≥ 100		25000	
FOE-Thiadone	*Folsomia candida*	NOEC 1.8	0.004	450	5
	Hypoaspis aculeifer	NOEC 32		8000	

* endpoints corrected to allow for log P_{ow} > 2

1) Calculated product PECsoil, considering the PECsoil for flufenacet (0.240 mg a.s./kg) and a concentration of 32.1 % flufenacet in DFF+FFA SC 600

2) Calculated product PECsoil, considering the PECsoil for flufenacet (0.160 mg a.s./kg) and a concentration of 32.1 % flufenacet in DFF+FFA SC 600

For DFF+FFA SC 600, flufenacet and the relevant metabolites the TER values exceed the critical trigger value of 5, demonstrating a low risk to Collembola and soil mites if the product is applied up to 0.6 L DFF+FFA SC 600/ha (240 g flufenacet/ha) in winter cereals.

CP 10.4.2.1 Species level testing

Report:	**CP 10.4.2.1/01 [redacted], R.; 2002**
Title:	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)
Document N°:	M-061660-01-1
Guidelines:	SECOFASE, Final Report. Development, improvement and standardisation 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 (certified laboratory)

Materials and Methods:

Flufenacet & Diflufenican SC 600 (active ingredient FOE 5043 and Diflufenican, 32.6 and 16.5 % respectively: 612.28 g/l, TOX no.: 05803-00, Batch no.: 07205/0024(0006)) was mixed homogeneously through standard soil (LUFA 2.1) at five nominal rates, viz. 3.2, 5.6, 10, 18 and 32 mg a.s./kg dry soil. The control was treated with deionised water. Dimethoate at a rate of 4.50 mg a.s./kg dry soil was used as toxic reference.

The bioassay was initiated within 1 hour after application by confining 20 protonymphs of *Hypoaspis aculeifer* per mortality unit (inert glass material). Five units were prepared for the water control, 4 units for each test rate of Flufenacet & Diflufenican SC 600 and 3 units for the toxic reference.

Fourteen days after initiation mortality was assessed. 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. Reproduction units were kept for egg hatch determination for an additional 4-5 days.

Mortality in the treatment groups was compared pair-wise to the water control group using Fisher's Exact test. Egg production (fertile eggs/female/7 days) was compared to the water control group using ANOVA techniques.

Findings:

Low control mortality (10%) and high reproductive performance (24.2 fertile eggs/female/7 days) in the control treatment indicated that test animals were in good condition. The toxic reference, dimethoate, caused 100% corrected mortality. This showed that test animals were sufficiently sensitive and that potential adverse effects of exposure to test item residues could be detected with the set-up used in this experiment.

Summary of findings

Test item	Flufenacet & Diflufenican SC 600			
Test organism	*Hypoaspis aculeifer*			
Test substrate	sandy soil (LUFA 2.1)			
Nominal application volume	150 ml/kg dry soil			
	Mortality after 14 days		Reproduction (fertile eggs/female/7 days)	
Deionised water control	10 %		24.2	
Application rates of Flufenacet & Diflufenican SC 600:	Corrected mortality after 14 days		Reproduction after 7 days (% reduction relative to control)	
3.2 mg a.s./kg dry soil	-3 %	P= 0.608	Not assessed	
5.6 mg a.s./kg dry soil	3 %	P= 0.639	Not assessed	
10 mg a.s./kg dry soil	1 %	P= 0.811	Not assessed	
18 mg a.s./kg dry soil	10 %	P= 0.128	24.0 (99.1 %)	P= 0.843
32 mg a.s./kg dry 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 dry soil		**NOEC ≥ 32 mg a.s./kg dry soil**	

* Statistically significantly different from deionised water control
Statistical analysis: Fisher's Exact test for mortality data and ANOVA/Fisher's LSD test for reproduction data.

Conclusion:

The NOEC for *Hypoaspis aculeifer* based on reproduction and mortality is calculated to be ≥ 32 mg a.s./kg dry soil.

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Report:	**CP 10.4.2.1/02 [redacted], U.; 2011**
Title:	Diflufenican + flufenacet SC 600 (200+400) G: Influence on the reproduction of the collembolan species *Folsomia candida* tested in artificial soil.
Document N°:	M-415903-01-1
Guidelines:	OECD 232 adopted, September 07, 2009: OECD Guidelines for Testing Chemicals - Collembolan Reproduction Test in Soil
GLP	Yes (certified laboratory)

Objective:

The purpose of this study was to assess the effect of Diflufenican + Flufenacet SC 600 (200+400) on survival and reproduction of the collembolan species *Folsomia candida* during an exposure of 28 days in an artificial soil comparing control and treatment.

Materials and Methods:

Diflufenican + Flufenacet SC 600 (200+400) G (analytical findings: 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; density: 1.246 g/mL (20°C), batch ID: EV56002670, sample description: FAR 01538-00, specification no.: 102000007948-03, material no.: 05700094.
Toxic standard: Boric acid.
Control: same application as test item but with deionised water only.
Ten collembolans (9-12 days old) per replicate (8 replicates for the control group and 4 replicates per treatment group) were exposed to control (water treated), 100, 178, 316, 562 and 1000 mg test item/kg artificial soil dry weight at 18 – 22°C, 400 – 800 Lux, 16h light : 8h dark, 5 % peat in the artificial soil. During the test they were fed with granulated dry yeast.

Mortality and reproduction were determined after 28 days.

Findings:
The results can be considered as valid, as all validity criteria of the test were met. Mortality in the control was ≤ 20% (5.0% in this study), reproduction of the control was ≥ 100 juveniles per control vessel (1539.3 juveniles in this study) and the coefficient of variation of reproduction in the control was ≤ 30% (7.6% in this study).

Test item	**Diflufenican + Flufenacet SC 600 (200+400)**		
Test object	***Folsomia candida***		
Exposure	**Artificial Soil**		
mg test item/kg soil dry weight nominal concentration	Adult mortality (%)	Mean number of juveniles±SD	Reproduction (% of control)
Control	5.0	1539.3 ± 117.0	-
100	7.5	1566.0 ± 110.1	101.7 n.s.
178	7.5	1490.0 ± 123.3	96.8 n.s.
316	30.0	1228.0 ± 160.7	79.8 *
562	27.5	335.3 ± 87.6	21.8 *
1000	42.5	155.0 ± 59.3	10.1 *
NOEC (mg test item/kg soil dry weight)			178
LOEC (mg test item/kg soil dry weight)			316

* Statistically significant (Williams' –t-test one-sided-smaller, α = 0.05)
n.s. = statistically not significant (Williams' –t-test one-sided-smaller, α = 0.05)

Observations:
Concerning the number of juveniles statistical analysis revealed statistically significant difference between control and the treatment groups from 316 up to 1000 mg test item/kg artificial soil dry weight.
Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 178 mg test item/kg artificial soil dry weight. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 316 mg test item/kg artificial soil dry weight.

Conclusions:
$NOEC_{reproduction}$: 178 mg test item/kg artificial soil dry weight.
$LOEC_{reproduction}$: 316 mg test item/kg artificial soil dry weight.

CP 10.4.2.2 Higher tier testing

In view of the risk assessment presented above, no higher tier testing is necessary.

CP 10.5 Effects on soil nitrogen transformation

Only endpoints used for the risk assessment are presented here. For an overview of all available endpoints for flufenacet and its metabolites please refer to the respective section of the MCA document.
For the second active substance in the representative formulation, diflufenican, references is made to the EU agreed endpoints according to the EFSA Scientific Report (2007) 122.

Table 10.5- 1 Endpoints for flufenacet and its metabolites used in risk assessment

Test substance	Test species	Endpoint		Reference
DFF+FFA SC 600	Nitrogen transformation, 28 d	No influence	0.6 and 3.0 L/ha	[redacted], 2009, M-357934-01-1 KCP 10.5/01
Flufenacet		No influence	0.62 and 3.1 kg a.s./ha	[redacted], 1994 M-003871-01-2
FOE oxalate		No influence	1.86 kg p.m./ha	[redacted] & [redacted], 2005 M-250511-01-1 KCA 8.5/04
FOE sulfonic acid		No influence	2.455 kg p.m./ha	[redacted] - [redacted], 2005 M-250265-01-1 KCA 8.5/03
FOE methylsulfone		No influence	0.451 and 4.51 kg p.m./ha	[redacted] (2010) M-398568-01-1 KCA 8.5/05
TFA		No influence	0.24 and 1.2 kg p.m./ha	[redacted] (2013) M-444423-01-1 KCA 8.5/06
FOE 5043-trifluoroethane sulfonic acid		No influence	0.123 and 0.615 kg p.m./ha	[redacted] (2013) M-457331-01-1 KCA 8.5/08
FOE-Thiadone		No influence	0.112 and 0.562 kg p.m./ha	[redacted] (2013) M-457326-01-1 KCA 8.5/07

Table 10.5- 2 Endpoints for the mixing partner diflufenican

Test substance	Test	EU agreed endpoints acc. to EFSA Scientific Report (2007) 122, 1-84	
Diflufenican	N-cycle	no influence	test rate not mentioned
AE B107137	N-cycle	no influence	test rate not mentioned
AE 0542291	N-cycle	no influence	test rate not mentioned

Risk assessment for Soil Nitrogen Transformation

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.

Report: **KCP 10.5/01; [redacted], W., 2002**
Title: Diflufenican + flufenacet SC 600 (200+400) G: Determination of effects on nitrogen transformation in soil
Document No: M-357934-01-1
Guidelines: OECD Guideline 216, Adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test.
GLP yes (certified laboratory)

Material and Methods:
Diflufenican + Flufenacet SC 600 (200+400) G (analytical findings: diflufenican, 191.4 g/L, flufenacet, 394.5 g/L; specification No.: 102000007948, batch No.: EV56001418, TOX-No.: FAR 01403-00), Density: 1.229 g/mL was used in the test. A loamy sand soil (according to DIN 'mittel lehmiger Sand') was exposed for 28 d to 0.8 µL and 4.0 µL test item/kg dry weight soil. Application rates were equivalent to 0.6 L and 3.0 L test item/ha. Lucerne-grass-green meal was added to the soil (5 g/kg dry weight soil) to stimulate nitrogen transformation.
The coefficient of variation in the control at the end of the study was 10 %. Therefore the validity criteria for the

Results:
During the 28-day test, 0.8 µL Diflufenican + flufenacet SC 600 (200+400) G/kg dry weight soil and the 5-fold dose of the test item had no relevant influence on nitrogen transformation in a loamy sand soil supplemented with Lucerne-grass-green meal. In none of the time intervals analysed during the 28 day exposure the difference in the daily nitrate-N rates exceeds the trigger value of 25 %.

Effects on non-target soil micro-organisms

Time Interval (days)	Application rates										
	Diflufenican + flufenacet SC 600 (200+400) G										
	Control			**0.8 µL/kg dry weight soil**				**4.0 µL/kg dry weight soil**			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	-1.86	±	0.11	-1.93	±	0.04	**4** n.s.	-1.80	±	0.09	**3** n.s
7-14	1.16	±	0.30	1.13	±	0.07	**2** n.s	1.03	±	0.15	**11** n.s
14-28	1.83	±	0.13	1.79	±	0.08	**3** n.s	1.68	±	0.01	**8** n.s

1) Rate: Nitrate-N in mg/kg dry weight soil/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t Test, two-sided, α = 0.05).

Conclusion: If used as recommended, Diflufenican + Flufenacet SC 600 (200+400) G should not have an impact on nitrogen transformation in soils.

CP 10.6 Effects on terrestrial non-target higher plants

In the first Annex I listing process non-target plant data for a different formulation of flufenacet were submitted and evaluated. The formulation FFA WG60 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 will be presented with this dossier. For the Annex I listing process of diflufenican, also the formulation Flufenacet + Diflufenican SC 600 (DFF+FFA SC600, 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.

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area. Spray drift from the treated areas may lead to residues of a product in off-crop areas.

Flufenacet & Diflufenican SC 600 (Herold SC 600)

Test organism	Study type	Test duration	Lowest ER_{50}	Most sensitive species	References
Terrestrial non-target plants; 6 species	vegetative vigour; Tier 2 dose response	21 days	23.82 g a.s./ha	*Allium cepa*	[redacted], 2002, M-071692-01-1 KCP 10.6.2701
Terrestrial non-target plants; 6 species	seedling emergence; Tier 2 dose response	21 days	190.43 g a.s./ha	*Lycopersion esculentum*	[redacted], 2002, M-072308-01-1 KCP 10.6.2702

RISK ASSESSMENT FOR TERRESTRIAL NON-TARGET HIGHER PLANTS

For herbicides and plant growth regulators, it is considered unprofitable to conduct tier 1 studies as it is inevitable that these will lead to tier 2 or dose response studies in order to generate data suitable for deterministic or probabilistic risk assessments, i.e. ER_{50} values for 6-10 species, representing a broad range of plant species.
Survival, shoot length and fresh weight were assessed. In all species the EC_{50}-figures based on fresh weight were the lowest. These endpoints are used for the risk assessment. In both studies the rates and endpoints are reported as g sum of active ingredients/ha. In order to avoid any confusion these endpoints were not converted to mL product/ha.

Crop	Timing of application (range)	Number of applications	Maximum label rate (range)	Maximum application rate, individual treatment (ranges) [g/ha]		g sum of DFF + FFA/ha
			[L/ha]	Diflufenican	Flufenacet	
Cereals	11-13	1	0.6	120	240	360
Cereals	11-13	1	0.4	80	160	240
Cereals	00-22	1	0.3	60	120	180

In course of the risk assessment these endpoints were compared to application rates converted in g as sum of DFF + FFA/ha (right most column in table above).

DFF+FFA SC 600			
Plant species	**Lowest ER_{50} [sum of g a.s./ha]**	**Parameter**	**Reference**
Vegetative vigour			
Oilseed rape	92.07	Shoot fresh weight	[redacted], 2002; M-071692-01-1 KCP 10.6.2/01
Cucumber	27.75		
Soybean	55.14		
Oat	227.54		
Tomato	**23.82**		
Onion	>332.3		
HC_5 [sum of g a.s./ha]	**11.549***		
Seedling emergence			
Oilseed rape	214.22	Shoot fresh weight	[redacted], 2002, M-072308-01-1 KCP 10.6.2/02
Cucumber	218.41		
Soybean	>332.3		
Oat	207.88		
Tomato	>332.3		
Onion	**190.43**		
HC_5 [sum of g a.s./ha]	**185.685***		

Bold letters: Values considered relevant for risk assessment
*calculated based on [redacted] & [redacted] (2000); greater-than figures were omitted

Risk assessment for Terrestrial Non-Target Higher Plants

Exposure

Effects on non-target plants are of concern in the off-field environment, where they may be exposed to spray drift. The amount of spray drift reaching off-crop habitats is calculated using the 90th percentile estimates derived by the *BBA (2000)*[3] from the spray-drift predictions of *Ganzelmeier & Rautmann (2000)*[4]. Only a single application was considered as factors such as plant growth will reduce residues per unit area between multiple applications. For a single application to a variety of arable crops, 2.77% of the application rate was assumed to reach areas at the edge of the crop (0 meter buffer zone; worst-case scenario). For a 5 m buffer zone a drift rate of 0.57% is assumed.

[3] BBA (2000) Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

[4] Ganzelmeier H., Rautmann D. (2000) Drift, drift-reducing sprayers and sprayer testing. Aspects of Applied Biology 57, 2000, Pesticide Application. Public domain.

Deterministic Risk assessment

According to the Terrestrial Guidance Document[5], the risk to non-target plants is evaluated by comparing the lowest ER_{50} observed in the laboratory studies with the drift rates ($PER_{off\text{-}field}$) including a safety factor of 5. In addition, the usage of drift reducing nozzles is considered.

Table 10.6- 1: Deterministic risk assessment for DFF+FFA SC 600 based on effects on seedling emergence

arable field crops, one application**, 360.0 g sum of a.s./ha**; lowest ER50 = 190.430 g sum of a.s./ha						
Distance	Drift	PER	TER			
[m]	(%)	no drift reduction [g sum of a.s./ha]	No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	9.972	19.10	38.19	76.39	190.96
5	0.57	2.052	92.80	185.60	371.21	928.02
10	0.29	1.044	182.40	364.81	729.62	1824.04

The calculations above clearly show that already for the highest use rate of DFF+FFA SC600 an acceptable risk (i.e. TER>5) can be demonstrated. Hence, no calculations for the lower dose rates are presented here, as they can as well be considered to demonstrate an acceptable risk.

Table 10.6- 2: Deterministic risk assessment for DFF+FFA SC 600 based on effects on vegetative vigour

arable field crops, one application, **360.0 g sum of a.s./ha**; lowest ER50 = 23.820 g sum of a.s./ha						
Distance	Drift	PER	TER			
[m]	(%)	no drift reduction [g sum of a.s./ha]	No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	9.972	**2.39**	**4.78**	9.55	23.89
5	0.57	2.052	11.61	23.22	46.43	116.08
10	0.29	1.044	22.82	45.63	91.26	228.16

arable field crops, one application, **240.0 g sum of a.s./ha**; lowest ER50 = 23.820 g sum of a.s./ha						
Distance	Drift	PER	TER			
[m]	(%)	no drift reduction [g sum of a.s./ha]	No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	6.648	**3.58**	7.17	14.33	35.83
5	0.57	1.368	17.41	34.82	69.65	174.12
10	0.29	0.696	34.22	68.45	136.90	342.24

[5] Anonymous (2002b). Guidance Document on terrestrial ecotoxicology under council directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.

arable field crops, one application, **180.0 g sum of a.s./ha**; lowest ER50 = 23.820 g sum of a.s./ha						
Distance	Drift	PER	TER			
[m]	(%)	no drift reduction [g sum of a.s./ha]	No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	4.986	**4.78**	9.55	19.11	47.77
5	0.57	1.026	23.22	46.43	92.87	232.16
10	0.29	0.522	45.63	91.26	182.53	456.32

According to EU requirements the risk for non-target terrestrial plants is considered acceptable, if a 5 m buffer zone is kept without drift reduction or no buffer zone and a 75% drift reducing spray equipment, if 600 mL product /ha (360 g sum of DFF + FFA/ha) is applied. At lower application rates (400 and 300 mL product/ha; 240 and 180 g sum of DFF + FFA/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.

Probabilistic Risk assessment

In addition to the deterministic risk assessment the Terrestrial Guidance Document recommends the use of the HC_5 (the concentration below which less than 5% of the species will be harmed above the EC_{50} level) which can be calculated from the data sets of ER_{50} growth inhibition levels. The EU guidance document for terrestrial ecotoxicology states: "If the ED_{50} 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 HC_5 itself (TER =1) can be regarded to be protective.

The HC_5 was calculated from the datasets of EC_{50}-growth inhibition levels. As the EC_{50} of shoot fresh weight was the lowest endpoint in all species of both studies, HC_5 calculations were conducted with the two datasets on growth inhibition from the seedling emergence and vegetative vigour.

The HC_5 is calculated according to the following equation ([redacted], T. & [redacted], J.S.; 2000[6]):

HC_5 = 10 exp(avg-ks*std)

With
avg=mean of log10 transformed EC_{50} values
std=standard deviation of log10 transformed EC_{50} values
ks = extrapolation factor

The HC_5 calculation for the seedling emergence and vegetative vigour studies leads to mean values of **185.685** and **11.549 g sum of DFF + FFA/ha**, respectively. The probabilistic risk assessment has been conducted for the lower vegetative vigour endpoints only. The TER calculation is summarised in the following table.

[6] [redacted], T. & [redacted], J.S.; 2000: Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicology and Environmental Safety 46: 1-18 (M-047079-01-1)

Table 10.6- 3: Probabilistic risk assessment for DFF+FFA SC 600 based on effects on vegetative vigour

arable field crops, one application**, 360.0 g sum of a.s./ha**; HC5 = 11.5490 g sum of a.s./ha						
Distance	Drift	PER	TER			
[m]	(%)	no drift reduction [g sum of a.s./ha]	No drift reduction	50% drift reduction	75% drift reduction	90% drift reduction
1	2.77	9.972	1.16	2.32	4.63	11.58
5	0.57	2.052	5.63	11.26	22.51	56.28
10	0.29	1.044	11.06	22.12	44.25	110.62

According to EU requirements the risk for non-target terrestrial plants based on the probabilistic risk assessment is considered acceptable even without any risk mitigation measures, if 600 mL product/ha (360 g sum of DFF + FFA/ha) is applied.

CP 10.6.1 Summary of screening data

For herbicides and plant growth regulators, it is considered unprofitable to conduct tier 1 screening studies as it is inevitable that these will lead to tier 2 or dose response studies in order to generate data suitable for deterministic or probabilistic risk assessments, i.e. ER_{50} values for 6-10 species, representing a broad range of plant species. Therefore, no screening studies were conducted for flufenacet or its representative formulation.

CP 10.6.2 Testing on non-target plants

This study was already submitted and evaluated for the Annex I listing process of diflufenican. Nevertheless, a full study summary will be presented below.

Report: **CP 10.6.2/01** [redacted], **W., 2002**
Title: Flufenacet & Diflufenican SC 600: Vegetative Vigour Test on terrestrial non-target plants of 6 families (2 Monocotyledoneae, 4 Dicotyledoneae).
Document No: M-071692-01-1
Guidelines: OECD Guideline for the Testing of Chemicals, Proposal for updated Guideline 208: "Terrestrial (Non-Target) Plant Test 208 B: Vegetative Vigour Test", Draft Document, July 2000
GLP yes (certified laboratory)

Material and Methods:
Seeds of two monocotyledoneous species (*Allium cepa, Avena sativa*) and four dicotyledoneous species (*Brassica napus, Cucumis sativus, Glycine max, Lycopersicon exculentuni*) were planted in a standard loamy sand, LUFA Sp2.2, and were allowed to emerge and grow until the two-leaf stage was reached. Then 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 test containers. The concentration of the test item in the highest test solution was analytically verified. Following application of the test substance, the development of the plants was observed for 21 days.
The test was performed in a growth chamber at a temperature of 22 ± 3 °C and lighting of 13000 ± 2000 lx (16 hours per day). The test containers were placed randomly at the beginning and were re-arranged several times during the incubation period. 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.

Deviations: Steinberg nutrient solution was used instead of Hoagland solution as proposed by the draft guideline. The organic carbon content of the soil was higher than the recommended value and the number of plants per species and treatment level was reduced (30 - 32 instead of 40).

Findings:
The validity criterion was met. Mean number of control plants that died during the test should be < 10% (0% in this study). 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 ingredients).

	Plant species					
	Monocotyledoneae		Dicotyledoneae			
21 days after 50 % emergence of controls	***Allium cepa***	***Avena sativa***	***Brassica napus***	***Cucumis sativus***	***Glycine max***	***Lycopersicon esculentum***
	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]	270.30	102.44	> 332.3 [1]
NOEC	≥ 332.3 [2]	32.1	32.1	10.0	> 332.3 [2]	32.1
LOEC	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	23.82
NOEC	≥ 332.3 [2]	32.1	32.1	3.2	3.2	32.1
LOEC	n.d.	103.4	103.4	10.0	10.0	103.4

[1] EC_{50} could not be calculated because of less than 50 % effect, therefore estimated to be > 332.3 g a.s./ha.
[2] no significant effect within the range tested
n.d. not determined

In summary the NOEC for the monocotyledoneous species was 32.1 g a.s./ha and the LOEC 103.4 g a.s./ha. Among the two species, *Avena sativa* was more sensitive. Its fresh weight was reduced by 50 % at 227.54 g a.s./ha, whereas a 50 % inhibition of *Allium cepa* was not observed within the range of concentration tested.
Among the dicotyledoneae *Cucumis sativus* and *Glycine max* were the most sensitive species. The NOEC on the fresh weight was 3.2 g a.s./ha and the LOEC 10.0 g a.s./ha. The other species, *Brassica napus* and *Lycopersicon esculentum* were less sensitive: the NOEC on the fresh weight was 32.1 g a.s./ha and the LOEC 103.4 g a.s./ha. The EC_{50} for the fresh weight ranged from 27.75 to 92.07. Thus, the dicotyledoneae were more sensitive than the monocotyledoneous species tested. The fresh weight was the more sensitive endpoint compared to the shoot length.

Observations:
Effects were observed soon after application. At day 7 following application the dicotyledoneous species were stronger affected than the monocotyledounous ones. Chlorosis was the most frequently observed effect. Even at 3.2 g a.s./ha, the lowest application rate one third of *Lycopersicon esculentum* and two third or even more of the other dicotyledoneous species showed chlorosis. Leaf deformations or wilting was most pronounced with *Cucumis sativus*. The monocotyledoneous species showed only chlorosis with *Avena sativa* being the more sensitive species.
At day 14 and 21 chlorosis as well as deformations or wilting were both observed frequently. The monocotyledoneous species showed different patterns: *Allium cepa* had mostly wilted leaftips,

whereas chlorosis was more frequent with *Avena sativa*. At 32.1 mg a.s./ha or higher most plants of the dicotyledoneous species were affected. At lower rates, chlorosis was more often observed than wilting or deformations of the leaves.
In general, effects on the fresh weight were more pronounced than on the shoot length. An EC_{50} 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 (27.75 g 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).

Conclusion:
Most sensitive parameter was the fresh weight followed by shoot length. The most sensitive species was *Lycopersicon esculentum* with an EC_{50} of 23.82 g a.s./ha (fresh weight) followed by *Cucumis sativus* (EC_{50} of 27.75 g a.s./ha – fresh weight). Phytotoxic effects appeared as mainly chlorotic spots.

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This study was already submitted and evaluated for the Annex I listing process of diflufenican. Nevertheless, a full study summary will be presented below.

Report:	**CP 10.6.2/02; [redacted], W., 2002**
Title:	Flufenacet & Diflufenican SC 600: Seedling Emergence and Seedling Growth Test on terrestrial non-target plants of 6 families (2 Monocotyledoneae, 4 Dicotyledoneae).
Document No:	M-072308-01-1
Guidelines:	OECD Guideline for the Testing of Chemicals, Proposal for updated Guideline 208: "Terrestrial (Non-Target) Plant Test 208 B: Vegetative Vigour Test", Draft Document, July 2000
GLP	yes (certified laboratory)

Materials and Methods:
Seeds of two monocotyledoneous species (*Allium cepa, Avena sativa*) and four dicotyledoneous species (*Brassica napus, Cucumis sativus, Glycine max, Lycopersicon esculentum*) were planted in a standard loamy sand, LUFA Sp2.2. 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. Soils were supplied with water or nutrient solution by glass fibre wicks. The test was performed in a growth chamber at a temperature of 22 ± 3 °C and lighting of 13000 ± 2000 lx (16 hours per day). The test containers were placed randomly at the beginning and were re-arranged several times during the incubation period. At day 7 and 14 after 50% of the control seedling had emerged, a visual inspection was done. At day 21 the plants were counted and, visually inspected and harvested to determine their shoot length and biomass (fresh weight).

Deviations: Steinberg nutrient solution was used instead of Hoagland solution as proposed by the draft guideline. The organic carbon content of the soil was higher than the recommended value and the number of plants per species and treatment level was reduced (30 - 32 instead of 40).

Findings:

As less than 10 % of the control plants died and most control plants developed healthily, the quality criteria of the draft guideline and the study plan have been fulfilled.

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).

	Plant species					
	Monocotyledoneae		Dicotyledoneae			
21 days after 50 % emergence of controls	***Allium cepa***	***Avena sativa***	***Brassica napus***	***Cucumis sativus***	***Glycine max***	***Lycopersicon esculentum***
	Flufenacet & Diflufenican SC 600 (g a.s./ha in 300 L/ha)					
survival 1)						
EC_{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						
EC_{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						
EC_{50}	190.43	207.88	214.22	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.

1) no. of surviving plants
2) EC_{50} 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.

The NOEC for both monocotyledoneous species was 32.1 g a.s./ha and the LOEC 103.4 g a.s./ha. Among these two species, *Allium cepa* was slightly more sensitive and its fresh weight was reduced by 50 % at 190.43 g a.s./ha.

Among the dicotyledoneous species *Brassica napus* was the most sensitive one. The NOEC on both fresh weight and shoot length was 3.2 g a.s./ha and the LOEC 10.0 g a.s./ha. The EC_{50} could only be determined for the fresh weight of *Brassica napus* and *Cucumis sativus* and was in a similar range as for the two monoeotyledonous species.

Observations:

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). Only very few dead plants of *Allium cepa* and *Cucumis sativa* were found.

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. Few additional dead plants of *Allium cepa* were found at the highest concentration and of *Glycine max* at 10.0 and 103.4 g a.s./ha.

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. Few dead plants of *Allium cepa* and *Avena sativa* were found at the highest application rate.
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.

Conclusion:
The most sensitive parameter was the fresh weight followed by shoot length, then survival. Effects of the test item on seedling emergence and growth were not severe and an EC_{50} could not be derived for all species and endpoints where effects were less than 50 %. The most sensitive species was *Allium cepa* with an EC_{50} of 190.43 g a.s./ha (fresh weight) followed by *Avena sativa* (EC_{50} of 210.99 g a.s./ha). Phytotoxic effects appeared as mainly chlorotic spots.

CP 10.6.3 Extended laboratory studies on non-target plants

In view of the results presented above, no further studies are deemed necessary.

CP 10.6.4 Semi-field and field tests on non-target plants

In view of the results presented above, no further studies are deemed necessary.

CP 10.7 Effects on other terrestrial organisms (flora and fauna)

No studies are required.

CP 10.8 Monitoring data

No ecotoxicological monitoring data available.