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Ŷ Version history Document identifier and Data points containing amendments or additions¹ and Date [yyyy-mm-dd] brief description Gersion number 2021-03-25 Original MCP as submitted by applicant Ĩ M-766068-01-1 The segment and the applicable beam indication and the segment and th M-76 2021-07-05 Addition of reliability assessments for aquatic organisms Ą and update of aquatic macrophyte endpoint and risk



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CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION **PRODUCT**

Fluopyram was included in Annex I to Council Directive 91/414/EEC in 2013 (Regulation (EU) 802/2013 into Force on August 22nd 2013). This Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of Fluopyram under Council Directive 91/414 EEC and which were therefore not evaluated during the first EU review. All data which were already? submitted by Bayer AG (former Bayer CropScience) for the Annex I inclusion under Council Directive 91/414/EEC are contained in the Draft Assessment Report (DAR) and its Addenda and are included in the Baseline Dossier provided by Bayer.

The formulation fluopyram + trifloxystrobin SC 500 (250+250 g/L), aboreviation FLU+TFS SC (250+250), is a Suspension Concentrate (SC) formulation containing 250 g/L of fluopyram and 250 g/L trifloxystrobin. This formulation is registered throughout Europe under trade usines such as Luna Sensation, Luna Sensation SC and Moon Sensation. ELU+TES SES500 (250+250) was not a representative formulation of Bayer AG for the Antex I indusion of Fluopyram under Council Directive 91/414/EEC.

FLU+TFS SC 500 is an end use preduct proposed for use in the field on games and for soil-less cultivation in greenhouse based on the application pattern shown below.

Fable 10.1- 1: Latended application pattern 5		
Crop Of application (range) (days)	Vaxinfum Mabel rate (range)	Maximum application rate, individual treatment (ranges) [kg a.s./ha] Fluopyram
Grapes 3° BBCH 53 3° 2° 0° 7°	0.2	0.050
Lettuce (soil-less cultivation, high-tech greenhouse)	0.8	0.200



Definition of the residue for risk assessment

The definition of the residue for risk assessment has been derived in the environmental fate chapter (see MCA 7.4.1). For ecotoxicology only soil, surface water and sediment are elevant environmental compartments. The residue definition for risk assessment is therefore given as

Table 10.1- 2:	Definition	of the residue	for risk	assessment

compartments. If	ie residue definition for fisk assessment is therefore given as
Table 10.1- 2:	Definition of the residue for risk assessment
Compartment	Residue definition for risk assessment
Soil	Fluopyram, Fluopyram-7-hydroxy, Trifluoroacetic acid (TFA)
Groundwater	Fluopyram, Fluopyram-7-hydroxy, Trifluorogeetic aeid (TFA)
Surface water	Fluopyram, Fluopyram-7-bydroxy, Prifluoroacetic acid (TFA)
Sediment	Fluopyram
Air	Fluopyram

EFSA (2019) provided guidance on how to document the results of metabolism and residue studies in plants and animals for consideration in the ecotoxic@ogical_risk assessment. Ô

As part of this guidance, a template was provided for a "question are" for the ase of residue data extracted from Vol. 3 B.% to support the cotox cological assessment of pesticides.

According to EFSA (2019), The respective RMS may consider this question are as useful in their Ş assessments. Ô

Therefore, the questionnaire with the information from the relevant studies with fluopyram is provided on the following page Ø

Ø

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Data Points	KCP Section 70/01 C C
Report Author:	
Report Year:	$2021$ $\swarrow$ $\checkmark$ $\checkmark$ $\checkmark$
Report Title:	Pluopyram: Residue information supporting the ecotoxicological assessment
Report No: 🖉 🦂	EnSterior Co
Document No:	<u>M2763894001-1</u> 0 0 0
Guideline(s) followed in	
study: 🔬	
Deviation from current	Current Guideline: pot applicable
test guideline:	$\sqrt{2}$ $\sqrt{2}$ $\sqrt{2}$
Prexious evaluation	No, not previously submitted
GLP/Officially	not applicable
recognised testing	
facilities:	
Acceptability/Reliability:	Yes



Metabolism in primary crops

Reference material: Test No. 501: Metabolism in Crops (OECD, 2007a)

**Question 1:** Are the provided metabolism studies in primary crops submitted in the residue section sufficient to depict a metabolic pathway of residues? If yes, which are the crop groups covered by the available metabolism studies?

Is a metabolism study available in a crop that belongs to the same metabolism crop group than GAP(s) under assessment? Please provide an overview of the available information.¹

#### Applicant response:

The metabolism studies are compliant with the use patterns sought (type of appreciation, dose pite, BBCH growth stage, PHI). They are presented in Vol. 3 B.7 of the DAB. No data gas is identified as 3 crop groups are represented (foliar) and the metabolism is similar. A metabolism study grapes is available in a crop that belongs to the same metabolism crop group as the GAP(s) under assessment (grapes and apple).

Report reference	Author, Year	Crop Crop	Crop	Application	Fluopysäm label
<u>M-282177-01-1</u>	2006	Fruit (19)	Grages	Roliar 2	[UL) ¹⁴ C-phenyl]
<u>M-282460-01-1</u>	2006 ~				Q2,6-14 pyridyl]
<u>M-286400-01-1</u>	2607	Root cops	O S		[Uto ¹⁴ C-phenyl]
<u>M-286531-01-1</u>	20079	(R) (R) (C)		Fonar 2	2,6- ¹⁴ C-pyridyl]
<u>M-283161-02-1</u>	2001 ×	Pulses and .		d alian a	[UL- ¹⁴ C-phenyl]
<u>M-299067-010</u>	2908			^v rollar	[2,6- ¹⁴ C-pyridyl]
<u>M-298790701-1</u>	2,008	Espit (F)	Bell .	Drip	[UL- ¹⁴ C-phenyl]
<u>M-298741-01-1</u>	2008		pepper	IIIgation	[2,6- ¹⁴ C-pyridyl]
<u>M-345948-091</u>	2009	Coeal/Gross	Wheat	Seed treatment	[UL- ¹⁴ C-phenyl] & [2,6- ¹⁴ C-pyridyl]
<u>M-615284,01-1</u>			Dias	Falian	[UL- ¹⁴ C-phenyl]
<u>M-615282-01-1</u> *	2018	wuscentaneous	Rice	Follar	[2,6- ¹⁴ C-pyridyl]

The following metabolism studies are available for Fluopyram:

UL : uniformly labelled. *For information only, not relevant for the crops during AIR review, will not be detailed further.

Metabolism Fudies have been conducted in three crop groups with foliar applications, namely fruit (F), root (R) and Fulses and oilseed (PO). Since the metabolism is similar in all three crop groups thus all three crop groups are covered. Additional studies are available covering rice, the drip

¹ The metabolism study should be conducted on a crop which belongs to the crop category representative of the GAP/intended use/representative use (e.g., a metabolism on fruit crops should be provided to support the GAP on pome fruit). It is also relevant to highlight that the metabolism study should be compliant with the GAP in terms of type of application (foliar, soil treatment, etc.), location, covering the dose rate of application, BBCH growth stage at application, PHI.



irrigation and seed treatment uses. Apart from the wheat seed treatment study, and the rice study, all of the foliar applied metabolism studies have been previously reviewed at the EU level; the following, conclusion was drawn from these studies:

all of the second secon EFSA Journal 2013;11(4):3052: "After foliar applications, fluopyram constitutes the prajor component of the radioactive residues, accounting for more than 85% TRR in grape, potate leaves and bean leaves, collected 4 to 51 days after the last application. Fluopyram was however observed in lower proportions in potato tubers and bean seeds, representing 5% to 21% TRR. In these matrices





**Question 2:** Which are the plant metabolites recovered in the study(s) in relative amount and absolute amount (greater than 10 (TRR %) and/or 0.05 mg/kg)² addressing the metabolic pathway of the representative use(s) ³?

#### **Applicant response:**

The metabolite pattern can be regarded as similar in all primary crops and under different application techniques (spraying, drip irrigation, seed treatment (see below, question 5)). Parent (see below, question 5)). Parent (see below, question 5)).

The metabolism of fluopyram consists as a first step, of the hydroxylation of the patent compound to the metabolites fluopyram- 7-OH (M08) and fluopyram-8-OH (M18), which undergo finither loxose conjugations. Cleavage of the hydroxylated metabolites and subsequent oxidation give two distinct groups of metabolites; those containing the trithuoromethyl-prenyl moiety [fluopyram-benzamude (M25), fluopyram-benzoic acid (M33)] and those containing the pyridyl moiet [fluopyram-DAA (M40), fluopyram-PCA (M43)].

	$\sim$		
	Overall	Maximum Conc	en@ation.(foliar and drip)
Metabolite	%JRR	fyg parent eq./kg	Comment 5 5 2
	A.0	0.42	Grape leaves
	0.35		愛raper Summer Cut: Faves BBC與71)、②
Fluopyram-7-hydroxy	J.6 🔊	0.60	Bean folizage
AE C656948-7-hydroxy / M08	1.1	<b>62</b> 0 0	Bean straw 5
BCS-AA10065		90.63 5 0	Pepper (interprediate plant) drip irrigation
	6.8	0,24 5	Depper (Sest of plant) drip irrigation
	04.80	×9.36 🏷 🛓	Potato leaves
	0.7	0.35	Grape leaves
Fluopytam-7-hydroxy-glc	0.2	\$12 J	Grape (Summer Cut: leaves BBCH71)
AE C656948-7-hydroxy-glc M11	<u>8.9                                    </u>	0.31 2	Pepper (rest of plant) drip irrigation
	0.6 🔊	0.25	Bean foliage
	0.0	Ø.14 O	Bean straw
Fluopyram-7-hydroxy-glc-AQA	3.2 0	1.220	Bean foliage
AE C656948-7-hydroxy-glc-MA M12 (conjugate of M08)	4.7	-0.590	Bean straw
	Ø8 ×	0.34	Grape leaves
Fluopyram-8-hydroxy AE C656948-8-hydroxy / M18	0.2	0.13	Grape (Summer Cut: leaves BBCH71)
	0.5	0.21	Bean foliage
	Ģ		

² These trigger values of 0.05 mg/kg or 10%TRR of total radioactive residues are only meant as guidance. In some circumstance, generally governed by toxicological concerns, it may be necessary to identify terminal metabolites, which are present a concentrations lower than 0.05 mg/kg or <10%TRR of total radioactive residues (European Commission, 1997). ³ For the ecotox section, a selection of the relevant metabolites should reflect only the representative uses. It is not necessary to cover the residue situation for consumer risk assessment but the expected residue situation in the field for the use(s) under assessment. It is recommend consulting whether metabolism studies were summarized following harmonized templates for further assessment (I.e. EFSA/OECD templates).



	0.0	0.17	D (
	0.9	0.17	Bean straw
Fluopyram-hydroxy-glyc-gluc AE C656948-hydroxy-glyc-gluc M22	10.4	0.013	Dry beans
	51.6	0.04	Succulent bean
Fluonvram-benzamide	64.0	0.08	Dry bean
AE C656948-benzamide	0.5	0.17	Bean foliage
AE F148815	0.6	0.10	Bean draw
BCS-AA10014	10.1	0.36	Pepper (rest of plant drip in gation
M125	16.1	0.006	Pepper (fruit) drip irrigation
	0.5	\$ <del>2</del> 3	Potato Reaves
Fluopyram-hydroxyethyl-glc AE C656948-hydroxyethyl-glc M35	0.2	0.062 0	Beany foliage
Fluopyram-hydroxyethyl-di-glc	7.00 .	B 16 ~	PepperQrest of plant) Arip irrigation
M36	$\langle , , , \rangle$		
Fluopyram-pyridyl-acetic acid	29,5%	0.05	Succulent bean
AE C656948-pyridyl-acetic acid	2296	0.07	Dry Dean J J J
Fluopyram-PAA-glycoside AE C656948-PAA-glycoside (7942	38.0	0423	Pepper (fruit) dop irrigation
	3.0	0.05 0	Succulent bean
	\$32.5 S	0.14 ~	Dry bean
L 5 19	0.60	Dil v	Beangstraw
Fluopyram-pyridyl-carboxylic acid		0.19	Bean foliage
AE C656948-pyrid -carbo ylic acid /	43.5~	0.028 2	Pepper (fuit) drip irrigation
PCA / AE C657 138 / M43	0.8	033	Orape Vaves
	0.3	0.21	Graße (Summer Cut: leaves BBCH71)
	49.8	QQ906 , S	Potato tuber
	A 1	Y U A	· · · · · · · · · · · · · · · · · · ·

Based on the metabolism date and field residue trials, the definitions of residues in plants were established by EFSA:

× 4	<b>Residue</b> definition		Reference				
Food of a pat	Monitoring ~	Duopyram (parent only)	EFSA Scientific				
rood of prain	Diate accodement	fluopyram and fluopyram-benzamide (M25)	Report EFSA Journal				
		expressed as fluopyram	2013;11(4):3052				
· \							
Ø							
RMS comment:	RMS comment:						
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	L'						
Č ^{O.}							
-							



**Question 3:** Is any translocation of pesticide residues observed in the different parts of the plants? Could it be drawn a general conclusion on translocation of residues based on the available data?

I.e. is there any particular distribution of the residues observed in specific plant tissues (leaves, grans, roots, etc.)? Is this occurring over time?⁴

#### Applicant response:

A transport via the xylem moves a chemical into regions with high water losses, particularly to the solder leaves. On the other hand, phloem mobility moves a chemical to sizes of utilization of products from photosynthesis, particularly to roots, growing points, developing seeds and frues.

Following application of radiolabelled Fluopyram to grapevine, potatoes, beans, red bell pepper and wheat employing both ¹⁴C-labels, the highest radioactive residue levels (PRR values) were observed in leaves and foliage of the treated plants, whereas the fruits (grapes, potato tubers, beans, bell pepper fruits and wheat grain) contain comparable low TRR levels. The major residue component of the TRR is the parent substance Fluopyram. Therefore, high Fluopyram levels were observed in feaves, and foliage; low levels were observed in fluits, tubers and seeds. This residue pattern shows that Fluopyram is xylem mobile, at least to a vertain extent.

However, it should be admitted that most of the residue in on the leaves after foliar application is assumed to consist of immobilized tesidue on the plant surface. This behaviour can be derived from the relative high residue levels in on foliage of graperine, potatoes and beans (foliar application) compared to the far lower residue levels in foliage of bed bell pepper and wheat (or papelication and seed treatment). On the other hand, the Flue pyram residues in succeeding crops (uptake via the roots) were higher in foliage than in seeds and roots suggesting a certain xylem transport (see next question).



#### Metabolism in rotational crops

Reference materia: Test No. 502: Metabolism in Rotational Crops (OECD 2007b), Test No. 504: Residues in Rotational Crops (OECD, 2007i)

**Question 4:** Do results of the rotational crops show any translocation of residues (uptake from soil) from roots to the aerial parts of the plant⁵? If so, which metabolites might be of relevance?

Is there any indication of accumulation of residues over time occurring in the rotational crop scenario? If so, in which crop categories (heafy, roots, cereals)/crop parts is the accumulation observed?

#### Applicant response:

The metabolism of Thropyram (AE C656948) was investigated in rotational crops (spring wheat, Swiss chard and turning) following soil application of either [phenyl-UL-14C] or [pyridyl-2,6-14C]

⁴ Special attention must be given to compare results at same BBCH/sampling time; particularly, for avoiding erroneous assessments due to crop growth and dissipation.

⁵ It must be noted that this information may not only refer specifically to the succeeding crops/crops growing in rotation; but also, it may be useful to give indications on a possible residue situation for the new emerging plants in the crop area after certain uses. For instance, the data can be used to disregard a possible residue situation to non-target organisms originated due to the consumption of contaminated seedlings /residues in weeds.



radiolabelled active substance. The application rates (534 and 514 g a.s./ha, respectively) were slightly higher than in agricultural practice (2x250 = 500 g a.s./ha was the anticipated maximum seasonal application rate).

The plant back intervals were 30, 139 and 280 days for all crops.

TRR accumulated at  $\geq 0.01$  ppm in all rotated crop matrices from all PBIs, except turnip roots from the 280-day PBI. TRR ranged from 0.009 ppm in turnip roots planted 280 days after soil application to 6.156 ppm in mature wheat straw planted 30 days after soil application. TRR generally declined with the later plantback intervals, except in wheat forage which increased at the 139-day PBJ, but decreased at the 280-day PBI, to ~2x the initial value. The TRR values for all FACs are given in the following table (Table B.7.9-1 from DAR).

Table B.7.9-1 : Total Radioactive Residues (TROS) in the different Racs of the three rotations (expressed as parent compound equivalents ing/kg)

		- N - O				× ·	/
TRR	Wheat		Û.	Ø	Swiss 0	turnip	à l
[mg/kg]	forage	hay	straw	Grain 4	char	leaves	Toots (
1 st rotation (30 days)	0.100	1.783	156 C	0.167	0.590	0.884	0.065
2 nd rotation (139 days)	0.78	1120	3.450	0.09	\$377	0.113	0.013
3rd rotation (280 days)	0,007 %	1.527	1.052	00923	0.164	0.10	0.009
	al (//)¥						

### Excerpt from DAR, Vol 3 B.7

"Parent AE C656948 accounted for the major part of the residues in all RACs of all rotations and covered 56 – 84% of the TRR in the RACs of the 1st rotation, 33 – 78% of the TRR in the RACs of the 2nd rotation and 28 – 59% of the TRR in the RACs in the 3rd rotation. In general, the levels of the parent compound decreased with subsequent plantback intervals. AE C656948-7-hydroxy and its various conjugates with glucose, malonic acid 22 isomers) and support acid were important metabolites mainly in Swiss chard, where the AE C656948-7-hydroxy yielded 21% of the TRR in the 1st rotation increasing to about 35% of the TRR in the following rotations. In the other RACs, the amount of AE C656948-7-hydroxy was distinctively lower; 10% TRR, except in wheat hay and straw from the 3rd rotation increasing from 7% of TRR in the 1st rotation to 46% and 12% of the TRR in the 2nd and 3rd rotation, respectively AE C656948-7-OH-SA was also a prominent metabolite in Swiss chard increasing from 7% of TRR in the 1st rotation to 46% and 12% of the TRR in the 2nd and 3rd rotation, respectively AE C656948-7-OH-SA was also detected at low levels in turnip leaves (0.7-1.0% TRR; 30 and 129-day PBIs), but not in the other rotated crop RACs.

AE C656948-8-hødroxy and its conjugate were only of minor importance. Both or at least one of them were detected in all RACs but at very low devels of <2.7% of the TRR in sum. AE C656948-phenolglc was detected in turnip Gaves only, where it amounted to 10%, 16% and 10% of the TRRs of the 1st, 2nd and 3rd rotation, respectively. Two label specific metabolites were identified: AE C656948benzamide and AE C656948 benzoic acid AE C656948-benzoic acid accounted for 0.6-6.9% TRR in wheat forage, hay and grain, and turnip leaves and roots from the 30-day PBI; 0.3-0.4% TRR in wheat forage and hay, and 13.6% TRR in wheat grain from the 139-day PBI; and 13% TRR in wheat grain from the 280-day PBI. AE C656948-benzamide accounted for 2.8-9.7% TRR in wheat forage, hay, straw and grain, and turnip leaves and roots, and 11.1% TRR in Swiss chard from the 30-day PBI; 3.2-7.4% TRR in all RACs from the 139-day PBI; and 5.9-8.0% TRR in wheat forage, hay, straw and grain, and turnip leaves and turnip leaves from the 280-day PBI.

The metabolism of [phenyl-CL-¹⁴C]AE C656948 in confined rotational crops corresponds very well with the metabolism of confined rotational crops after application of [pyridyl-2,6-¹⁴C] AE C656948."

Apart from parent (main component found), the metabolites (greater than 10 (TRR %) and/or 0.05 mg/kg) are described in the table below:



<b>17</b> , <b>1 1</b> ,	Overall Maximum Concentration (CRC)					
Metabolite	%TRR	mg parent eq./kg	Comment <i>Q</i> °			
Fluopyram-phenol-glc AE C656948-phenol-glc / M06	10.4	0.092	Turnip leaf			
Fluopyram-7-hydroxy	12.6	0.193	Wheat Hay			
AE C656948-7-hydroxy / M08 / BCS-AA10065	7.4	0.494	Wheat Straw			
Fluopyram-7-OH-SA AE C656948-7-OH-SA / M10	16.8	0.058	Swiss chard Swiss chard			
Fluopyram-7-hydroxy-glc AE C656948-7-hydroxy-glc / M11	3.1	0.203	Wheat Straw			
(conjugate of M08)	3.4		Wheat Day 6			
Fluopyram-7-hydroxy-glc-MA AE C656948-7-hydroxy-glc-MA / M12 (conjugate of M08)		0.448 0 5	Wheat Hay 🔨 💭 Wheat Straw			
Fluopyram-8-hydroxy AE C656948-8-hydroxy / M18		0.087	Wheat Straw			
Fluopyram-benzamide AE C656948-benzamide		90.095 7 5 0 169 0 0	Wheat Hay			
AE F148815 / BCS-AA10014 / M25 0	1101 5	1008 <u>(</u> ) )	Swess chard			
Eluonurom honzoio ocid	9.7	0.086	Turnip leaf			
AE C656948-benzoic acid M33			Wheat grain			
Fluopyram-pyridyl-catorylic@cid	₩.9 ° °	10.088 × <u>k</u>	Wheat forage			
AE C656948-pyrid carboxylic actor / PCAC	0.9 .9	00060	Wheat straw			
AL COST 1887 1945	35.9	0.230	Wheat grain			
Fluopyram-methyl-suffoxide AE C656948-methyl-sulfoxide	49.07	0,035	Wheat grain			
RMS comment:		0°				
õ						



**Question 5:** If the GAP is for a seed treatment or other pre-emergency⁶ treatment, is any information related to the magnitude of residues at early post-emergence (BBCHs<10) for the crop(s) under assessment?

#### **Applicant response:**

Although the soil spray + incorporation use or seed treatment uses are not included among the representative uses sought for the Fluopyram renewal, the seed treatment study is presented here for the sake of completeness.

The metabolism of fluopyram was investigated in wheat after seed treatment with [paenyl  $L^{-14}$ C]AE C656948 and [pyridyl-2,6-¹⁴C]AE C656948 formulated as SC 500. Due to the few intended dressing rate of 1 g a.s./dt (decitonne = 100 kg) in apricultural practice, only an overdose experiment has been conducted with a dressing rate of approx 10 g a.s./dt

Wheat forage and hay were collected as intermediate plant samples and wheat so aw and grain were harvested at maturity.

Parent compound was the predominant residue in all plant matrices. Hydroxytation of the test item was detected as the main metabolic path, resulting in AE C6569487-hydroxy and AE C65694848-hydroxy. Subsequent conjugation of the hydroxylated metabolites with glucose and malonic acid followed. As a consequence, AE C656948-7-hydroxy-glc MA and AE C656948-8-bydroxy-glc-MA were detected. Hydrolytic cleavage of the hydroxylated metabolites was observed, as welloAE C656948-benzamide was identified as direct cleavage product of AE C656948-9-hydroxy. Subsequent hydrolysis of the metabolite resulted in AE C656948-benzamide acid, AE C656948-9-hydroxy. Subsequent hydrolysis of the metabolite resulted in AE C656948-benzamide. AE C656948-benzamide. AE C656948-benzamide.

Because this is a seed freatment, the parent comported fluopyram (AE C656948) is also subjected to metabolic conversion in the soil. Detabolites formed by molecule cleavage may also be related to the degradation of the set it m in the soil. Uptake of these metabolites via the foots could be - at least in part - the reason for their occurrence in the plant matrices.

			<u> </u>	
	Nº V	Overall M	taximum Co	ncentration (seed treatment)
Metabolit			mg	
A state		STRR 🔬	parrent 🔬	Comment
× ¥			eq./kg	
Fluopyram-7-hyd	roxy a 65	N. 1		
AE C656948-7-h	droxy M08	10.1 ×	0.053	Wheat straw (seed treatment 10X overdose)
BCS-AA1006		Ŭ O		
Fluopyram, 7-hyd	roxy-glc-the A	12,2	¢0.017 O	Wheat forage (seed treatment 10X overdose)
AE C656948-7-h	ydroxy-glc-MA	15.2 3	0.073	Wheat straw (seed treatment 10X overdose)
M12 (conjugate	of <b>M08</b> ) 🕺 💊	11.9	0 <b>0</b> 34	Wheat hay (seed treatment 10X overdose)
Fluopyram-benza	mřelě 💭 👡		C C	
AE C656948-ben	zamide 💞 🖉	10.4	0.01	Wheat grain (seed treatment 10X overdose)
AE F148815: BC	Š-AA10014 (20125 .			
J. J				
RMS comment				
Ĵ, Ĝ	A S			
	T ST			
Q				

⁶ Consideration for the seedling scenario, relevant for bird & mammals and the guttation water scenario for bees might be necessary.



Magnitude of the residues in supervised residue trial Reference material: Test No. 509: Crop Field Trial (OECD, 2009); Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs (European Commission, 2017)

Question 6: From the supervised residue trials, is there any indication of a sesidue decline over time?^{7,8} If so, please indicate the reference to the residue trial and the part of the plants where the decline was observed.

Were the residue determinations performed at 0 days after the last application or at a given time close to the last application(s)?⁹

#### **Applicant response:**

Residue trials were conducted for all representative uses for grapes and lettuce and all present decline data that can be relevant for the ecotoxicology risk assessment. These supervised esidue trials are summarised and referenced within Appendix 2 of this document.

In the 16 trials performed for grape (bunch of grapes), samples were taken at BBCH O (in Smials)  $\swarrow$  79, 85 and 89. The last sampling was performed between O and to 4 days after the last of two applications. Samples were taken immediately after the last application, with exception of 4 trials, in which the last application was performed at BBCH 69.  $\bigstar$  gradual decline in the readule level of fluopyram was observed in all trials with a light upturn observed in some cases at the last sampling. Residue levels of the metabolite AP C656948-benzamide were <LOQ (<0.01 mg/kg) with only few exceptions.

Residue levels of fluopyram were also found to desine gradually in all 12 trial sperformed for lettuce. Two applications were performed 14 and 7 days before harvest and samples were taken prior and after the last treatment (days -0 & 0) and 3, 7 44 and 21 (in Finals) days after that While the residue level of the parent compound remained above the LOQ, AD C656 48-benzamide was often below or slightly above the LOQ (<0.01 ang/kg), with no clear decline. Exceptions to this pattern were observed in the 6 trials performed in 2014, in which becline was observed after the last application, with some uptures 7 days after last application, followed by a fall to values below the LOQ.

Samples of lettuce were taken after the normal commercial harvest in order to assess the decline of residues after the proposed pre knyvest interval (PHI). By the time of the last sampling, lettuce plants BBCH 49 (typical size, form and firminess of head reached) and vere appropriate for consumption.

The residues field totals were performed according to the guidance in place at the time when they started. All of the trials were conducted at rates and timings comparable to the requested GAPs for the fluopyram renewal. The residue data are supported by validated methods of analysis and procedural (concurrent) recovery data. The deep frozen storage stability periods for the samples (from the time of sampling to residue expaction), were covered by separate storage stability studies.



⁷ Please report if the residue trials were fully validated in terms of storage stability, GAP compliance, etc.

⁸ It is mensioned in the EU data requirement that when planning residue trials, it shall be borne in mind that information on the residues in ripe or unripe crops may be of interest with respect of the risk assessment in other areas like ecotoxicology and worker safety. Please include this information if available.

⁹ Residue determinations close to the application(s) and/or the last application may provide relevant information for certain non-target taxa that can forage in the crop area at a time close to the application(s).



Question 7: On which crops were field residue trials performed? ¹⁰ Has an extrapolation been suggested and is it considered appropriate?¹¹

#### Applicant response:

Residues trials have been submitted to support the representative uses on grapes and lettuce for the purposes of the renewal, no additional uses for extrapolated commodities have been sought. Therefore, an extrapolation to other crops was not suggested. 

#### RMS comment:

#### Metabolism studies in animals (livestock, fish). Reference material: Test No. 503: Metabolism in Livertock (OECD, 2007c) Residues in Livestock (OECD, 2007e), Test No. 305, Bioacounulation in Fish (@ECD

Question 8: Is a metabolism study in fish/broaccumulation study part of the residue Section? If the fish metabolism study is available, does at indicate an occumulation of residues in fish tissues?¹²

m

#### Applicant response:

A fish metabolism study has not been undertaken for Fluopyram. According to the current EU guidance (SANCO/11187/2013 rev. 3) the met folism in fish is not require for the Annex I Renewal because the trigger value of dictary borden was not exceeded with the representative uses.

However, a fish boconcentration study is svailable for fluopyram (M-298506-01-1). The bioconcentration potential of fluopyram from the aqueous environment into Gluegill sunfish (*Lepomis* macrochirus) was determined in a continuous flow(through exposure system. The bioconcentration part of the story included a 28-da uptake period and a 14-day depuration period. The fish were dissected into edible and non-edible tissues. 

The average percent light over the entire study period ranged from 8 to 11%, from 5 to 10%, and from 5 to 11% in the whole fish samples in the solvent control, in the low treatment, and high treatment, respectively. The overall mean percent lipid content in samples from aquaria A, B, and C on day 0 and 28 was 7.03%. The kinetic bioconcentration factors based on TRR (BCFTRR,) were 47.6 (edible tissue) and 87.9 (whole fish) for the low treatment (6.0  $\mu$ g [pyridyl-2,6-¹⁴C]fluopyram/L) and 359 (edible tissue) and 65.7 (whole fish) for the high treatment (60 µg [pyridyl- $2,6^{-14}C$ -fluopyram/L). Ő

The steady-state BCF for parent fluopyram based on whole fish (wet weight) was calculated to be 18 and the steady-state BCF for parent fluoporam normalized to 6% lipid content was 16.



¹⁰ The non-mum number of super Gred residue trials considers for MRL setting might not be applicable for the ecotox. We might build a performe curve with less than 4 residue data points. For this consideration, please do not disregard the resider data may based on the commum number of residue trials. If the residue trials are compliant with the GAP table, ecotox experts might use them for further refinements.

¹¹ Ecotor Selleagues might need advice on questions such as e.g. can residue decline studies in tomato be used to refine the residues entering throughout diet of frugivorous birds when the representative use is on pome trees? And can we use residue data generated in the SEU for refinements in the NEU zone when the representative use is in whole EU?

¹² If we observe any accumulation in tissues, it might help in case that further assessment of bioaccumulation and/or biomagnification (accumulation throughout trophic chain) are necessary.



The parent compound fluopyram accounted for > 97% of the radioactivity in the profiles of all water samples after SPE and concentration. In the samples collected during the later exposure phase of fish  $\circ$  the metabolite AE C656948-7-hydroxy was detected with ca. 1 – 2 % of the TRR. Total radioactive residues (TRR) measured were 0.753 mg/kg in edibles (day 7), 1.533 mg/kg in edibles (day 4), 3.221 mg/kg in viscera (day 7) and 12.597 mg/kg in viscera (day 14).

The metabolic profiles for both time points were similar for edibles and viscera, respectively. In edibles the major part of the residue was represented by the parent compound followed by the metabolite fluopyram-7-hydroxy. Samples of viscera exhibited significant higher proportions of conjugates compared to edibles. In viscera, the major compounds were parent compound and fluopyram-7-OH (glucuronic acid conjugate of fluopyram-7-hydroxy). Minor metabolites detected were fluopyram-8-hydroxy (edibles and viscera), fluopyram-8-OHcOA and fluopyram-pyridyl acetic acid (both in viscera, only).

Fluopyram accumulated in bluegill sunfish with a total residue bioconcentration factor of about 65.7 to 87.9 for whole fish (sum of radio labelled compounds, fluopyram parent, metabolites and mineralization products) (see table below)

	6			, ° ×			
Damanastan	6.0 µg [руна fluopyrada/I	dyl-2,6 ⁻ [™] C <u></u>	Ş D	69.µg [pwidyl-236- ¹⁴ C]@ Muopyram/L			
(based on TRR)	Edible	Non- () Sedible () tissue,	Whole Bish	Edible tissue	Non- Ó edible & tissue O	Ŵhole ∕fish	
Kinetic bioconcentration factor (BCF _{TRR} )	Ø\$7.6 O	1,505.4	87.9	35.9	ý21.6	65.7	
Time to reach 95 % of steady state [days]	30.6 30	8.1 0	0 ⁴ .8		4:6	7.7	
t _(1/2) for clearance	47.1 S		3.45 6	[,] 4.2	2 ^{1.1}	1.8	
Uptake rate constant (k)	4.67 🖌 🐇	58.2	A.7.8 🔊	5,96	78.7	25.6	
[1/Day]	(€0.42) 0*	$(\pm Q, \theta)$	$J(\pm 1.0)$	and <u>(0.57)</u>	(± 3.62)	(± 1.59)	
Depuration rate	0.098 (±0.03)	037 (± 0.16)	0.20 (\$ 0.08)	0.17 ( <b>1</b> 9.06)	0.65 (± 0.26)	0.39 (± 0.175)	

Substance uptake and depuration constants and bioconcentration factors

The OriginTM calculated kinetic BCFTRR values for edible parts and whole fish (calculated as the ratio of uptake and depuration the constant) for espond well with the respective bioconcentration factors (calculated as the ratio of concentration in fish and in water) 48.8 X (edible parts) and 97.2 X (whole fish) for 6.0 µg [pyridy]-2,6  4 C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridy]-2.6  4 C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridy]-2.6  4 C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridy]-2.6  4 C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridy]-2.6  4 C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridy]-2.6  4 C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridy]-2.6  4 C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridy]-2.6  4 C]-fluopyram/L for 60 µg [pyridy]-2.6  4 C]-fluopyram/L for 60 µg [pyridy]-2.6  4 C]-fluopyram/L for 60  4 C]-fluopyra

These values correspond to the calculated total residue levels of 0.292 mg/kg edible parts and 0.581 mg/kg whole fish for 6.0  $\mu$ g [pyrid/1-2,6 ⁴C]-fluopyram/L and of 2.49 mg/kg edible parts and 4.75 mg/kg whole fish for 60  $\mu$ g [pyrid/1-2,6 ⁴C]-fluopyram/L, respectively.

Taking into account that in edible parts of the fish 24.7% of the TRR (sample day 14) were identified as parent compound and in viscer 21.9% of the TRR (sample day 14), the steady-state-BCF for parent (based on whole fish, wet weight) is 18, the steady-state-BCF for parent (normalised to 6% lipid content) is 56.

RMS comment



Question 9: Can the metabolism in animals (mammals/fish/hens) bring any information on accumulation/exposure¹³ to different metabolites in addition to those present in the plants? Is  $\mathcal{M}$ possible to observe an accumulation of residues in fatty tissues/other animal tissues considering all available metabolism studies?

#### **Applicant response:**

Based on the livestock metabolism studies, fluopyram was extensively metabolised in apimals and the main metabolite was fluopyram-benzamide (M25) (49% to 99% in fat and muscle). Olefins of fluopyram (M02 and M03) were also detected. The lives tock metabolism studies were performed of 2 mg/kg bw/d, corresponding to 21N for ruminant and \$3N for poulted with the regression of the uses

However, in the feeding studies, more parent was recovered compared toghe metabolists studies and the only anticipated residues in animal matrices are parent and benzamide M25. No olefing (M-02 and M03) are expected above the LOQ with the representative uses 4

There is no potential for accumulation (goat, hen). This was also the conclusion based on rat (ABME), studies (results of repeated dose study did not show accumulation).

Excerpt from DAR, Vol 3 B.7

"For laying hen and lactating goat, metabolism studies were conducted with each [poridyl-26-14C] or [phenyl UL-14C] labelled fluoporam at nominal rates of 2 rog/kg bw/day. One pretabolism study was conducted with [pyridyl-2₆-¹⁴C], fluopyram in rish [see question &. All Studies were well performed and fulfilled the acceptability criteria of EC and DECD guidelines. The metabolic pathways of fluopyram in livestock consisted of the following principal metabolic reactions that are also observed in the rat:

- Hydroxylation of the ettorene bridge Of the molecule resulting in fluopyram-7- hydroxy, fluopyram-8-hydroxy, and a dihydroxylated compound,
- hydroxylation of the phenyl ring leading to fluopycam-phenol
- conjugation of the hydroxylated metabolities with gluce poinc acid
- elimination of water from compounds hydrox stated to the ethylene bridge leading to fluopyram-Z-olefine and E@lefine (E- and Z-olefine can isomerise into each other),
- molecular cleavage of fluopyram & hydroxy to fluopyram-pyrdyl-hydroxyethyl (pyridyl label spectric) followed by either conjugation with guicurous acidor oxidation to fluopyram-pyridylacetic acid (PAA)  $\bigcirc$ K, ~ « n
- molecular cleavage of fluop@am-& hydroxy to fluopyram-benzamide (phenyl label specific) and formation of fluopyam-benzamide sulfate or floopyran-benzoic acid.

Parent fluopy am is intensionly metabolised in the animal. Main metabolites in the goat and hen were fluopyram-benzamide (M25) and fluopyrang E- and Z-olefins (M02 and M03). In the goat, fluopyrand 7-OH-GA (M09; sum of Komers) and Jluopyram-8-OH-GA (M20b; isomer 2) exceed



¹³ If there is information of new metabolites in the excreta, it might be relevant for the environment. Non-target organisms might be exposed to these new metabolites if there is a release in the environment after animal metabolization.



Magnitude of residues in pollen and bee products

Reference material: Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (EC, 2018); Guidance on the risk assessment to plant protection products on bees (Apis mellifera, bombus spp. and solitary bees (EFSA, 2018).

**Question 1:** Are data on the magnitude of residues on pollen and bee products part of the tesidue section? If so, please indicate which data are available and sampling times?

#### **Applicant response:**

Residue trials were conducted aiming to determine the concentration of Fluopyram in honey. Two spray applications of 250 g Fluopyram/ha were performed in a 6-7 day interval onto full flowering *Phacelia tanacetifolia* in tunnels that contained bee colonies. Test area were located in northern and southern European zones. Honey samples were collected 2-10 days after the last application and residue analysis was performed for the amounts of fluopyram (AE 6656948) and its metabolities fluopyram-benzamide (AE F148815, FLU-benzamide), fluopyram-pyrioyl-catboxylic-acid (AE C657188, FLU-PCA), fluopyram-pyrioyl-acetic-acid (BC AA 10139, CLU-PAA) and fluopyram-7-hydroxy.

No residues of fluopyram and its five metabolites (FLU-benzami@, FLU-PCA, FLU-PAA, FEU-7OH and FLU-methylsulfoxide) were found above the 400Q (FOQ = 0.01 me/kg) and any honey samples originating from treated or untreated tunnels. Detailed data on test methodology and indings from these trials are presented in section CA 6 0.1.

Residues of fluopyram and its metabolites fluopyram-pyridylacetic acid (BCS-AA10189) and fluopyram-benzamide (AE F148815) were also analysed in flowers, bee-collected nectar and beecollected pollen as part of a honey bee semi-field trial. The study involved two applications of FLU+TFS SC 500 onto the bee attractive crop *Phaedia taracetifolia* at rates of 560 mL product/ha (corresponding to 140 g fluopyram ba per application). The 1st foliar application was performed at BBCH 59-61 and the 2nd at full flowering (BBCH 64-65), while bees were actively foraging on the crop. Monitoring of residues occurred in 3 out of 6 test item application and the following day. Residues of fluopyram in pollen ranged from 3 to 30 me/kg. Residues of FLU-PAA did not exceed 0.01 mg/kg pollen, while those of FLU4benzauride ranged between LOQ and 0.017 mg/kg pollen (LOQ = 0 01 mg/kg). Detailed information on the methodology of sample collection, residue analysis and the findings of this study are presented in the formulation specific section CP 10.3.1 (M-435338-01-1).



¹⁴ Residue section may contain information of residues in pollen, leaves and flowers. For residues assessment, data on nectar and pollen would be also useful for deriving a more realistic MRL/PF for nectar/honey and pollen/honey. Specific residue data can be used for refinement of higher tier studies in the risk assessment for bees if considered representative of the situation under assessment.



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										Fluopyra	nm + Trifloxy	strobin SC	500 (250 +	- 250 g/L)
									د.	A.	_^C	ð	2 10	¢°
							Appendix	1 . BÖ	Jer p	3	perty	at 10th	2091	) OLDO
Metab	olites seen in the (	confined	rotationa	l crop stud	lv ( <mark>M-24</mark> 0	707-03-1)	) .	O ^L	<b>Å</b> °	J.P.	· t			ð
Pheny	l label - metabolites			- or op stat	- <u>j (</u>	<u> </u>	, the second sec	- to	F V		<u> </u>	- PULE	-tok	Q.
Plot	Crop Part	PBI	DALA	M-04	-		PI-01	110	Lecu 1	AM-06	3 J. 10	00 ² 0	Fluoperan	· • • • • • • • • • • • • • • • • • • •
1100		(days)	(days)	%TRR	mg/kg a.s. equivs	₩ Mag/kg	%TRR	, nægdeg a.s. Dequivs	ng/kg	A TRR	mgakg a.s. Dequivs	mg/kg x	© [≫] %TRR	mg/kg
	Lettuce	29	83	-	- , 6	- 6 )	81.2 0	0.82	0.407	- 45	- *		11.1	0.112
	Radish Tops	29	74		- "	0 ^y	<u>E.</u>	381	<u>2</u> ,170		CI.	- CI	24.5	1.644
29 Radis Whea	Radish Roots	29	71	- 010	- 3	- 9 ¹	43.2	0.062	0.031	- CUL	- CIT	- WILL	47.9	0.069
	Wheat Forage	29	68	32,700	1.619	sQ. \$0	6.5 5	0. <b>2</b> 2	10855 ·	₫₽°	A 0.049	< 0.051	36.6	1.812
	Wheat Grain	29	93				3.6	0.006	0.003	13.1 SC	0.021	0.022	27.3	0.043
	Wheat Straw	29	93 G	13.6000	1.84	0,990	3.4 0	0.401	0.22	10	-05	-	23.1	3.132
	Lettuce	133	C216	- 4			60.9	0.070	0.035	<u>p</u>	-	-	26.6	0.031
	Radish Tops	133	196	- 5	- <u> </u>	- E D	77.3	0.186	0.092	- apri	-	-	15.1	0.036
133	Radish Roots	133	196	te s		1- ×	\$4.9	JQ.013	0.006	<u>(-)</u>	-	-	28.2	0.006
155	Wheat Forage	133	281	28.9	0.065	0.035	5.1	0.011	0.006	-	-	-	23.3	0.052
	Wheat Grain	133	335	23-3	0.004	0.003	19.00 ⁴⁸	0003	0.003	-	-	-	7.0	0.001
	Wheat Straw	133	Q335 _C	94.6	0 123 🔬 🕻	0.067 🔬 📿	25.5	0.215	0.107	-	-	-	15.5	0.131
	Lettuce	365	421	- 000	- 200	- 25	87.0	0.53900	0.267	-	-	-	2.1	0.013
	Radish Tops	365	421	0,0		100	087.5	A.755	0.869	-	-	-	3.8	0.076
365	Radish Roots	365	421	- 10"	- 2	- 010	60.9	0.022	0.011	-	-	-	24.2	0.009
505	Wheat Forage	365	ALON .	159.5	5515	<b>\$276</b>	Ø:8"	0.128	0.063	-	-	-	4.8	0.042
	Wheat Grain	365	449	24.5	0.013	0.007 C	17.9	0.010	0.005	-	-	-	7.3	0.004
	Wheat Straw	301	449	280	0663	0:350	5.1	0.121	0.060	-	-	-	7.2	0.172
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#### Document MCP – Section 10: Ecotoxicological studies Fluopyram + Trifloxystrobin SC 500 (250 + 250 g/L)

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													PG						Ś, ^w	A Du
Pyridy	l label- met	abolites											<u>\$</u>		<u> </u>	^{بل} ار		<u>~</u>	<u> </u>	
		DDI	DALA	M-08			M-05			M-02		adi	M-09	eC	j2 ^{e.}	M-06	ų Č		Fluopyra	m
Plot	Crop Part	(days)	(days)	%TRR	mg/kg a.s. equivs	mg/k g	%TRR	mg/kg a.s. equivs	mg/k g	%TRR	mg ag ag equivs	mg/k	%TRR	meske a.s. equivs	mg∕k ∯°	%TRR®	Ong/kg a.s. equiv	mg k g	NRR D	mg/k g
	Lettuce	29	83	-	-	-	13.0	0.039	0.026	17,4	0.053@	0.031	53 ⁰	0,010	0.008 🔦	<u>67</u>	- 8	- 10	35.8	0.108
	Radish Tops	29	71	-	-	-	3.3	0.069	0.046	210.4	0.217	Q.138	4.8	0.100	\$0.052	3103	- (	¢	EL.P	1.072
•	Radish Roots	29	71	-	-	-	9.6	0.011	<b>2</b> 007	3385	0.020	0.023		4	-	-		ner	41.1	0.048
29	Wheat Forage	29	68	-	-	-	3.8	6463	0408	43.0	1.844	×C087	- ×0'	- <u></u> )	0 <u></u> »"	1.Q'>	0.060	0.063	33.7	1.445
	Wheat Grain	29	93	-	-	-	13.1	0.34	0 225	\$69.6	4899	1.060		QC _	QŪCIU.	- 5	-	Ъ°	1.8	0.046
	Wheat Straw	29	93	-	-	- DEL	7.7 8	0.544	0 \$59	7.0C ^T	0.494	0.291	2210	20C2	- 11	C.P.	OW	-	34.9	2.462
	Lettuce	133	217	-	C	<u> </u>	6 ³		- C	No.	¢	- %	- 6	<u> </u>	0 ^{C°°}	1,50	-	-	79.9	0.027
	Radish Tops	133	197	-	0.0	1.6 ₀₇	- 2	<u>9</u> .	<u>a</u> e	-100	- 01	»_" s	CD1 -	, ĝ	- J	-	-	-	72.2	0.171
	Radish Roots	133	197	The star	-	- ×	£ <del>2</del> 9	0.001	0.001	9.6	0.002	8062	19.1%	0.005	<b>Ş0</b> .003	-	-	-	54.9	0.014
133	Wheat Forage	133	282	-	-	200	4105	0.064	0.042	C54 2	0.000	0.005 (	D10.5	0.916	0.008	-	-	-	26.2	0.041
	Wheat Grain	133	336	-		- j	66.6	0.064	6042	10.9	0.010	9.006	n ^e			-	-	-	3.2	0.003
	Wheat Straw	133	336	₹4C	0.033	0.019	ela ^{to}	0.904 1	0.003	21	0.007°	0.004	21.5	0.075	0.039	-	-	-	25.7	0.089
	Lettuce	365	421	9.0	<b>G</b> Ø05	0.000	7.8	0.005	0.003	11.8	0.007	≫0.004	3.7	0.002	0.001	-	-	-	41.5	0.024
	Radish Tops	365	421	-	- ð	0	js.P	030252	0.015	27.1	<b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b> <b>1</b>	0.067	6.0	0.025	0.013	-	-	-	25.2	0.106
265	Radish Roots	365	421	9.5	0.003	£1092	5.3	0.002	00.001	19.0	0.003	0.002	-	-	-	-	-	-	55.8	0.018
365	Wheat Forage	365	410	6.3	0.015	0.009	18.3	0.045	0 Câr	8 2	0.020	0.012	9.9	0.024	0.012	-	-	-	27.8	0.068
	Wheat Grain	365	- HOIL	- J 1	-	sj).	-8 ^{3°}	0.116	0.077	14.2	0.025	0.015	-	-	-	-	-	-	2.9	0.005
	Wheat Straw	365DC	449	4.8	019948	0.028	14.2	0.143	0.094	41	0.042	0.025	-	-	-	-	-	-	27.5	0.277
	E ^J	,ODBE	OLU OL	A A A A A A A A A A A A A A A A A A A	10 ¹² ^E	Ŷ	~\$~ }													



# Appendix 2

		Appendix 2			0	
					<u>e</u>	ð
					N.	S
Summary of the r	esidue decline trials	for fluopyram treat	ted grape	<u>s 🗞</u>		-
Report No.	Application	Portion analysed	PHI	Re Re	esidues 🎸 🌧	
(Document No.)			(days)	"0" (n	ng/kg)	Ļ
I Mai No.				AL_C656948	So benzamide	Q
Report No	2 x 0 038 kg a s /ha	Bunch of grapes	-0	\$ 0.078	Socialization of the second se	
RA- 17-2112	2 A 0.050 Kg u.s./hu	Bunch of grapes	0	0.22	Q.015 %	L.
( <u>M-653354-02-1</u> )		Bunch of grapes	17 _ Ô	0.028	~<0.01~~	,0″
Trial No		Bunch of gapes	805	<0.01 0	_~~<0,0℃	X
17-2112-01		Bunch of grapes	104/	<u>© &lt;0.01</u>	<0.01	-
Report No	2 x 0.038 kg a.s./ha	Bunck of grapes				
KA-1/-2112 (M 652254 02 1)		Bunch of grapes				
$\frac{(M-03334-02-1)}{\text{Trial No}}$		Bunch of grapes	42	0.043	<0.01 & <0+4.1 °	
17-2112-02		Bunch @ grapes	-Q2	0.63		
Report No	2 x 0.038 kg a.s./ha	Burch of grapes	°r -0 €	× 00052 «/	₹0.01,	
RA-17-2112		Bûnch of gropes		20.11	× <0.04	
( <u>M-653354-02-1</u> )	Q.	Burnch of grapes	45	0.024	o.01 کې د	
Trial No	L. L.	Bunch of grapes	 ∂7	0.008	<b>40</b> .01	
1/-2112-03	2 - 0.029 1	Bunch of grapes	$3^{93}$		<ul> <li>≪0.01</li> <li>≪0.01</li> </ul>	
Report No	2 x 0.038 kg a.s./na	Bunch of grapes		0.02	× <0.01	
(M-653354-02-1)		A Runch of grapes	Ű,	© 0.018	○ <0.01 ○ <0.01	
Trial No		Bunch of grapes	⁴ 62	× ≤0.01 ⊘	< 0.01	
17-2112-04		Burch of grapes	∖ 76√	~@0.01 <u>~</u>	< 0.01	
Report No	2 x 0.1 kg a s ha (last	Banch of grapes	29	0.039	< 0.01	
RA- 16-2157	treatment of BBCH?	Bunch Ograpes	0 ³⁴	0.024	< 0.01	
( <u>M-614532-02-1</u> ) Trial Na	69	Bunch of grapes	65 (	0:0:0:14	<0.01	-
16 2157 01	2 x 0. Hkg a.s./Ma)(last	Bunch of grapes		0.12	<0.01	
10-2137-01		Brinch of grapes		× v 0.30	<0.01	
~~		& Bunch of grapes	36	0.085	< 0.01	
Report No	0 x 0.1 kg a.s./ba (last	Buneb of grapes	^O 35 K	0.028	< 0.01	
RA- 16-2957	treatment at BBCH	Bunch of grapes	510	0.021	< 0.01	
( <u>M-614632-02-1</u> )	<u> </u>	Bunch of grapes	- 78	< 0.01	< 0.01	
'Bital No	2 x 0.1 kg a.s./ha (last	Bunch of grapes	0	0.33	0.042	
10-2157-02	O treatment at Bac H	Bunch of grapes	A 0 16	0.63	0.044	
Č)		Bunch of grapes	43	0.28	0.040	
Report No @	200 0.1 kg/a s/ha (fast	Bunch @ grapes	18	0.15	<0.01	
RA- 16-219	Otreatment at BBCH	Bunch of grapes	44	0.052	< 0.01	
( <u>M-614533-02-1</u> )	O 69)~O 4	Burch of grapes	74	0.087	< 0.01	
TriatNo	2 x 9.1 kg a 3 ha (lag	Bunch of grapes	-0	0.16	< 0.01	
16-2957-03	*tseatment at BBCH	Bunch of grapes	0	0.38	< 0.01	
A Contraction of the second se		Bunch of grapes	22	0.070	<0.01	
Report No	2 x 19 kg a Cha (last	Bunch of grapes	30	0.002	<0.01	
RA- 16-2157	treatment at BBCN	Bunch of grapes	85	0.07	<0.01	
(M-614532-02-1)		Bunch of grapes	94	0.052	< 0.01	
Trial No.	2 x 0 Kg a.s./ha (last	Bunch of grapes	-0	0.20	< 0.01	]
16-2157-04	treatment at BBCH	Bunch of grapes	0	0.46	< 0.01	
	790	Bunch of grapes	53	0.13	< 0.01	
Alan ant Man		Bunch of grapes	02	0.11	<0.01	-
RA- 16-0157	treatment at RRCH	Bunch of grapes	51 84	0.10	<0.01	
(M-6145)2-02-1)	69)	Bunch of grapes	93	0.041	< 0.01	
Trial No	2 x 0.1 kg a.s./ha (last	Bunch of grapes	-0	0.16	< 0.01	1
16-2157-05	treatment at BBCH	Bunch of grapes	0	0.36	< 0.01	
	79)	Bunch of grapes	53	0.14	< 0.01	
		Bunch of grapes	62	0.14	< 0.01	



(Document No.) Trial No.         (days)         (mg/kg)         (mg/kg)         (mg/kg)         (mg/kg)           Report No.         2 x 0.1 kg as 3/a (last RA-16-2157.0         Bunch of grapes         36         0.073.         0.003.         0.001.           Midel 552-02-1)         69)         Bunch of grapes         79         0.015         -0.017.           Trial No.         1x 0.1 kg as 3/a (last reatment at BBCH         Bunch of grapes         -0.         0.13         -0.017.           Midel 552-02-1)         69)         Bunch of grapes         -0.         0.13         -0.017.           Report No.         Treatment at BBCH         Bunch of grapes         -0.         0.13         -0.017.           Report No.         2 x 0.1 kg as 3/a (last reatment at BBCH         Bunch of grapes         -0.         -0.056.         -0.064.           Midel 552-02-1)         69)         Bunch of grapes         -0.         -0.056.         -0.064.           Midel 552-02-11         79         Bunch of grapes         -0.         -0.056.         -0.064.           Report No.         2 x 0.1 kg as 3/a (last)         Bunch of grapes         -0.         -0.064.         -0.014.           R4-152157.08         2 x 0.0 kg as 3/a (last)         Bunch of grapes         -0.014.	Report No.	Application	Portion analysed	PHI	Re	sidues	1
Trial No.         AE C55048         AE C55048         AE C55048           Report No.         RA : 16:2157.06         1 kg as 3 ha (last treatment at BBCH treatmentreatment at BBCH treatmen	(Document No.)			(days)	(n	ng/kg) ₀	
Report No         2 x 0.1 kg a.s./ha (last         Bunch of grapes         36         0.073         0.064           Mold 552.02.1)         0.90         Bunch of grapes         57         0.054         0.064           Mold 552.02.1)         0.90         Bunch of grapes         0         0.15 $\sim$ 0.067           Ireatment at BBCH         Bunch of grapes         0         0.15 $\sim$ 0.067           Report No           Report No         16-2157.06         90         Bunch of grapes         29         0.11 $\sim$ 0.067 $\sim$ 0.01           Ite-2157         09         Point No         Report No <td< td=""><td>Trial No.</td><td></td><td></td><td></td><td>AE C656948</td><td>AE C656948</td><td>ð</td></td<>	Trial No.				AE C656948	AE C656948	ð
Report No.         RA-16-2157         Council at BBCH         Bunch of grapes         79         0.035         -0.005           16-2157-06         22.0.1 kg a.s./n (tast treatment at BBCH         Bunch of grapes         0         0.15         -0.005           16-2157-06         20.0 1 kg a.s./n (tast treatment at BBCH         Bunch of grapes         0         0.15         -0.005           Report No.         20.0 1 kg a.s./n (tast treatment at BBCH         Bunch of grapes         2         0.1 1 kg         -0.005           Report No.         20.0 1 kg a.s./n (tast Bunch of grapes         2         0.1 1 kg         -0.005           Report No.         20.0 1 kg a.s./n (tast Bunch of grapes         0         0         0.055         -0.005           Report No.         20.0 1 kg a.s./n (tast Bunch of grapes         0         0         0.014         -0.005           Report No.         20.0 1 kg a.s./n (tast Bunch of grapes         -0         0         0.014         -0.001           Report No.         16-2157-07         12 a.0 1 kg a.s./n (tast Bunch of grapes         -0         0.026         -0.014           Report No.         16-2157-08         12 a.0 1 kg a.s./n (tast Bunch of grapes         -0         -0.035         -0.014           Report No.         7.0 0 fr         0 fr						benzamide	
RA         16-2157         0034         -9901         -9001           Trial No         12 × 0.1 kg a.s./n (last         Bunch of grapes         -9         0.015         -0.014           16-2157.06         treatment at BBCH         Bunch of grapes         -0         0.015         -0.014           16-2157.06         treatment at BBCH         Bunch of grapes         -0         0.015         -0.016           Report No         2 × 0.1 kg a.s./n (last         Bunch of grapes         21         -0.18         -0.010           Model 532-02.1         690         Bunch of grapes         29         -0.010         -0.000           Trial No         2 × 0.1 kg a.s./n (last         Bunch of grapes         -0         -0.000         -0.000           Report No         2 × 0.1 kg a.s./n (last         Bunch of grapes         -0         -0.000         -0.011           Report No         2 × 0.1 kg a.s./n (last         Bunch of grapes         55         -0.012         -0.014           Report No         2 × 0.0 kg a.s./n (last         Bunch of grapes         54         -0.035         -0.014           Report No         2 × 0.0 kg a.s./n (last         Bunch of grapes         57         -0.017         -0.014           RA-16-2157         Gras s./n (la	Report No	2 x 0.1 kg a.s./ha (last	Bunch of grapes	36	0.072	<0.00	0
(Model 53/2021)         (P)	RA- 16-2157	treatment at BBCH	Bunch of grapes	57	0.03		
If all No         22.0.1 kg a.s.hn (last breatment at BBCH         Bunch of grapes Bunch of grapes         -0         6.0.15         0.000           Report No         79)         Bunch of grapes         21         6.0.18         0.011           Report No         70.01 kg a.s.hn (last Bunch of grapes         29         0.011         6.0010           Main I State         Bunch of grapes         29         0.011         6.0010           Main I State         Bunch of grapes         29         0.011         6.0010           Main I State         Bunch of grapes         20         0.000         6.0000           Main I State         Bunch of grapes         70         8.0010         70.000           Trail No         16-2157.07         12.01 kg a.s.hn (last         Bunch of grapes         70         8.011         70.001           Report No         Ra.s./ha (last         Bunch of grapes         40         0.011         8.001         70.001         70.001           Main I State         Bunch of grapes         40         0.011         90.01         70.001         70.001         70.001         70.001         70.001         70.001         70.001         70.001         70.001         70.001         70.001         70.001         70.001	( <u>M-614532-02-1</u> )	69)	Bunch of grapes	/9	0.099	< 0.01	-
10-2137-00         Treatment at BBCH         Bunch of grapes         21         0.18         Constrained           Report No         2 x 0.1 kg as./hu (last treatment at BBCH         Bunch of grapes         22         0.12         Col 1           RA-16-2157         (M-614532-02-1)         69)         Bunch of grapes         29         0.011         <0.014	Irial No	$2 \ge 0.1 \text{ kg a.s./ha}$ (last	Bunch of grapes	-0	0.15		Ô
17)         Bunch of grapes         21         0.18         2001           Report No         2 x 0.1 kg a.5/ha (last freatment at BBCH         Bunch of grapes         29         0.11         40.01           Meid 4532-02-1         G90         Bunch of grapes         85         -0.054         -0.061           Trial No         16-2157.07         Report No         2 x 0.1 kg a.5/ha (last freatment at BBCH         Bunch of grapes         50         0.036         -0.061           Report No         2 x 0.1 kg a.5/ha (last freatment at BBCH         Bunch of grapes         55         -0.037         -0.011           Report No         2 x 0.1 kg a.5/ha (last freatment at BBCH         Bunch of grapes         55         -0.037         -0.011           Trial No         1 fail No         1 fail No         1 fail No         -0.047         -0.047           16-2157.08         2 x 0.0 kg as/ha (last freatment at BBCH         Bunch of grapes         -0.037         -0.017           1731 No         2 x 0.0 kg as/ha (last freatment at BBCH         Bunch of grapes         -0.037         -0.017           16-2157.08         2 x 0.0 kg as/ha (last freatment at BBCH         Bunch of grapes         -0.037         -0.011           1731 No         2 x 0.0 kg as/ha (last freatment at BBCH         Bunch of grapes	16-215/-06	treatment at BBCH	Bunch of grapes	0	× 10.30		Į į
Report No RA- 16-2157         2 x 0.1 kg a.s./ha (last treatment at BBCH         Bunch of grapes Bunch of grapes Bunch of grapes (M-614532-02-1)         0.01         - 0.001           Model 653-02-10         69)         Bunch of grapes Bunch of grapes (M-614532-02-1)         0.005         - 0.005         - 0.005           Trial No 16-2157-07         12 x 0.1 kg a.s./ha (last treatment at BBCH         Bunch of grapes Bunch of grapes (M-614532-02-1)         - 0.01         - 0.01         - 0.01           Report No RA- 16-2157         2 x 0.1 kg a.s./ha (last treatment at BBCH         Bunch of grapes Bunch of grapes         55         - 0.036         - 0.01           Report No RA- 16-2157-08         2 x 0.1 kg a.s./ha (last treatment at BBCH         Bunch of grapes         55         - 0.036         - 0.01           Trial No 16-2157-08         2 x 0.1 kg a.s./ha (last treatment at BBCH         Bunch of grapes         58         - 0.03         - 0.01           Report No RA-17-2128         2 x 0.05 kg a.g./ha (27)         Bunch of grapes         58         - 0.02         - 0.01           Model S233-01-11         Trial No 17-2128-01         2 x 0.05 kg a.g./ha (27)         Bunch of grapes         - 0.02         - 0.01           Report No RA-17-2128         2 x 0.05 kg a.g./ha (27)         Bunch of grapes         - 0         - 0.01         - 0.01           Report No RA-17-2128 </td <td></td> <td>79)</td> <td>Bunch of grapes</td> <td>21 42</td> <td>0.18</td> <td></td> <td>Ø</td>		79)	Bunch of grapes	21 42	0.18		Ø
RA:         Local         Particle         Par	Donort No.	$2 \times 0.1$ line a c /ha (last	Bunch of grapes	43			Ś
RA-1432101       Idealinetit at BOC1       Bunch of gapes       0050 $-0.056$ $-0.064$ Model 3322-02-10       69)       Bunch of gapes       0       0.056 $-0.064$ Iteratiment at BBC1       Bunch of gapes       0       0.056 $-0.064$ Report No       Rx - 16-2157       2x 0.1 kg as./ha (dast       Bunch of grapes       39 $-0.13$ $0.011$ RA-16-2157       2x 0.1 kg as./ha (dast       Bunch of grapes       40 $0.0986$ $-0.041$ Model 332-02-10       69)       Bunch of grapes       40 $-0.0916$ $-0.041$ Report No       reatment at BBCH       Bunch of grapes       40 $-0.0916$ $-0.041$ Report No       reatment at BBCH       Bunch of grapes       57 $0.012$ $-0.011$ RA-17-2128       days imgobil last       Bunch of grapes $0.026$ $-0.011$ Trial No       2 x 0.05 kg ag/ha (12)       Bunch of grapes $0.026$ $-0.011$ Report No       Report No       Report No       Report No $0.0356$ $-0.011$ Report No       2 x 0.05 kg ag/ha (12)       Bunch of grapes $0.6$ $-0.011$	Report NO DA 16 2157	2 X 0.1 Kg a.s./ha (last	Bunch of grapes	²⁹		$\sim$ < 0.01 $\sim$	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	$(M \ 614532 \ 02 \ 1)$	60)	Bunch of grapes	00×5	0.085 O		×
116:11:0       2 A 0.1 kg as. M (as.)       Bunch of grapes       0       0       0.21       Color         16:2157-07       17 addition and abCH       Bunch of grapes       39       0.13       0.011         Report No       2 x.0.1 kg as./ha (dast       Bunch of grapes       30       0.13       0.011         RA-16:2157       17 addition and the BCH       Bunch of grapes       40       0.0988       -0.012         Trial No       12 x.0.1 kg as./ha (dast       Bunch of grapes       41       -0.012       -0.012         Trial No       12 x.0.1 kg as./ha (dast       Bunch of grapes       41       -0.012       -0.012         Report No       Report No       2 x.0.05 kg ag./ha (2)       Bunch of grapes       53       -0.012       -0.011         M-648233-01-11       treatment at BECH       Bunch of grapes       54       -0.012       -0.011         Report No       2 x.0.05 kg ag./ha (12)       Bunch of grapes       57       -0.017       -0.011         M-68823-01-11       treatment at BECH       Bunch of grapes       68       -0.021       -0.011         M-68823-01-11       treatment at BECH       Bunch of grapes       69       -0.011       -0.011         Trial No       17-2128-01       2 x.00	( <u>M-014552-02-1</u> ) Trial No	$2 \times 0.1 \log a s /ba (last)$	Bunch-of grapes			×0.01	
No. 11.9       Difference       Difference <thdifference< th="">       Difference</thdifference<>	16-2157-07	2 X 0.1 Kg a.s./lia (last treatment at BBCH	Bunch of grapes			\$ \$ 0.01	
Bitch of grapes         58         0.0087         0.001           Report No (M-614532-02-1)         2 x 0.1 kg a.s./ha (last treatment at BBCH)         Bunch of grapes         61         0.03         -0.004           Trial No (M-688233-01-1)         2 x 0.1 kg a.s./ha (last treatment at BBCH)         Bunch of grapes         88         0.03         -0.004           Report No (M-688233-01-1)         2 x 0.1 kg a.s./ha (last treatment at BBCH)         Bunch of grapes         -0         -0.012         -0.01           Report No (M-688233-01-1)         2 x 0.05 kg a.g/ha (2)         Bunch of grapes         53         0.01         -0.01           Trial No 17-2128         2 x 0.05 kg a.g/ha (2)         Bunch of grapes         0         -0.024         -0.01           Report No (M-688233-01-1)         2 x 0.05 kg a.g/ha (2)         Bunch of grapes         68         -0.021         -0.01           Trial No         2 x 0.05 kg a.g/ha (2)         Bunch of grapes         67         0.007         -0.01           Report No (M-688233-01-1)         2 x 0.05 kg a.g/ha (12)         Bunch of grapes         67         0.01         -0.01           Trial No         2 x 0.05 kg a.g/ha (12)         Bunch of grapes         67         -0.01         -0.01           Report No RA-17-2128         2 x 0.05 kg a.g/ha (12)	10 2157 07	79)	Busch of gropes	° 39 ∞	× 013	°≫ 0.01 ř	
Report No RA-16-2157 (M-61452-02-1)         2 x 0.1 kg a.s./ha (last treatment at BBCH         Dunch of grapse Bunch of grapse (b)         30         0.01         0.01         0.01           M-61452-02-1)         69         Bunch of grapse treatment at BBCD         Bunch of grapse Bunch of grapse (b)         64         0.03         0.04         0.04           M-61452-02-1)         79         Bunch of grapse treatment at BBCD         Bunch of grapse Bunch of grapse         60         0.12         0.04         0.04         0.04         0.04         0.04         0.04         0.04         0.06         0.01         0.06         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0.01         0		())	Burch of grapes	52	© 0 098	¢ 0.011	
RA: 16-2157 (M-61432-02-1)         Trial No.         2 x 0.05 kg ag/n (21)         Bunch of grapes Bunch of grapes (P)         6 1         9055         x 0.01           Trial No.         2 x 0.15 kg a s/n (last treatment at BBCH)         Bunch of grapes Bunch of grapes (P)         0         0.15         90.01           Report No.         2 x 0.05 kg ag/n (21)         Bunch of grapes (P)         0         0.05         0.01         90.01           Report No.         2 x 0.05 kg ag/n (21)         Bunch of grapes (P)         58         0.096         -<0.01	Report No	2 x 0 1 kg a s /ha (last	Bunch & grapes	-90°	0.11		
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	RA- 16-2157	treatment at BBCH	Bunch of grapes		0-065		
Trial No         12 x 0.1 kg a.s./ha (last) treatment at BBCB         Bunch of grapes Bunch of grapes         40         0.090         <0.061           Report No         2 x 0.05 kg a.g./ha (21) days integral, last treatment at BBCH         Bunch of grapes         58         0.096         <0.01	(M-614532-02-1)	69)	Bunch of grapes	88	°∼003 ~	« « <0.01»	
16-2157-08         treatment at BBCH 79         Bunch of grapes Bunch of grapes Bunch of grapes Bunch of grapes         0         0.15         0.01           Report No RA-17-2128         2 x 0.05 kg ag/ha (21 days interval, last asystement at BBCH         Bunch of grapes Bunch of grapes         00         0.005         < 0.01	Trial No	$2 \ge 0.1 \text{ kg a s/ha}$ (last	Bunch of wrapes	-000	× 0 091	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	16-2157-08	treatment at BBC	Bunch of grapes	ar	~ 0.12	\$9.01	
Report No         2 x 0.05 kg a(2/ha (2) days interval, last treatment at BBCH         Burch of grapes (M-688233-01-1)         58         00.085 (0.0685)         <0.01           Trial No         7.7         0.007         <0.01		79)	Bunch of grapes	31	P 0.11	₹ ≪≉0.01	
Report No         2 x 0.05 kg a 2/ha (2)         Baifch of grapes         0         0         0.068         6         0.01           (M-688233-01-1)         Trial No         73)         0         Bunch of grapes         68         0.025         0.01           17-2128.01         2 x 0.05 kg a s/ha (12         Bunch of grapes         68         0.024         -0.01           17-2128.01         2 x 0.05 kg a s/ha (12         Bunch of grapes         67         0.01         -0.01           17-2128.01         2 x 0.05 kg a s/ha (21)         Bunch of grapes         67         0.01         -0.01           Report No         2 x 0.05 kg a s/ha (21)         Bunch of grapes         68         0.01         -0.01           Report No         2 x 0.05 kg a s/ha (21)         Bunch of grapes         24         0.018         -0.01           Report No         2 x 0.05 kg a s/ha (21)         Bunch of grapes         25         -0.01         -0.01           Report No         Report No         2 x 0.05 kg a s/ha (21)         Bunch of grapes         2         -0.016         -0.01           Report No         Report No         73)         2 x 0.05 kg a s/ha (21)         Bunch of grapes         0         0.11         -0.01           Report No <t< td=""><td></td><td></td><td>Bungh of grapes</td><td>58,0</td><td><u>م</u> 0.096 م</td><td>`≪≫&lt;0.01</td><td></td></t<>			Bungh of grapes	58,0	<u>م</u> 0.096 م	`≪≫<0.01	
RA-17-2128 (M-688233-01-1)       days initified, last treatment at BBCH       Bunch of grapes       20       0.035       <0.01         17-2128-01       2 x 0.05 kg a.s./na (12 days interval, last treatment at BBCH       Bunch of grapes       57       0.097       <0.01	Report No	2 x 0.05 kg a@/ha (21	Burch of spapes	00	00.068	<i>‱</i> <0.01	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	RA- 17-2128	days interval, last	Bunch of grapes	. ØG ^V	© 0.035	◎ <0.01	
Trial No         273         Bunch of grapes         68         C021         -0.01           17-2128-01         2 x 0.05 kg as /ha (12)         Bunch of grapes         0         v.0.094         -0.01           days interval, last         Bunch of grapes         26         0.025         -0.01           Report No         7.3         Bunch of grapes         26         0.025         -0.01           RA- 17-2128         Constructure at BBCH         Bunch of grapes         68         0.01         -0.01           Trial No         2 x 0.05 kg as /ha (21)         Bunch of grapes         68         0.01         -0.01           RA- 17-2128         Constant BBCH         Bunch of grapes         67.3         -0.01         -0.01           Trial No         2 x 0.05 kg as /ha (12)         Bunch of grapes         57.         -0.01         -0.01           Trial No         2 x 0.05 kg as /ha (12)         Bunch of grapes         57         -0.01         -0.01           Constant Rate         BBCH         Bunch of grapes         24         0.016         -0.01           Constant Rate         BBCH         Bunch of grapes         57         -0.01         -0.01           RA- 17-2128         days interegit last         Bunch of grapes	(M-688233-01-1)	treatment at BBCH	Bunch of grap	√57 °∕	y 0. <b>6</b> 07	< 0.01	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Trial No	¢ي 73) 0 °	Bunch of grapes	68		< 0.01	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	17-2128-01	2 x 0.05 kg a.s./ha (12	Bunch of grapes	× 0 ~	£0.094	< 0.01	1
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		days interval, last	Banch of grapes	26	0.02	< 0.01	
(73) $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$ $(73)$		DeatmenOat BBCH	Bunch of grapes	Q ₃₇	<0.01	< 0.01	
Report No       2 x 005 kg a x ma (21)       Brach of grapes       0       0.031       <0.01		73)	Bunch of grapes	@ 68 [°]	0.01	< 0.01	
RA-17-2128 (M-688233-04-0)       cdays interval, last, treatment at BBCH       Bunch of grapes, Sector of grape, Sector of grapes, Sector of grape, Sector of grapes, Sector of grape, Sector of gr	Report No 🔊	° 2 x 0@5 kg a`s∳ha (21%	Bounch of grapes		0.031	< 0.01	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	RA- 17-2128 🔘	days interval, last	Brinch of grapes ∾	2F	0.018 💞 🖉	< 0.01	
Trial No $7/3$ Bunch of grapes $57$ $<0.01$ $<0.01$ 17-2128.022 x 0.05 kg as fla (12)Burch of grapes $0$ $0.036$ $<0.01$ days integral, lastBunch of grapes $24$ $0.016$ $<0.01$ days integral, lastBunch of grapes $23$ $0.011$ $<0.01$ Report No2 x 0.05 kg as fla (21)Burch of grapes $63$ $0.011$ $<0.01$ Report No2 x 0.05 kg as fla (21)Burch of grapes $66$ $0.011$ $<0.01$ M-688233-01-1drys intered, lastBurch of grapes $56$ $0.038$ $<0.01$ 17-2128-032 x 0.05 kg as fla (12)Burch of grapes $56$ $0.021$ $<0.01$ days intered, BBCHBurch of grapes $56$ $0.074$ $<0.01$ days interest, lastBurch of grapes $56$ $0.077$ $<0.01$ days interest, lastBurch of grapes $53$ $0.024$ $<0.01$ days interest, lastBurch of grapes $32$ $0.057$ $<0.01$ days interest, lastBurch of grapes $80$ $0.027$ $<0.01$ days inte	( <u>M-688233-04</u> )	creatment at BBCh	& Bunch of grapes	_3¥3	0.011	< 0.01	
17-212892       2 x 0.05 kg as fla (12 - 1)       Bitten of grapes       0       0.036       <0.01	Trial No 🤎	@ ^v <\(73)	Bunch of grapes	0 57	< 0.01	< 0.01	
days interval, last         Bunch of grapes         21         0.016         <0.01           Report No         2 x 0.05 kg a s/ha (21)         Bunch of grapes         57         0.01         <0.01	17-2128402	2 x 0.05 kg a.s.7ha (12≉	Bumen of grapes		0.036	< 0.01	
Report No       2 x 0.05 kg a, sha (21)       Bunch of grapes       43       0.011       <0.01	ACT I	days interval, last	Bunch ot grapes	21	0.016	< 0.01	
Report No       2 x 0.05 kg a s ha (21)       Bunch of grapes       0       0.11       <0.01	K, v	Creatment at BBCH	Bunch & grapes	× 43	0.011	<0.01	
Report No       2 x 0.05 kg a.s. tha (2)       Bugen of grapes       0       0.11       <0.01	D ())		O' Bunch of grages	2.21	0.01	<0.01	
KA-17-2128       days interval, last       Bunch of grapes       56       0.038       <0.01	Report No	2 x 0.05 kg a.s Ma (21	Burgeh of grapes		0.11	<0.01	
Image: constraint of the set of the	KA-1/-2128	days interval, last	Bunch of grapes	14	0.063	< 0.01	
1111 1 YEC       (12) (12) (12) (12) (12) (12) (12) (12)	$(\underline{\text{IVI-088233-U1-1}})$	Academicat BBC	Bunch of grapes	50 00	0.038	<0.01	
17-2120-05       2 x 0.05 kg a sind (12)       Butch of grapes       0       0.21       <0.01	17_2128 02	(12)	Duncingol grapes	98	0.028	<0.01	-
Arys microg, tax       Bunch of grapes       14       0.11       <0.01	17-2120-05	days interval last	Butch of arapes	14	0.21	<0.01 <0.01	
Argument of boch       Summer of grapes       50       0.074       <0.01	Q"	streatment & BRCH	Bunch dearanes	56	0.11	<0.01	
Report No       2 x 0.05 kg a.s. pa (21)       Burch of grapes       0       0.32       <0.01			Bunch of grapes	98	0.074	<0.01	
RA-17-2128       Jack interval (21)       Burch of grapes       32       0.057       <0.01	Report No	X 0 05 10 2 5 10 1 (21)	Bingh of grapes	0	0.32	<0.01	1
(M-688233-01-0)       treatment w BBCL       Banch of grapes       53       0.024       <0.01	RA- 17-2128	daw interval last	Burch of grapes	32	0.057	<0.01	
Trial No       A       Bunch of grapes       80       0.027       <0.01         17-2128.04       2 x 0.05 kg a.s./ha (12       Bunch of grapes       0       0.67       <0.01	(M-688233-01-12)	treatment & BBCH	Bunch of grapes	53	0.024	< 0.01	
17-2128 or     2 x 0.05 kg a.s./ha (12 // Bunch of grapes     0     0.67 // <0.01	Trial No 🖴	1 8 5	Bunch of grapes	80	0.027	< 0.01	
dage interval, last     Bunch of grapes     32     0.074     <0.01	17-2128-0	2 x 0.0 x g a.s./ha (12	Bunch of grapes	0	0.67	< 0.01	1
treatment of BBCH Bunch of grapes 53 0.03 <0.01 Bunch of grapes 80 0.033 <0.01		da interval last	Bunch of grapes	32	0.074	< 0.01	
J         J         Bunch of grapes         80         0.033         <0.01		treatment at BBCH	Bunch of grapes	53	0.03	< 0.01	
	N D	A. ~	Bunch of grapes	80	0.033	< 0.01	
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Report No.	Application	Portion analysed	PHI	Res	sidues 🔊 o	A
(Document No.)			(days)	(m	g/kg)	ð
Trial No.				AE C656948	AE C656948-	S
				(Fluopyram)	benzamide	0
Report No	2 x 0.2 kg a.s./ha	head	-0	1.50	<0.01	
RA-2620/07	14 and 7 days before	head	0	5.8	0.01	
(<u>M-308622-01-1</u>)	harvest	head	3	<u>4</u> .7	\$ 0.0 kg	Ô
Trial No		head	7	2.1	∞ 0 . 00 x	j l
R 2007 0266/3		head 🦉	14	0.73	≪ ≈0,01 ~S	a
			21	0.51		, L
Report No	2 x 0.2 kg a.s./ha	head	-0 🦿	0.81 🗸	~ ^{30.02} ~	, Oʻ
RA-2620/07	14 and 7 days before	head		5.5	× 0.020	X
(<u>M-308622-01-1</u>)	harvest	head	3 ~~		λy 0.02 χ	
I rial No		head		0.92		
R 2007 0642/1		head o	×014			
D (N	2 0 2 1 //			<u> </u>	0.01	-
Report No	$2 \times 0.2 \text{ kg a.s./na}$	head (0.39 10	\sim	
(M 308622 01 1)	14 and / days before	hand N				
(<u>IVI-508022-01-1</u>) Trial No	narvest	Kead a				
R 2007 0644/8						
12007 0011/0			2.10	~~ 0.07		
Report No	2 x 0.2 kg a.s. Ava	head	.~	0.69 ~	× 0.01	1
RA-2620/07	14 and 7 days before	a gread o	$\delta 0$	Õ.5 Õ	∾≪ 0.01	
(M-308622-01-1)	harves 🔬 🔬	head	Ø 3 Ó	⁰ 0.63 ~	≪ < 0.01	
Trial No		ACY head	₹ 7.0 [°]	0.23	© [∞] < 0.01	
R 2007 0645/6		O ^v héad 🔗	18	× 0.19	< 0.01	
			<u>21</u>	× ~0,07 ~	< 0.01	
Report No	2 x 0.2 kg a.s./ha	head of	S -0	≪ ^v 1.6 <i>2</i> S	< 0.01	
RA- 14-2028	14 and 7 days before	O head		§ 5.8	0.014	
(<u>M-534623-01-1</u>)	hadvest	head si	<u>y</u>	\bigcirc ^v 2 6 $\overset{\circ}{}$	< 0.01	
Trial No	\$~ ~ ~ ^ <i>`</i>	S head ~		1.6 Ød 0.4	0.011	
14-2028-01		heads y		2.0	< 0.01	-
	d and 7 days hat a	nead	-0	× 2.0	0.015	
$(M_{-534623}, 0) = 1)$	harvest	hand a	02		0.039	
(<u>INI-354025-01-1</u>) Trial No	Marvest	head O	a. 7 m	3.6	0.027	
14-2028-02		head \sim	14	1.1	0.011	
Report No	. Q x 0.2 Q a.s./ha	∼ bead w	\$~ <u>~</u> ₽	0.42	< 0.01	
RA- 14-2028	14 and 7 days before	head &	Sõ	5.3	0.014	
(<u>M-534623-01-1</u>)	harves	Mead O	3	1.5	< 0.01	
Trial No 🔌		, ≪ head	P 7	0.83	< 0.01	
14-2028-03	N N C	head m	14	0.47	< 0.01	
Report NO	© 2 x 03 kg a,s./ha	Negad 🚬	-0	1.2	< 0.01	
RA- 14-2028	14 and 7 days before	S Shead	0	7.6	0.030	
(<u>M-534623)01-1</u>)	harvest of	* head	3	5.5	0.016	
Treat No		head	14	0.94	0.010	
14-2028-04		shead	14	0.17	< 0.01	
Report No	2 X UZ Kg a syna	hand	-0	0.0 12	< 0.01	
$(M_{-534623-01})$	hartest	head	3	13	0.023	
Trial No		head	7	12	0.014	
14-2028-05		l head	14	71	< 0.013	
Report No	2. 20 2 kg 3 /ha	head	-0	1.1	< 0.01	1
RA-14/2028	14 and 7 days before	head	Ő	5.7	0.017	
(<u>M-524623-01</u>)	A harvest	head	3	5.4	0.011	
Trial No.		head	7	3.9	0.010	
24-202 8 ,06		head	14	0.85	< 0.01	
Report No	2 x 0.2 kg a.s./ha	head	-0	2.9	< 0.01	
RA= \$8-2048	14 and 7 days before	head	0	9.1	< 0.01	
(<u>M-675904-01-1</u>)	harvest	head	3	6.7	< 0.01	
Trial No		head	7	5.5	< 0.01	
18-2048-01	1	head	14	13	< 0.01	1

Summary of the residue decline trials for fluopyram treated lettuce



Report No. (Document No.)	Application	Portion analysed	PHI (days)	Res (m	idues g/kg)	
Trial No.				AE C656948 (Fluonyram)	AE C656948)) ,
Report No	2 x 0.2 kg a.s./ha	head	-0	2.2	< 0.0	
RA- 18-2048	14 and 7 days before	head	0	6.6		
$\frac{(M-673904-01-1)}{\text{Trial No}}$	narvest	head	3 7	م بر چین	~ 0.01 ~ · · · ·	
18-2048-02		head	14	4 .6	\bigcirc \bigcirc $^{\vee} < 0.00$	
Report No R A - 18-2048	2 x 0.2 kg a.s./ha 14 and 7 days before	head	, -0 0		(1, 0, 0)	,Ø
(<u>M-675904-01-1</u>)	harvest	head	3		30.010	1
Trial No		head	7			
18-2048-03		nead	14~			
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#### Assessment of other residue studies of potential relevance for birds and wild mammals

In MCA section 8.9, studies are submitted and summarised which provide information on residue decline in matrices relevant for bird and mammal risk assessment:

- Residue decline in arthropods: 5 experimental studies and 2 kinetic evaluation reports providing 12 DT₅₀ values for foliage dwelling arthropods (3 DT₅₀s in vines, 3 DT₅₀s in OSR and 6 DT₅₀s in apple orchards), 9 DT₅₀ values for flying insects (3 DT₅₀s in vines, FDT₅₀ in OSR and 5 DT₅₀s in apple) and ground dwelling arthropods (1 DT₅₀ extended lab, 6 DT  $\delta$  in apple). Ø
- Residue decline in foliage: 143 trials and 6 kinetic evaluation Peports providing Do values for various types of vegetables (surrogates for pon-grass weeds: 118 DT₅₀s) and young cereals (surrogates for grass and cereals: 25  $DT_{50}$ s). Due to the size of the kinetic evaluation reports, these DT₅₀s are reported in 4 reports for the vegetables and 2 reports for the coreals.  $\sim$

The arthropod residue studies in this evaluation were especially conducted for the purpose to inform the bird and mammal risk assessment. Ø

The plant residue trials for this evaluation have been compiled from all potentially relevant residue decline trials conducted with fluopyram in the EU (e.g., irrespective of the applied formplated product).

However, only trials were selected where the sampled matrix corresponds with the EFSA bird and mammal food categories "grass & cereals" and non-grass words", and where the type of plant matrix and growth stage matched those behind the RUDs for these matrices in the EFSAGED 2009 App. F (e.g., cereals only up to BBCH 30 at application m

It should be noted that the data set of shrrogates for non-grass weeds also includes onions and leek, which are monocots. However, onion and leek are not grosses (do not belong to the botanical order Poales which includes both grasses and cereals), and were conducted under conditions more similar to the other vegetables for these reasons it is proposed to include onions and leek with the other vegetables into the group of sprroga@s for non-grass weeds. S

In the summaries for these studies, an attempt is wade to visualize and assess the influence of rainfall on the residue time ourse according to the recommendations of EFSA 2019. For that purpose, the DT₅₀ values from the trials have been assigned to 3 categories:

Category 1: no discernible in Plaence of precipitation

Category 2: influence possible /

Category 3: marked influence

# Influence of rainfall on arthropod residue decline

1

The evaluation of the arthropod residue decrine trials demonstrated that rainfall occurred in the majority of trials. Thus, rainfall (and/or insignition) is a prical element for exposure assessment in realistic bird and wild mammakscenarios under EFSA GD 2009. However, there was hardly any discernible impact of rainfall on the insect residue decone, so that nearly all trials can be assigned to rainfall category 1. The difference between the geomean  $D\mathfrak{D}_{50}$  for category 1 trials and for both category 1 & 2 trials is negligible  $\langle \xi \rangle$  Therefore, it is proposed to pool all trials per foliage dwelling arthropods (n= 12), flying insects ( $n^{(2)}$ 9) or groun Odwelling arthropods (n= 7), respectively.

DT30 of floopyram in arthropods

The geometric mean  $DT_{50}$  for foliage dwellers is 3.10 days (n= 12), for flying insects it is 3.03 days (n= 9) and for ground dwellers it is 6.39 days (n= 7).



Group	Crop	Zone	Kinetic	DT50	Cat	Rainfall	Source	ð
Plot			model				DT	Ş
Edition no.						Ô		0
Ground dweller	Bare	na	HS slow	5.58	1	none	A <u>M-</u>	
Extended lab	soil		phase				<u>545010-</u>	
<u>M-545010-02-1</u>			2016					Q
Foliage dweller	Vines	Ν	FOMC	5.94 ^{a)}		No discernible influence of	EnSa-	,
plot l			$DT_{90}/3.32$			rainfall op residue time course	<b>3</b> 5-093 <b>4</b> *	
<u>M-453376-01-2</u>	x 7°	ЪT	dE0		× 1			Ő
Foliage dweller	Vines	Ν	SFO	5.57	* 1	No discernible influence of $Q$	Enga-	1
plot 2						raintall on residue tume course	19-0934	
<u>M-4333/0-01-2</u>	Vinag	N	EOMC	- 287	2			
Foliage dweller	vines	IN	FUNIC	2.37	oZ	Consistent as a lation with the	EnSa	
piot 5			D190/3.32	)" _@`		rasidita tire acuration with the	13-0934	
<u>M-435570-01-2</u>	Vince	N	SEO 着	598		Noticeerpible intruence	Enso av	
riving insects	vines	IN	SFU X	2 C 2 C		The second secon	15 6924	
M_453376_01_2			Ű,	₹¥ .©			13-00-34	
Flying insects	Vines	N	H	2850	r	Nædiscern ble interence of	sEnSa-	
nlot 2	v mes	1	DT 0/3 0		\$V	raynfall or residue time sourses	15-0934	
M-453376-01-2		d		<i>6</i>	× 4		15 0751	
Flying insects	Vines	N.Ø	DFOP	3 38	2	Frequent early rainfail without	EnSa-	
nlot 3	v mes		$DT_{00}/3$ 80%	5.5900	-4	consistent@orrelation with the	15-0934	
M-453376-01-2		$\sim$		a s	[®]	residue time course	10 0751	
Foliage dweller	Oilseed	₽ _N (	SFO	00 685.	1	No discernible influence of	EnSa-	
plot 1	rape.	A			Ô	raigafall on residue time course	16-0035	
M-544190-01-1				, ^o	N.	O 4 N		
Foliage dweller	Queseed	N Å	HS and	49078 a	Sĭ -	No discernible influence of	EnSa-	
plot 2	ape Ó		DT563.32~		Ĩ, ĝ	rainfall on residue time course	16-0035	
<u>M-544190-01-1</u>		~~	& <u>&gt;</u>		N N			
Foliage dweller,	Oilseed	Ø	OS &	1,594	ð	No discernible influence of	EnSa-	
plot 3	rape		DT ₉₀ (3.32			rainfall on residue time course	16-0035	
<u>M-544190-01-1</u>	×.	s s						
Flying insects	Oilseed	N	SEO N	2.13	K)	V v little rain	EnSa-	
plots 1+2+3	rape			NY (	4.	A Y	16-0035	
<u>M-544190-01-1</u>	S i			¢ C				
Foliage dweller	Apple	N	SFO	4.1		No discernible influence of	<u>M-</u>	
plot l	orchand	õ		, Ô ^y	- Or	rainfall on residue time course	<u>644049-</u>	
<u>M-644049-QOI</u>	O A	o a			<b>.</b>		<u>01-1</u>	
Foliage dweller	Apple (	rn 🄊	FOME	\$ ^{2.7} _©	1	No discernible influence of	$\frac{M}{(14040)}$	
plot 2	orchard	Q.	$D_{0}/3.32$			rainfall on residue time course	<u>644049-</u>	
<u>M-644699-01-1</u>		NT .	Found		2		<u>01-1</u>	
Follage dweller	apple			283	2	Slight influence of rainfall	<u>IVI-</u>	
M 644040 01 1	orcharg	″٢, ا	D190% 32	) [′]		from day 8	<u>644049-</u> 01_1	
<u>M-044049-01-1</u>	1 1 1 min 1 2	NO NO	NOMC Q	2.4	1	No discornible influence of	<u>01-1</u> M	
rlying insects	Apple	Č,		2.4	1	rainfall on residue time course	<u>1v1-</u> 644040	
M-644040		<u>ک</u> ک					01-1	
Flying inspects	Annle	NÔ	SEO	22	1	No discernible influence of	<u> </u>	
nlot 2	orchard		510	2.2	1	rainfall on residue time course	<u>644049-</u>	
M-644049-00-1		К [°]					01-1	
Fixing insects	Annle	N	SFO	1.9	1	No discernible influence of	M-	
plot 3 0	orchard	- 1	~~~~		1	rainfall on residue time course	644049-	
M-644049-01-1							01-1	
Ground dweller	Apple	Ν	Pseudo	8.3	1	No discernible influence of	EnSa-	
plot 1	orchard		SFO DT ₅₀			rainfall on residue time course	20-0891	

Table 10.1- 3:	DT ₅₀ of fluopyram in arthropods per stratum and rainfall category
----------------	-------------------------------------------------------------------------------



Group	Crop	Zone	Kinetic	DT ₅₀	Cat	Rainfall	Source
Plot	_		model				DT50 °
Edition no.							
M-644049-01-1							N A
Ground dweller	Apple	Ν	Pseudo	4.4	1	No discernible influence of	EnSa-
plot 2	orchard		SFO DT ₅₀			rainfall on resiductime course	20-082 ·
M-644049-01-1							
Ground dweller	Apple	Ν	Pseudo	9.4	1	No discernible influence of O	Ensa-
plot 3	orchard		SFO DT ₅₀		Ĉħ	rainfall on tesidue time course	20-0891
M-644049-01-1					The second secon		
Foliage dweller	Apple	S	SFO	6.1	<u>,</u> 1	No discornible influence of	<u>M-</u>
plot 1	orchard			a O	r	rainfall on residue time course	<u>649048-</u>
<u>M-644048-01-1</u>							01-1
Foliage dweller	Apple	S	FOMC	5R\$	1	No discernible influence of	© <u>M-</u>
plot 2	orchard		DT ₉₀ /3.32	k, Ö	° •	rainfall on residue time course	<u>644048-</u>
<u>M-644048-01-1</u>			C	) [*] [*]	×.		<u>04-1</u>
Foliage dweller	Apple	S	SFO 🛒	4.4		Nodiscernible influence of	M-
plot 3	orchard		K, Y		$\sum$	rainfall on residue time course	<u>644048-</u>
<u>M-644048-01-1</u>			an in	V O	× 4		<u>01</u>
Flying insects	Apple	S	SEQ	4.9 🔊		No discernible influence of	<u>M</u> -
plot 1	orchard				L,	rainfall on residue time course	<u>¢ø44048-</u>
<u>M-644048-01-1</u>		d		à ?	~		<u>01-1</u>
Flying insects	Apple	S _Q	SFQ	3.8	1	No discernible influence of	<u>M-</u>
plot 3	orchard	~~~		1 Or	- K	rainfall on residue time course	<u>644048-</u>
<u>M-644048-01-1</u>		<i>w</i>	k Š	L.		N N O	<u>01-1</u>
Ground dweller	Apple	¢S (	Pseudo	Į.	٦	No discornible influence of	EnSa-
plot 1	orchard	1 1	SFO DT50	Š . 67	Ś	rainfall on residue tione course	20-0890
<u>M-644048-01-1</u>	Ś		<u> </u>	ð	s O		
Ground dweller	Apple	Ś ŝ	Seudo	55	¢2″	Moderate rainfals on days 4	EnSa-
plot 2	@chard	f , Č	SFQ DT ₅₀		p" a	and 5 coincide with a visible	20-0890
<u>M-644048-01-1</u>		L, Y		t N	Š	droppin residures, influence	
			× , @	×,×		likery 🖤	
Ground dweller	Apple .	S .	Pseudo	, <b>5</b> QS ,	S.	No discornible influence of	EnSa-
plot 3 🧳	orchard		SFQ DT50 7	S C	a A	rainfall on residue time course	20-0890
M-644048-01-1							

(a it is proposed to use FOMC as the best fit (instead of OFOP as selected in EnSa-15-0934) because the visual fit

rating is identical but the  $\chi^2$ -error is lower ^(b): it is proposed to use HS as the loss fit for flying insects on plot 2 (instead of DFOP as selected in EnSa-15-0934) because the visual fit rating is identical but the  $\chi^2$  error is lower ^(c): it is proposed to use DFOP is the best fit for flying insects on plot 2 (instead of SFO as selected in EnSa-15-0934) because the visual fit rating is identical but the  $\chi^2$  error is lower ^(c): it is proposed to use DFOP is the best fit for flying insects on plot 2 (instead of SFO as selected in EnSa-15-0024).

0934) because the visual fit running is menticable the  $\chi^2$  -error is lower ^(d) it is proposed to use the pseudo-SPO DTF of 5.5 days instead of the FOMC DT₉₀/3.32 of 7.9 days as suggested in the original report. Just Acation both the pseudor-SFO of 5.5 days and the FOMC DT₉₀/3.32 of 7.9 days are used here as surrogate for the real best fit kinetic with the FOMC parameter alpha = 1.6093 and beta = 3.4342 (which is difficult to apply without a suitable calculator like TREC, Ebeling & Hammel 2020). However, the surrogate SFO- $DT_{50}$  calculated as FOMIC  $DT_{60}/3.32$  of 7.9 mays is an overestimation as it results in a 21-d  $f_{TWA}$  much larger than the 21d-  $f_{TWA}$  calculated with the FOMIC parameter alpha and beta. The 21-d  $f_{TWA}$  calculation with the pseudo SFO-DT₅₀ of 55th days stull overestimates the 21-d  $f_{TWA}$  but is much closer to the best fit 21-d  $f_{TWA}$  with the FOMC parameter alpha and beta: R

Appresch	, Calculated with	Parameter values	<b>Resulting 21-d f</b> _{TWA}
FOMC-DT 3.32	Surrogate SFO DT ₅₀	7.9 days	0.46
Pseudo SFG-DT50	Surrogate SFO DT ₅₀	5.5 days	0.35
Best fit parameter	FOMC alpha & beta	1.0693 & 3.4342	0.30

Therefore, the pseudo SFO- $DT_{50} = 5.5$  days can be considered as a more accurate kinetic parameter than the FOMC-DT₉₀/3.32 = 7.9 days, which is still conservative compared with the best fit FOMC kinetic.



#### Influence of rainfall on foliage residue decline

The evaluation of the foliage residue decline trials demonstrated that rainfall occurred in the majority of trials (in vegetables often supplemented by irrigation). Thus, rainfall (and/or Grigation) is a typical element for exposure assessment in realistic bird and wild mammal scenario under EFSA GD 2009. The comparison of DT₅₀s for the 3 rainfall categories indicate slower residue dissipation of category 1 than in categories 2 or 3, which is not surprising since rainfall may influence residue decline by various gory of the second seco mechanisms beside wash-off (e.g., allowing dilution by plant growth, promoting metabolic activity of microflora on leaf surfaces).

Table 10.1- 4:	Summary of DT50s in plan	nt foliage per rainfat	category	and feed category
14010 1001 10				

Cate	gory 1	Cate	gory 2	Qite	of a france of a state	e 0
young cereals	non- grass herbs	young cereals	non- grass herbs @	young % Gereals	grass heeds	
4.60 d	3.39 d	3.58 d	3.22 d	2.50 đ	276 d geomean DEso	
11	34	6	36	80	V48 number of trials	
44%	29%	24%	31% «	32%	41 de la company	
		d	\$ X	~C		

Table 10.1- 5:	Overview on	foliage	residue	decline DT	50 sorted 1	ner rainfa	al inflorence	categories
	0 . 01	- or and -						entegoi les

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Trial	Crep	Zone -	Kinetic	DT50	Cat	Inforence kain and or	Source
Edition no.	Û,		model	mod	No.	irrigation 🔗	DT50
R 2006 0655/9	Beans O	N S	SFQ .	~ <b>2</b> ,729.,~	Q1	bate raise no influence	Ensa-20-
<u>M-290825-01-1</u>		Ş	<u>%</u>	ÝŴ			8029
R 2006 0722/9	Beaus	ÔN	OŠFO ⟨⟨ [′] O [®]	13,88	10	vers little rain, no influence	Ensa-20-
<u>M-291180-01-1</u>	Or K		4		S		8029
R 2006 0723/7	Beans	NŚ	SFO	<b>©</b> .636	1	Vate rafo, no influence	Ensa-20-
<u>M-291 00-01-1</u>			¢,	- A			8029
08-2096-01 T1	Beans	∖S″ - ≼	SFO	2.969	دا ا	irrigation d5 and d11, no	Ensa-20-
<u>M-365542-01-1</u>	\$° 4	S S	~~		ŏ× .	discernible influence	8029
R 2006 0378/9	Beans	S	ઙાજ્	8.872	1	pho rain, no influence	Ensa-20-
<u>M-290827-01-1</u>	Å.	Ň	ê ô		Ĩ		8029
R 2006 06575	Beans	S a	∀HS 🥎	0,883	J.	no rain, no influence	Ensa-20-
<u>M-290827-01-1</u>	Õ	~0"	<u>A</u>	<u>s</u>			8029
R 2006 0658/3	Beans	Sa	SEO X	≫3.548≪	1	no rain, late irrigation, no	Ensa-20-
<u>M-290827-01-1</u>	Š)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~				influence	8029
R 2007 0550/6	Beans	S 🔊 🖉	"SFO@"	0 7695	1	no rainfall, no influence	Ensa-20-
<u>M-297564-01-1</u>	ð	Č,	_~~\{	0 [×]			8030
R 2007 0551/4	^{\Seans}	S	STO A	¥8.169	1	no rainfall, no influence	Ensa-20-
<u>M-297564-01</u>	A`.		y 'v				8030
R 2007 05522	Beans S	S S	DF Q4	11.166	1	nearly no rainfall (1.2mm day	Ensa-20-
<u>M-297564 01-1</u>		Š	¥			7), no influence	8030
R 2007 0599/9	Cabbage	N)	SFO	1.979	1	marked decline, unlikely to be	EnSa-
<u>M-30%101-016</u>						influenced by very little early	20-0832
	Ø N	-				rainfall	
R\$006 05\$4/7	Cabbage	S	FOMC	3.148	1	Little rainfall until day 14, no	EnSa-
<u>M-2931@2-01-1</u>						influence discernible	20-0832
R 2006 0605/2	Lettuce	Ν	HS	3.587	1	very little rain, no influence	Ensa-20-
<u>M-292048-01-1</u>							8029



Trial	Crop	Zone	Kinetic	DT ₅₀	Cat	Influence rain and/or	Source
Edition no.	-		model	mod		irrigation	DT50 _ °
R 2007 0244/2 M-304280-01-1	Lettuce	N	SFO	3.09	1	late rain, no marked influence	Ensa 0- 8030
R 2007 0540/9 M-304280-01-1	Lettuce	N	SFO	1.368	1	late rain, no discernite	Ensa-20, \$030
14-2029-02 <u>M-534202-01-1</u>	Lettuce	N	SFO	2.892	1	little rainfall, irrigation without discernible impact. Influence	Ensa-20- 803 Y
14-2029-04 <u>M-534202-01-1</u>	Lettuce	N	SFO	2.034	17	no rainfall antil day 6, without discernible impact. Influence	Énsa-29- 8034 0
18-2086-01-T1 M-675005-01-1	Lettuce	S	SFO	8.248	1	nearly no rate, no influence	Ensa-20 8022
18-2086-01-T2 M-675005-01-1	Lettuce	S	SFO	Ø.754 O	ġ°	pearly for rain, no influence	Ensa+20- 8029
18-2086-02-T1 M-675005-01-1	Lettuce	S	SFO	3.419		neady no ram, no influenco	ænsa-20+ 8029
18-2086-02-T2 M-675005-01-1	Lettuce	S	SFO	° <b>4,</b> 04		Apearly no rain, no influence	Ensa-20- 8029
18-2086-03-T1 M-675005-01-1	Lettuce	S	SFO ở	4.378	15	nø rain, no milluente	Ensa-20- 8029
18-2086-03-T2 M-675005-01-1	Lettuce	S	SFO ²	\$.339 A	M L	no rain no influence	Ensa-20- 8029
18-2086-04-T1 M-675005-01-1	Lettuce	<b>Š</b> (4 ) O	SFO OF	1.186	1 ₀	nearly no rein until d7, no	Ensa-20- 8029
18-2086-04-T2 M-675005-01-1	Lettuce V	SA	SFO ©	\$.174 °	×1	Snearly no rainfuntil de no	Ensa-20- 8029
R 2006 0376/2 M-292050-01-1	Lettuce	Š.S	SFO @	1.5		little rain, Date irrigation, no	Ensa-20- 8029
R 2006 0608/7	Letture	S	SFO ~	2.57	1	very little rath no influence	Ensa-20- 8029
R 2006 0610/9 M-292050-001-1	Lettuce «	JS J [©]	SFO SFO	3. <b>52</b> 9	Ê,	late rain, to influence	Ensa-20- 8029
R 2006.0611/7 M-292650-01-1	Lettuce	89	STO C	3.02		late ram, no influence	Ensa-20- 8029
14-2030-01 M-534595-01-1	A ottuce 4	S	SFQO	4304	Ø,	Vertually no rain, no influence	Ensa-20- 8031
14-2030-02 <u>M-534595-01</u>	Lettuge	S ×	Stro à	5.522		no rain, no influence	Ensa-20- 8031
14-2185-02 M-536963-01-1	Lettuce	S S	SFQ	40578	ð	virtually no rain, no influence	Ensa-20- 8031
14-218503 M-536963-01-1	Lettuce		SFO S	4.78	1	no rain until day 9, no influence	Ensa-20- 8031
R 2007 0568/9 M-302325-01-1	Onion S	S	SFQ	2 <b>2</b> 03	1	No rainfall and no influence from irrigation day 10	EnSa- 20-0832
18-2951-02 M-678413-001	Young cereals		SFO Q	3.214	1	very little rain, no influence	EnSa- 20-0834
18-2951-03 M-678412-01-1	Young cereals	N 🖑	SFQ	3.523	1	no rain, no influence	EnSa- 20-0834
E19R0102-01	Young cereals.	(N)	SFO	6.419	1	no rain, no influence	EnSa- 20-0834
E <b>A9</b> ŘP102-02 M-758824-01-1	Young	Ν	SFO	8.185	1	no rain, no influence	EnSa- 20-0834
15-29 <b>\$2</b> -01 M-566830-01-1	Young cereals	Ν	SFO	3.37	1	rain only late, no influence	EnSa- 17-0484



Trial	Crop	Zone	Kinetic	DT ₅₀	Cat	Influence rain and/or	Source	
Edition no.			model	mod		irrigation	DT ₅₀ °	
15-2952-02	Young	Ν	SFO	7.59	1	no rain, no influence	EnSa	
<u>M-566830-01-1</u>	cereals					<u>^</u>	17,0484	6 ⁷
18-2954-03	Young	S	SFO	3.607	1	no rain, no influence	Ensa-	
<u>M-675129-02-1</u>	cereals						20-083	
E19RP087-01	Young	S	SFO	10.18	1	no rain, no influence	EnSa	<i>R</i> o
<u>M-758649-01-1</u>	cereals						2060834	
E19RP087-02	Young	S	SFO	1.782	1 🖒	rain d4 and d5 but no	ÉnSa- 🖉	<i></i>
<u>M-758649-01-1</u>	cereals				Ŵ	discernible influence O 🖓	<u>)</u> 20-083,4	, L
15-2952-04	Young	S	SFO	4.19	J.	very little rain, no influence	EnSa	,0″
<u>M-566830-01-1</u>	cereals			4	V″		17-0484	X
15-2953-03	Young	S	SFO	4.64	1	no rain, no influenço	EnSa- 🖉	
<u>M-566828-01-1</u>	cereals			- Q°			©17- <b>04\$4</b>	
R 2006 0377/0	Beans	Ν	SFO	4.674	63°	Date rain, influence possible 🛯 🥎	Ensa 20-	
<u>M-290825-01-1</u>				0′@	Ň		<u>80</u> 29 。	
R 2006 0656/7	Beans	Ν	SFO 🔬	2.84	20	frequent rainfall, slight	@Ensa-2∯≁	
<u>M-290825-01-1</u>			st s	Ň	$\sim$	influence possible 4	8029	
R 2007 0546/8	Beans	Ν	SFO	\$ <b>2,969</b> (	ØŽ 🔬	frequent but little rain onfluence	Epsa-20-	
<u>M-297562-01-1</u>			Q V			possible of a f	8030	
R 2007 0547/6	Beans	N ,	SFO 🔊	2.744	2≪∛	late frigation and orinfall	Ensa-20-	
<u>M-297562-01-1</u>		- Q		Ĩ.	$\gg$	possibly gight influence 🖓 🔬	8030	
R 2007 0548/4	Beans	N	HS	<i>@</i> .639 🗸	2	Juttle early and more rain on day	Ensa-20-	
<u>M-297562-01-1</u>		$\sim$		- 0	4	6, normarked influence	8030	
R 2006 0347/9	Cabbage	¥ %	∛ل HS	4.729	200	frequent rainfall but in small	EnSa-	
<u>M-292103-01-1</u>		) ()			~	amounts which are unlikely to	20-0832	
	~~	A		Å,		Shave marked Minfluenced		
	Ś	S"	Q (			residue levels.		
R 2006 0543/9	Cabbage	"N 🔍	SFO Ø	5.78	2	Little rainfall until day 8, no	EnSa-	
<u>M-292103-01-1</u>	S A				õ	fulluence discernible	20-0832	
R 2006 0348/7	Cabbage	S	FOMC ≈	3.693 🔿	2 🐔	Little Cainfall Ontil day 8, no	EnSa-	
<u>M-293182-01</u>	Č,	Ô	× 10			influence discernible	20-0832	
R 2007 0079/2	Cabbage	JS .	FOMC	4.084	L,	norked deeline until 2nd	EnSa-	
<u>M-302044-01-1</u>	×.,	~~?	A	°,	°	sampling but little early rain	20-0832	
	Ŭ.	ŝ		S	ŝ	until day 7 (influence		
<u> </u>	<u> </u>	0)				questionable)		
R 2007 0600/6	Cobbage (	S	SFQO	3.981	S2/	Moderate early rainfall but no	EnSa-	
<u>M-302044-01-1</u>	2 1	Ű	Ŵ,		Ρ 🦓	marked decline (influence	20-0832	
		- S	× °		Ş	unlikely)		
10-2099-01	Epcive	Ň 🦻	SFO O	2.252	2 "0"	frequent heavy rainfall,	Ensa-20-	
<u>M-423901-01-1</u>	8		$Q^{\prime}$	Q.	Õ	influence not discernible but	8029	
4	~	Š	and the second		<i>v</i>	likely		
R 2006 0343/6	Leek	₩Ç [®]	SFO 🔊	8.282	2	frequent rainfall after day 5 did	EnSa-	
<u>M-292101-02-1</u>						not seem to have any	20-0832	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	N S		-Q	S,		discernible influence on residue		
	\'O'	<u> </u>				dissipation		
R 2006 0466/1	Leek	ð j	SFO Q	, 5.836	2	frequent rainfall after day 7 did	EnSa-	
<u>M-292101-02)ř</u>	S é					not seem to have any	20-0832	
	V Ö	×.	~Q~			discernible influence		
R 2006 0468/8	≫Leek©	N°	SFO	8.99	2	Frequent late rainfall and heavy	EnSa-	
<u>M-292191-02</u>	A	Ç,				irrigation coincide with a	20-0832	
						moderate drop of residue levels		
		~	97.6	6.01		on day 15		
R 2006 (\$44/4	Leek	S	SFO	6.01	2	rainfall on day 6 and 7 may	EnSa-	
<u>M-292082-01-1</u>						have slightly influenced residue	20-0832	
D 2005 045015	T 1	G	050	7.051	-	dissipation	F <i>C</i>	-
R 2006 0469/6	Leek	S	SFO	7.054	2	trequent irrigation and	EnSa-	
<u>M-292082-01-1</u>						occasional rainfall may have	20-0832	



Trial	Crop	Zone	Kinetic	DT ₅₀	Cat	Influence rain and/or	Source	
Edition no.			model	mod		irrigation	DT50 °	~
						markedly influenced residue		^o
						dissipation, although this is not		0 ⁷
						discernible in the dechine pattern	Ũ A	
R 2006 0604/4	Lettuce	Ν	SFO	1.409	2	rain after 75% already declined,	Ensa-20-	
<u>M-292048-01-1</u>	.		97.0			at most slight influence	8029	Ĉo
R 2006 0606/0	Lettuce	Ν	SFO	2.452	2	some rain after and sampling O	Enosa-20-	e) i
<u>M-292048-01-1</u>	T 11	N T	(TEO)	1.0.40	<u> </u>	but no visible influence	80129	<i>(</i>)
R 2007 0011/3	Lettuce	Ν	SFO	1.048	2%	little rain during first days	DEnsa-29-	Å
<u>M-304280-01-1</u> D 2007 0527/0	Latteras	N	EOMC	1.040 @	£	Little Ma conference	8030×	\sum_{k}
K 2007 055779	Lettuce	IN	/ DEOD	1.949	2	nossible any rain, indudence	EASa-20-@	ŗ
P 2007 0520/5	Lattuco	N	/ DFOF	2 1000	2	Vor orly of the line of the li	\mathcal{O} Enco	
M_30/280_01_1	Leuuce	IN	510	2.1%	2 &	the cornible	8030	
14_2029_01	Lettuce	N	SEO	[∞] 021 Ø		traquent irrightion and fainfall	8030 Emsa_20	
$M_{-534202-01-1}$	Lenuce	1	510			Slight influence possible	20^{13}	
14-2029-03	Lettuce	N	SEO	1 682	.57	ktle rainfall untid av 6 (8 mm)	Fnsa 0-	
M-534202-01-1	Lettuce	11				Slight influence possible	803	
14-2029-05	Lettuce	Ν	SEO /	M.8 ×	2	several rainfails without	Ensa-20-	
M-534202-01-1					J.	discernible impact Flight &	2 8031	
		Ŵ		Ře	~	inDiuence cannot be excluded 🔬		
14-2184-02	Lettuce	N _@	SEO	A.71 L	×2	painfall coincides with light	Ensa-20-	
<u>M-536965-01-1</u>		~~~	°~y	ŭ 🖓	4	drop residue levels. Influence	8031	
		Ψ° γ	, d	4		possible. 🔊		
14-2184-03	Lettucę 🖉	N O	SFQ	2,406	2	irrigation coincides with slight	Ensa-20-	
<u>M-536965-01-1</u>	~~	A		ŝ,		Arop of residue levels Influence	8031	
	×,	S.			No.	possible 🌾 😽		
R 2006 0609/5	Letrúce	`S 🛒	SFO Ø	0.8344	29	little rain Ouring flyst days, but	Ensa-20-	
<u>M-292050-01-1</u>	S d				Ô,	Miluence possible	8029	
R 2007 0012/1	Lettuce	S 🎸	SFO A	1.813	2 🐔	ho rate but dayly irrigation.	Ensa-20-	
<u>M-3042/8-01-0</u>		0.			ð	Inference likely.	8030	
R 2007 0245/0	Lettuce «	JS Ø	SFO "	1.204		howe but very early rain after	Ensa-20-	
$\frac{M-3042/8-01-1}{D 2007.05}$	Latture	S S		0° 5 707@		application, influence possible	8030 Emai: 20	
K 2007 VS4177	Leilice	S, v		3.1910		nequent but nulle rain, innuence	Elisa-20- 8030	
14-2030-03	Jothice of		SEOO	3/233	\$ 0.	Repeated to the second	Ensa_20_	
M-534595-01-1		° S	5100	W V	Ď ø	sprinkler irrigation Marked	8031	
<u> </u>					-C	influence not discernible but	0051	
Ø	,Ô¥	õ 🔹	Û O	, Ô ^v	- Or	slight impact likely		
14-2185-04	Lettuce	S 🚿	SFQ	20403	ð	Several rainfalls around 2nd	Ensa-20-	
M-536963-01-1	. 0.		A.Y.	Q (P-	sampling, no influence	8031	
		Â,				discernible		
R 2006 0339/8	Oxion _	S	SFO SFO	4:448	2	Irrigation coincides with a	EnSa-	
<u>M-292098-01-1</u>	s S		-Q	S		moderate drop of residue levels,	20-0832	
, (0).	\ \	<u> </u>	a, Å			slight influence likely		
R 2007 0555/7	Peas∿	ð í	SFO Q	5.468	2	Only little rainfall but	Ensa-20-	
<u>M-298639-001</u>						coinciding with residue decline.	8030	
	× [°]			7.000		Influence possible.	D C	
R 2007 0856/5	r Peas ∪	Jose -	SFO	7.032	2	many days with little rainfall.	Ensa-20-	
<u>M-298099-01-07</u>			GEO	2 275		Influence possible	8030	
K 200/ 055/5	reas N	- 5	SFO	3.275	2	early irrigation and rainfall,	Ensa-20-	
$\frac{1}{10}$	Vauna	N	SEO	7.01	2	milluence possible	8030 EnSc	
$M_758874_01_1$	i oung	IN	510	/.01	2	influence	20-0834	
$E19RP102_04$	Young	N	FOMC	4 3 1 9	2	moderate rain d2_slight	EnSa-	
M-758824-01-1	cereals	1	1 01010	1.517	-	influence	20-0834	
			I	1	1		==	1



Edition no.modelmodirrigationDT50 °13-2950-01YoungNSFO1.952little early rainfall, very littleEnSaM-471216-01-1cerealsImpact on DT5017/048417/048415-2953-02YoungNSFO4.232heavy rain day 5, visible butEnSa-M-566828-01-1cerealsImpact on DT5017/0484Impact on DT50Impact on DT50E19RP087-04YoungSSFO2.9562"moderate rain d3, d4; slightEnSa	
13-2950-01 M-471216-01-1Young cerealsNSFO1.952little early rainfall, very little impact on DT50Ensage 17,048415-2953-02 M-566828-01-1Young cerealsNSFO4.232heavy rain day 5, visible but slight influenceEnsage 17,0484E19RP087-04Young YoungSSFO2.9562"moderate rain d3, d4; slightEnsage Ensage	- G F
M-471216-01-1 cereals impact on DT50 17,0484 15-2953-02 Young N SFO 4.23 2 heavy rain day 5, visible but slight influence Ensa- virona M-566828-01-1 cereals SFO 2.956 2 "moderate rain d3, d4; slight Ensa- virona) }
15-2953-02 M-566828-01-1Young cerealsNSFO4.232heavy rain day 5, visible but slight influenceFor Sa- V7-0484E19RP087-04YoungSSFO2.9562"moderate rain d3, d4; slight V7-0484EnSa	
M-566828-01-1 cereals slight influence 7-0484 E19RP087-04 Young S SFO 2.956 2 "moderate rain d3, d4; slight EnSa	
E19RP087-04 Young S SFO 2.956 2 "moderate rain d3,"d4; slight reference SFO	
	à
<u>M-758649-01-1</u> cereals influence"	2
15-2952-03 Young S SFO 2.86 2 no rain before day 5, only shift EnSa-	Ø,
$\frac{M-566830-01-1}{2}$ cereals influence 0 017-0484	Å
08-2034-01 T1 Beans N SFO 4.053 3 moderate rain d3, d5, marked 8000 8000	/
08-2034-02 T2 Beans N SFO 3.992 3 moderate rate d3 d5 market Ensa-26	
$\frac{M-365530-01-1}{M}$	
R 2006 0380/0 Beans N SFO \$7.32 3° Date rain but induced Rely ~ Ensu 20-	
<u>M-291180-01-1</u>	
R 2006 0654/0 Beans N SFO \swarrow 0.7187 3 \heartsuit marked influence by early \heartsuit \checkmark \checkmark marked influence by early \heartsuit	
<u>M-290825-01-1</u> <u>M-290825-01-1</u> <u>M-290825-01-1</u>	
R 2007 0014/8 Beans N SF6 2,733 03 Heavy fain on days around 2nd Epsi-20-	
M-297562-01-1 Q & Sampling, influence likely 8030	
R 2007 0549/2 Beans N SFO 7 3.172 34 marked influence of rainfall Ensa-20-	
<u>M-297562-01-1</u> Q A BO30	
08-2096-02 T2 Beans S SEO 3:648 3 Jurrigation d5 and d11 Garked Ensa-20-	
$\frac{M-365542-01-1}{M}$	
\mathbb{R} 2006 0620/6 Beans S \mathbb{S} SFO \mathbb{C}^{*} 0.7254 $3_{\mathbb{C}^{*}}$ matched introduce of early Ensa-20-	
$\frac{M-290827-01-1}{2}$	
$\mathbb{R}^{2007}(0035/0)$ Beans \mathbb{R}^{3} SFO $\mathbb{R}^{3.176}$ Marge rainfall days 4 and 5, Ensa-20-	
$\frac{M-297564-01-1}{D} = \frac{1}{2} + $	
R 200/00/8/4 Coopbage N (SFO 2.962 2 early raintall coincides with EnSa-	
$\frac{M-302101-01-1}{5}$	
10-2099-02 O Endeve N POMCO 2.659 3 early rainfall, marked decline, Ensa-20-	
$\underline{M-423901-019}$	
10-2099-03 Endive, N SFO, 3228 3 gearly rainfall, marked decline, Ensa-20-	
M-42390 201-1 0 8029	
10-2099-04 Endeve SFO 1.489 3 early rainfall, marked decline, Ensa-20-	
$\underline{M-423901-01-1} = \sqrt[3]{2} \sqrt$	
11-2029-01 Veek N V SEO 279 3 Heavy rainfall coincides with a EnSa-	
$\underline{M-442996-01-1} \qquad \qquad$	
influence likely	
11-2029-02 Leek N SFQ Z457 SFQ Heavy rainfall coincides with a EnSa-	
$\frac{M-442996-01-1}{M-442996-01-1}$	
influence likely	
11-2029-03 Each AN SFO 25070 3 Early rainfall coincides with a EnSa-	
$\frac{M-442996-01-1}{M}$	
11-2029-04 Eleck OF 2.543 3 Early rainfall coincides with a EnSa-	
M-442996-001 marked drop of residue levels, 20-0832	
P 2006 (25/2 - V and C NOV SEC 2.246 2 Encrement and a minimized Enclosed	
M 2020 1 02 M Leek STO 2.540 5 Frequent early rainial coincides EnSa-	
with a marked drop of residue 20-0852	
R \$907.00\$6/3 Peek & N DEOP 4.184 3 Early rainfall coincided with a EnSa	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	
likely)	
R 2007 0249/3 Leek N HS 3 392 3 early rainfall coincides with EnSa-	
<u>M-304276-01-1</u> marked drop (influence likely) 20-0832	



Trial	Crop	Zone	Kinetic	DT ₅₀	Cat	Influence rain and/or	Source	
Edition no.	_		model	mod		irrigation	DT50 °	
R 2007 0569/7	Leek	N	FOMC	3.551	3	early rainfall coincides with	EnSa	, Ô
<u>M-304288-01-1</u>						marked drop (influence likely)	20-0832	ð.
R 2007 0570/0	Leek	Ν	FOMC	3.675	3	early rainfall coincides with	Ensa-	
<u>M-304288-01-1</u>						marked drop (influence likely)	20-083	
R 2007 0571/9	Leek	Ν	SFO	2.557	3	early rainfall coincides with	EnSa-	Ĉo
<u>M-304288-01-1</u>			6 70			marked drop (influence likely)	2060832	e) i
R 2007 0573/5	Leek	Ν	SFO	3.321	3	early rainfall coincides with	EnSa-	a)
<u>M-3042/6-01-1</u> D 2007 0574/2	T 1	N	GEO	2.016	·¥*	marked drop (influence likely)	D20-0832	Š
K 2007 0574/3	Leeк	IN	SFU	2.916	P	market drop (influen alikely)	200022	\bigvee
R 2007 0057/1	Leek	S	FOMC	5 241	3	early itrigation coincides with	EnSa &	, °
M-302775-01-1	LUCK	5	TOME	2.2 - Age v	5	marked drop (influence likely	20-08	
R 2007 0250/7	Leek	S	HS	¢1.434	ra [°]	early in gation coincide with ~	EnSa-	
M-302780-01-1		~		Ő,		moderate droff influence likely)	20-0832	
R 2007 0572/7	Leek	S	SFO 🔬	1.952	3 @	earl@irrigation coincides with	EnSa-	
M-302775-01-1			s s		\sim	marked drop (inforence likely)	20-08.32	
R 2006 0375/4	Lettuce	Ν	HS	2,198	Ø3 🔬	learly ranifall and springer 🖉	Ensa-20-	
<u>M-292048-01-1</u>			Q V			virrigation, marked influence	8029	
R 2006 0607/9	Lettuce	N	SFO 🔗	1.129	3≪∛	marked influence by sprinker	Ensa-20-	
<u>M-292048-01-1</u>		R	È ch	Ô	ð	in gation	8029	
R 2007 0538/7	Lettuce	NØ	SEO	@ .72554	3	marked influence of early	Ensa-20-	
<u>M-304280-01-1</u>	T	×,		1.065	~		8030	
14-2184-01 M 52(0(5,01,1	Lettuce	N %	SFO C	1.0%	-70°	Early raints I concides with	Ensa-20-	
<u>M-550905-01-1</u>		4	Ŵ.	S I	¢ .	marked drop of desidue levels.	8031	
14-2184-04	Lettade	NO Y	SFO (1 598	3.00	frequent early rainfall may have	Ensa_20_	
M-536965-01-1	S	de la				markedly fluenced residue	8031	
<u></u>	S S	. 6	, <u>~</u> ~		n n n n n n n n n n n n n n n n n n n	evels , a	0001	
R 2007 0246/9	Letture	S	,SFÕ ∧	0.8952	3 🐔	Fearly and Arrigation,	Ensa-20-	
<u>M-304278-01</u>	Č,		× 1,0			influence likely	8030	
14-2030-04	Lettuce	,s	FOMC	1.928	Â,	Early hearly rainfall, marked	Ensa-20-	
<u>M-534595-01-1</u>	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	L?	A	S.Y	0°	ginfluence likely	8031	
14-2030-03	Lettuce	SS	St O	3.779	3 ~~	Heavy rainfall before 3rd	Ensa-20-	
<u>M-534595-01-1</u>		∀			1,	sampling, marked influence	8031	
14 2195 01				<i>©</i> <i>©</i> 057		Meely	E	
14-2185-01 M 526062 01 1	Lettuce	2	SKU .	(¹ <u>9</u> .057		bearly rainfall and irrigation,	Ensa-20-	
R 2006 0337/1	Onlan	λ «	Ê	710	30	Irrigation and rainfall coincide	EnSa-	
M-292996-0121				6	Š	with a moderate drop of residue	20-0832	
	O,	, \$°	A.	Q.	Į	levels, influence likely	20 0052	
R 2006 0504/8	Onion	Ŕ	SFO 🔊	4.91	3	Irrigation coincides with	EnSa-	1
<u>M-292996-01-1</u>			n á,	°2		moderate drops of residue	20-0832	
~~~	Ŵ.Ś	× ~	Q,	S"		levels, influence likely.		
R 2007 0567/0	_{\\$} Onion ["] "	N.C	ŞFO 🦼	2.992	3	early rainfall coincides with	EnSa-	
<u>M-302330-01-1</u>		Ů,	Ç Q	2		marked drop (influence likely)	20-0832	
R 2006 05050	Onion	S S	SFO	3.282	3	Irrigation coincides with	EnSa-	
<u>M-292098691-1</u>	je v		~Ŷ			moderate drops of residue	20-0832	
P 2007 2042/1~	nice .	<u>j</u>	FOMC	1 501	2	I likely marked influence from	EnSc	
M_362225 0		S.	FUMC	4.384	5	irrigation at day 3	EIISa- 20-0832	
R 2907 0000 /0	Peas &	N	SEO	5 287	3	large rainfall days 4 and 5	20-0032 Ensa_20.	
M-298679-01-1	1 Cus ar	1	510	5.207		influence likely	8030	
R 200 0553/0	Peas	N	HS	3.401	3	Rain on day 2, influence likely	Ensa-20-	
<u>M-298639-01-1</u>							8030	



Trial	Crop	Zone	Kinetic	DT ₅₀	Cat	Influence rain and/or	Source	
Edition no.	•		model	mod		irrigation	DT50 _ °	~
R 2007 0554/9	Peas	Ν	FOMC	9.837	3	Large rainfall on days 2 and 3,	Ensa 30-	Ì
M-298639-01-1						influence likely	8030	
15-2030-01	Peas	Ν	SFO	3.346	3	Heavy rainfall coincides with a	Ensa-20	,
M-566823-03-1						marked drop in restatue levels,	8031	
						impact likely		~
R 2007 0037/7	Peas	S	SFO	3.329	3	Large rainfall on day 3, $\mathbb{O}^{\mathbb{V}}$	Enosa-20-	Q.
<u>M-297487-01-1</u>					ĈĄ	influence likely.	8030	
15-2030-04	Peas	S	SFO	2.928	3 💎	Rainfall op days 3 and 4 🖉 🔌	DEnsa-20-	Ľ
<u>M-566823-03-1</u>					L.	coincides with a drop in residue	803	O''
					$V^{\nu}$	levels frafluence likel		1
18-2951-01	Young	Ν	SFO	2.74	3	early rain, marked decline	EnSa- 🖉	
<u>M-678413-01-1</u>	cereals			- Qo			20-0834	
13-2950-02	Young	Ν	HS	Q.03	Ċ3°	fonnfall day0, marked deline 🕎	En <b>Sa</b> -	
<u>M-471216-01-1</u>	cereals			o" "@			17-0484	
13-2950-03	Young	Ν	HS 🔬	1.2	3 @	earl@rainfall, marked decline	EnSa- 🐓	
<u>M-471216-01-1</u>	cereals		st v	Ň	$\sim$	$\gamma \rightarrow \gamma \gamma$	17-0484	
13-2950-04	Young	Ν	SF	×1,25 (	C3 .	Agarly rainfall, marked docline	Ensa-	
<u>M-471216-01-1</u>	cereals		Q V				17-0484	
15-2953-01	Young	N	CHŚ 🔊	3.48	3≪∛	early rain marked decline	EnSa-	
<u>M-566828-01-1</u>	cereals	- Q		<i>i</i> a	$\gg$		17-0484	
18-2954-01	Young	S	SEO	<b>A</b> .201 (	3	heavy thin d4, marked decline	EnSa-	
<u>M-675129-02-1</u>	cereals	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		· '0	4		20-0834	
18-2954-02	Young	°S (	SFO O	3.599	3	heavy rain d4, mated decline	EnSa-	
<u>M-675129-02-1</u>	cereals 🖉				~		20-0834	
E19RP087-03	Young 🏷	SA	SFŐ	3.415 n	¥3 /	Sheavy rain d3, marked decline	EnSa-	
<u>M-758649-01-1</u>	cereals	S.			N N		20-0834	

E19KPU8/-U3 Young Y SA SFO SI:415 @ 3 Aleavy rain d34marks


#### Influence of the residue zone on foliage DT₅₀

A comparison of the  $DT_{50}$  values from trials conducted in the Northern EU residue zone with the  $DT_{50}$ values from trials conducted in the Southern EU residue zone shows comparability within each of the rainfall categories.

It is therefore proposed to pool the foliage residue decline DT₅₀s from trials conducted in the Northern EU residue zone with the  $DT_{50}$  values from trials conducted in the Southern EU residue zone.



In a part of the residue trials evaluated here for the parpose of informing the bird and mammal risk assessment, the metabolite fluopyram benzamide (BNZ) was included as analyte since it is part of the residue definition in the toxic of ogical assessment for plant material.

Based on the metapolism data and field residue trials, the definitions of residues in plants were established by EKSA: m

	Residue definițion		Reference
Food of plant	Monitoong	floopyraps (parent only)	EFSA Scientific Report
origin	Rickassesment	Duopyram and Huopyram-benzamide (M25)	EFSA Journal
ongin 🖉	KISK assessment	expressed as Buopyram	2013;11(4):3052
			<u> </u>
	- A		



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However, the comparison of the foliage  $DT_{50}$  of fluopyram alone with the foliage  $DT_{50}$  of the combined residues of fluopyram and its benzamide-metabolite shows that this metabolite contributes very little to the potential exposure of herbivorous birds and mammals (typically less than 5%) which may be considered negligible.

It is therefore proposed that the definition of the residue for herbivorous birds and mammals can be limited to fluopyram alone.

		1	, A		
Kinetic		# of trials	Geomean	Geomean	Difference in
evaluation	Matrix	with analysis	DT ₆₀ FLU	Dra FLU+BNZ	
report		for BNZ			
EnSa-20-0829	Vegetables	37	2 765	Q.857 <u>°</u>	<u>~ 3% C</u>
EnSa-20-0830	Vegetables	26	2.845	2.926	<b>∅~ 3%⊘</b>
EnSa-20-0831	Vegetables	20	2.673.	2,697 0 0	~188 28
EnSa-20-0832	Vegetables	35	3.921	A.144 S	~ 5%
EnSa-20-0834	Young cereals	8	4.820 C	94.8210 ^{°0}	₩1% Å " [°]



### Applicant assessment on effects on biodiversity

According to Regulation 1107/2009 potential effects on biodiversity and ecosystems shall be considered in the renewal process for an active substance. However, at present EU-agreed guidance is lacking on how to address this topic and there is no technical assessment scheme available on how to perform any assessment. Therefore, to formally address these topics the following information is provided by the applicant.

The risk assessments for bird and mammals result in acceptable outcomestat screening of tier 1 Pevel

The risk assessment for aquatic organisms based on active substance is acceptable when considering FOCUS step 2 PECsw. The aquatic product-based risk assessment considering spray drifts acceptable with a 5 m no-spray buffer and 90% drift reducing nozzles.

The risk assessment for bees does not indicate a need for higher ther assessment nor mitigation measures.

The non-target-arthropod in-field and off-field risk assessment resulted in acceptable outcomes at tier 1 and tier 2 level, respectively, without the need for risk mitigation of the second se

The risk assessment for soil organisms resulted in acceptable outcomes with darge margins of safety.

The non-target-terrestrial-plant off-field risk assessments resulted in acceptable automes considering tier 1 and tier 2 data, without the need for risk mitigation.

Therefore, the applicant concludes that the use of the representative lead formulation FLU+#FS SC 500 has low potential to cause unacceptable effects on biodiversity and the ecosystem via trophic interactions. To the best of our knowledge and with the presented safety profile of the active substance fluoyram and the representative lead formulation. The applicant close has forece any effects on biodiversity and the ecosystem.



#### **CP 10.1** Effects on birds and other terrestrial vertebrates

The risk assessment has been performed according to "European Food Safety Authority; Guid@uce Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009) 7(12):1438. doi:10.2903/j.efsa.2009.1438), referred to in the following as "EFSA GD 2009" GD 2009".

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#### **CP 10.1.1** Effects on birds

		r muopyrain and en			
Test substance	Test design	Test species		Fadpoint	Reference
	Acute oral	Bobwhite quail			(2011) (2011) (2011)
	toxicity	virginiants)			M-263049-64-1 KCA 8.1.19/01
			~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	>2,000 mg as./kg by	2008) <u>M-30©71-02-1</u> KGA 8.1.1.1/02
	Acute oral toxicity	Zwora fosch @Taen&pygia			Extrapolated acc. to chapter
	5		LD ₅₀	$= 3036 \text{ mg as./kg bw }^{\text{A}}$	2.1.2 of EFSA Journal 2009;
		A 9 5			7(12):1438
	Acute or al	Chieken			(2011)
	toxoity	o Granus Ogmestficits)		> 5000 mga.s./kguw	M-446344-01-1 KCA 8.1.1.1/03
	Dietary	Bobychite qually		> 50 0 mg z /kg feed	(2007)
	toxicy	(Colimue O'	6 LDD	> 1845.4 mg a.s./kg	M-264902-02-1
, Og	(shor@term)~	Surginianus)	Ň		KCA 8.1.1.2/01
	Dietar	Malleel duck	Sc. S	> 500 mg a s /kg feed	(2005)
Fluopyram	toxix ty `*	Anas C	YLDQ50	⊿1643 mg a.s./kg bw/d	<u>M-262710-01-1</u>
ieen.	(slipsy term) y		Í O'	<i>y</i>	KCA 8.1.1.2/02
	20-weeds	SBoby Ate quar	NOECO	< 250 mg a.s./kg feed	(2008)
¢	9 chronic, 0	Aroini Que	NOSD	< 23 mg a.s./kg bw/d	<u>M-299245-02-1</u>
A	reproduction			20	KCA 8.1.1.3/01
L.	22-weeks	Botwhite of all	NOAED	7.2 mg a.s./kg bw/d	(2008)
L.	chronic.	Colings	y .		<u>M-298723-01-1</u>
	reproduction	virginianus) O	NOED	50 mg a.s./kg feed	KCA 8.1.1.3/02
			NOED	500 mg a.s./kg feed	
	19 Weeks	Mallar Oual	NOED	40 mg a.s./kg bw/d	(2008)
, Ó	Feding	(Anas			<u>M-299277-01-1</u>
	Seproduction	platyrhynchos)	NOEC	200 mg a.s./kg kg feed	KCA 8.1.1.3/03 DAR
			NOED	18 mg a.s./kg bw/d	
	Chronic	Bobwhite quail		-	
Ċ	reproduction:	(Colinus	Lowest	7.8 mg a.s./kg bw/d	(2019)
	EC ₁₀	virginianus) –	EC ₁₀	(14-day survivors per	<u>M-667209-01-1</u>
	calculation	studies combined		eggs set)	кса 8.1.1.3/04

Table 10 1 1 1. Studios for fluonyra and and d in



Test substance	Test design	Test species	Endpoint	Reference
	Chronic, reproduction: EC ₁₀ calculation	Mallard duck (Anas platyrhynchos)	EC ₁₀ 78.6 mg a.s./kg bw/d (eggs laid per hen)	(2019) M-66723,201-1 KCA & 1.1.3/05
FLU+TFS SC 500	Acute oral toxicity	Japanese quail (<i>Coturnix</i> <i>japonica</i>)	$LD_{50} > 2000 \text{ mg prod./kg bw}$ $LD_{50} = 3228 \text{ mg prod./kg bw}^{B}$	(2012) MQ42022 01-1 CA 8.11.1/08 Extraordated acc to chapter 2.1.2 of EPSA Journal 2009; J 7(12):4438
Fluo- pyram + Trifloxy- strobin	Acute	Bobwhite quail	I_{2050} \rightarrow 2009 mg total a.s. f_{20} f_{2050}	See Table

Note:

Studies referring to KCA are filed in the dossignor the active substance where studie in black type are Studies written in grey type are referring to so dies in the corresponding Baseline dossier 12 instruidual Cand Legertalite) studies of the Supplemental dossier a.s. = active substance, prod. = product \bigcirc

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Ô A Factor 1.518 for 10 birds/dose level with a single modality (sudy result

В Factor 1.614 for 5 birds/dose level for no mortality.



Û	× 10 L	. 6 . 7		, 	Shortcut	value (SV)
Crop		¹ Indicato	or species	2°	Acute RA Sbased on RUD ₉₀	Long-term RA based on RUD _m
Vineyards (grapes)	2	Small omn	ivorous bird	Ő	¢ 95.3 €	38.9
×	st i	Kľ al	0,	Ø,	. 107	

Table 10.1.1-5. Scelevany generative local species for miss-tier rist	k assessment	
	Shortcut	value (SV)
Crop C Generic focal species	Acute RA based on RUD ₉₀	Long-term RA based on RUD _m
\bigcirc Small insectivorous species "redstart" \bigcirc BBCH ≥ 200	Not needed	9.9
Vineyards (grapes) BBCH 53-73 BBCH 240	Not needed	3.4
\mathcal{S} malf of mixor of us bird "lark" BBCH ≥ 40	Not needed	3.3
Greenbouse use in lettuce 25		

According to the guidance provided in EFSA Supporting publication 2015:EN-924 for permanent greenhowses, a risk assessment is only necessary for active substances that have a log $P_{OW} > 3$ and only for the sk of secondary poisoning if exposure to soil and water is anticipated. Therefore, for soil-less cultivation, a dietary and drinking water risk assessment for the use in lettuce is not considered below.



ACUTE DIETARY RISK ASSESSMENT

Table 10.1.1- 4: Screening acute risk assessment for birds (fluopyram)								
	Indianton	DDD				L2050		#0°
Сгор	species	Appl. rate [kg a.s./ha]	SV90	MAF ₉₀	DDD) [mg a.s./ kg bw]	TERA Trigger	Ĉa
Vineyards (grapes)	Small omnivorous bird	0.050	95.3	ۍ 1.4	6.67	> 2000	300 7 10.5	
			Â,	¢	<u>,</u> , , ,			
Table 10.1.1- 5:	Screening acute ris	k assessment	t for bir	ds (FLU+	ŤFS SØ	500)Q [°] (Ĉ		

Table 10.1.1- 5:	Screening acute risk a	assessment/for birds	(FLU+ŤFS	SC 500)Q [*]
1 abic 10.1.1- 5.	Servening acute risk a			

Сгор	Indicator species	Appl. rate C [kg:total.] CSV 90 a.s./ha C	LD ₅₉ [mggotal a.s./ Xg bw]	Trigger
Vineyards (grapes)	Small omnivorous bird	Q 0.100 Q 5.3	> 2000 715	0 0 10

values are above the trigger For fluopyram and the calculated LL by min and required of 10. Therefore, a Tier 1 risk assessment is not required For fluopyram and the calculated LDsymix of FLU

Combined toxicity risk assessment

According to current requirements when a product contains more than one active substance, an additional assessment on Combined toxicity risk of the product has to be done.

For the assessment of scute offects (mortality), a surrogate LDs (mix) can be calculated for the mixture risk assessment. The EFSA GD 2009 indicates that the following equation should be used for deriving a surrogate LD₅₀ (mix) for a mixture of active substances with known toxicity assuming dose additivity:

$$LD_{50} (mix) = \left(\sum_{i=1}^{n} X(a, \xi_{i}) \atop DD_{40}(a, s, \xi_{i}) \atop Q \in Q } \right)^{-1} \int_{Q}^{1} \int_{Q}^{1}$$

where:

= Fraction of active substance (i) in the formulation mixture $X(a.s._i)$

for the active substance (i) LD50 (a.S.i Acute to

The active substance content of the formulation FLU+TFS SC 500 addressed in this dossier is 250 g fluopyram/L product and 250 g trifloxystrobin/L product, making up a total of 500 g a.s./L product.

The table below shows the calculation of the predicted LD50 (mix) of fluopyram and trifloxystrobin when moved in these proportions (step 1 in Appendix B of EFSA GD 2009).

Please note that the following calculation is based on the endpoint for Bobwhite quail for fluopyram (whereas the dietary risk assessment for fluopyram has been conducted with the lowest extrapolated LD_{50} for Zebra finch) as this species has also been used for the study with trifloxystrobin.



0

Table 10.1.1- 6:	Avian LD50 (mix) for fluopyram and trifloxystrobin when combined as FLU+TFS	
	SC 500 (step 1 in Appendix B of EFSA GD 2009)	

	Fluopyram	Trifloxystrobin
Content of a.s. in product [g a.s./L prod.]	250	250 0 5
Fraction in the a.s. mixture	0.50	0.50
LD ₅₀ of a.s. [mg a.s./kg bw]	> 2000	
Fraction / LD ₅₀	0.00025 °	Č 0.00035 Č
Sum	<u>کې</u> کې د 0.0	
1/sum = predicted LD ₅₀ (mix) [mg total a.s./kg bw]	A 9 8 2	
A LD ₅₀ for Bobwhite quail given in EFSA Journal 20	Q15(10):4989	

It is obvious from the comparison of the (low) acute or toxicity of the active substances and their relative proportions within the formulated product FLU+TFS SC 590, that neither fluopyrant nor trifloxystrobin contributes to more than 0 % to the predicted acute nixture toxicity (see yext table).

Table 10.1.1- 7:	Avian	"tox per i	fraction"	forFL	LU+TFS	SC 500	(step 1	inAppe	n dix B g	of EFSA	GD
	2009)		×~	~	°C'	L	a.s		ð Å	×	

6 Flugpyram ¹⁰ Trifloxystrobin 9	"Mix"
Content of a.s. in product [g'a.s./L-prod.]	500
Fraction in the a.s. mixture of the gradient of the second	1
LD_{50} of a.s. [mg a.s. g bw] \sim	> 2000
Tox per fraction 3 4000 3 4000	> 8000
Contribution to prediged toxicity	100 %

A LD₃₉ for Bobwhite quail given in EFSA Journal 2017;15(10):4982

EFSA GD 2009 recommends as next step (2a and 2b in Appendix B) to check the predicted toxicity against measured poxicity from D_{50} studies conducted with the formulation.

According to FSA 6 2009 the following equation should be used for the comparison:

$$\sum_{i} \frac{X(a;s_{i})}{Lb_{50}(a,s_{i})} = \frac{1}{LD_{50}(a,x)}$$
With:

$$X(a,s_{i}) = \text{Fraction of a crive substance [i] in the mixture}$$

$$LD_{50}(a,s_{i}) = \text{A crite toxicity value for active substance [i]}$$

$$LD_{50}(a,s_{i}) = \text{Measured acute toxicity value for the mixture}$$

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known



toxicity). This may be due to, e.g. further toxic co-formulants, toxicokinetic interaction or synergism/potentiation of effect. It may also reflect the inherent variability of toxicity testing. In all these cases, the use of the LD₅₀ for the formulation is recommended for the first-tier assessment, because λ it cannot be excluded that such effects would also occur after exposure of animals to residues in the environment. Dismissing the LD_{50} of the formulation from the risk assessment would only be acceptable at a higher tier if any observed greater toxicity in the test could be clearly and anambiguously ascreded to a factor that would not be relevant under environmental exposure conditions.

If, in contrast, the measured toxicity of a formulation is hower than predicted, the predicted mixture toxicity according to Step 1 should be used in the first-tier risk assessment.

N^Y N

Left side of the equation:
$$\sum_{i} \frac{X(a.s._{i})}{LD_{50}(a.s._{i})} = \underbrace{\frac{0.5}{2000 \text{ mg } a.s}}_{kg \text{ bw}} \underbrace{\frac{0.5}{2000 \text$$

< 0.0005 appears lower than $\sqrt[6]{0.001}$

£ L 1

The numerically larger value on the right side (< 0.001) than on the left side (< 0.0005) should not be misinterpreted as to indicate that the formulation FLU+TFS SC 500 would be more toxic than predicted from the toxicity of the individual components thropyram and trifloxystrobin. There are only unbound LD50 values for the active substances (> 2000 mg bs./kg bw) and for the formulation (> 2000 mg prod./kg bw) included in the equation, so no precise ratio can be calculated. Actually, the outcome of the calculation simply reflects the fact that the limit dose five of 2000 mg/kg bw corresponds only with 1000 mg a.s. sum for a product wah 50% active substance content.

Therefore the LD50 min for the formulation is used if the seven in fevel risk assessment (please refer to S Table 10.1.1-5).

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

For the fungicidal use in props under assessment in this evaluation (grapes) the leaf scenario is not considered relevant according to the EFSA GD 2009

Acute risk assessment for birds drinking contaminated water from puddles

Due to the characteristics of the experience scenario in connection with the standard assumptions for water uptake by anomals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate on g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≱500 L∰g). 2

With a K(foc of 232.1 Likg, fluopyram belongs to the group of less sorptive substances.



0

Table 10.1.1- 8:	Evaluation of potential concern for exposure of birds from drinking water (acute,
	escape clause)

	1 ,						
Сгор	Compound	K _{oc} [L/kg]	AR _{eff} (Appl. rate × MAF _m) [g a.s./ha]	LD ₅₀ [mg a.s./ kg bw]	Ratio (AR _{eff} / LD ₅₀)	"Escape clause" No concern if ratio	Conclusion
Vineyards (grapes) 2×0.050 kg a.s./ha	Fluopyram	232.1 ^A	100 ^B	2000	< 0.050	≤ 50	No concert
Vineyards (grapes) 2×0.100 kg total a.s./ha	FLU+TFS SC 500	232.1 ^C	200 ^D	> 1000	©0.200	\$50	No concerne

Koc value given in MCP 9.2.4.1 (Table 9.2.4-1)

Koc value given in MCP 9.2.4.1 (Table 9.2.4-1) MAF_m = 2.0, calculated for 2 applications with a 7-day interval considering the D_{50} in soil of 2981 days given in QCP В 9.2.4.1 (Table 9.2.4-1)

С Koc of fluopyram

MAF_m calculated considering the DT₅₀ in soil of 298.1 days for fluopyram D

According to the EFSA GD 2009 "nospecific calculations of exposure and TER are recessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg b) does not exceed 50 in the case of less sorptive substances (Kog < 500 J/kg). This is the case for Huopyram and Juopyram + trifloxystrobin. Therefore, the acute risk for burds from drinking water that may contain residues from fluopyram and trifloxystrobin is acceptable

LONG-TERM REPRODUCTIVE BISK SSESSMEND

EFSA GD 2009 recommends not to conduct a combined reproductivorisk assessment for compounds not sharing the same mode of action (step 3). Therefore, no combined reproductive risk assessment is required for FLUG TFS SC 500 in this AIR-evaluation but may be conducted post-AIR according to the respective zonal guidance.

Screening step

L i Table 10.1.1-9: Screening long-term reproductive risk assessment for birds (fluopyram)

Crop	Indicator Species	Appl. rate [kg a.S./ha]	DDD D	ftwa	DDD	NOEL [mg a.s./kg bw/d]	TERLT	Trigger
Vineyards (grapes)	Small S ommerorouspird	0.050	38.9 1.6	0.53	1.65	4.5	2.73	5





O

Tier 1

Table 10.1.1- 10:	First-tier long-term reproductive risk assessment for birds (fluopyram)							, G		
	Conoria facal		DDD				NGEL			0°
Сгор	species	Appl. rate [kg a.s./ha] SV _m		MAF _m	f _{TWA}	DDD	[mg a.s./ kg bw/d]	TERAT	Frigger	Ĉ
	Small insectivorous species "redstart" BBCH ≥ 20	0.05	9.9	\$ ₹ \$	0.53	0 ^{4/20}	4.5 Č			
Vineyards (grapes) BBCH 53-73	Small granivorous bird "finch" BBCH ≥ 40	0.05	9.4	1.6	~Q, [*] ≈Q,53 Ø	00/44	4.5 O			
	Small omnivorous bird "lark" BBCH ≥ 40	0.05			Q3	0.140	4.5 Ó	¥ 32.2	54 °	
		J. in	, l		, K			Ś Ć) }	

The TER_{LT} values calculated in the long-torm risk assessment exceed the a-priori-acceptability trigger of 5 for all evaluated scenarios. This, the long-term risk to buds can be considered as acceptable.

Long-term risk assessment for birds drinking contantinated water from puddles

Ò Evaluation of potential concern for exposite of birds from dritking water (long-term, Table 10.1.1-11: × escape clanse) 📣 \bigcirc Ň s V a

	~~~ -			J '¥	
	Koc	AReff (Appl. rate	NO(AJEL R	ation "Escape clause"	Conclusion
	[ <b>b</b> ]kg]	(@MAKu) [g ass./ha]	Leg bw/df NG	(Kerry) (FD) No concern if ratio	Conclusion
Vineyard's (grapes) Fluopyram	232	100 в 🔊		$2.2 \leq 50$	No concern
A Kockslife given in MC @ 2.4 10 Tab	le 9 7 4- 1	$1b^{\circ}$	w . O		

Koc value given in MC 29.2.4, 1 2 able 9.2.4-1)

7-day utervakconsidering the DT50 in soil of 298.1 days given in MCP В  $MAF_m = 2.0$ , calculated for 2 capplications with  $O^2$ 9.2.4.1 (Table 9.2.4)

According to the EFSA GO 2009, no specific calculations of exposure and TER are necessary when the ratio of effective application rate for g/ha to relevant endpoint (in mg/kg bw/d) does not exceed does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances  $(100 \times 500 L/cg)$ ." This is the case for fluopyram. Therefore, the long-term risk for





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### **RISK ASSESSMENT OF SECONDARY POISONING**

According to the EFSA GD 2009, substances with a log  $P_{OW} > 3$  have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains.

Table 10.1.1- 12:	Log Pow value of fluonvram
1 abic 10.1.1-14.	Log I ow value of huopyram

	15	Æ	
Substance	Log Pow	Compartment	Reference
Fluopyram	3.3 (20 °C)	Soil, surface water	(2006) <u>M-280089-01-1</u> O MCA 2.7 O

The log Pow value of fluopyram is 3.3 and thus, effects on secondary poisoning have been assessed.

<b>T</b> 11 10 1 1 10			- Č	× ×	$Q \leq $	
Table 10.1.1-13:	Avian generic focal	species for the	her I risk	assessment	of secondary	y poisoning "

Generic avian indicator species	L.	Body weight (g)	Example	FJR/bw
Earthworm eater $Q$		2 2 100 Å	hrush	م الم
Fish eater 🦧 🦓	Ô	2 1000 Q	Heron	<b>€</b> ∕ 0.159
				*



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

The risk assessment for earthworm-eating birds is only presented for the field use in vineyards. The exposure of earthworms to residues of fluopyram following the greenhouse use in lettuce can be excluded as lettuce is a soil-less cultivation and thus, soil is no compartment of concern.

Important remark by the applicant: The PEC_{soil} and TER values as presented below are interior values and are therefore subject to change until final modelling input parameters can be established. The applicant intends to provide final PECsoil values and revised TER calculations lates by end of March 2022.

Table 10.1.1- 14:	Tier 1 long-term DDD and TEI	R calculation for	earthworz	n-eating	rds in vinev	ard

	$\bigcirc$				
		X D	<b>OF</b> luop <b>y</b> ram		d,°
		Tier 1		Refinement	Ŵ
Kow		2060 °C		2060	
K _{OC} [mL/g]		×232,1××		© [™] 232 [™] A O	
foc		<u>~~~ 0.62 (</u>		9.02 ×	
BCFworm		<b>(5</b> .51 )		€ 0.85 ك	
PECsoil, accu (mg/kg)		0.238 C		о 0.23,8 ^в	
PECworm (mg/kg)	×, 8	£ 1 <u>3</u> 1	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.202	
FIR/bw		1.05		× 1.05	
DDD (mg/kg bw/d)		1.38	S S	0.212	
NO(A)EL (mg/kg bw/d)		Q 4.7	O' & L	<b>∀</b> 4.5	
TERLT		^y 3.3 0		21.2	
Trigger		<u>مَ</u> مَّ 5 مَ ²		5	
	W/	185.7	1 57 25 1		

В

- **Tigger** Koc value gix for MCO9.2.4.1 (Table 52.4-1) PEC_{soil, accu} value gix for MCP 9.1.3, Table 9.1.3-3 (vities, 2 × 69 g a.s.fa): 21-6ay-TWA of 0.053 mg a.s./kg + plateau The source of the
- С





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

Important remark by the applicant: The PEC_{sw} and TER values as presented below are interine values and are therefore subject to change until final modelling input parameters can be established. The applicant intends to provide final PEC_{sw} values and revised TER calculations latest by end of March ineyateds 2022.

Table 10.1.1- 15:	Tier 1 long-term DDD and TER	calculation for	fish-cating b	irds in vineya
	8			

	e V	al .	<u> </u>	~ <u>.</u>	
	L.	Q.	Fluopyram		0, 0
BCFfish	Ø"	~ ľ	16 ^A Q		
FOCUS Step 2 PECsw (twa, 21 d) (mg/L)	, , , , , , , , , , , , , , , , , , , ,		🖌 0.00 <b>5</b> 76 ^в ≽		
PEC _{fish} (mg/kg)	y R		0,092		~
FIR/bw	, N		0.159 °°	Å (	A L°
DDD (mg/kg bw/d)			0.0147		
NO(A)EL (mg/kg bw/d)			×4,5	/ «,	S.
TERLT			×307 Ø		0
Trigger			<u>555</u>		þ
		- 1			

Measured BCF resulting from a bioconcentration study in fish, please refer to MCA 802.3 В 21 d twa PECsw value given in MCP 9.2, 27 Table \$2.5-6 Fines, 2550 g as ha), FOCUS Sep 2, Southern Europe, spring application scenario as worst case

		<b>DD</b>		12 1			~~	<b>N</b>
Table 10.1.1-16:	Tier I long-term	DDD and	ITER	calculat	tion for	fish-eati	ng/birds-ù	í lettuce
	(anonhoused)		LS .	. 01	Nº 1	1.	S X	
	TO PERMINALING INCOMENT	Re (	3		()	V .	())	

Griuopyram
BCFfish
$\mathbf{GEM PEC}_{sw} (\mathbf{mg}) = \sqrt{\sqrt{2}} $
PEC _{fish} (mg/kg) 0 0 0 697
FIR/bw 0 2 0.159
DDD (mg/kg bw/d) 🔬 💭 🖉 🖉 🖉 0.111
NO(A) K (mg/kg bw/d) A A A A A A A A A A A A A A A A A A A
$\mathbf{TER}_{LT} \qquad \qquad$
Trigger 5
NO(A) E (mg/kg bw/d)         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A         A

А Measured BCP resulting from a bioconcentration study in fish, pease refer to MCA 8.2.2.3 PEC_{sw} value given in MCP 9.2.5, Table 9.2.5 25 (lettine, 2 × 290 g a.s./ha) calculated with exposure assessment В model GBM, soildess cultivation, with rease of filter cleaning water (0% mitigation), application dates 22.04. + 29.04, as worst case





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## CP 10.1.1.1 Acute oral toxicity

Data Point:	KCP 10.1.1.1/01
Report Author:	
Report Year:	
Report Title:	Acute oral toxicity of fluopyram & trifloxystrobin SC 500 ( $250 + 250 \text{ g/L}$ ) to
	Japanese quail (Coturnix japonica) (according to OE 223)
Report No:	EnSa-12-0495
Document No:	<u>M-442023-01-1</u>
Guideline(s) followed in	OECD-Guideline for the Testing of Chemical Stor. 223; US EPA OCSPP not
study:	applicable; Regulation (EC) of 1107/2009 of the European Parliament and of the
	Council
Deviations from current	Current Guideline: OECO 223 (2016)
test guideline:	Deviations: The photoperiod was 12 hours light, above the 8 tours light as $\sqrt{2}$
	recommended. No information on medication prior to test start is given in the
	report. This deviation and the missing information are not expected to have an
	impact on the study results. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes v v v v

Note: this study was conducted in order to meets egulatory requirements in countries outside of the EU.

## Executive summa

FLU + TFS SC 500 (250+250 g/L) was administered orally to Japanese Quails (2 females and 3 males) at the limit dose of 2000 mg product/kg bw. Birds were field at an average temperature of 20.8 °C with a humidity of 48 % and 12 hours light per day. Birds were observed for 21 days for mortality and sublethal symptoms. Body weight and average feed consumption were measured for each dosage and control group. Gross percorps were performed or all survivors at test termination.

The study fulfilled all validity criteria of OECD 223 goideline.

There were no mortalities in the compol group and fir the 2000 mg a.s./kg bw treatment. From day - 1 to day 3, a small weight loss was measured for the birds of the dosed group. From the fourth day onwards, the body weights were comparately to those of the compol animals. Two female and two male birds had a reduced feed consumption on day. I which was more expressed for the females. Thereafter, no significant differences were seen between the animals of the dosed and control group. At sacrifice, one female bird of the dosed group had yellowish discoloured lower part of the esophagus and stomach contents, probably triggered by terlux of bile that. No pathological findings were observed for all other animals.

Based on this study the acute oral  $LD_{50}$  for Japanese Quail exposed to FLU + TFS SC 500 (250+250 g/L) as a single oral dose was determined to be > 2000 mg product/kg bw.

LE DE CONTRACTOR



#### I. MATERIAL AND METHODS

<u>Test item</u>: FLU + TFS SC 500 (250+250 g/L), specification No.: 102000012886 - 03; origin Batch D.: 2011-002700; Sample Description: TOX09383-00; analysed a.s. contents: fluopyram: 21.4 % w/w: trifloxystrobin: 21.4 % w/w.

Test design: Japanese quail (*Coturnix japonica*, adult, 2 females and 3 males) were single orally desed with FLU + TFS SC 500 (250+250 g/L) at the limit dose of 2000 mg product/kg bw. The administration was done via gelatine capsules filled with the test item. Control birds received empty gelatine capsules. The treatment group comprised 5 adult birds (2 females and 3 males) and the control group 10 adult birds (5 females and 5 males) which were housed individually. Each case had floor space that measured approximately  $38 \times 50$  cm with a ceiling of 23 cm.

Birds were acclimatized for approximately 14 days prior to being randomized into test groups. At the start of acclimation, the quails were well developed and similar to birds from wild population. Water and feed were provided ad libitum during acclimation and during the test, except during periods of fasting prior to testing. Birds were starved for 16 hours prior to oral administration. Birds were held at an average temperature of 20.8 °C with a humidity of 48%. The photoperiod was 12 hours light per day during acclimation and throughout the test.

Observations on mortality and signs of intoxication were made continuously during the first two hours and approximately hourly on the day of dosing and at least once work-daily throughout the 21 days observation period. Body weights were coorded at da 1 prior to do sing of study days 3,7, 14 and at test termination (day 21). Food consumption was determined by per for each dosage group and control group daily until day 3, there for days 3-7 7-14 and 14-21. Gross necropsies were carried out on all survivors at the end of the study.

Statistics: In this limit test a LD calculation was not necessary. & Dates of experimentar Work: September 1th to October 16th 2012

Validity criteria:

Validity coveria (according to OBCD 223, adopted 26 July 2016) Table 10.1.1.1-1:

Validity criteria	Obtained
Control mortality $\mathcal{O}^{\mathcal{O}}$ $\mathcal{O}^{\mathcal{O}}$ $\mathcal{O}^{\mathcal{O}}$ $\mathcal{O}^{\mathcal{O}}$ $\mathcal{O}^{\mathcal{O}}$ $\mathcal{O}^{\mathcal{O}} \leq 10 \%$	0 %

. RESULTS AND DESCUSSIC

clinical observation

On the day of administration all five birds of the dosed group excreted soft excrements or had diarrhoea. On day 1, 4 of these birds excreted soft excrements and one bird had diarrhoea and excreted uric acid.

On day 2, all birds excreted soft excrements. From the third day onwards, the excrements appeared normal.



Treatment level [mg product/kg bw]	Overall mortality (females and males)	Number dosed	Clinical symptoms
Control	0	10	
2000	0	5	diaPrhoea, soft excrements, uric acid

Table 10.1.1.1- 2:	Summary of mortalities and clinica	l symptoms
	Summary of mortantics and chinea	i symptoms

Body weight and feed consumption:

From day -1 to day 3, a small weight loss was measured for the birds of the dosed group. From the four day onwards, the body weights were comparable to those of the control animals

Table 10.1.1.1- 3:	Mean body weight o	of surviving birds
1 abic 10.1.1.1- 5.	mean bouy weight (	n sugaring on us

Treatment level			Mean body weig	hit in the second	
	Day -1 🖉	Day 3	Dax	Day 14	Day 21
Control	209 🖓	e 2 kg	3- 299 C		م محمد المحمد محمد المحمد محمد محمد محمد محمد محمد محمد محمد
2000	218 ->	2912 ô	218	22	221
Day-1: 1 day prior to dosi	ng. 🗸 🐇				

Day of oral administration. Day 0:

O CONTRACTOR Two female and two frale blids had a reduced feed consumption on day 1 which was more expressed for the females. Thereafter, no significant differences were seen between the animals of the dosed and control group.

Table 10.14.1-4:	Mean feed consumption	\$ \$	× ~		
Treatment level	× × ×	Mean food	consumption		
[mg product/		jg/bir	·d/day]		
kg bw] 🖉	Dax 0 - 1 Dax 1 - 2	🔊 Day 2 - 3	🏷 Day 3 - 7	Day 7 - 14	Day 14 - 21
Control 🥡	26.3 26.0	× Ô21.7 ô	24.7	23.2	23.7
2000		^م ر 23.20	26.0	24.2	22.9
A					

Gross_pathology:

At sacrifice, only one fonale bird of the dosed group had yellowish discoloured lower part of the oesophagus and stomach contents, probably triggered by reflux of bile fluid. No pathological findings

were observed for all other animals of the dosed and the control group.



### **Biological findings:**

Table 10.1.1.1- 5:         Acute oral toxicity to Japanese Quail	
Test substance	FLU + TFS SC 599 (250+250 g/L)
Test object	Japanese Quail (male, female)
LD ₅₀ [mg product/kg bw]	> 2000
No observed effect dose (NOED) [mg product/kg bw]	$\geq 2000$ $\swarrow$ $\checkmark$ $\checkmark$ $\checkmark$
The second se	
III. CONCEPSION	
Based on this study the LD ₅₀ for Japanese Quail exposed to	FLU + TFS SC \$00 (250+250 g/L) was
determined to be $> 2000 \text{ mg product/kg bw}$ . The NOED ₆ was	≥2000 mg product/kg bw. ~~ ~~
Assessment and conclusion by applicant?	
The study and its data are considered as acceptable and relia	ble foouse in risk assessment.
The endpoint is: $(2^{4}, 3^{4}, 3^{4})$	
$LD_{50} > 2000  mg product/kg bw. Bases on zero motalities extrapolated to 3228 mg a.s./kg/bw.$	$\int_{2}^{\infty} do S do S do Birds, the D D_{50} can be$
CP 10.1.1.2 Higher ther data on birds	
Insect and foliage residue decline studies and kinetic valuate assessment on birds and mampals are in the MCA point 8 and	She to generate a $DT_{50}$ for higher tier risk
CP 10.1.2/ Effects on terrestrial vertebrates of	ier than birds
	à M
Table 10.1.2-1: DEndpoints used in the risk assessment for a	ňammals

		N P	S S	•	
Test ~Ç substance	Testdesign	Test species		Endpoint	Reference
	Acetororal	Rat	**** ***** ****** ********************	> 2000 mg a.s./kg bw	(2005) <u>M-259398-01-1</u> KCA 5.2.1/01
Fluopyram	Taxo- generation study	Rat Q	NOAEL	= 14.5 mg a.s./kg bw/d	(2008) <u>M-299334-01-1</u> KCA 5.6.1/02
FL\$#+TFS®	Acute organ	v Rat	LD ₅₀	> 2000 mg prod./kg bw > 1000 mg total a.s./kg bw ^A	(2007) <u>M-287410-01-1</u> KCA 7.1.1/01
Fluopyram + Trifloxystrobin	Acute oral	Rat	LD ₅₀ (mix)	> 2857 mg total a.s./kg bw	Table 10.1.2- 6

Considering 25% fluopyram and 25% trifloxystrobin within the product



able 10.1.2- 2: Re	levant indicator species for screening risk as	ssessment				
		Shorteut	value (SV)			
Сгор	Indicator species	Acute RAS based on RUD ₉₀	Long-term RS based on RUD _m			
Vineyards (grapes)	Small herbivorous mammal	126.4	2 723 X			
Table 10.1.2- 3: Relevant generic focal species for tirst-tier risk assessment						
Сгор	Generic focal species go	Acute RA based on RUD	alue (SV) Long-term RA based on RUD _{in}			
Vinevards (grapes)	Large herbivorous taammal 'agomorph' BBCH ≥40 Small insectivorous mammal 'shrees' & BBCH ≥ 20 '	Not needed	3.3 5 3.3 5 5 5 5 5 9			
BBCH 53-73	Small herbivænus mænmal "Øðle" $BBCH \ge 40$ Small onnivorous mænmal "mouse" $BBCH \ge 40$	Not needed	21.7			
reenhouse use in left			<u> </u>			

According to the guidance provided in EFSA Supporting publication 2015:EN-924 for permanent greenhouses, a risk assessment is only necessary for active substances that have a log  $P_{OW} > 3$  and only for the risk of secondary poisoning if exposure to soil and water is anticipated. Therefore, a dietary and ACUTE DIETARY RESK ASSESS TENT Table 10.1.2-4. Sereening acute risk assessment for mammals (fluopyram)

Crop	Andicator species	Apple rate	DDD SV90	MAF ₉₀	DDD	LD50 [mg a.s./ kg bw]	TERA	Trigger
Vineyards (grap	Small herbiverous	0.050	136.4	1.4	9.55	> 2000	> 209	10

The second secon



Vineyards (grapes)

as./

fog bw

> 2857

	······································							
		DDD						
Сгор	Indicator species	Appl. rate [kg total	SV90	MAF90	DDD	(product) [mg_total	TERA	Ørig

Table 10.1.2- 5:	Screening acute risk assessment for mammals (FLU+TFS SC 500)
------------------	--------------------------------------------------------------

a.s./ha]

0.100

	L	,Õ [¥]	×,	Nº S	, , ,
The TERA values for fluopyram and the produce	ct ( CLU+TFS	SG 500 are	above the	trigger, of	10°
Therefore, a Tier 1 risk assessment is not required.	.		Q. A	in the second se	Å.

136.4

1.4

19.1

#### Combined toxicity risk assessment

According to current requirements wheth a product contains more than one active substance an additional assessment on combined toxicity risk of the product has to be done.

For the assessment of acute effects (portality), a surrogate ED₅₀ (mix) can be carculated for the mixture risk assessment. The EFSA GD 2009 indicates that the following equation should boused for deriving a surrogate LD₅₀ (mix) for a mixture of active substances with now toxicity assuming dose additivity:

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

where:

substance (i) in the formation mixture action of active X(a.s.i)

cuto toxicity for the active substance (i)  $LD_{50}(a.s._{i})$ Ò

Small herbivorous

mammal

the formulation FIGU+TES SC 500 addressed in this dossier is 250 g The active substance content of fluopyram/L product, and 250 g trifloxystropin/L product, making up a total of 500 g a.s./L product.

0

The table below shows the calculation of the predicted LD₅₀ (mix) of fluopyram and trifloxystrobin when mixed in these proportions (step, I in Appendix B of SFSA GD 2009).

L'a	
Table 10.12 6:	Manamalian 2. D.50 (maix) for Aluop ve an and trifloxystrobin when combined as
	ELAU TES C. 500 (days the day day D of EESA CD 2000)
	FEG+1FS SC 500 (step an Appendix B of EFSA GD 2009)

	Fluopyram	Trifloxystrobin		
Content of a.s. ir@roduct.[g a.s.fr prod	250	250		
Fraction in the a.s. mixture	0.50	0.50		
LD ₅₀ of a stand mg as kg bŵ	> 2000	> 5000 ^A		
Fraction LD S	< 0.00025	< 0.0001		
Sum Sum < 0.00035				
$1/sum = \mathbf{D}$ dicted LD ₅₀ (mix) [mg total a.s./kg bw]	> 2857			
A $M_{12}$ for ret given in EESA Journal 2017:15(10):4090	)			

LD₅₀ for rat given in EFSA Journal 2017;15(10):4989



It is obvious from the comparison of the (low) acute oral toxicity of the active substances, and their relative proportions within the formulated product FLU+TFS SC 500, that neither fluopyram nor trifloxystrobin contributes to more than 90 % to the predicted acute mixture toxicity (see next table).

Table 10.1.2- 7:	Mammalian "tox per fraction" for FLU+TFS SC 5	00 (step 1 m A	Appendix B of EF	
	GD 2009)	A	. 8	

		-20	
	Fluopyram	Trifloxystrobin	S" "Mix" O
Content of a.s. in product [g a.s./L prod.]	250 بر	0250	
Fraction in the a.s. mixture	0.5	Q 0.5 °	
LD ₅₀ of a.s. [mg a.s./kg bw]	>2000	>>000	₹2857 ~~~
Tox per fraction	<u>√</u> 4000	10000	>14000
Contribution to predicted toxicity	A 71.4%	Q 28.6%	O 100%

EFSA GD 2009 recommends as next step (2a and 2b in Appendix B) or check the predicted toxicity against measured toxicity from LDS studies conducted with the form Dation

against measured toxicity from LD5 studies conducted with the formulation  
According to EFSA GD 2009 the following equation should be used for the comparison  

$$\sum_{i} \frac{X(a.s._{i})}{LD_{50}(a.s._{i})} = \frac{4}{LD_{50}(mix)}$$
With:  

$$X(a.s._{i}) = Fraction, \rho = Active substance [i] in the prixture of the mixture of the following equation of the mixture of the mixture$$

×,

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). This may be due to, e.g. further toxic co-formulants, toxicokinetic interaction or synergism potentiation of effect. It may also reflect the inherent variability of toxicity testing. In all these cases, the use of the LD₅₀ for the formulation is recommended for the first-tier assessment, because it cannot be excluded that such effects would also occur after exposure of animals to residues in the environment. Dismissing the LQ₃₀ of the formulation from the risk assessment would only be acceptable at a higher tier Wany observed greater toxicity in the test could be clearly and unambiguously ascribed to a factor that would not be relevant under environmental exposure conditions.

If, in contrast, the measured to ficity of a formulation is lower than predicted, the predicted mixture toxicity according to Step 1 should be used in the first-tier risk assessment. A. 67

Left side of the equation: 
$$\sum_{i} \frac{X(a. s._{i})}{LD_{50}(a. s._{i})} = \frac{0.5}{\frac{>2000 \text{ mg a. s}}{\text{kg bw}}} + \frac{0.5}{\frac{>5000 \text{ mg a. s}}{\text{kg bw}}} = < 0.00035$$



Right side of the equation: 
$$\frac{1}{\text{LD}_{50}(\text{mix})} = \frac{1}{\frac{>1000 \text{ mg total a.s.}}{\text{kg bw}}} = < 0.001$$

$$< 0.00035 \text{ appears lower than } < 0.001$$
The numerically larger value on the right side (< 0.001) than on the left side (< 0.00035) should not be misinterpreted as to indicate that the formulation FLU#TFS SC 500 would be more toxic than predicted.

from the toxicity of the individual components fluon from and triffer system. There are only another the toxicity of the individual components fluon from the toxicity of the toxicity of the individual components fluon from the toxicity of the toxicity of the individual components fluon from the toxicity of toxicity of the toxicity of the toxicity of LD50 values for the active substances (> 2000 mg/a.s./kg bw and > 5000 mg/a.s./kg/bw) and for the formulation (> 2000 mg prod./kg bw) included in the equation; so no precise ratio can be calculated. Actually, the outcome of the calculation simply reflects the fact that the limit dose level of 2000 mg/kg bw corresponds only with 1000 mg a.s. sum for a product with 50% active substance content

Therefore, the LD_{50 mix} for the formulation is used in the screeping level essment (please refer to Table 10.1.1-5).

### Acute risk assessment for mammals deinking containinated water from puddle

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

/kg, fluopyram belongs to the group of less sorptive substances. With a K(f)oc of

Table 10.1.2-8:	Evaluation of po	tential c	oncern for e	posure of r	natormals fro	om drinking v	vater (acute,
	escape clause	<b>A</b>	07		Y Y		
Cron		Kos	AReff (Appl. rate		Ratio	"Escape clause"	Conclusion
		[LAkg]	XMAFm) Yg a.s. (ha]	ke bw]	(AReff/ LD50)	No concern if ratio	Conclusion
Vineyards (grapes) $2 \times 0.050$ kg a.s./ha	Flugyram	232,04	фоо в С	۶ > 2000	< 0.050	$\leq 50$	No concern
Vineyards (grapes) $2 \times 0.100$ kg total a.s./ha	Puopyram + ~	232.1	2000	> 1000	< 0.200	≤ 50	No concern

Koc value given in MCP 9.2.4.1 (Rable 9.2.4-1) В  $MAF_m = 2.0$ , calculated for 2 applications with a day interval considering the DT₅₀ in soil of 298.1 days given in MCP 9.2.4.1 (Table 9.2.4- 4)

С Koc of fluggram 🔬

 $\kappa_{oc}$  of fluopyram  $Margin F_{50}$  in soll of 298.1 days for fluopyram D

According the BFSA GD 2009 "no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)." This is the case for fluopyram and fluopyram and trifloxystrobin. Therefore, the acute risk for mammals from drinking water that may contain residues from fluopyram and trifloxystrobin is acceptable.



### LONG-TERM REPRODUCTIVE ASSESSMENT

EFSA GD 2009 recommends not to conduct a combined reproductive risk assessment for compounds not sharing the same mode of action (step 3). Therefore, no combined reproductive risk assessment is required for FLU+TFS SC 500 in this AIR-evaluation but may be conducted post-AIR according to the respective zonal guidance. 

Table 10.1.2- 9:	Screening long-term re	eproductive risk asses	sment for mammals 🔬
	8 8	•	<i>µ</i> =

	Indicator	DDH		¢ NÇ	ÉXEL /	\$ 	
Сгор	species	Appl. rate [kg a.s./ha]	MAF _m Frwa	BDD [mg	a.s./kg w/dl	TERIA	Trigger
Vineyards (grapes)	Small herbivorous mammal	0.050 .7293		3.07	₩.5 ₩	4.72	
		<u> </u>			a di la companya di l	Ĉ.	Õ

r 1 tûsk assessment is required. The screening level TER_{LT} value is below the trigger  $\mathcal{R}_{LT}$ 

#### Tier 1

First-tier long-term reproductive risk assessment for manimals Table 10.1.2-10:

Crop	Generic focal mecies	Appl. rate [kg a.s:/ha]	SV.	MAR	0 frw5		《NOEL [mg a.s./ kg bw/d]	TER _{LT}	Trigger
	Large herbjvorou mainsmal "lagomorph" BBCH 240	20.050 Š	3.3 ( 5	1.6 2 2 2 3	0.53*	© 0.140	14.5	104	5
Vineyards (grapes) BBCH 53-73	Small insectivorous mannal "slacew" BBCH 20	5 5 7 0.050 7 7	1.9 6	1.6	0.53	0.081	14.5	180	5
	Small herbivorous mammal "vore" BBCH 2040	0.050 G	21 TC	1.6	0.53	0.920	14.5	15.8	5
	Sorall opinivorous mamma "mouse" BBCH ≥ 40	Q.050	2.3	1.6	0.53	0.098	14.5	149	5

The Tier 1  $ER_{LT}$  alues exceed the trigger of 5. Therefore, the long-term risk to mammals is considered acceptable.



#### Long-term risk assessment for mammals drinking contaminated water from puddles

Table 10.1.2-11: Evaluation of potential concern for exposure of mammals from drinking water (longterm. escape clause)

	ter in, escape t					~	
Crop	Compound	Koc	AReff (Appl. rate	NOAEL	Ratio	Descape Clause"	Conclusion
Стор	Compound	[L/kg]	× MAFm) [g a.s./ha]	kg bw/d]	NOAEL	No concern if ratio	
Vineyards (grapes)	Fluopyram	232.1 ^A	100 ^B	14.5	6.20	$\leq 50^{\circ}$	No concetta
A Koc value given in	MCP 9.2.4.1 (Ta	ble 9.2.4-	1)	a la	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		Q O K

 $MAF_m = 2.0$ , calculated for 2 applications with a 7-day interval considering the DT₅₀ in soil of 298.1 days given in MCP 9.2.4.1 (Table 9.2.4-1)

According to the EFSA GD 2009 "no specific calculations of exposure and TER are specessing when the ratio of effective application rate (in the application of effective application rate (in the application rate (in th does not exceed 50 in the case of less sorptive substances (Kock 500 L/kg) or 30004 in the case of more sorptive substances (Koc > 500 L/kg); This is the case for thropyram. Therefore the long-terminisk for mammals from drinking water that pay contain residues from fluopyram is acceptable 

#### POISONING **RISK ASSESSMENT OF**

Office of the second sturstances with  $\log \hat{R}_{W} > 3$  have potential for bioaccumulation and According to the EFS GD 2009, should be assessed for the tisk of formagin fication in aquatic and terrestrial food chains.

#### Table 10.1.2- 12 Log Pow value of Huopyram

Şubstanco [°] [«]	Log Pow N	Compartment	Reference
Fluopyram	3€3 (20 °C)	Soil Surface water	(2006) <u>M-280089-01-1</u> MCA, 2.7
<u> </u>			

The log Pow value of Dropyram is and thus, effects on secondary poisoning have been assessed.

#### Manimalian generic focal species for the Tier 1 risk assessment of secondary Table 109.2-13: poisoning, Ő, L 1 (n)

Generic mammalian in action opecies	Body weight (g)	Example	FIR/bw
Earthworm eater A	Q 100	Common shrew	1.28
Fish eater &	<i>a</i> [*] 1000	Otter	0.142



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

The risk assessment for earthworm-eating birds is only presented for the field use in vineyards. The exposure of earthworms to residues of fluopyram following the greenhouse use in lettuce can be excluded as lettuce is a soil-less cultivation and thus, soil is no compartment of concern.

Important remark by the applicant: The PEC_{soil} and TER values as presented below atenterin values and are therefore subject to change until final modelling input parameters can be established. The applicant intends to provide final PEC_{soil} values and revised TER calculations lateer by end of March 2022.

Table 10.1.2- 14:	Tier 1 long-term DDD	and TER	calgulation	for earth	worm?e	atingma	mmals	in≰
	vinevards	Č.	01 X	Š –		S	4	n

VIIIe	eyarus	Û		õ.	0° 6	ç di	L.	A
				Ø f	Tuopyran	1	0"	ê û
			∼y ^y Tierył	, D	$\mathcal{A}$		øfineme	nt 🔗
Kow			× 2060			U Ô	2060	Ő
Koc [mL/g]			×282.1 {		Ŏ , Ţ		282.1 A	, Ôj
foc	, Q		<u>ک</u> 0.02	2			0.02	<b>9</b>
BCFworm	Q	, L' U	r <u>5</u> S1	, O)		ð ₂ 0	0.85 [°]	
PECsoil, accu (mg/kg)			0.238 ^H	3 ~ J	Ø Å	<i>l</i> a	0.238 в	
PEC _{worm} (mg/kg)	à Ő		© 1.31 ⁰	У ⁷			Q0.202	
FIR/bw	× 1		\$ 1,28	Å.		U ₂ S	1.28	
DDD (mg/kg bw/d)		Q O	Ø1.68	N C	× 4	ŝ\$	0.259	
NO(A)EL (mg/kg bw/d			ని 14 ప్ర	, ,	0		14.5	
TERLT			× \$			<b>V</b>	56.0	
Trigger			× 5	». Ś	Ç V	Ŷ	5	

Α

- PEC_{soil, acceptalue given in MCP 9.1.3} (Table 9.2.4-1) concentration (5 cm) of 01485 mg 3.3/kg В
- С





#### Long-term DDD and TER calculation for fish-eating mammals

Important remark by the applicant: The PEC_{sw} and TER values as presented below are interine values and are therefore subject to change until final modelling input parameters can be established. The applicant intends to provide final PEC_{sw} values and revised TER calculations latest by end of March Svards 6 2022.

Table 10.1.2- 15:	Tier 1 long-term DDD and TER	calculation f	for fish-eating	mammals in	ı vinevards
		Y			Q. N

	1			Fluopyram	, l	Ç, Q
BCF _{fish}			, ()	8 16 Q ,	Ň Ň	Ĩ,
FOCUS Step 2 PECsw (twa, 21 d) (mg/L)		<u> </u>	<u>```</u>	0.00576 ^B	v v v	Ś
PEC _{fish} (mg/kg)			- L	0.092	~~	, [©]
FIR/bw			Ø (	0.142 ⁰⁰		L L
DDD (mg/kg bw/d)			<u>~</u>	0.04971		<u>v</u>
NO(A)EL (mg/kg bw/d)			× ×	MA.5	«	A.
TERLT			*	×1108		0
Trigger	<u>~</u> ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		0	7 5 Ş		)

Ā Measured BCF resulting from a bjoconcentration study in fish, please refer to MCA 8@2.3 21 d twa PECsw value given in MCP 9.2, Table 92.5-6 Gines, 2050 g as ha), FOCUS Sep 2, Southern Europe, В spring application scenario as forst case

Tier 1 long-torm DDD and TER calculation for fish-eating manimals in lettuce Table 10.1.2-16: (greenhouse use)

Fluopýram
<b>BCF</b> _{fish} $\mathcal{N}$
GEM PECsw (mg/L) ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
$PEC_{fish} (mg/kg) \qquad \bigcirc \qquad $
FIR/bw @
DDD (mg/kg bw/d) $\sim$
NO(A) (mg/kg bw/l) $(14.5)$
$TER_{LT} \qquad \sqrt[4]{7} \qquad \sqrt[4]{7} \qquad \sqrt[4]{7} \qquad \sqrt[4]{7} \qquad \sqrt[4]{7} \qquad 146$
Trigger N Q V V D 5

Ā

Measured BCF resulting from a bioconcentration study on fish, please refer to MCA 8.2.2.3 PEC_{sw} value given on MCPO.2.5, Table 9.2. 25 (lettice, 2 × 200 g a.s./ha) calculated with exposure assessment в model GEM, soil ess autovation, with rease of filter clearing water (0% mitigation), application dates 22.04. + 29.04 as worst case





#### **CP 10.1.2.1** Acute oral toxicity to mammals

Table 10 1 2_ 17.	Mammalian toxicity	v data of the formulated	product FLU+TES SC 500
1 abic 10.1.4 - 17.	mannanan toxicit	y uata of the for mulated	

Table 10.1.2- 17:	Mammalian to	xicity data	of the formulated product FLU+	-TFS SC 500
Test substance	Test design	Species	Endpoint	<b>Reference</b>
FLU+TFS SC 500	Acute	Rat	$LD_{50} > 2000 mg prod./kg W$	(2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2007) (2

#### **CP 10.1.2.2** Higher tier data on mammals?

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

Information on effects of fluopy fam on reptiles or amphibians is not available. No guidelines for studies





#### **CP 10.2** Effects on aquatic organisms

Table 10.2- 1: Endpoints used in risk assessment

Table 10.2- 1:	Endpoints used in ri	isk assessm	ent 💦	Ľ,			
Test substance	Test species	Time scale/ Study type	Endpoint		A Contraction	Reference	
	Fish, acute Oncorhynchus mykiss	96 h / static	LC&= 0.09	ymg pfod./L (no	m) 0	M-294350-04 KEP 10.2 <u>4</u> /01	
	Fish, acute Oncorhynchus mykiss	96 h / semi- statec	LC30= 0.08	<b>84</b> mg fröd./L.	nom)	(2018) <u>M-636236-6</u> K&P 10.2.1/02	
	Invertebrate, acute	48 h /	EC = 0.08	Sing prod./L (ps	25 th) 6	(2007) M-292365-01- KCP 10.2.1/03	<u>-1</u> 3
	Invertebrate, acute & Daphnia magna	480h / Semi- StatigS	¥EC50≇0.05	1 mg prod./L (a	om)	(2018) M-636231-01- KCP 10.2.1/04	- <u>1</u> I
FLU+TFS SC 500	Green algae Proudokirchnerielia Subcapitata	0 -72 P/	$\mathbf{E}_{\mathbf{f}} \mathbf{C}_{50} = 0 2$	2 mg prod./L(1	() () nom)	(2021) M-292579-02- KCP 10.2.1/05 Recalculation	<u>-1</u> 5 by
	Currently known as Raphidocetis subcapitata	static	$E_yC_{50} = 0.04$	125 mg prod./L (1	nom)	(2020) <u>M-757720-01-</u> KCP 10.2.1/06	) - <u>1</u> 5
	Greefalgae Pseudokirchheriefa subcapitata (currently known as Raptudocelic subcapitatu	to -72 kg	$\begin{array}{c} & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\$	Sing prod./L (noi 5 mg prod./L (noi 59 mg prod./L (noi 69 mg prod./L (noi 60 mg prod./L (noi	n) om) om) om)	(2018) <u>M-615579-01-</u> KCP 10.2.1/07	- <u>1</u> 7
	Green algae Pseudskirchnoriella subcapitata (curently ariown as Raphidocelis Subcapitata)	0 72 h 7 Q statie	$\begin{array}{c} & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\ & & \\$	9 mg prod./L (no 30 mg prod./L (1 405 mg prod./L (1 32 mg prod./L (1	om) nom) nom) nom)	(2018) M-636234-01- KCP 10.2.1/08	- <u>1</u> 3
		Q					

Table 10.2- 1:	Endpoints used in risk assessmen
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Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Fish, acute Oncorhynchus mykiss	96 h / static	LC ₅₀ > 1.89 mg a.s./L (nom)	(2678) <u>M-277740-02-1</u> KCA&2.1/01
	Fish, acute Lepomis macrochirus	96 h / static	$LC_{50} > 68 \text{ mg a.s./L (10m)}$	(2007) M-278441702-1 CCA & 1/02
	Fish, acute Pimephales promelas	96 h / static	$L_{50}^{4} > 4.95 \text{ mg} \text{ Q}_{5}^{3}/\text{L} \text{ (mm)}^{A}$	(200%) (200%) M-298918-01-1 NCA § 2.1/03
	Fish, acute <i>Cyprinus carpio</i>	96 h	$L \mathbf{O}_{50} = 35.3 \text{ mg fs}/L (pon)^{B}$	M ₂ 280108-01-1 6 ^A 8.25 ^P 04 4
	Fish, acute Cyprinodon variegatus	96 h-	$L(\mathcal{Q}_{0} > 0.25 \text{ mg a OL} (\text{mg})$	(2006) <u>M577916791-1</u> & A 8.¢1/05
	Fish, acute Geometric mean	96 h / Static	Geometric poran L(50 = 4,39 mg as /L	
	Fish, cheoric (ELS)	Jr3 d / Q floys	NQEC = $0.135 \text{ mg/s}$ .s./I- $0$ mm)	(2006) <u>M-279440-01-1</u> (KCA 8.2.2.1/01 Recalculation by
Fluopyram tech.		through		(2020) <u>M-758375-01-1</u> KCA 8.2.2.1/02
	Fish CF flov- through Lepomizmacroadirus	28 dy exposure 44 d glepurato n / flow-	CF (whole fig., wet weight) = 18 BOG (whole fish, permalized to 6% lipid ontent = 16	(2008) <u>M-298506-01-1</u> KCA 8.2.2.3/01
₿ _y v	In Ortebraty, acuto	48 h / static	ECO > 20 mg a.s./L (nom)	(2006) <u>M-278709-01-1</u> KCA 8.2.4.1/01
	Sedi Cht dverler, C sub chroniO Leptocherius plumulosus (spiked sediment)	Olod G staric	C = 100 mg a.s./kg (mm) K EC = 100 mg a.s./kg (mm)	(2008) <u>M-297751-01-1</u> <u>KCA 8.2.4.2/01</u>
	Invertebote, acore 2 Crassostrea Arginica	Q 96 h floty- thrQugh	$EC_{50} > 0.44 \text{ mg a.s./L (mm)}$ (shell deposition and mortality)	(2006) <u>M-282691-01-1</u> KCA 8.2.4.2/02
	Awericamysi Qahia	96 h / flow- through	$LC_{50} > 0.50 \text{ mg a.s./L (mm)}$	(2007) <u>M-282839-01-2</u> KCA 8.2.4.2/03
	Invertebrate, acute Geometric mean	-	Geometric mean $EC_{50} = 1.638 \text{ mg a.s./L}$	
	Scometre metin	1	2039 1000 mg 0.5/12	L



		Time		
Test substance	Test species	scale/ Study type	Endpoint	Reference
	Invertebrate, chronic Daphnia magna	21 d / static- renewal	NOEC = 1.25 mg a.s./L (nom) EC ₁₀ : not determined ^C	(2009) <u>M-282142-02-1</u> KCA 2.5.1 Recoculation by (2920) <u>M-758376-01.4</u> KCA 2.5.1/02
	Sediment dweller, chronic (54 d, Life cycle) <i>Chironomus tentans</i> (spiked sediment)	54 d / & statice renevor	NOEC = 26 ag a.s ag (mm) ECa: not determined c	M.298809-01-1 RCA § 5.3/0 Recalculation by (2020) 758550-01-14 KCA § 5.3/0 KCA § 5.3/0 (2020)
	Sediment dweller, chronic (28 d, Life Cycle) <i>Chironomus riparios</i> (spiked water)	C 2 & A Ortic	N( $EC = 1.89 \text{ ms}(3.5/L (n)m)$ $EC_{10} = 0.64 \text{ mg}(3.5/L (n)m)$ $EC_{15} = 1.37 \text{ mg}(a.s./S nom)$ $EC_{10} > 32 \text{ mg}(a.s./Q nom)$	©008) <del>298266-01-1</del> RCA 52.5.4/01
	chronic Leptocheinys (spiked sediment)	Static- 0 Tenevge	NOE $G = 38 \text{ mg a.s.} \text{Ky (ma)}$ $LC_{60} > 94 \text{ mg a.s.} \text{Ky (ma)}$	(2008) M-298810-02-1 KCA 8.2.5.4/02
	Gen al de Seud Ourchnediella Subc Ottata D (curgently & yown as Raphidocelis	0 C h / static.	$F_{r}C_{50} = 3^{2} \text{ mg } 2^{2}/L \text{ (mm)}$ $F_{r}C_{10} = 3.97 \text{ mg } a. s./L \text{ (mm)}$ $E_{b}C_{b}C_{b}C_{b}C_{b}C_{b}C_{b}C_{b}C$	(2007) <u>M-286541-01-1</u> KCA 8.2.6.1/01 Recalculation by
ÊÇ ⁴			F.C. 50 08 mg a s /L (mm)	M-757659-01-1 KCA 8.2.6.1/03 (2007) M-289899-01-1
	Freshware diatony Navicula pelliciposa	P 72 ho statio	$E_{r}C_{50} = 5.23 \text{ mg a.s./L (mm)}$ $E_{r}C_{50} = 5.62 \text{ mg a.s./L (mm)}$ $E_{r}C_{50} = 5.64 \text{ mg a.s./L (mm)}$	KCA 8.2.6.2/01 Recalculation by (2020) <u>M-757699-01-1</u> KCA 8.2.6.2/04
	Marine Chatom	0 - 72 h / static	$E_rC_{50} > 1.13 \text{ mg a.s./L (mm)}$ $E_rC_{10} > 1.13 \text{ mg a.s./L (mm)}$ $E_bC_{50} > 1.13 \text{ mg a.s./L (mm)}$ $E_yC_{50} > 1.13 \text{ mg a.s./L (mm)}$	(2007) <u>M-287289-01-1</u> KCA 8.2.6.2/03 Recalculation by
	Aquatic macrophyte, Lemna gibba	7 d / static	$E_rC_{50} = 2.51 \text{ mg a.s./L (nom)}$ $E_rC_{10} = 1.58 \text{ mg a.s./L (nom)}$ $E_yC_{50} = 2.12 \text{ mg a.s./L (nom)}$	M-757680-01-1 KCA 8.2.6.1/06 (2021) M-283647-02-1 KCA 8.2.7/01



Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Invertebrate, acute Daphnia magna	0 - 48 h / static	EC50 > 88.7 mg p m./L (nom)	(2020) <u>M-759029-01-1</u> KCA.&.2.4.1.02
Fluonyram-7-	Green algae Pseudokirchneriella subcapitata ^D	0 -72 h / static	$E_rC_{50} = 20.9 \text{ mg p m./L. (nom)}$ $E_rC_{10} = 20.2 \text{ mg p m./L. (nom)}$ $E_yC_{50} = 13.0 \text{ mg p m./L. (nom)}$	
hydroxy	(currently known as Raphidocelis subcapitata)	0 -96 h / static	$E_{r}C_{50} = 21.1 \text{ mg p m/L (nom)}$ $E_{r}C_{10} = 20.4 \text{ mg p m./L (nom)}$ $E_{r}C_{50} = 13.7 \text{ mg p m./L (nom)}$ $E_{b}C_{50} = 12.6 \text{ mg p m./L (nom)}$	M-758708-01-4 KCA-8.2.6 905
	Aquatic macrophyte Lemna gibba	7 d static	$E_rC_{50} = 9.2$ ing p to /L (mm) $E_rC_{10} = 5.2$ mg p to /L (mm) $E_rC_{50} = 7.1$ mg p m./L (mm)	20208 <u>M₇759030-01-1</u> KCA 8.25702
	Fish, acute Brachydanio rerio	96 h	LC ₅₀ > 1200 mg p mAL (nom Na- TFA) > 1008 mg p m./L (nom V Y EPA) ^E	(1992) <u>M2947889901-1</u> XCA 8.201/06
	Invertebrate, acute	9 - 48 13 static	EC. > 12000 mg p $p/L$ (nof Na- TFA) $>$ 1008 mg p m./L (nom $>$ TFA) $=$ $TFA$	(1992) <u>M-247890-01-1</u> KCA 8.2.4.1/03
	Invertebrate, chronic Daphara magaa	21 d Seroi- otatic	NOEC $\geq$ 30 mg p m/L (nom Na ₋ TRA) $\geq$ 25.2 mg p m/L (nom TFA) $\leq$ TFA)	(2010) <u>M-615126-01-1</u> KCA 8.2.5.1/03
Trifluoro- acetic acid (TFA)			$\begin{array}{c} F_{7}C_{50} > 1,2 \text{ mg ppn./L (nom Na-TFA)} \\ 1.01 \text{ mg p m/L (nom TFA)}^{E} \end{array}$	
	Green algae S Pseudokirchnerielta sabcapitata ^D	0°,″ ,∼0°-72 hoj	F.C. ₁₀ > 1.9 mg p.m./L (nom Na- FEA) $\bigcirc$ FEA) $\bigcirc$ $\bigcirc$ 1.01 mg p.m./L (nom $\bigcirc$ TFA) $\stackrel{E}{\longrightarrow}$	(1993) <u>M-247818-02-1</u> KCA 8.2.6.1/06
~ A	(currently known as Raphoocelis C subcapitato	staffe	$F_{5}C_{50} > 4.2 \text{ mg p m./L (nom Na- T_{6}A)> 1.01  mg p.m./L (nom  TFA)E$	Re-evaluation by (2021) <u>M-762268-02-1</u> KCA 8.2.6.1/07
			ESC50 > 1.2 mg p m./L (nom Na- TFA) > 1.01 mg p.m./L (nom TFA) ^E	



Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Green algae Pseudokirchneriella subcapitata ^D (currently known as Raphidocelis subcapitata)	0 -72 h / static	$ \begin{array}{c} E_r C_{50} = 160 \ \text{mg p m./L} \ (\text{nom Nie} \\ TFA) \\ = 134.4 \ \text{mg p.m./L} \ (\text{nom} \\ TFA)^E \\ E_r C_{10} = 2.239 \ \text{mg p m./L} \ (\text{nom} \\ \text{Ma-TFA}) \\ = 1.881 \ \text{mg part/L} \ (\text{nom} \\ TFA)^E \\ E_r C_{50} > 4.8 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ > 4.02 \ \text{mg p an./L} \ (\text{nom} \\ TFA) \\ > 4.02 \ \text{mg p an./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \\ TFA) \\ = 3.52 \ \text{mg p m./L} \ (\text{nom} \ TFA) \ (n$	C1992) M-24-320-01-1 KCA8.2.6.008 R&revaluation by 20221) M-762-208-02-1 KCA 8.2.6.1/09
Note:	Green algae Pseudokirchneriellä subcapitata (currently known as Raphidocolis subcapitata) Aquanc macrophyte Lellina gibba	0 -72 H/ Sortic	$E_rC_{50} = 237 \text{ (mg p.m./L (form))}$ $E_rC_{10} = 240.95 \text{ mg p.m./L (nom)}$ $E_rC_{10} = 5.99 \text{ mg p.m./L (nom)}$ $E_rC_{10} = 5.80 \text{ mg p.m./L (nom)}$ $E_rC_{50} = 26.866 \text{ mg p.m./L (nom)}$ $E_rC_{50} = 18.956 \text{ mg p.m./L (nom)}$ $E_rC_{50} = 18.956 \text{ mg p.m./L (nom)}$ $E_rC_{50} = 100 \text{ mg p.m./L (nom)}$ $E_rC_{50} = 2016 \text{ mg p.m./L (nom)}$ $TFA)^{E}$ $F_rC_{50} = 308 \text{ mg p.m./L (nom)}$ $E_rC_{10} = 308 \text{ mg p.m./L (nom)}$	(2017) M-613180-01-1 KCA 8.2.6.1/12 Re-evaluation by (2021) M-762267-01-1 KCA 8.2.6.1/13 (1993) M-247900-01-1 KCA 8.2.7/03 Endpoint recalculation by (2021) M-768038-01-1 KCA 8.2.7/06

Studies referring to KCA are filed in the doser for the active substance. Studies written in rey to are referring to studies in the corresponding Baseline-dossier, whereas studies in black type are studies of the Supplemental dossier a.s. = active substance, pm = pure metapolite, prod. = product ______ mm = measureasured; nom @ nominal studies of the Supplemental dossier a.s. = active substance, pm = pure metabolite, prod. mm = mean measured; nom @ nominal Bold values used in risk, assessment

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old values used in risk assessment Practical limit of vater solubility In all test levels precipitations were observable of the LC50 is clearly above the water solubility of the test item. Not determined due to mathematical casons Formerly known as Selena trum cappicornuum As the sudy was conducted with sodium pitluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to Trifluoroacetic acid with factor 0.84. E



### Metabolites

Α

Metabolites Fluopyram-7-hydroxy and Trifluoroacetic acid (TFA) are relevant for the aquatic cisk assessment. No metabolite is relevant for sediment risk assessment. The EFSA AGD (2013) stepwise approach was used for all metabolites to be addressed in the risk assessment:

Step 1: Are the studies with the active substance adequate for assessing the potential effect of the metabolites?

No: Go to step 3.

Step 3: Is it clear that the toxophore has been lost from the molecule? No or unclear: Go to step 4.

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Step 4: Identify the species or taxonomic group determining the lowest tip 1 RAC_{sw,ac} for the parent compound. Is the acute metabolite  $L(F)C_{50} > 10$  times the a.s.  $L(F)C_{50}$  (on a motar basis)?

Studies on green algae are available for Euopyiam and its metabolites Floopyram-7-hydroxy and Trifluoroacetic acid (TFA), they are used for the comparison (see table below).

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	Ů, V
Substance name S Fluopyram 7-hydroxy	Ç TFA
Endpoint (mg/L)	>1.01
Molecular Worght (genol) 396.9 412 1	114.0
Parent endpoind recalculated on a molar basis (mg/L) $\sim$ $Na^{+}$ $\sim$ $92.59$	25.58

The bound value for green above is used for this assessment. The unbound value of >1.13 mg a.s./L from *Skeletonema* costatum is not a direct comparison to the green algae studies available for the metabolites and is not an EU data requirement. Therefore, the more direct comparison and more discret value is used.

The green algae endpoints for TFA and Fluopyran 7-hydroxy is much greater than 10 times the parent endpoint recalculated on amolar basis Step 200

For TFA and Fluopycam 7-hydroxy "No: Go to step 5"

For metabolites TFA and Fluopyram 7-hydroxy:

Step 5: Identify the species or taxonomic group determining the lowest tier 1 RACsw;ch of the a.s. ISRACsw; ac OPECsw and RACsw; ch > PECsw?

For the metabolite OTFA and Fluopyram 7-hydroxy, a risk assessment is performed with available data on hish as the most sensitive organism, using the geometric mean. When metabolite endpoints were not available, the parent endpoint divided by 10 is used.



Spacing	Endpoints [mg/L]				
Species	Fluopyram-7-hydroxy	Trifluoroacetic acid (TTA)			
Acute fish	$LC_{50} = 0.437 *$	$C_{50} > 1008$ (5)			
Acute invertebrates	$EC_{50} > 88.7$	$EC_{50} > 1008$			
Chronic fish	NOEC = 0.0135 *	₩ NOEC = 9 135,* ×			
Chronic invertebrates	NOEC = $0.12$	$\mathbb{Q}^{\vee}$ NOF $\mathbb{Q}^{\vee} \ge 252^{\vee}$ $\mathbb{Q}^{\vee}$			
Algae	$E_r C_{50} = 20.9$	$ \begin{array}{cccc} & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & &$			
Macrophyte	$E_r C_{\theta} = 9.2$	$\begin{array}{c} & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\$			
* 1 st tier parent endpoint divid	led by 10 🔬 🖉 🏅				

#### Table 10.2- 2: Summary of the metabolite endpoints used in risk assessment

### Selection of endpoints – Tier 1

#### Acute toxicity to fish

The acute toxicity of fluopyram to fish has been investigated in tota with five different fish species. The 96 h LC₅₀ values observed for the different fish species, including freshwater and marine as well as cold water and warm water species, differed by a factor of 30 (LC₅₀ values ranged from 0.98 mg a.s./L to 30.5 mg a.s./L, whereas the standard test organism used for its known high sensitionty, the trout, resulted in a 96 h LC₅₀ of >1.98 mg a sol. Ó

As acute toxicity date are available for five different fish species a geometric mean endpoint was derived according to the Der 2 Oapproach of the Aquatic Guidance Document (EFSA PPR Panel Guidance, 2013; 11(7):3290). Therefore, a Tie 2A Geomean-EC50 of 4.37 org a.s. W was calculated and used for the fish acute ask assessment in connection with an assessment factor of 100.

Fable 10.2- 3:     Simmary of acute endpoints for fish				
Test species	Test system	Endpoint		
Fish, acute	96 h DC 50	> 1.89 mg a.s./L (nom)		
Fish, actor Pimephales promelas	867 h LC ₅₀	> 4.95 mg a.s./L (mm) ^A		
Fish acute	96 h LC ₅₀	> 5.68 mg a.s./L (nom)		
Fish, acute	96 h LC ₅₀	30.5 mg a.s./L (mm) ^B		
Marine figh, acute V V	96 h LC ₅₀	> 0.98 mg a.s./L (mm)		
Geometric mean	96 h LC ₅₀	4.37 mg a.s./L		

Α Practical limit of water solubility В

In all test levels precipitations were observable so the LC₅₀ is clearly above the water solubility of the test item.



One of the metabolites (Trifluoroacetic acid (TFA)) was acutely tested using Zebra fish. The Trifluoroacetic acid (TFA) had a  $LC_{50}$  value >1008 mg p.m./L. This metabolite is far less toxic than the parent molecule to the Sheepshead minnow by >1029-fold.

The existing acute fish study investigating the toxicity of the fluopyram metabolite revealed learly lower fish toxicity of metabolites compared to the active substance fluopyram

#### Chronic toxicity to fish

According to the AGD, EC10 values are preferred over NOEC and should be used for risk assessment. when robust values are available. In the fish ELS study, the NOEC is 0.135 mg/L based on length and morphological and behavioural effects, the lowest E670 is 0.162 mg a.s./L based on fry survival. It proposed to use the NOEC for risk assessment (refer to MCA for further explanations)

#### Acute toxicity to invertebrates

The acute toxicity of fluopyram to invertebrates has been nvest gated on Daphnids as well as on the estuarine species Mysid Shrimp and Eastern Oyster. In addition, subchronic tests with spiker sediment have been conducted on two sediment dwelling organisms, Chironomus tentans and Leptocheirus plumulosus. Chronic testing was done with Daphnids and Chironemus riperius &

The EC₅₀ for the standard species  $\beta aphnic magna was > 20 \text{ mg/a.s./I} The <math>\beta \beta_{50}$  for the mysid shrimp Americanysis bahia was > 0.50 mg a.s.  $\Delta$ . The set of the Eastern O ster Q. virginica) resulted in an LC50 > 0.44 mg a.s./L for mortality (i.e. no effects on mortality up to the highest cest concentration) and shell deposition. m

As acute toxicity data are available for three different aquatic invertebrate species a geometric mean endpoint was derived according to the Tier A approach of the Aquatic Guidance Document (EFSA PPR Panel Guidance 2013; At(7):3290). Therefore, a Tier 2A Geomean-EC of 1.638 mg a.s./L was calculated and used for the aquatic invertebrate acute risk assessment in connection with an assessment factor of 100.

The subchroni Gests with sediment dwellers C. tentans and L. dumulosus showed a very low toxicity of fluopyram towards these species with NOEC values of 26 and 38 mg a.s./kg, respectively.

The metabolites fluopyram-7-hydrox @and trifluoroacetic acid (SFA) were of low acute toxicity to invertebrates with EC@values of >88.7 mgp.m./LQand to 1008 mg p.m./L, respectively.

### Chronic toxicity to invertebrates

Chronic testing on Dophnia magne resulted in NOE of 1.25 mg a.s./L. The life cycle test with Chironomus tentans revealed a NOEC of 7.39 mg a.s./L and an EC₁₀ of 0.54 mg a.s./L that is considered for use in the risk assessment.

### Toxicity to algae

Following current state of science, the test guidelines OECD TG 201 and 221, the EU-Method C3, the Regulation for Classification and Labelling (Regulation (EC) No 1272/2008), the PPR Opinion (EFSA Journal 467, 1-44, 2007 The ERSA supporting publication 2015 (EN-924 published 22 December 2015) and also the EDSA Aquatic Guidance Document (AGD, 2013, noted by SCFCAH on July 10-11th, 2014 Jist growth fate as the relevant endpoint of the algae and the Lemna growth inhibition test. Therefore, the risk assessment is based on the  $E_rC_{50}$ , when available.

Extensive testing has been done on green algae, blue-green algae, freshwater algae and marine diatoms.



In total four studies on algae/diatoms are available for the parent compound fluopyram with  $E_rC_{50}$  values ranging from > 1.13 mg a.s./L to 9.08 mg a.s./L. The most sensitive species was the marine diatom *Skeletonema costatum*.

The green algae were tested with the two metabolites fluopyram-7-hydroxy and trifluoroacetic acid (TFA). Comparison of the 72-hour  $E_rC_{50}$  values demonstrates that only trifluoroacetic acid (TFA) showed a similar toxicity to the parent only in one study with a 72-hour  $E_rC_{50}$  of >1.01 mg p.m./L; however, the 72-hour  $E_rC_{50}$  values ranged up to 237.07 mg p.m./L. The metabolite fluopyram-7-kydroxy showed lower toxicity than the parent with 72-hour  $E_rC_{50}$  values of 20.9 mg p.m./L.

#### Toxicity to aquatic macrophytes

The aquatic plant *Lemna gibba* showed a comparable toxicity as for algae for the parent with a 7-day  $E_yC_{50}$  value of 2.12 mg a.s./L and an  $E_rC_{50}$  value of 2.51 mg a.s./L.

The aquatic plant *Lemna gibba* was also tested with two metabolites (fluoryram-7-hydroxy and trifluoroacetic acid (TFA)). Consistent with metabolite testing in algae, the metabolites (fluoryram-7-hydroxy and trifluoroacetic acid (TFA)) were far less toxic than the patent (by a factor of ca 3.3 to 475 or 3.7 to 803).

The 7-day  $E_yC_{50}$  values of the metabolities were 7.1 mg p.m./L (fluopyram-7 hydroxy) and >1008 mg p.m./L (trifluoroacetic acted (TFA)) and the 7-day E.  $G_0$  values of the metabolities were 9.2 mg p.m./L (fluopyram-7-hydroxy) and >2016 mg p.m./L (trifluoroacetic acted (TFA)).

# Uncertainty factors for tsomer composition of metabolites

The metabolite Fluopyram-7-by droxy has a Chiral center. Ecotox pological testing was performed with the racemic mixture therefore, for this metabolite an additional uncertainty factor of 2 will be applied to the RAC of the *Daphnia magna* acute and of the algae and aquatic plant studies in consideration of enantiomers.

# Predicted environmental concentrations used in the risk assessment

Predicted environmental concentrations of fluopyram and it metabolites in surface water were calculated according to FOCUS Steps 1-20 or the use in grapes

For the application in lettuce predicted environmental concentrations in surface water (PECsw) were estimated after use in bigh-tech greenhouses in the Netherlands and Europe. The exposure assessment model Greenhouse Emission Model 3.3.2 (GEM 3.3.2) was used.

**Important remark by the applicant:** The PEC, values as presented below are interim values and are therefore subject to change until final modeling input parameters can be established. The applicant intends to provide final PEC_{sw} values latest by end of March 2022.

intends to provide final PEC_{sw} values latest by end of March 2022.



#### Application in grapes, 2 x 50 g a.s./ha

Table 10.2- 4: Initi	al max PEC _{sw} valu	es – FOCUS Steps 1 and 2 – Mu	Itiple application in graphs
(3)11		PECs	w, max
Compound	FOCUS Scenario	Grapes - Spring (Mar May) 2 × 50 g a.s./ha,	Grapes 7 Summer (Jung Sepa) 2 50 g.s./ha. 7 Opport 1 sec
Fluopyram	STEP 1 STEP 2 – North STEP 2 - South	28.1 3.96 5.96	2 28.1 3.96 4.96
Fluopyram-7-hydroxy	STEP 1 STEP 2 – North STEP 2 - South (	$\begin{array}{c} & & 1.70 \\ \hline & & 0 \\ \hline \end{array}$	√ 0.141
Trifluoroacetic acid (TFA)	STEP 1 STEP 2 – North STEP 2 – South		5 192 0 5 0.113 0 0 5 0.169
Bold values used in risk asse	ssment		

Initial max PEC_{sed} values - KOCUS Steps 1 and 2 Multiple application in grapes (spring and summer, 2 x 50 g a.s./ka) Table 10.2- 5:

Compound	FOCKS Grocks Grapes - Ω	ed, myx g/LJ, S Grapes –
	Scenario Spring (Mar May) 2 × 50 g a.s./ba, BBCH 53-73	<ul> <li>Summer (Jun Sep.)</li> <li>2 × 50 g a.s./ha,</li> <li>BBCH 71-89</li> </ul>
Fluopyram	STEP 2 - North 8.82 STEP 2 - South 4.4 STEP 2 - Sou	63.8 8.82 11.1
Fluopyram-7-hydroxy	A STEP 2 - South 2 - 0.283	1.78 0.142 0.212
Trifluoroactic acid (TFA)	STEP 1         0.001           STEP 2         0.001           STEP 2         0.001           STEP 2         0.001	<0.001 <0.001 <0.001

The set of the set of


## Application in lettuce, 2 x 200 g a.s./ha



Risk assessment for aquatic organisms

According to the Aquatic Guidance Document (EFSA PPR Panel Guidance, 2013), the risk to aquatic organisms is evaluated based on the derivation of Regulatory Acceptable Concentrations (RACs) as follows



Acute risk assessme	<u>nt:</u>				
$RAC_{sw, ac} = LC_{50} \text{ or } I$	EC ₅₀ / 100				2° do
The risk is considered	ed acceptable, if the RAC	$C_{sw, ac} \ge PEC_{sw, max}.$		^y	
Chronic risk assessn	nent:			6	
$RAC_{sw, ch} = NOEC o$	or EC ₁₀ / 10	(Č4)	L'Y	L'Y	
$RAC_{sw ch} = E_r C_{50} / 1$	0	To a start of the	Q	Ô	
The risk is considered	ed acceptable, if the RAC	$C_{\text{sw, ch}} \geq \mathbb{P}^{\mathbb{C}}_{\text{sw, max}} \qquad $			
To summarise, these	e abbreviations are used	in subscript following	the term PEC	Con RAC.	
ac: acute, ch: chroni ACUTE RISK ASS Important remark and are therefore su applicant intends to 2022. Application in grape Table 10.2–8:	c, sw: surface water, ma SESSMENT FOR AQU by the applicant: The ubject to change until f provide final PECsw val	ATIC ORGANISSIS	as presented parameters c calculations	tion in grap	terim values blished. The hd of March
Compound	Spegies &	Endpoint	RAC	PEC _{sw,max}	$RAC \ge REC$
		pring application	[[µg/L]	[[µg/L]	
FLU+TFS C 500	Fish, avute Oncorhynchus myknys	LQ50 8894	0.884	11.317 ^A	No
	hvertebrate, acute	EC 51	0.51		No
Fluopyram tech $\sqrt[6]{2}$	Fish, acute C	€C ₅₀ 4370	43.7	5.96	Yes
	Invertebrate, acute Geometric mean ~9	EC ₅₀ 1638	16.38		Yes
Fluopysam-7-bydroxy	Fish, acute	LC ₅₀ 437 ^B	2.19 ^C	0.282	Yes
	Inversebrate, acute Daphnia magna	EC ₅₀ >88700	>443.5 ^C		Yes
Trifluor acid (TFA)	Fish, acute Oncorhynchus mykiss	LC ₅₀ >1008000	>10080	0.226	Yes



Compound	Species	Endpoint [µg/L]	RAC [µg/L]	PECsw,max [µg/L]	$\begin{array}{c} \mathbf{RAC} \geq \\ \mathbf{PEC}_{\mathbf{sw}}  \bigcirc^{\circ}  & \\ \end{array}$
	Invertebrate, acute Daphnia magna	EC ₅₀ >1008000	>10080	<b>A</b> .	Yes
	Su	mmer application			
	Fish, acute Oncorhynchus mykiss	LC ₅₀ 88.4	0.884	11 217	No
FLU+1FS SC 500	Invertebrate, acute Daphnia magna	EC ₅₀ 51	0.51		
	Fish, acute Geometric mean	LC ₅₀ 4570	¢#3.7 °		Yes
Fluopyram tech.	Invertebrate, acute Geometric mean	EC 50 1638 · 5	16,38	¢4.96 ()	Yes S
Eluonurom 7 hudrouu	Fish, acute		2.19	0 2 1 2	Yes of
Fluopyram-7-hydroxy	Invertebrate, acute	EC50 >88700 -	9443.5 È		Yes
Trifluoroacetic acid	Fish, acute	LC ₅₀ >1008000	>10080		Wes
(TFA)	Invertebrate@acute	EC50 2008000	\$10080	0009	Yes

В

Ô 1st tier parent endpoint divided by 10 L For the metabolite fluopyram Ahydroxy the RAC has been С corrected in addition according to an uncertainty factor of 2 in consideration of two enantiomers; 0

For the application in grapes (spring and summer application) at 2, 50 g a.s./ha the acute trigger is met for all aquatic species for fluopyran and its metabolites.

in grapes (spring and summer oplication) at 2 × 50 g a.s./ha Therefore, a refinement is presented below considering drift mitigation measures. Š X

500 considering drift mitigation - grapes (2) Refined risk assessmer  $\times 0.2 \text{ L prod}$ 

0

Table 10.2- 9:	Refined tisk assessment for the formulation based on mitigation measures- grapes
A Contraction of the second se	$(2 \times 0.3)$ prod ha) $\sqrt{2}$

Compound Species	Endpoint [µg/L]	RAC [µg/L]	Mitigation	PEC _{sw,max} [µg/L]	RAC≥ PEC _{sw}
FLU+755 SC	$ \begin{array}{c}                                     $	0.884	5 m drift buffer + 90 % drift reducing nozzles	0.504 Å	Yes
500 Jack Invertebrate acu Daphnia magna	te EC ₅₀ 51	0.51	5 m drift buffer + 90 % drift reducing nozzles	0.504**	Yes

Formulation-specific PEC_{sw}

A



Based on the refined risk assessment for the formulation an acceptable use in grapes at 2 x 0.2 L prod./ha can be concluded considering a 5 m drift buffer in connection with 90 % drift reducing nozzles.

# Application in lettuce, 2 x 200 g a.s./ha

Since the application in lettuce is intended for a greenhouse use no exposure of the formulated product via drift is expected. Furthermore, the soil metabolite fluopyram-7- hydroxy was not iden in watersediment systems and thus, no exposure assessment could be carried out for the soil-less use. Therefore, no acute risk assessment is presented for the formulated product and the metabolite floopyran-7hydroxy.

hydroxy.					
Table 10.2- 10:	Acute risk assessment mitigation (2 × 200 g a	based on FOGUS Step 2 f s./h@soil-les cultivation	or the applica	tionom lette	içe — BO
Compound	Species	Findpoint Carlos	RAG [µg/L]	PECsw,max [µgAL]	$\mathbf{RAC} \geq \mathbf{O}^{Y}$ $\mathbf{PEC}_{sys}$
	Nør	euse of filter cleaning wate			Ő
Fluonyram tech	Fish, acute Geometric mear	LC ₅₀ 4370	43.00 0		₩ês ≯
Fluopyram tech.	Invertebrate & Cute &	EC ₅₀ 1698	\$6.38 \$		No
Trifluoroacetic acid	Fish, acute	2 LC -1008000	>10080	10 10 10 10 10 10 10 10 10 10 10 10 10 1	Yes
(TFA)	Invertebrate acute	EC ₅₀ 1008000	510 <b>080</b>	8337 7	Yes
~	With	reuse of filter Cleaning wat	er Ø		
Fluonyram tech	Fish, acute Ceometri©mean O	\$ 0 \$ 50 \$ \$ 70 \$ \$	43.7 ×	43 59	Yes
Fluopyram tech.	Invertebrate, acate <u>s</u> Geometric mean		¥6:38	-5.57	No
Trifluoroacetic acid	Fish, acute Oncorhknchus mykiss	5 LC 50 - 1008000 3 -	>10080	0 895	Yes
(TFA)	Invertebrate scute Dagonia mogna		>10080	0.095	Yes
~\$					

For the application in lattice without relise of filter cleaning water) at  $2 \times 200$  g a.s./ha the Ô acute trigger was not met for aquatic invertebrates for fluopyram. A risk assessment for fluopyram using





Refined acute risk assessment for fluopyram using mitigation removal fraction of 0.95 - lettuce (soilless cultivation, 2 x 200 g a.s./ha)

### Acute risk assessment based on mitigation removal fraction of 0.95 for letture Table 10.2-11: less cultivation, 2 x 200 g a.s./ha)

Compound	Species	Endpoint [µg/L]	RAC [µs_]	Mitigation	PECswimax	$\begin{array}{c} \mathbf{R}\mathbf{A}\mathbf{C} \geq \\ \mathbf{R}\mathbf{E}\mathbf{C}_{sw} \end{array}$
		No reuse of filter c	leaning v	water 🔗		
Fluopyram tech.	Invertebrate, acute Geometric mean	EC ₅₀ 1638	16.38	95 % (end-of-pipe reduction)	9.996 <u>(</u>	Y&
		With reuse of fifter	cleaning	mater 🔊 👦		, <u>,</u> ,
Fluopyram tech.	Invertebrate, acute Geometric mean	EC50 1638	16.38	95 % (end-of-pipe reduction)	Q.178 £	YAS .

For the 2 x 200 g a.s./ha application in Qettuce (with and without reuse of filter cleaning water) the acute 0 trigger was met for aquatic invertebrates for fluopy am based on mitigation reproval fraction of 0.95.

#### ÓR OU¶⁄I CHRONIC RISK ASSESSMENT ORX , O O Carlo Ő

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Important remark by the applicant: The PEC, and TER values as presented below are interim values and are therefore subject to change until final modelling input parameters can be established. The applicant intends to provide final PEC_{sw} values and revised TER calculations latest by end of March 2022.

Chronic risk assessment based on FOCKS Step 2 for the application in grapes (2 × 50 g a.s./ka) Table 10.2-12:

Compound	Species 2 5 5 5	Endpoi [µg/L]	nt	RAC [µg/L]	PECsw.max [µg/L]	RAC≥ PEC _{sw}
	Spring appli	cation				
FLU+TFS SC 500 \	Algae C O PseudoferchnerGlla subeapitata	ErC ₅₀	292	29.2	11.317 ^A	Yes
	Fish Shronic ^y Pimephales prometis	NOEC	135	13.5		Yes
	Invertemate, chronic Daplyna magna	NOEC	1250	125	- 0.6	Yes
Fluoperam tech?	Invertebrate, chronic Chironomus riparius	EC ₁₀	540	54	5.90	Yes
Č	Algae Skeletonema costatum	$E_rC_{50}$	>1130	>113		Yes



Compound	Species	Endpoint [µg/L]	RAC [µg/L]	PECsw.max [µg/L]	RAC≥ PEÇ _{sw}	
	Aquatic macrophyte <i>Lemna gibba</i>	ErC ₅₀ 2510	251		Nes /	G.
	Fish, chronic Pimephales promelas	NOEC 13.5 ^B	0.675 ²	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Yes	
Elizanor 7 hudrour	Invertebrate, chronic Daphnia magna	NOEC 125 ^B	6.25 ^C		Yes 4	
Fluopyram-7-nydroxy	Algae Pseudokirchneriella subcapitata	ErC ₅₀ 20900	1045 °	0.282 ¥	Stes (	
	Aquatic macrophyte	ErC ₅₀ 9200	460.5 ⁶		Yes	,*
	Fish, chronic	NOEC 13.5	¥35 5		Yes	
Frifluoroacetic acid	Invertebrate, chronic	NOEC 23200	≥2 <b>500</b>		Yoos	,
(TFA)	Algae Pseudokirchnertella subcapitata	ErC 5 >1010	¥101 ©	0.226	Yes	
	Aquatic macrophyte	EyC ₅₀ 924000 DrC ₅₀ 5-2016900	92400 201600℃		Yes	
	summer app	lication 8	° °	Ő	I	
FLU+TFS SC 500	Algae	ErC ₅₀ 292		11.317 ^A	Yes	
Å	Fish, chronic & O Pimephales promelas, S	NØEC 135 &	13.5~		Yes	
	Invertebrate hronie	NOE© 1250	<b>Q</b> 25		Yes	
Fluopyram tech	Phyertebrate, chronic, D	EQ10 AD	54	4.96	Yes	
	Algae 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	$E_r G_{G_r} > 1$	>113		Yes	
	Aquatic macterphyte	ErC ₅₀ 2510	251		Yes	
	Pinephales promelas	NØEC 13.5 ^B	0.675 ^C		Yes	
Ą	Invertebrate chrouge	NOEC 125 ^B	6.25 ^C		Yes	
Fluopyrad -7-hydroxy	Algae Psehänkirchiperiellaubcahitata	E _r C ₅₀ 20900	1045 ^C	0.212	Yes	
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Aquatic macrophyte	ErC ₅₀ 9200	460.5 ^C		Yes	1
	Fish Thronic Pimephalesprometas	NOEC 13.5 ^B	1.35		Yes	1
Trifluoroscetic act	Invertebate, chronic Daphna magna	NOEC ≥25200	≥2520	0.169	Yes	1
	Algæ Pseudokirchneriella subcapitata	ErC ₅₀ >1010	>101		Yes	1



Compound	Species	Endpoi [µg/L]	nt	RAC [µg/L]	PEC _{sw.max} [µg/L]	RAC≥ PEÇ _s ŵ	~
	Aquatic macrophyte Lemna gibba	<mark>EyC50</mark> ErC50	<mark>924000</mark> >2016000	<mark>92400</mark> >201600		Nes /	
A Formulation mass	fin DEC			· _ O'	())	i A	

rmulation-specific PEC_{sw} В 1st tier parent endpoint divided by 10

J. S Step 2 for the start С For the metabolite fluopyram-7-hydroxy the RAC has been corrected according to an uncertainty 2 in consideration of two enantiomers.

Table 10.2- 13:	Chronic risk assessment for	sedim <i>en</i> t organism	s based	on FOC	🕼 Step 🕯	2∦for t
	application in in grapes (2 ×	50 g a.s./ha)	R.	\$° Â	1 4	(

			\sim	î ~~~	, .O <i>a</i>	n (7 1)
Compound	Species	Endpoint₀ µg/kg/		RAC [µg/kg]	PECsed.max	RAC≥ PECsed
		Spring cerea				
Fluopyram tech.	Sediment dweller, <i>Chironomus tentan</i>	NOEC	26000	2690	چ ۲3.5 م م	a ses
		Wintercerea	15° ~ 70			ò.
Fluopyram tech.	Sediment dweffer, Chironomu@tentants	NOEC OF	260000	2690	13.5	Yes
		¥ (× 0×		0	<u>. </u>

Ø For the application in grapes (spring and summer application), at 2 ×50 g a S/ha the chronic trigger was met for all aquatic species for thopyram and its metabolites.

Application in lettuce

Since the application of lettuce is intended for a greenhouse use no exposure of the formulated product via drift is expected. Furthermore, the soil metabolite floopyram-7- hydroxy was not identified in watersediment systems and thus, no exposure assessment could be carried out for the soil-less use. Therefore, is presented for the formulated product and the metabolite fluopyram-7no acute isk assessment hydroxy. S

Coronic Fisk assessment based on FQCUS Step 2 for the application in lettuce - no Table 10.2mitigation (2, 200 gas./ha, soil-less cultivation) 4

Composit	Species	Endpoint [µg/L]	RAC [µg/L]	PECsw.max [µg/L]	RAC≥ PECsw
~~~ .	No reuse of file	ter cleaning water			
, de la companya de l	Fish, chropite	NOEC 135	13.5		No
	Invertoriate, Oronic S Daphhia magna	NOEC 1250	125		Yes
Fluopyram tech?	Invertebrate, chronic Chiroromus riparius	EC ₁₀ 540	54	20.75	Yes
	Algae [®] Skeletonema costatum	ErC ₅₀ >1130	>113	-	Yes
)	Aquatic macrophyte Lemna gibba	ErC ₅₀ 2510	251		Yes



Compound	Species	Endpoint [µg/L]	RAC [µg/L]	PECsw.max [µg/L]	RAC≥ PEC _∭ °
	Fish, chronic Pimephales promelas	NOEC 13.5 ^A	1.35		Yey
Trifluoroacetic acid	Invertebrate, chronic Daphnia magna	NOEC ≥25200	≥2520 இ	0.557	Yes S
(TFA)	Algae Pseudokirchneriella subcapitata	ErC50 >1010			Yes
	Aquatic macrophyte <i>Lemna gibba</i>	<mark>EyC₅0</mark> 𝒱 <mark>924000</mark> ErC₅€y ≥2016000	<mark>∕ <mark>92400</mark> <mark>≯201600</mark> ≤</mark>		Yes
	With reuse of fi	lter cleaning water	6	, , , , ,	
	Fish, chronic	NOEC 1350	13.5 0		No
	Invertebrate, chronic Daphnia magna	SOEC 250 Q		Ó É	res $\int_{0}^{\circ}$
Fluopyram tech.	Invertebrate, chronic	EC@ 540 0		¥3.59 %	Yes
	Algae Skeletonema constitum	ErC ₅₀ -1130	A13 2		Yes
	Aquatic macrophyte C 7	E4650 2510	251 °		Yes
	Fish, chronic	NOEC 13.5 A	1.35		Yes
Trifluoroacetic acid (TFA)	Invertebrate Phronics O Daphnia Magna	©OEC`~≥25206	\$2520 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0 805	Yes
	Algae ( ) Pseudokirchneriella subcapitata	E, C,	>100	0.075	Yes
	Aquatic macrophyte	<mark>€_yC₅₀ </mark>	[™] 92400 ≯201600		Yes
¹ 1 st tier parent er	ndpoint divided by 10				

For the application in lettice (with and without reuse of filter cleaning water) at 2 × 200 g a.s./ha the chronic trigger was not net for filtoryram. A risk assessment for fluopyram using mitigation removal fraction of 0.95 is presented below



Refined chronic risk assessment for fluopyram using mitigation removal fraction of 0.95 - lettuce (soilless cultivation, 2 x 200 g a.s./ha)

## Refined chronic risk assessment for fluopyram based on mitigation removal traction Table 10.2-15: of 0.95 for lettuce (soil-less cultivation, 2 x 200 g a.s./ha)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]Û	Mitigation		PEC, w, max > [µg/L]	RAC × PEC
		No reuse of filt	ter cleani	ng water 🔗			
Fluopyram tech.	Fish, chronic Pimephales promelas	NOEC 135	↓3.5	95 % (Ond- reduction)	of-pipe	0,496	Yes
		With reuseof fi	ilter¢clear	ning water			Ś
Fluopyram tech.	Fish, chronic Pimephales promelas	NOEG 135	13.5	95 % (end- reduction)	oppipe	2078	Yes
							0 North

For the application in lettuce (with and without refise of the cleaning water) at 2 200 ggs.s./ha the chronic trigger was met for fish foo fluopyram considering a margation removal fraction of 0.95.

According to the EESA "Guidance on pered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2016, 11(7), 3290; chapter 10.3.11), for products containing more than one active substances, the mixture toxicity shall be addressed via the Concentration Addition (CA) Model. And, following the recommendations of the EFSA "Outcome of the pesticides peer review meeting on general recurring issues in octoxicology" (EFSA Supporting publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parents. When the endpoint of the PPP (expressed in terms of fluopyram) is at least three times lower than the equivalent endpoint for the active substance, it should be considered to be more toxic.

The measured toxicity data (ECx) available for the given endpoint is shown in the table below for the





# Is the formulation three times more toxic than fluopyram?

Table 10.2- 16:	Comparison of SC 500 and th	f endpoints av e active substa	ailable for the fo nce fluopyram	ormulated product (	PPP) FEX+TFS
Test species	Endpoint and test system	Measured toxicity of PPP [mg prod./L]	Fluopyram [mg.a.s./L]	Formulation endpoint recalculated for & fluopyram ^A [mg a.s./L]	Fluopyram Oendpoint / Recalculated formulation Endpoint
O. mykiss	LC ₅₀ , acute, 96 h	0.0884	> 1.89	of calconated	
D. magna	EC ₅₀ , acute, 48 h	0.05		nef@alcul@ed	
Pseudokirchneriella subcapitata	E _r C ₅₀ , short- term, 72 h	0.292	8.94 8.94	0,06 yr	47 U 47 142 G

Amount of fluopyram in the test icin used in formulation studies 21.4 % please refer to study  $\frac{1-292579}{02-1}$ 

Regarding fish, aquatic invertebrates and algae, endpoints are available for both, formulation (ECx_{PPP}) and a.s. (ECx_{as}).

No meaningful comparison can be performed for fish and opuatic invertebrates the to unbound values for the active substance fluopyram.

for the active substance fluopyram. For algae the formeration (endpoint expressed in terms of fluopyram) is more than 3 times more toxic than fluopyram. Therefore, a risk assessment for the formulation (provided.

# MDR calculation

Th calculation is performed for fish and aquatic invertebrates due to the unbound values for the active substance fluopyram As a conservative approach the lowest endpoints for fish and aquatic invertebrates are used in the calculation, therefore different species are considered.

Table 10.2-47:

Overview of endpoints available for the formulated product (PPP) FLU+TFS SG 500 and the active substances fluopyram and trifloxystrobin

Test species Test system	m m m m m m m m m m m m m m m m m m m	FLU [mg a.s./L]	TFS ^A [mg a.s./L]
O. mykiss (PPP / TES) C. variegaus (ELU)	96 a 0.0884	> 0.98	0.015
D. magna (PPP) TFS) C. Dirginiqu (FLU)	48 h 0.051	> 0.44	0.016

^A Please offer for endpoints to EFSA Journal 2017;15(10):4989: Peer review of the pesticide risk assessment of the active substance triflow strobin



A

Table 10.2- 18:	Summary of results obtained in the studies with the formulated product (PPP)
	FLU+TFS SC 500 and comparison of calculated and measured mixture toxicity $\circ$

Test species	Endpoint and test system	Measured toxicity of PPP (converted to be a.s. based) (LC _{50 PPP} or EC _{50 PPP} ) [mg total a.s./L]	Calculated mixture toxicity ^A (a.s. in product) LC _{50 mix-CA} or EC _{50 mix-CA} [mg total a ./L]	Model deviation ratio (MDR = EC 50 mixed / EC 50 PPP)
Fish	LC50, acute, 96 h	0.038	0.029	2 Q.77 5 0
Aquatic invertebrates	EC50, acute, 48 h	0.025	~ 0.030 [°]	

The mixture toxicity of the formulation was re-calculated based on the measured contents of Huopy from (251 st g/L), and trifloxystrobin (253.5 g/L) within the form Dation and the product deposity (1, 104 g/mb) (please refer to study Me 294350-01-1).

The calculated MDR values are between 0.2 and 5 for fish and acpartic invertebrates, indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances for °∼ these organisms. No synergisms or additional toxicity occurs and to the co-formulants.

Therefore, the evaluation of the safety of the formulation can be based on the risk assessment of

Therefore, the evaluation of the safety of the formulation can be based on the risk assessment of fluopyram. Nevertheless, a formulation-based risk assessment has also beeoperformed for fish and aquatic invertebrates and was presented above.



#### **CP 10.2.1** Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes A)

Data Point:	KCP 10.2.1/01
Report Author:	
Report Year:	2007
Report Title:	Acute toxicity of fluopyram & trifloxystrobin SC 506 (250+250) G @ fish @
	(Oncorhynchus mykiss) under state conditions
Report No:	EBGMP030
Document No:	<u>M-294350-01-1</u>
Guideline(s) followed in	EPA-FIFRA § 72-1/SEP-EP 2540/9-85-006 (1982/1985)
study:	OPPTS 850.1075 (Public Braft, 1996)
	Directive 92/69/EEC, C. (1992)
	OECD No. 203 (rev. 1992); Equivalenção US EPA OPPTS Guideline No.
	850.1075 SUPP O' O O O O O
Deviations from current	Current Guideline 203 (2019) $\mathcal{O}$ $\mathcal{O}$ $\mathcal{O}$
test guideline:	Deviations: The fish length at test start was not reported. The missing information
	was not expected to have impacted the study esults. All validity criteria were met.
Previous evaluation:	No, not prevously submitted
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$\underline{Yes}  \underbrace{ \bigvee  \bigcup^{v}  \underbrace{ \bigvee  \bigcup^{v}  \bigvee^{v}  \underbrace{ \bigvee  \bigcup^{v}  \underbrace{ \bigvee^{v}  \underbrace{ \underbrace{ \bigvee^{v}  \underbrace$
s	

# Executive Summary *st*

An acute toxicity testwas performed with the Randow Fout (Oncorh Phchus mykiss) in a static system. Juvenile fish were \$\$\$ posed to FLU + RFS SC 500 (2\$0+250 g/L) is groups of 10 (one replicate of 10 fish per test level to an aqueous solution of the product at hominal concentrations of 0.0313, 0.0625, 0.125, 0.250 and 0.500 mg@rod./D for & period of 96 hours Additionally, a control was included. Observations of mortality and other signs of toxicity were made approximately 4, 24, 48, 72 and 96 hours after test initiation

Concertizations of fluopyram were verified by OPLC WMS/MS on days 0, 1, 2 and 4 for each concentration and control Measured concentrations of fluopyram were in the 95 - 113 % range of nominal concentrations and no residues were found in the control samples above 0.521 µg a.s./L, which was used as the lowes Qstandard concentration during the study. The mean measured concentrations were: 7.26, 126, 264, 53.1, 403 µg/luopyram a s/L

The study Alfils all validity criteria of GECD 203 guideline.

There were behavioural abnormalities of the fish of the three highest test concentrations (0.125, 0.250 and 0500 mg prod (1). In the concols not not applies or sub-lethal finding were observed.

The endpoints based on nominal product concentrations of FLU + TFS SC 500 (250+250 g/L) were:

LC₅₀ – 96 hours (95 % C.I.) (0.091) mg product concentrations of PEO + FFS SC 300 (230+230 g/L) LC₅₀ – 96 hours: 0.0625 mg prod./L.



# I. MATERIALS AND METHODS

	Q° ~
Test material	FLU + TFS SC 500 (250+250 g/L)
	Specification No.: 102000012886
	Batch No: 2007-000441
	Content of a.s.: 21.4 % w/w fluopyram
	21.6 % w/w trifloxystrobin
Guideline(s) adaptation	None specified
Test species	Rainbow trout ( <i>Oncorhynchus mykissy</i>
Acclimation	At least 14 days to test conditions Health during acclimation: less than 5 % mortality in the 48-hour acclimation period before testing, all unsuitable fish (e.g. injured deformed, etc.) were eliminated prior to the assignment of test groups.
Organism	Mean length: $55 \pm 6$ mm at test start $\sqrt{2}$ $A$ $\sqrt{2}$
age/size	Mean body weight: $1.6 \neq 0.6$ g at test gart $4$
Test solutions	Nominal concentrations: 0.0313 - 0.0625 0.125 0.250 0.500 mg prod/L
	Mean measured recoveries based on a.s. content ranged from 95 to 113 % of neurinal a.s.
	concentrations v v v v v v v v v v v v v v v v v v v
	Control: water
	Evidence of undissolved material. During the whole exposure period no test material was observed and the lest material was
Doplication	No. of Vocaldara concentration (routh otoc) (1)
Replication	No. of vessels per control (replicate) 1 2 2 2
Oneniana	No of resonant Server 110
replicate	
Exposure	State
	Tratal exposure duration: 96 hours
Test Vessel	0.40 ostish/L řest medaum
Loading	
Feeding during	None in the of the state
test	
Test conditions	Temperature 11.7 92.2 cr S
	Photoperiod: 16 hours light, 8 hours dark
A.	Light intensity on treported of w
Ö,	pH: 628 - 7.2
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Water hardness: 49/- 60 mg CaCO/L
No.	Dissolved oxygen: 88 200 % of saturation (aeration was added on study to reach oxygen
"Ø	Saturation)
Ô	Conductivity. < 0, \$ μ 5/cm
Demons at 64	Tick and the second for the second se
Measured /	once daily (Pay 1 to 4)
Observations	Discrete weasurements of dissolved oxygen, water temperature and pH were obtained at test
ST O	mitiation and after 24, 48, 72, and 96 hours. Temperature was additionally measured hourly
r s s s s s s s s s s s s s s s s s s s	by a calibrated data logger in one control replicate.
Sampling for	Samples of test solutions were taken at test initiation (0 hour), after 48 hours and at test
Chemical	termination (96 hours) for analysis of test substance. In case of 100 % mortality prior to the
anarysis	end of the test, the analytical determinations were made at the respective times.



	The chemical analyses were performed by using a High-performance liquid chromatograph (HPLC – MS/MS)
Data analysis	The LC ₅₀ values and the 95 %-confidence intervals were calculated every 24-hour using one of three statistical techniques: moving average, logit analysis or probit analysis. The b hour $-$ LC ₅₀ value was determined by probit analysis. All calculations were carried out using Microsoft Excel data sheets. All statistical evaluations were done using the commercial program ToxRat Professional (ToxRat® version 2.09).

II. RESULTS A	NEDISCUSSION
III ILSULIS	

		II. RESULTS AND DI	SCUSSION		
Table 10.2.1- 1:	Validity criteria	Q ^D Y			
Validity criteria		Č ⁴ V	Required	S Obtained	
Mortality in control	ol during test		$\sqrt[m]{2} \leq 10\%$	\$ 00 % \$	<u> </u>
Dissolved oxygen	saturation		\$60 % \$ * * *	88 - 400 %	

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU, regulatory requirements outlined within SANTE/2020/12830, Rev 4 SANTE/2020/12830, Rey. Ô

Recoveries of fluopyram on day 0, day 1, day 2 and day 4 were between 95 and 105 % (see table below). Biological results are based on nontral product concentrations of FLU + TFS SC 500 (250+250 g/L). No residues of flugpyran were found in the control samples above 0.521 og a.s./L, which was used as the lowest standard concentration during the study

Č. V		añ	ŐŇ		~ ~	O ^v	Ľ	. 0			
Nomir concentr	al of ation		lean na concen lagg a	oasured tration .s./LY			% of n	ominal	l	Mean measured	Mean
[mg prod./L]	^{¶μg} a.s./L]	Day 0	Day 1	Day 2.0	Day 2 4	Day 0	Day 1	Day 2	Day 4	conc. [μg a.s./L] ^A	% of nominal
0.03130	6.70	\$ 38	ê B	1 9.5 6	\$\$5	110	-	113	102	7.26	108
0.0623	13.4 🔊	14.2	[♥] B ∧	13.4	\$ 1 3.3.	\$ 06	-	100	99	13.6	102
Q.125	26.8%	25.	-~ <mark>B</mark> ∕⊘	26,6	- %	9 5	-	99	-	26.1	97
0.250	53.5	54.0	5Ô.1	C [♥]	JO'	101	97	_	_	53.1	99
0.500	. 1 07	403	CΥC	C ^C	^C ^C	96	-	-	-	103	96

Analytical gesults for fluopyram & Table 10.2.1-2:

A Not given in report Calculations based on measured concentrations on each sampling day.

В No messurements performed.

С lo measurements performed as all fish were dead.

Observations:

In the controls no mortalities or sub-lethal finding were observed.



In the three highest test concentrations (0.125, 0.250 and 0.500 mg prod./L) behavioural changes were observed during the exposure period. \swarrow°

					N R
Table 10.2.1- 3:	Mortality				
			Dead fish		
Nominal			No. (%)	J.	
[mg prod./L]			Exposure time	Î Î	
	4 h	24 h	48 h	72 h 🔊	2 96 kg 0
Control	0 (0)	0(0)	0 (0)	· 0 (00	
0.0313	0 (0)	0 (0)	0 (0)	<u>v</u> 0(6) vC	
0.0625	0 (0)	0(0)	° (5,60) x		
0.125	0 (0)	4@40) 🔬	بر (100)	~010 (100)	(10 <u>4</u> 100) 。
0.250	3 (30)	A0 (1000)		10,(100)	10 (100)
0.500	10 (100)	10 (190)		* + (100)	10 (100)
The study meets the va	lidity criteria act	MI. CONCLU Fording & OEC	uston 2019) and	the endpoints b	ased on nominal
product concentrations	wele.)
L	C50 96 hours (95	5% C.I.		6.091 mg pro	d./L (n.d. ^A)
highest concentration	NOBE - 964bo on without an effe	urs: ct (based on mort	athrity and	0.0625 mg	prod./L
A Not determined due	to mathematical/ter	asons of star		×.)	
Assessment and conc	<u>Iusion by appli</u>	<u>cant</u> r ~			
The study and its data	are considered,	as acceptable an	d reliable for use	in risk assessn	nent.
The endpoint is: LC50	(96 how s) = 0.0	91 mg prod L	Å		
			*		



Data Point:	KCP 10.2.1/02
Report Author:	
Report Year:	2018
Report Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L) - Acute toxicity to rainbow
	trout (Oncorhynchus mykiss) in a 96-hour semi-static test 🖉
Report No:	134621230
Document No:	<u>M-636236-01-1</u>
Guideline(s) followed in	- Commission Regulation (EC) No 440/2008, Annex, Part C, C.1: "Acute Texicity,"
study:	for Fish", Official Journal of the Ryropean Union May 30, 2008
	- EPA Guideline 712-C-16-007: OC SPP 850.107," Freshwater and Sabyater Fish
	Acute Toxicity Test" October 2016
	- Japanese MAFF, Notification No. 12-Nousan-8147, JMAP Test Guideline, 2-7
	1-1, Fish acute toxicity studies, 2005
	- OECD Guideline for Testing of Chemicals, Section 2, No. 203. "Daphria sp
	"Fish, Acute Toxicity Test" adopted July 17, 1992
	- SANCO/3029/99 r. 4 11/07/00: Residues Guidance for Benerating and
	reporting methods of Analysis in Support of preregistration data equirements for
	Annex II (part A Section 4) and Annex III (part A; Section 5) of directive 91/434
Deviations from current	Current Guide Me: 203 (2019)
test guideline:	Deviations: The temperature ranged Detween 13.9 and 14.8 C and thus above the
	maximum 14 °C recommended in ØECD 203. This deviation was not expected to
	have impacted the study results. All valuatity crateria were met
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability	Yes v v v v

Executive Summary

An acute toxicly test was performed with the Ranbow Frout (*Incorhynchus mykiss*) in a semi-static system. Juvenile fishower exposed to FLU + TES SC 500 (250+ 250) in groups of 10 (one replicate of 10 fish per test level) to an aqueous solution of the product acnominal concentrations of 0.0156, 0.0313, 0.0625, 0.125 and 0.250 mg prod./L for a period of 96 hours. Additionally, a control was included. Observations of mortality and other signs of toxicity were made at the beginning (0h) and approximately 2, 24, 48, 72 and 96 hours after test initiation.

Concentrations of fluopyram and trittoxystrobin were verified in the freshly prepared and aged test media by using HPLC – MSCMS detection on days 0, 1, 2, 3 and 4 for each concentration and control. Measured concentrations were for trifloxystrobin in the 79 - 116 % range and for fluopyram in the 97 - 109 % range of nominal concentrations and no residues were found in the control samples above the LOQ (floopyram and trifloxystrobin: 0.4 µga.s./L

The study fulfils alk validity criteria of the OECP 203 guideline.

In the control and in the three lowest test concentrations (0.0156, 0.0313 and 0.0625 mg prod./L), all fish survived phtil the end of the experiment and no signs of intoxication occurred. In the two highest test concentrations (0.125 and 0.250 mg brod./L) all fish were dead after 48 hours of exposure.

The endpoints based on hominal concentrations were: $LC_{50} - 96$ hours (95 % C.I.): 0.0884 mg prod./L (0.0625) 0.125 mg prod./L 30 LOEC - 96 hours: 0.125 mg prod./L and NOEC - 96 hours: 0.0625 mg prod./L.



I. MATERIALS AND METHODS

	° ~
Test material	FLU + TFS SC 500 (250 + 250)
	Specification No.: 102000012886
	Batch No: PAIS 005173
	Content of a.s.: 21.3 % w/w fluopyram
	21.4 % w/w trifloxystrobin
Guideline(s)	None specified
adaptation	
Test species	Rainbow Trout (Oncorhynchus mykis)
Acclimation	At least 12 days to test conditions of the testing.
Organism	Mean length: 46.8 ± 2.5 mm a test start $\sqrt{2}$ $\sqrt{2}$
age/size	Mean body weight: 1.11 ± 0.18 g as test start Q
Test solutions	Nominal concentrations $0.0156y^2 0.03y^3 - 0.00625 \text{ f} 0.1250^v 0.250 \text{ mg prod./L} $
	Mean measured recoveries based on as. content ranged from 81 to 26 % commina a.s.
	concentrations for totoxysteobin and between 97 and 109% for thopyran
	Control: water Q
	Evidence of undissolved material: There were no remarkable observations and the test
	medium appeared clear in all test concentrations during the whole exposure period.
Replication	No. of vessels per concentration (replicators): 1
	No. of xessels per control (replacates): (1
Organisms per	No of organisms pervessel Q0
replicate	
Exposure	Semi-static (daily renewal)
	J Total exposure duration: 96 hours
Test Vessel 🔊	0 gg fish test medium of of of o
Loading	
Feeding during	Nonco de la constance de la consta
test «>	
Test conditions	Semperature: 13.9 - 14.8 °C
(© Photoperiod: to hours fight, Shours dark; with 30 minute transition periods
	Light intensity: $560-680$ @x \circ \circ
.1	pH: $/.4 \approx 8.0$ \sim $0^{\prime\prime}$ $0^{\prime\prime}$ $0^{\prime\prime}$ $0^{\prime\prime}$
Į į	Water naroness? 250 fing 62603/Ly
. Å	Dissolved oxygen: 93 - 104% of saturation (the test media were slightly aerated during the
L.	Condustryity: A 0 uStern
, " 	Alkalinity: 0.8 mmol
Doromators	Eith wars bear ad for mortalities and sub lathal babayioural affects at test start and after 2
Measured	24.48 and 96 hours
Observations	The temperature, dissolved oxygen and pH were determined daily in the freshly prepared
	and aged test media of each treatment group.
Sampling for	Daplicate samples of test solutions were taken from the freshly prepared test media of all
Chemical	test concentrations and the control at test start and each test medium renewal (day 1, 2, and
analys	3). The aged test media were sampled in duplicate out of all test media and the control at
	each test medium renewal (day 1, 2, and 3) and at test end (96 hours). In case of 100 %
	mortality prior to the end of the test, the analytical determinations were made at the
	respective times.



	The chemical analyses were performed by using liquid chromatography with MS/MS detection. Q_{a}°
Data analysis	The LC ₅₀ and the corresponding confidence intervals were estimated by Weibull analysis and as the geometric mean of the highest concentration showing 0 % mortality and the lowest concentration showing 100 % mortality for the observations at 48, 72 and 60h. The NOEC, the LOEC, the LC ₀ and the LC ₁₀₀ were determined directly from the raw data. The statistical analysis was performed with ToxRat Professional (Version 3.2.1, ToxRat Solutions GmbH).

II. RESULTS A	AND
III IIIS C DIS I	

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		П. Разна на на м			
		II. KESULTS AND	ØISCUSSION &	Õ	× 0. ×
Table 10.2.1- 4:	Validity criteria				
Validity criteria			Required	Ob Ob	tained
Mortality in contr	ol during test		<u></u> ≤16%₀ _{{)% ([*] ([*])
Dissolved oxygen	saturation		× 260 % ×	\$93 -	101 % S
		Q VY X			

Analytical results: Full details and acceptable walldation data to support the analytical methodoare presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANTE/2020/12830, Rev &

Recoveries in freshly prepared and aged test media weach simpling (day 0, 1, 25) and 4) were between 97 and 110 % of nominal a.s. conceptrations for trifloxystrobin and between 77 and 116 % for fluopyram

(250 + 250). No residues of fluopyram and trifloxystrobin were found in the control samples above their limit of quantification (LOQUOT fluopyram: 0.4 μg a.s. 4, LOQUOT trifloxystrobin: 0.4 μg a.s./L).



Nomi concenti	nal ration			Mea	n measure [µg a	ed concentr .s./L] ^A	ation			
[mg	[µg	Day 0	Da	y 1	Da	y 2	Đa	y 3	Day 4	<i>'0</i> '
prod./L]	a.s./L]	New	Aged	New	Aged	New	Aged	New	Age	
0.0156	3.316	3.471	3.427	3.645	3.393	3.597	3.484	3.392	3,3,14	Ĉo
0.0313	6.653	7.074	6.913	6.891	6.613	7.039	<i>N</i> .126	6.891	s .6 .461 ≾	l l
0.0625	13.285	14.002	14.089	13.567	13,393	14.132	14.306	14.610	¥13.61	
0.125	26.571	28.083	27.649	28.171	26.693	- ^B	- ^B %	С - ^В Д		, Ó [×]
0.250	53.141	54.422	54.769	- ^B	<u>Ф_</u> В	Q.	<u>- в С</u>	B S	Ô ^B	
				<i>Q</i>	^w % of n	ominal ^B	, Q		n di	
0.0156	3.316	105	104	£10	الم 104 م	0° 108	1005	گ ^م 103	\$00	
0.0313	6.653	107	104	Ø04 🛒	© [®] 100 ي	106	Ø 107 🔗	104	_ 97	Þ
0.0625	13.285	106	106	🐴 102 T	104	Q107 (108	Φíο _k	¢″103	
0.125	26.571	106	104	<u>1</u> 06	N 101			₽ - _₹ ,]
0.250	53.141	103	103	<u> </u>		K.	<u>8</u> - 0	<u>í</u> ş]

Table 10.2.1- 5: Analytical results for fluopyram

Not given in report. Calculation based on measured concentrations В

No measurements performed as all fish died. L.

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Table 10.2.1- 6:	Analytical results/fo	or trifloxystrobin 🛷

				\sim		<u> </u>	¥		
Nomi	inal	N A		Mea	n measure	d concentr	ation 🛒	7	
concent	ration s		, Q	0 8	γ̃ {µga	.s./167 A			
[mg	[µg_	Day0	S Da	¥1 🔊	🔊 Da	y 2 0	s Da	y 3	Day 4
prod./L]	a.s./	New >	Age	New .	Aged	New	Aged	New	Aged
0.0156	3943	3.859	2%622	3 .742	2.733	€641 ¥	J 2.897	3.523	2.711
0.0313	06.707	6.848	5.285 %	6.910	5,968	6.96 4 €	5.638	7.065	5.302
0.0625	13.392	14.365 x	10.455	12,201	₽0.42 b	13.526	11.780	15.372	11.277
0.125	26.784	Q28.043	22\$03	28.026	× 24.270	. O ^Y ^B	_ B	- ^B	_ B
0.250	53.568	53 586	49 .558	- ^B	K, B	б́У - ^В	_ B	- ^B	_ B
	2 2 7	.1 .	D L	<u>_0</u>	0% of p	ominal ^B			
0.0156	3.343	Q*1165	<i>7</i> 9	گې 112	* 825	109	87	106	81
0.0313	Q 6.707 Ĉ	102	^≫79 ∽	1035	~77	104	84	105	79
0.0625	13.392	908 🐔	784	\$\$ 00	Ø 78	101	88	115	85
0.125	26.784 🦕	🖗 105-Q	84	×105	93	-	-	-	-
0,250	53.568	1.00	93		-	-	-	-	-

Not given in report. Calculation based of measured concentrations of 2 replicate samples. A В

Biologica

Observations

In the control and in the three lowest test concentrations (0.0156, 0.0313 and 0.0625 mg prod./L), all fish survived until the end of the experiment and no signs of intoxication occurred. In the two highest test concentrations (0.125 and 0.250 mg prod./L) all fish were dead after 48 hours of exposure.

not given in report. Cardination based on the asturned as all fish died.



Nominal			Dea	d fish					
concentration	Exposure time								
[mg prod./L]	0 h	<u></u> 96 b ↔							
Control	0(0)	0(0)	0(0)	0(0)	0 (0)	5 029X			
0.0156	0 (0)	0(0)	$0(0) \approx 1$						
0.0313	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)				
0.0625	0 (0)	0 (0)	Ø\$(0)	0.00	0,490	0 40 5			
0.125	0 (0)	0 (0)	A (80)	10 (100) °	10/(100)	10(100)			
0.250	0 (0)	8 (80)	0 10 (100)	>10 (109)		¥0 (100)			
e study meets the va oduct concentrations	lidity criteria were:	according to	одсяетода. ФЕСБ 203 (2 	©19) and the	Adpoints bas	ed on nominal			
LC ₅₀ -	– 96 hours (9	5 % CA .):			884 mg prod	M rod /L)			
]	LOEC _ % ho	urs?			125	то ц ., Ш)			
lowest co	oncentration w	ith an effect	ý o		. i a mg prod./	L			
] 	NOEC -96 ht	ours:			0625 0 prod.	/L			
nignest cor									
					, V				
ssessment and con	usion by a	mlicant,							
ne study and its data	are consider	ed as accepta	ble and reliab	le for use in ri	isk assessmer	nt.			
ne endooint is: LC ₅₀	96 hoars) =	0.00884 mg p	rod L	\sim					
				<u>z</u>					



Data Point:	KCP 10.2.1/03
Report Author:	
Report Year:	2007
Report Title:	Acute toxicity of AE C656948 + trifloxystrobin SC 250 + 250 G to the water free
	Daphnia magna in a static laboratory test system
Report No:	EBGMP031
Document No:	<u>M-292365-01-1</u>
Guideline(s) followed in	OECD guideline 202,(2004); EEC Directive 92/69/270G, part C.2.(992); 6.
study:	EPA Pesticide Assessment Guidel@es, Subdivision E, § 72 2 (1982), OPPTS
	Guideline 850.1010 public draft 1996 (modified) MAFF 12 Nousan No. 8147
	(2000); Equivalent to US EPA OPPTS Guideloe No. 850.1010 SUPP
Deviations from current	Current Guideline: 202 (2004)
test guideline:	Deviations: None. All validate criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP/Officially recognized testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes of y y y y

Executive Summary

An acute toxicity test was performed with daphrids (*Daphnic magna*) under static conditions to determine the 48-hour EC₅₀. First-instar neonate daphnids (< 24-hours old) were exposed to FLU + TFS SC 500 (250+250 g/L) in group of 30 (6 replicates of 5 organisms per test level) to the nominal concentrations of 0.010, 0.020, 0.040, 0.080 and 0.160 mg prod./L. Additionally, acontrol was included. Immobilisation and sub-lethal behavioural effects were determined after 24 and 48 hours.

Concentrations of thopyram were verified by HPLC \rightarrow MS/MS on day 0 and 2. Measured concentrations were in the 96 – 104 % range of nominal concentrations and no residues of fluopyram were found in the control samples higher than 0.2085 at a sol, which was used as the lowest standard concentration during this study.

The study fulfils all validity criteria of DECD 202 guideline

No immobility or other effects on behaviour were observed in the control within 48 hours of exposure. Immobilisation was observed after 24 hours at the highest test concentration (160 μ g prod./L) and after 48 hours in the two highest test concentrations (80 and 160 μ g prod./L).

The endpoint based on noninal concentrations was: $EC_{30}^{\circ} - 48$ hours (95 % C.I.): 0.086 mg prod./L (0.077 - 0.095 mg prod./L)

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4	S A MATERNAL AND METHODS
Test material	FLU TFS SC 500 (250+250 g/L) Specification No: 102000012886 Batch No: 2007-000441
	Content of a \$21.4 % w/w fluopyram 21.6 % w/w trifloxystrobin
Guideline(C) adaptation	None specified
Test species	Water flea (Daphnia magna)



Organism age/size at study initiation	First instar neonates, less than 24 hours old
Test solutions	Nominal concentrations: 0.010 – 0.020 – 0.040 – 0.080 – 0.160 mg prod./L Corresponding mean measured concentrations: not relevant Control: water Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 6
Organisms per replicate	No. of organisms per vessel: 5
Exposure	Static Total exposure duration: 48 hours
Feeding during test	None
Test conditions	Temperature: 20.9 – 21.29°C Photoperiod: 16 hours fight, 8 hours dark Light intensity: max 0,200 hrs pH: 8.1 (at test start) Water hardness: 281 mg/L as CaCO ₃ (at test start) Dissolved oxygen: 8.3–8.8 mg/L (92–99 % of saturfation) Conductivity 579 µ8/cm (at test start) Alkalinity: 53 mg/L as CaCO ₃ /L (at test start)
Parameters	Observations for mobility and sub-lethal behavioural effects were made after 24 and 48
Measured /	hours offexposure.
Observations	Prior to test unitiation conductivity total hardness, pH and alkalinity of the dilution media (Effordt M7) were determined. Additionally, the dissolved oxygen and pH values were measured in the peshly prepared test solutions of each treatment level and control and repeatedly in the pooled replicates of the aged medicat test formination (day 2). Temperature of the test media was measured inside one vessel of the control and of the highest test concentration at start and end of the fudy. Light intensity was measured at start of the study as diffuse light immediately above the exposure vessels.
Chemical analysis	Duplicate samples of the freshly prepared test media were taken from bulk preparation for all lest levels and the control immediately after preparation and again from pooled replicates of the cotresponding age media at the end of the 48 hours exposure interval.
Q1	The chemical analyses were performed by high-performance liquid chromatograph (HPLC - MQMS. S
Data analysis	For EC ₅₀ determination a dose response relationship curve (displayed as sigmoid, shaped over the logarithm of the concentration) was modelled by Probit Analysis after Finney fitted by an iterative weighed linear regression according to the Maximum Likelihood principle which allows computation of EC ₅₀ and 95 % confidence limits for immobility rates if
4	possible unathernatical finits based on quality of the dose-response pattern).
de la companya de la comp	Calculations (mean and standard deviation) were performed using Microsoft Excel The statistical analysis was carried out using the ToxRat Professional® Software (Vers.2.19), ToxRat Solutions GmbH, Germany).



II. RESULTS AND DISCUSSION

Table 10.2.1- 8:Validity criteria		
Validity criteria acc. to OECD 202	Required	Obtained 6
Mortality in control during test	$\leq 10 \%$	
Dissolved oxygen concentration at the end of the test	$\geq 3 \text{ mg/L}$	8:3 - 8.5 mg/L ~
	, O.	

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outwide within SANTE/2020/12830, Rev.1.

Recoveries on day 0 and 2 ranged from 96 to 104 % of nominal values. The biologica Desult care based on nominal product concentrations of FLE+TES SC 500 (250) 250 g/t).

Given that the toxicity cannot be attributed to any one of the a.s. compounds but to the formulation as a whole, all results were based on nominal concentrations of FLU-0FS SC 500, 250+250 g/L

The other active ingredient trifloxystrobin was not analysed since it is present in the added formulation in a fixed ratio to the analysed fetive ingredient.

No residues of fluopyram were detected in the control samples higher than 0.2085 μ g a.s./L, which was used as the lowest standard concentration during this study.

				° a	
Nominal co	centration 4	Measured cor	reentration	S % of no	ominal
[mg prod./L]	Qug a.s.#L]	Day 0 (New)	Day 2 (Aged)	Day 0 (New)	Day 2 (Aged)
0.010	<u>ک</u> ۲4 ک	2.051	× 2051 ×	96	100
0.020	× 4.28 ×	4.204 ~	4.276	99	100
0.040	\$ 8.56	\$ \$611	8.383	101	98
0.080		17.07	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1	100	104
0.160	⁰³ 4.2 گ	<u>, 0</u> 33.92 .0	94.84	99	102

Table 10.2.1-9: Analytical results for floopyrain

Biological result Observations

No immobility of other effects on behavious were observed in the control within 48 hours of exposure. Immobilisation was observed after 24 hours at the highest test concentration (0.160 mg prod./L) and after 48 hours in the two highest lest concentrations (0.080 and 0.160 mg prod./L)

after 48 hours in the two highest test concentrations (0.080 and 0.160 mg prod./L).







Data Point: KCP 10.2.1/04 Report Author: • Report Year: 2018 Report Title: Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daphtia magna in a semi-static 48-hour immobilisation test Report No: 134621220 Document No: M-636231-01-1 Guideline(s) followed in study: - Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphniasp. Acute Immobilisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013; OCSPP 850, 100, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-81477, JMAFF TechGuideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 202; Daphnia sp., Acute Immobilisation Test' (Adopted Aprils 13, 2004 - SANCO/3029/92 rev.4 14/07/00/Residues: Guidance for generating and reporting methods of Analysis in Support of precedistration for Annex ff (part 4); Section 4) and Annex JII (part 4); Section 5) of directive 91/414 Deviations from current test guideline: Deviatione, All validity criteria were method. Deviatione, All validity criteria were method. Particus conduction: Deviations None, All validity criteria were method. Deviations from current toxicul provember and the device of the study of the		
Report Author: 2018 Report Year: 2018 Report Title: Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daphtia magna in a semi-static 48-hour immobilisation test Report No: 134621220 Document No: M-636231-01-1 Guideline(s) followed in study: - Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphnia2b. Acute Immobilisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013;OCSPP 850.1006, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 24, 2-1, Daphnia acute immobilization study, 2005 - OECD Guideline for Testing of Chemicals No. 202; Daphtia sp., Acute Immobilisation Test" adopted Aprils 43, 2004 - SANCO/3029/99 rev.4 1/ 07/007 Residues: Guidance for generating and reporting methods of Arraysis in Support of prefegistration for Annex H (part A; Section 5) pf directive 91/414 Deviations from current test guideline: Deviations from current Leviations from current Current Guideline Current guideline? 202 (2004) Deviations from current Deviations None, All validity criteria were met Deviations from current Deviations when the during were met Deviations from current Deviations when the during were met	Data Point:	KCP 10.2.1/04
Report Year: 2018 Report Title: Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daphma magna in a semi-static 48-hour immobilisation test Report No: 134621220 Document No: M-636231-01-1 Guideline(s) followed in study: - Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphmap. Acute Immobilisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013; OCSPP 850.100 0, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphmids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Cheenicals No. 202; Daphtia sp., Acute Immobilisation Test" (adopted Aprils 13, 2004 - SANCO/3029/9grev.4 1/07/00g Residues: Guidance for generating and reporting methods of Amaysis in Support of precisistration for Annex IF (part 4; Section 4) and Annex IF (part 4; Section 5) of thirective 91/414 Deviations from current test guideline: Deviations None All validity criteria were met Deviations from current Current Guideline Current guideline? 202 (2004) Deviations from current Deviations None All validity criteria were met Deviations from current Deviations None All validity criteria were met Deviations from current Deviations None All validity criteria were met	Report Author:	
Report Title: Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daphina magna in a semi-static 48-hour immobilisation test Report No: 134621220 Document No: M-636231-01-1 Guideline(s) followed in study: - Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphnia"sp. Acute Immobilisation Test", Official Journal of the European Ution (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013; OCSPP 850.1010, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4 - OECD Guideline for Testing of Chemicals No. 202; Daphnia sp., Acute Immobilisation Test" adopted Aprils 13, 2004 - SANCO/3029/99; rev. 4 1/07/00; Residues: Guidance for generating and reporting methods of Amaysis in Support of prefegistration for Annex If (part A; Section 5) of directive 91/414 Deviations from current test guideline: Durations Annex III (part A; Section 5) of directive 91/414 Deviations from current Current Guideline, Current guideline, 202 (2004) Deviations from current Deviations All validity criteria were met met	Report Year:	2018
magna in a semi-static 48-hour immobilisation test Report No: 134621220 Document No: M-636231-01-1 Guideline(s) followed in study: - Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphnia"sp. Acute Immobilisation Test", Official Journal of the European Union (EN), dated, May 30, 2008 - EPA Guideline 712-C-16-013:OCSPP 850.1006, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 2022, "Daphnia"sp., Acute Immobilisation Test" adopted Aprils 43, 2004 - SANCO/3029/99 rev.4 14/07/00; Residuos: Guidance for generating and reporting methods of Analysis in Support of prefegistration for Annex ff (part A, Section 4) and Annex JP (part A; Section 5) of directive 91/414 Deviations from current test guideline: Current Guideline: Current Studeline? 202 (2004) Deviations from current Current Guideline Current Studeline? 202 (2004)	Report Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daptinia
Report No: 134621220 Document No: M-636231-01-1 Guideline(s) followed in study: - Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphnia.pb. Acute Immobilisisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013:OCSPP 850.1000, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8 497, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 2022, "Daphnia sp.," Acute Immobilisation Test" Odopted Aprils 73, 2004 - SANCO/3029/99 rev.4 1/ 007/000 Residues: Guidance for generating and reporting methods of Analysis in Support of pretegistration for Annex IP (part A, Section 4) and Annex IP (part A, Section 5) of directive 91/414 Deviations from current test guideline: Current Guideline; Current guideline, 202 (2004) Deviations manufacture Deviations None, All validity criteria were met	-	magna in a semi-static 48-hour immobilisation test
Document No: M-636231-01-1 Guideline(s) followed in study: - Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphnia'sp. Acute Immobilisisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013: OCSPP 850.1070, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 2022, "Daphnia sp., "Acute Immobilisation Test" Odopted Aprils 43, 2004 - SANCO/3029/99 rev.4 1, 007/002 Residues: Guidance for generating and reporting methods of Analysis in Suppart of prefegistration for Annex IF (part A, Section 4) and Annex IIF (part A; Section 5) of directive 91/414 Deviations from current test guideline: Current Guideline, Current guideline, 202 (2004) Deviations methods Current Guideline, Current guideline, 202 (2004) Deviations in current Deviations, None, All valuely criteria were method.	Report No:	134621220
Guideline(s) followed in study: - Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Dephnia'sp. Acute Immobilisisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013; OCSPP 850.1000, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 202; "Daphnia sp., Acute Immobilisation Test" Adopted Aprils 43, 2004 - SANCO/3029/99 rev.4 1, 07/007 Residues: Guidance for generating and reporting methods of Analysis in Support of precegistration for Annex II (part 4, Section 4) and Annex III (part 4; Section 5) of directive 91/414 Deviations from current test guideline: Current Guidaline; Current guideline 202 (2004) Deviations we understore None All validity criteria were met	Document No:	<u>M-636231-01-1</u>
study: Acute Immobilisisation Test", Official Journal of the European Union (EN), dated May 30, 2008 - EPA Guideline 712-C-16-013 OCSPP 850.1000, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" Octobe 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 2022 Daphnia sp., Acute Immobilisation Test" Adopted Aprils 43, 2004 - SANCO/3029/99 rev.4 1/ 07/007 Residues: Guidance for generating and reporting methods of Ararysis in Support of prefegistration for Annex IF (part 4, Section 4) and Annex III (part 4, Section 5) of directive 91/414 Deviations from current test guideline: Current Guideline: Current buideline, 2002 (2004) Deviations acute May 30, 2008	Guideline(s) followed in	- Commission Regulation (EC) No 440/2008, Annex, Part C, C.2: "Daphnia p
May 30, 2008 - EPA Guideline 712-C-16-013; OCSPP 850.1006, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 2022 Daphnia sp., Acute Immobilisation Test adopted Aprils 43, 2004 - SANCO/3029/99 rev.4 1/07/000 Residues: Guidance for generating and reporting methods of Ariarysis in Support of prefegistration for Annex IF (part 4, Section 4) and Annex III (part 4, Section 5) of directive 91/414 Deviations from current test guideline: Deviations from current Mey and Annex III validity criteria were met Deviations from current No. and Annex III validity criteria were met	study:	Acute Immobilisisation Test", Official Journal of the European Union (EN), dated
 EPA Guideline 712-C-16-013 OCSPP 850.1000, "Aquatic Invertebrate Acute Toxicity Test, Freshwater Daphnids" October 2016 Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 OECD Guideline for Testing of Chemicals No. 2022 Daphna sp., Acute Immobilisation Test adopted Aprils 43, 2004 SANCO/3029/99 rev.4 1, 007/007 Residues: Guidance for generating and reporting methods of Anarysis in Support of prefegistration for Annex IF (part A, Section 4) and Annex IIF (part A, Section 5) of directive 91/414 Deviations from current test guideline: Deviations None, All validity criteria were met 	2	May 30, 2008
Toxicity Test, Freshwater Daphnids" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-4, 2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 202, "Daphtia sp., Acute Immobilisation Test adopted Aprils 13, 2004 - SANCO/3029/99 rev.4 1, 007/007 Residues: Guidance for generating and reporting methods of Analysis in Support of precedistration for Annex IF (part A, Section 4) and Annex IF (part A, Section 5) of directive 91/414 Deviations from current test guideline: Deviations methods of Analysis in Support of precedistration for Annex IF (part A, Section 4) and Annex IF (part A, Section 5) of directive 91/414 Deviations from current test guideline: Deviations from current Current Guideline: Deviations were met		- EPA Guideline 712-C-16-013; OCSPP 850.1000, "Aquatic Invertebrate Acutor [
 Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7, 2-1, Daphnia acute immobilization studies, 2005 OECD Guideline for Testing of Chemicals No. 202, Daphnia sp., Acute Immobilisation Test adopted Aprils 73, 2004 SANCO/3029/99 rev.4 1, 007/007 Residuos: Guidance for generating and reporting methods of Analysis in Support of prefegistration for Annex IF (part A, Section 4) and Annex III (part A, Section 5) of directive 91/414 Deviations from current current Guideline; Current guideline; 202 (2004) Deviations more All validity criteria were met 		Toxicity Test, Freshwater Daphnids" October 2016
2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 202, Daphnia sp., Acute Immobilisation Test'Odopted Aprils ¥3, 2004 - SANCO/3029/99 rev.4 1,007/00 Residues: Guidance for generating and reporting methods of Analysis in Support of prefegistration for Annex IF (part A, Section 4) and Annex IF (part A, Section 5) of directive 91/414 Deviations from current test guideline: Deviations from current Current Guideline; Current guideline; 202 (2004) Deviations No. and Annex III validity criteria were met		- Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-44
 OECD Guideline for Testing of Chemicals No. 202, Daphua sp., Acute Immobilisation Test adopte Aprils 43, 2014 SANCO/3029/99 rev.4 1/07/007 Residues: Guidance for generating and reporting methods of Aratysis in Support of prefegistration for Annex IV (part A, Section 4) and Annex IV (part A; Section 5) of directive 91/414 Deviations from current test guideline: Deviations. None, All validity criteria were met. 		2-1, Daphnia acute immobilization studies, 2005
Immobilisation Test Odoptet Aprils 13, 2004 - - SANCO/3029/99 rev.4 1, 07/007 Residues: Guidance for generating and reporting methods of Amaysis in Support of prefegistration for Annex IV (part A; Section 4) and Annex IV (part A; Section 5) of directive 91/414 Deviations from current Current Guidaline: Current guideline? 202 (2004) Deviations. None, All validity criteria were met. - Deviations No. not Oviauch submitted a		- OECD Guideline for Testing of Chemicals No. 202, Daphie sp., Acute
- SANCO/3029/99 rev.4 1,007/007 Residues: Guidance for generating and reporting methods of Analysis in Support of prefegistration for Annex 19 (part A, Section 4) and Annex III (part A, Section 5) of directive 91/414 2 Deviations from current test guideline: Deviations. None All validity criteria were met		Immobilisation Test adopte Aprils ¥3, 2004 a a
reporting methods of Analysis in Support of prefegistration for Annex IV (part 4; Section 4) and Annex IV (part 4; Section 5) of directive 91/414 Deviations from current test guideline: Current Guideline: Current Buideline? 202 (2004)		- SANCO/3029/99 rev.4 1 07/00 Residues: Guidance for generating and
Section 4) and Annex II (part A; Section 5) of directive 91/414 Deviations from current Current Guideline: Current guideline? 202 (2004) Deviations Deviations Deviations None All validity criteria were met		reporting methods of Analysis in Support of prefegistration for Annex fr (part A,
Deviations from current test guideline: Deviations None All validity criteria were met 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5		Section 4) and Annex III (part A; Section 5) of directive 91/41A 🖉
test guideline: Deviations None All validity critoria were met 2 5 6 9	Deviations from current	Current Guideline: Current guideline? 202 (2004)
Dravious avaluation: No. not Swiguely submitted a State of Control of State	test guideline:	Deviations. None All validity criteria were met 2 5
	Previous evaluation:	No, not Deviously submitted a a construction of the second s
GLP/Officially Yes conducted under GLP/Officially recognised testing faeilities	GLP/Officially	Yes Conducted under GLP/Officially recognised testing faeilities
recognised testing	recognised testing	
facilities: $Q = Q^* + $	facilities:	
Acceptability/Reliability: Yes A 2 2 2 2 2 2	Acceptability/Reliability:	Yes A S A S . S S
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	

# Executive Summa

An acute toxicity test was performed with daphnids (*Daphnia nogna*) under semi-static conditions to determine the 8-hout EC₅₀ Pirst-instar neonate daphnids (<2 thousald) were exposed to FLU + TFS SC 500 (250 + 250) in groups of 20 (4 replicates of 5 organisms per test level) to the nominal concentrations of 0.01 0.02, 604, 0.08 and 0.16 rag proc/L. Additionally, a control was included. Immobilisation and sub-lethal behavioural effects were determined after 24 and 48 hours.

Concentrations of Puopyram were verified by LC- MS/MS detection on day 0, 1 and 2. Measured concentrations were in the 93 105 % range of nominal concentrations and no residues were found in the control samples higher than the LOQ (Diopyram: 0.27 µg a.s./L, trifloxystrobin: 0.86 µg a.s./L).

The study fulfils all validity criteria of GECD 292 guideline.

After 48 bours of exposure normmobilisation of the test animals was observed in the control and in the lowest test concentration (001 mg prod./f). At the concentration of 0.02 mg prod./L one daphnid was immobile and 8 daphnids were immobile at the concentration of 0.04 mg prod./L. At the concentration of 0.08 mg prod./L 14 daphnids were immobile and at the highest test concentration (0.16 mg prod./L) all daphnids were immobile

The endpoints based on nominal product concentrations were:  $EC_{50} - 48$  hours (95 % C.I.): 0.051 mg prod./E (0.04  $\bigcirc$  - 0.063 mg prod./L), NOEC - 48 hours: 0.01 mg prod./L, LOEC - 48 hours: 0.02 mg prod./L.

0.02 mg prod



# I. MATERIAL AND METHODS

	° ~
Test material	FLU + TFS SC 500 (250 + 250 g/L)         Specification No.: 102000012886         Batch No.: PAIS005173
	Content of a.s.: 21.3 % w/w (248.7 g/L) fluopyram
	21.4 % w/w (250.7 g/L) trifloxystrobin
Guideline(s) adaptation	None specified
Test species	Water flea (Daphnia magna)
Organism age/size at study initiation	First instar neonates, less than 24 tours old
Test solutions	Nominal concentrations: 0.01 – 0.02 – 0.04 0.08 0.16 mg prod./L Corresponding mean measured concentrations: not relevant Control: water Evidence of undissolved material: There were no remarkable observations. The test medium appeared clear.
Replication	No. of vessels per control (replicates): 4
Organisms per replicate	No. of organizems per vessel: 5 0 4 0 0 0
Exposure	Semi-staric (renewal on day 1) Total exposure duration: 48 bours
Feeding during test	None of the of t
Test conditions	Temperature 20.8 , 21.0 °C Photoperiod: 16 hours light 8 hours dark Light intensity: 780 - 840 fux C
	Water hardness: 2.5 mmol/L as CaCO ₃ (= 250 mg/L (reconstituted water) Dissolved oxygen: 8.7 - 9.2 mg/L (99 - 104% of saturation) Conductivity: $<5 \mu$ S/cm
Parameters Measured & Observations	Observations for inactionality and sublethal behavioural effects were made after 24 and 48 hours of exposure. The water temperature, pH-values and dissolved oxygen concentrations will be determined in all freshly prepared and aged test media of each treatment group. The light intensity will be measured at least once during the test
Chemical analysis	Diplicate samples were taken from freshly prepared batch solutions at test start and on day 1. Duplicate samples from agentiest media were collected at day 1 (after 24 hours of exposure) and at the end of the test by pouring together the contents of the test beakers of each treatment.
Data analysis	The $24$ -hour and $48$ -hour EC ₅₀ , EC ₂₀ and EC ₁₀ and the 95 % confidence limits were calculated by probit analysis.
	The NOEC and LOEC after 24 and 48 hours were determined directly from the raw data. The software used to perform the statistical analysis was ToxRat Professional (Version 3.2.1, ToxRat® Solutions GmbH).



## II. RESULTS AND DISCUSSION

Table 10.2.1-11:       Validity criteria		
Validity criteria acc. to OECD 202	Required 💭	Obtained O
Mortality in control during test	$\leq 10 \%$	
Dissolved oxygen concentration at the end of the test	$\geq 3 \text{ mg/L}$	<u>^8</u> ,7 mg/₽
	Q,	

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outhred within SANTE/2020/12830, Rev.1.

Recoveries of freshly prepared and aged test media on each sampling (day 0, 1, and 2) were between 93 and 105 % of nominal a.s. concentrations for fluopyram and between 78 and 98 %, for triffoxystrobin (see table below). Biological results are based on nonfital product concentrations of FLUE TFS SC 500 (250 + 250 g/L).

No residues of fluopyram and trictoxystrobin were found in the control samples above the limit of quantification (LOQ for fluopyram: 0.2) µg and L, LOQ for friflox strobio 0.86 Qg a.s./L).

Nor concer	ninal 👘	S Mea	Sured con	centration			% of n	ominal ^A	
[mg		Day O	Da	y 1	Day 2	Day 0 @	) Da	y 1	Day 2
prod./L]	[µg as./L]	New 🤇	Aged	New	Aged	New	Aged	New	Aged
0.01	°€.126€	©190 ©	2.040	\$ 024	Q 2.007	<u>10</u> 3	96	95	95
0.02	a.251	4.347	<b>A</b> 064 ~	¥4.1230	3,948	a 103	96	97	93
0.04	8.503 Č	8,40,2	8.578	8.587	<b>%</b> .420	¥ 103	101	101	99
0.08	17.003	Å9.716≪	17 32	16.883	16.366	105	101	100	97
0.16	34.00	34.790	35,040	33.458	33.125	102	103	99	98

## Analytical results for Auopyram Table 10.2.1-12:





Nominal concentration		Me	asured con [µg a.s	centration ./L]	Α		% of no	ominal ^A		Å.
[mg	[µg	Day 0	Da	y 1	Day 2	Day 0	<b>⊘D</b> a	ny 1	Day 2	0°
prod./L]	a.s./L ^A ]	New	Aged	New	Aged	New	Aged	New 🖧	Ageo	
0.01	2.143	2.062	1.778	1.848	1.755	97	83	870	~\$2	Ĉo
0.02	4.285	3.957	3.398	3.610	3.351	92	⁽³⁾ 80	285	<b>\$</b> 78 🗶	j
0.04	8.571	8.360	7.720	7.450	<b>~~</b> 844	98 🖉	90	87	800	أم
0.08	17.142	16.410	15.029	14.962	13.614	26° ×	88 🦼	880	A CON	, Ô
0.16	34.284	33.555	29.310	31.096	27.424	Ø <b>9</b> 8	。 85 O	. 91	ی 80 °	,×

Table 10.2.1-13: Analytical results for trifloxystrobin

Not given in report. Calculated based on measured concernations of 2 replicate samples.

# **Biological results**:

## Observations

of the test animals where a test After 48 hours of exposure no immobilisation of the test animals was observed in the control and in the lowest test concentration (0.01 mg pod./L) At the concentration of 0.02 mg prod./L one daphnid was immobile and 8 daphnids were impobile at the concentration of 0.04 mg prod./L. At the concentration Table 10.2.1- 14: Impobilisation of daphnics

# Table 10.2.1- 14:

, QO.		
T-11. 10.2.1. 14. L		
Table 10.2.1-14: Immobilisation of	eaphnies of the k	
	ي من No. of immobilize المربي المربع الم	cumulative %)
Nominal concentration	Exposure Sector	time
	24 h	لا ^ب 48 h
Control		0 (0)
		0 (0)
0.02° 0° 4	$\overset{\sim}{\longrightarrow} \overset{\circ}{\longrightarrow} 1 (5) \overset{\checkmark}{\longrightarrow} \overset{\circ}{\longrightarrow} 1$	1 (5)
0.00	3 (53)	8 (40)
0908		14 (70)
~~~0.16°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°°		20 (100)
	AN BE O	





Endpoints 0 to	24 hours
EC ₅₀ – 24 hours (95 % C.I.):	> 0.16 mg prod./L
EC ₂₀ – 24 hours (95 % C.I.):	0.062 mg pod./L
EC ₁₀ – 24 hours (95 % C.I.):	0.036 mg prod./L
NOEC – 24 hours:	(0.022 - 9:061 mg prod/10)
LOEC – 24 hours:	√ J ⁰ 0.02 mg prot L 0 5 5 k
Endpoints	48 hours
EC 50 - 48 nours (93 % C.1.).	(0)041_0063 mg/prod/L)
EC ₂₀ – 48 hours (95 % C.I.):	(0.024 - 0.04) mg prod./L)
EC ₁₀ – 48 hours (95 % C.I.	0.024 mg prod 7L 0.018 - 0.033 pg prod 7E)
NOEC – 48 hours	J J Orol mg prod./5
LOEC – 48 hours:	0.02 mg prod./L &
Assessment and cover lusion by applicant:	
The study and its data are considered as acceptable a	Bod reliable for use in Gisk assessment.
The endpoint 6 : EC 3 48 hours) = 0.051 mg prod 1	



Data Point:	KCP 10.2.1/05
Report Author:	
Report Year:	2021
Report Title:	Amendment no. 01 to final report: Pseudokirchneriella subcapitata growth 🔊 👘
	inhibition test with fluopyram & trifloxystrobin SC 500 ($260 + 250$)G ($260 + 250$)G ($260 + 250$)G
Report No:	E 323 3111-4
Document No:	<u>M-292579-02-1</u>
Guideline(s) followed in	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Philipition Test
study:	(March 23, 2006); Equivalent to USEPA OPPTS Suideline No. 850.5400 SUPP
Deviations from current	Current Guideline: OECD 201 (2006)
test guideline:	Deviations: None. All validity criteria were mo 🖉 🖉 🖉
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GBP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes A & Q Q O' Q' A

Executive Summary

The green alga *Pseudokirchnerietta subcapitata* were exposed to FLU+TFS SC 500 (250+250 g/L) under static conditions for 72 hours. Algal cultures with an initial reminal cell count of approximately 1.0×10^4 cells/mL were used to test the nominal concentrations of 0286, 0.0916, 0.293, 0.938 and 3.00 mg prod./L. The study design included 3 replicates for each/test concentration and 6 replicates for the control. At 24 hour-intervals, the cells density (cells/mL) of each culture was counted.

Concentrations of fluopyram were verified by HPDC- MSMS for day 0 and 3 for each concentration and control. Measured concentrations were in the 90 - 98% range of rominal concentrations except on day 3 in the lowest test concentration where a recovery of 152% was found. This could most likely be explained by a handling error while taking samples, which rid not influence the outcome of the study negatively. No residues were found in the control samples above 0.5214 μ g a.s./L, which was used as the lowest standard concentration during the study. The bological results are based on nominal concentrations of FLU+TFS SG 500 (250+250g/L).

The study fulfils all varidity offeria of OECD 201 Quidelfine.

No physical abnormalities were deserved in the controls or any test concentration during the study.

The 72 hour-endpoints based on nominal product concentrations were: 72 hour – E_rC_{50} : 0.292 mg prod/L; 72 hour – E_bC_{50} , 0.0497 mg prod/L and 72 hour – E_yC_{50} : 0.0425 mg prod/L.

	I. MARERIAL AND METHODS
L.	
Test material	FLU ⁴ TFS SC 500 (250 +250 g/L)
L L	Specification Ng 10200 12886
l Q	Batch 10. 2007-00044
	Contact of a set 21.4% w/w fluopyram (251.5 g/L)
	21.6 % w/w trifloxystrobin (253.5 g/L)
<u> </u>	Pensity: 174 g/mL
Guidelines	Not specified.
adaptation	49"
Test species	Freshwater Green alga Pseudokirchneriella subcapitata
	Strain SAG 61.81



Culturing conditions	In-house 4-day old pre-culture held under test conditions.
Test solutions	Nominal product concentrations: 0.0286 – 0.0916 – 0.293 – 0.938 – 3.00 mg prod./L& Corresponding mean measured concentrations: not relevant Control: untreated medium Evidence of undissolved material: not reported
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 6
Exposure	Static Total exposure duration: 72 hours
Initial cell density	1×10^4 cells/mL in each test group \tilde{Q}
Test conditions	Temperature: 22.0 - 22.7 °C Photoperiod: 24 hours light Light intensity: 5620 - 7090 fux Type of light: bank light containing cool white fluorescent lamos pH of control: 8.0 - 9.4 Conductivity: not reported for the dium. Yes
Parameters Measured / Observations	pH- values were measured at test start, daily afterwards. At test end the pH was determined in composite samples of all replicates for each est concentration. Temperature was determined by a continuous measurement in one additional incubated glass vessel. Light intensity was measured; however time point was not reported. Cell density measurements and morphological examinations were done daily. Cell numbers per volume (as a surrogate for biomass per volume) were estimated by direct algae cell optiming ander a microgoope.
Sampling for chemical	Samples of test solution were taken at test initiation (Chour) in all treatment levels and the control Advest termination (72 hours) samples were collected from composite samples
analysis	of all replicates for each test concentration Samples were analysed bousing a high performance liquid chromatograph (HPLC) –
Data analysis	Probit analysis using tinear max. likelihood regression was used for EC _x -value estimation. LOEC/NOEC determinations were done using the ANOVA procedure and properly selected multiple t-tests Calculations were done with process to Excel sheets and the further statistical evaluations with the commercial program ToxRat Professional (version 2.09).

0.2.1-15: Validito criteria

Table 10.2.1-15:	Waliditocri
100201 100	Funding

Validity criteria acc. to OECP 201 (apopted 2006)	Required	Obtained
The biomass of the control contures should have increased exponentially by a factor that lease 16 within the 72-hour test period.	≥16	85.9
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control confideres must not exceed \$ %.	< 35 %	22.8 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	< 7 %	2.8 %



Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANTE/2020/12830, Rev.1.

Recoveries for fluopyram on day 0 and day 3 were in the range between 90 and 98 % (see table below). Only in the lowest test concentration on day 3, recovery was 152 % of nominal concentration. This could most likely be explained by a handling error while taking samples, which did not influence the outcome of the study negatively. Given that the toxicity cannot be autibuted to any one of the as compounds but to the formulation as a whole, all results were based on nominal concentrations of ULU+SFS S (250+250 g/L).

The other active ingredient trifloxystrobin was not malysed since it is present if the added formulation in a fixed ratio to the analysed active ingredient. ³ Ò

No residues of fluopyram were detected in the control samples in a concentration higher than 0.5214 µg a.s./L, which was used as the lowest standard concentration during the study.

		-0 /			s li
Nominal co	ncentration	Measored a.s.	concentration	nomei	p≹≫ຶ "nal
[mg prod./L]	[µg-a, š./L]			Day 0 O	Day 3
0.0286	<u></u> , @6.12		9,31 A	98 4	152 ^в
0.0916	19.6	¥8.3	17.8	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	91
0.293	Q Q.7 X	59.3	مَّنَّ ⁵ 90 مَ	¥ £ 95	94
0.938	ي 201 6	189	Ø184	<i>"</i> 94	92
3.00	642	~603	స్తో 578లో	,∽Ş 94	90

Table 10.2.1- 16:	Analytical	results 🐼 ř	fluopyram
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A Calculations based on 3 measured samples. Sample A was measured again, and sample B was also measured to confirm the first measurement.

Most thely an outlier based on a nandling error while sampling. в

Biological results

Observations:

observed in any test concentration and the control. No physical approximatives

Table 10.2.1-17: Cendensity			
Nominal concentration	C C Me	an cell density [x 10 ⁴ cells/1	nL]
[mg prod./L]	24 h	48 h	72 h
Controk	<u>م</u> ري مي3.7	14.0	85.9
@ ^v 0.0286 ^v C	3.2	11.7	53.7
چې 0 69 16	1.8	5.7	24.0
جمع (M.293 T	1.7	3.3	10.7
^{مرج} 0.938	1.5	2.2	3.0
C 3.00	0.7	2.0	1.7



Nominal concentration	Mean growth rate [1/d]	% Inhibition			
[mg prod./L]	0 - 72 h	🔊 0 - 72 h			
Control	1.482	- 4 A			
0.0286	1.324 *				
0.0916	1.059 *	28:5 28:5			
0.293	0.782 *	Q ^Y 47,2 X Q ^Y Q			
0.938	0.363 *	9 495.5 5 A O			
3.00	0.167 *	0 ^{88,7} 0 ⁶ 0 ⁷			
* Significantly (α=0.05, one-sid	ed smaller) reduced, based on Dunnett?	s Multiple Aest Procedure			
The study mosts the validity of	III. Conclusion				
72 hours were:	ineria and the endpoints based	on nonunar product concentrations after			
72 nours were.					
ErC50 -72 hou	rs 🛞 % Ç.I.): 🖉 🏷	5^{m} 0.292 mg 0.16^{m}			
E _r C ₂₀ -72 hou	₿\$ (95 %C.I.);	Not determined A			
E _r C ₁₀ -72 hou	rs (\$7 % C.15): 0	Not determined A			
E _b C ₅₀ -72 hou	EbCsy-72 hours (95 % C.I.)				
$\mathbb{E}[\mathcal{E}_{20} - 72 \text{ hours} (\mathcal{E})^{(n)} (\mathcal{E})^{(n)}$					
E_bC_{pp} -72 hours (95% C.I.) γ γ γ γ Not determined ^A					
Ey @ - 96 h ou	rs (95 % CI): ^B	0.0425 mg prod./L (0.0351 – 0.0500 mg prod./L)			
E_yC_{20} E_yC	F S (95 CI): ^B	Not determined ^A			
E of n - 96 hou	urs @5 % CO. B & A	Not determined ^A			
Jowest contra	Phours C C C	\leq 0.0286 mg prod./L			
highest concentration without an	72 hours: $\sqrt{3}$ affect (based on growth rate and 3^{3})	< 0.0286 mg prod./L			
A Nor determined due to mathem B Please refer to recalculation de Assessment and conclusion l	hatical reasons ocument M-201720-00-11 by applicant:				
The study and its data are con	sidered as acceptable and reliable $rs = 0.292$ mg prod /I	le for use in risk assessment.			

Table 10.2.1- 18:Algae growth rate

J.

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V (D) 10 2 1/0(
KCP 10.2.1/06	
2020	Ô
evaluation (non-GLP) of the study $M-292579-01-1$ (magnetic 2007, 3	ř
EBGMP032) on the chronic toxicity of fluopyram & triflog strobin SC 500 (250+	
250) G to Pseudokirchneriella subcapitata (currently known as: Raphidocelis	
subcapitata) under static conditions	
<u>M-757720-01-1</u>	2
M-757720-01-1	6
None 🕅 🗸	Ľ
	0″
Current Guideline: not applicable	1
Deviations: not applicable	
No, not previously submitted	
not applicable O , , , , , , , , , , , , , , , , , ,	
Yes $\mathcal{O}_{\mathcal{A}}^{\mathcal{A}} \rightarrow \mathcal{O}_{\mathcal{A}}^{\mathcal{A}} \rightarrow O$	
	KCP 10.2.1/06 2020 evaluation (non-GLP) of the study M-292579-01-1 EBGMP032) on the chronic toxicity of fluopyram & trifloo strobin SC 500 (250-250) G to Pseudokirchneriella subcapitata (currently known as: Raphidocellis subcapitata) under static conditions M-757720-01-1 M-757720-01-1 M-757720-01-1 None Current Guideline: not applicable Deviations: not applicable Monot previously submitted Yes

Summary

In the existing report M-2920 endpoints for yield were 9-01 (amended Peport ý, statistically determined at 72[°]H. Õ W

A statistical evaluation addressing the calculation of valid 72-h ECK, EC20, and EC50 values as well as NOEC values for yield was conducted to folfill the datorequirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 205 (2014). Ô 2

The recalculations were performed with the software To Rat Professional (Version 3.3.0) based on Ľ nominal concentrations (EFSA 2019). Ô

Models providing best fit to the respective data were selected and are as follows: In order to derive Effect Concentrations that have 10, 20 and 50 % effects of yield of the test subjects (EC10, EC20, and EC₅₀), a probit analysis using linear maximum-likelihood regression was performed.

21 NOEC was determined by William's Multiple Sequential t-test Procedure (one-sided smaller, p = 0.05). To test for normal distribution and variance homogeneity a Shapiro-Wilk's test and a Levene's test were performed/respectively

able 10.2.1-19: Rescalculated Eq. 6, EC. and NOEC values based on nominal concentrations			
Endpoint	FLU+TOS SC \$00 [mg.product/L]	Fluopyram [mg a.s./L] Yield	Trifloxystrobin [mg a.s./L] Yield
72 hours - C.I.	not defermined	not determined	not determined
72 hours EC ₂₀ 5 % CÛ.)	not determined	not determined	not determined
72 hours - EGs (95 % C.I.)	0.0425 (0.0351 - 0.0500)	9.10 (7.51 – 10.7)	9.18 (7.58 - 10.8)
272 hoors - NOEC	< 0.0286	< 6.12	< 6.18

Midence interval C.I.:



Assessment and conclu	ision by applicant:
The data are considered	as acceptable and reliable without use in risk assessment.

Data Point:	
Data Politi.	
Report Author.	
Report Fear.	2018 V V V
Report Thie:	resudokirchnerielia subcapitata growth innjorion test with praopyram +
Dama et Mari	
Report No:	
Document No:	
Guideline(s) followed in	EU Directive 91/4149EEU
study.	DECD Test Cuilding 200
	USEDA OCSERVISO CIL
Deviations from surrant	US EFA OCSOFF 050.4900 0 . VY O VY O
test guideline:	Deviation of the retring of (2000)
test guidenne.	Deviations. The phy increase in the control was as units and this singler main the
	avpaced to have imported the study regults. MI validate orithms were met
Providua avaluation:	Note that a submitter and the study of suits and values of the submitter o
Tievious evaluation.	Not previously submitted a set of the set of
CI P/Officially	Bas conducted Inder 6 P/Officially recognized testing facilities
CLF/Officially	Tres, conducted inder ed r/opincially recognized testing factaties
facilities:	
A coentability/P aliability	
Acceptability/Kellablogy.	

Executive Summary

The green alga *Pseudokirchneriella subcapitula* were exposed to FLU+TFS SC 500 (250+250 g/L) under static conditions for 96 hours. Algal cultures with arcmitial nominal cell count of approximately 1.0×10^{5} cells/mL were used to test the nominal concentrations of 0.00182, 0.00582, 0.0186, 0.0596, 0.191, 0.610, 1.95 (6.25 and 20.0 mg prod./L The study design included 4 replicates for each test concentration and the control. At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Ô

Concentrations of fluopyran and trifloxystrobin were verified by HPLC – MS/MS on day 0, 3 and 4 for each concentration and control for fluopyran measured concentrations were in the 91 - 113 % range of nominal concentrations. For trifloxystrobin the recoveries showed a decrease over time with recoveries ranging between 97 and 118 % of nominal concentrations on day 0, between 62 and 122 % on day 3 and between 15 and 80 % on day 4. No residues of fluopyram and trifloxystrobin were found in the control samples above the LOQ (0.6625 μ g a.s./L). As the analytical results for both active substances showed a correct dosing at test start (day 0) with recoveries in the 91 - 120 % range of nominal concentrations and since the toxicity has to be attributed to the tested formulation as a whole, all biological results were related to nominal concentrations of the formulation FLU+TFS SC 500 (250+250 g/L).

The study fulls all validity criteria of OECD 201 guideline.

No morphological change in algae was observed in any test concentration and the control over the whole testing period.



The 72 hour - endpoints based on nominal product concentrations were: 72 hour - E_rC_{50} : 4.25 mg prod./L (3.72 - 4.90 mg prod./L), 72 hour - E_bC_{50} : 0.069 mg prod./L (0.066 - 0.072 mg prod./L) and 72 hour - E_yC_{50} : 0.04 mg prod./L (0.038 - 0.041 mg prod./L).

	I. MATERIAL AND METHODS
Test material	FLU + TFS SC 500 (250 + 250 g/L)
	Specification No.: 102000012886
	Content of a s 21 1 % w/w fluons from
	21.6 % w/w trifloxystrobin
	Density: 1.167 g/mL
Guidelines	Not specified.
adaptation	
Test species	Freshwater Green alga <i>Eseudokaichneftella subcapitata</i>
Culturing	In-house 3-day old pre-culture held under test conditions.
conditions	
Test solutions	Nominal product concentrations: of 000182 0.00580 - 0.0566 - 0.0596 - 0.191 - 0.610
	$-1.95 - 6.250 + 20.04$ mg prod C/L 3° 3° 3° 3° 3° 3° 3° 3°
	Control: untracted modified
	Evidence of undissolved material: The highestytest concentration (200 mg prod /L) was
	turbid over the whole testing period All other test concentrations and the control showed
	clear medna?
Replication	No. of vessels per concentration (replicates): 4
Exposure	Static Fotal exposure duration 96 hours
Initial cello density	1×10^4 cells/m2 in each test soroup 0^7
Test conditions	Femperature: 23.1 - 23.78 - 23.78
	Photoperiod: 24 hours fight &
Ô	Light intensity: 4510 4820 fux
l 0	Type of light: bank light containing cool white fluorescent lamps
~\$	pH of control: 8.Y - 9.9
A	Conductivity not reported & W
Doromotoro	Growth meeting same as current reduction. Tes
Measured /	Temperature was determined by a continuous measurement in one additional incubated
Observations	glass Vessel C
l "Q"	Morphological examination of cells using a microscope were done daily. Cell numbers per
	volume as a surrogate for biomass per volume) were estimated photometrically by
	$\sqrt{2}$ determining the extinctions at a wave length of 578 nm. Cell numbers were computed
	from extinction values using the conversion formula. $\log_{10} (\text{cell no.}) = 6.5593 \pm 0.85507 \times \log_{10} (\text{extinction})$
Sandpling for	Suplicate samples were taken at test initiation (0 hour) from bulk solution. After 72 hours
chemical	and artest termination (96 hours) duplicate samples were collected from the pooled
analysis	replicates from each test concentration and the control.
Ŭ	Samples were analysed by using a high-performance liquid chromatograph (HPLC) –
	MS/MS.


Data analysis	Probit analysis using l LOEC/ NOEC determ selected multiple t-tes Calculations were dor	linear max. likelihood ninations were done us sts. ne with Microsoft Exc	regression was using the ANOVA el sheets and the t	sed for EC_x -value estimation. procedure and properly \int_{0}^{∞} further statistical evaluations
	with the commercial p	program ToxRat Profe	essional (version 3	3.2.10
	II. I	RESULTS AND DISC	USSION	
Table 10.2.1- 20:	Validity criteria	a C	~~ ``	
Validity criteria	acc. to OECD 201 (adop	pted 2006	Required	O Obtained
The biomass in the exponentially by a period.	e control cultures should factor of at least 16 with	have increased ° hin the 2-houriest	$\begin{array}{c} & & & \\ & & & & \\ & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\ & & & & \\$	
The mean coefficient specific growth ra cultures must not of	ent of variation for section tes (days 0-1, 1-2 and 2 exceed 35 %.	ort-by-section	2 5 % ×	2 2 2 2 2 3 1.6 % 2 3 1.6 %
The coefficient of during the 72-hou not exceed 7 %.	variation of average Spe r test period in repocate	control cultures must		5 5 × × × ×
				9 A

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4 which comply with the EU regulatory requirements outlined within S SANTE/2020/12830, Rev.J. Ő

Recoveries for floopyram on day 0, & and 4 were between 94 and 43 % of nominal concentrations (see table below). For trifloxystrobin the decoveries ranged between 2 and 120 % of nominal concentrations on day 0, between 62 and 122 % on day 3 and between 15 and 80 % on day 4.

The analytical results for both active substances showed a correct dosing at test start (day 0) with recoveries between 91 and 120% of nominal concentrations. Therefore, and since the toxicity has to be attributed to the tested formulation as a whole, all calculated results were related to nominal concentrations of the formulation FLUG TFS SC 500.





Nominal co	oncentration	Measur	red a.s. concer [µg a.s./L]	ntration	% of nominal			
[mg prod./L]	[µg a.s./L]	Day 0	Day 3	Day 4	Day 0	🖉 Day 3	bay 4	0°
0.00182	0.384	0.413	0.387	0.420	108 0	101	109	
0.00582	1.23	1.38	1.37	1.14	112	111 🔍 Ĉ	8	Ì,
0.0186	3.93	4.39	3.80	٢٩.41 گ	<u>₩</u> 2	97,6,5	×112 ~	0
0.0596	12.6	14.3	14.2	14.2	Q113	103	S 1130	Å
0.191	40.3	45.7	45.4	43.5	¢ 113	. 113 K	108	Ý
0.610	129	145	140	142	¢¢}2	109	110 x	<i>x</i>
1.95	405	437	419	422	108		104	
6.25	1319	1420	×400	1440	108	~~106 ×	109	
20.0	4220	4240	3830	4560	100	⁹¹	₩08 <u>~</u> °	
		Ŕ		Y &	1 5	, ,		-

Table 10.2.1-21: Analytical results for fluopyram

Table 10.2.1- 22:	Analytical	results for	trifloxystro	in
	•		<i>"</i> • • <i>"</i>	

	oncentration	Measured a.s. concentration			% of nominal		
[mg prod./L]	[µg a.s./L]	Day 0	Day 3	Day 4 0	Day 0	Dato 3	Da
0.00182	0.393	0.454	\$4 81	© 0.177 [°]	~~116 °~~	_¢3122	4
0.00582	1.26	1.49	\$0.915¢	0(193	× 112°	م م 73	1
0.0186	4.02	<u>4</u> 238 O	2.00	°∼1.47 °	&109 se	6 2	3
0.0596	Q12.9	\$15.1	. <u>9</u> .09	7.02	0 ₁₁₇ 🖗	70	5
0.191	N 41.D	√ 45 . 9 [×] ^	y 28.7,∽	23.3	\$ 1.0 <i>\$</i>	69	5
0.610		¥36	86.*	79.9	103	65	6
1.95	<i>6</i> ,421 <i>√</i>	408	263	230	<i>©</i> 97	62	5
6.25	1350, K	, 1620	∂ ⁷ 959 ¹⁰	63 8	<u>> 120</u>	71	6
2050	420 8	\$100 N	3230	3450 ⊘	118	76	8
bservations	0 6		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	、 、			



Nominal	Mean cell density				
[mg prod./L]	24 h	48 h	72 h	96 h	F
Control	8.2	31.3	103.7	2263	
0.00182	8.0	29.8	97.6	£13.6	\$
0.00582	7.8	28.3	90.3	× 209.9 × ×	Q I
0.0186	6.9	24.3	689	2 1587	
0.0596	6.4	16.1 J	A0.3	93.7 ×	
0.191	5.4	10.6	20.8	40.8	×
0.610	4.3	GOA		<u>181</u>	
1.95	4.4	6.1 °	8.7 0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
6.25	5.9	[™] 6.8 [™] ×	9.00	10.9	
20.0	13.0	A 1600	Q 12.5	O GO A	
Nominal concentration	Mean go	with rate 0 /d] 0 2 0		Dition	
[mg prod./L]	0, ∠,72 h	<u>~~ 0-96 h</u>	0,- 22 h	[©] 0 - 96 h	
Control	1.547	Q1.355		<u></u>	
0.00182		<u>\$ 1.34</u>		≥ 1.1 *	
0.00582	× 14,501 ×			1.4 *	
0.0186		J.267 5	<u>8.8</u> * **	6.6 *	
0.0596	0" 1.232" "	1.135	20.4	16.3 *	
0.191	<u>~ 1.012</u> <u>~</u>	0,927	34.6*	31.5 *	
0.610	0.836	× <u>0</u> .737	6 45 .9 *	45.6 *	
1,99	0.727	© 0.6490° Q	53.3 *	52.1 *	
£ \$ 2 5	Ŭ 05731 &	0,597 25	© [♥] 52.7 *	55.9 *	
ž0.0	<u>)</u> (9.818)	6 ⁵⁷	<u>4</u> 7.1 *	70.0 *	
* Significantly (5) Bonferroni-Holm	0.05, one-side smaller)	reduced Tased @ multip	le sequentially-rejective V	Welch- t-test after	

Table 10.2.1- 23:Cell density

<u>k</u> . v	(// n		~		<i>(</i> ())	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
* 20.0		% .818	K)	ð.) 0.407	,
Significantly	y (🔊 0.05, c	me-sided	maller)	reduced	based @	mu
Bonferroni-	Hôglĩm 🖂			, K		d
Ø1	. Ő¥	e de la companya de l	ς, ΟΫ́	Ő	. Ô ^v	Ô
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Ũ	~ ~ _		$\sim$	ž n	2
1		Ô _v Ç	) A	× 0	\$¢	/
Q, A	Ö.	Ň	Ø			
		~~~	$\sim$	£.	.~Q ⁷	
, K	J.	A.	°,	¢″	, s	
	- 1	$\tilde{\boldsymbol{z}}$		\$.Õ	A,	
C	7,1	4	Q	Å		
L.	A	Ű	4	~~		
	~~~ × *	ë.		Ø		
- S		Dî 🐥		Ņ		
	v v	Ő				
N R	A	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~				
	A.	$\sim$				
E A						
í "Ô						
$\bigcirc$						



Mean measured concentration	Bioma	ss ^A	% Inhibi biom	tion of
[µg a.s./L]	0 - 72 h	0 - 96 h	0 - 72 h 嶡	0 - 96 h
Control	2131.2	6067.0	- \$	~~ <u>~</u> ~
0.00182	2019.4	5730.0	5.2 *	\$ 5.6 *
0.00582	1890.6	5468.8	× 8.13	× 9.2 ×
0.0186	1515.5	4222	ØŽ8.9 *	\$0%4 * @` _ @
0.0596	961.6	2545.5	54.9 * J	≥58.0 ± 0°
0.191	574.3	A289.6	73.1 *	78 78
0.610	345.7	698.6	83.8 *Q	Ø¥ 888.5 * ∅¥
1.95	296.4	538.2	× 86,10* ~	· 91.1 2
6.25	353.5	\$ \$67.3 ×	\$0.5 * ³	90.6 *
20.0	800.6	<u>_</u> @~1008.€	62.4	D* \$9.4 * %

#### Table 10.2.1-25: **Biomass**

 20.0
 000.0

 Biomass is equal to the area under the growth curve,

 Significantly (α=0.05, one-sided smaller) educed based on multiple sequential t-teceprocedure for the 0-96

 Bonferroni-Holm for the 0-72 hour period and based on Williams multiple sequential t-teceprocedure for the 0-96

 hour period

		<i>R</i> o
Nominal concentration	[∞]	idition
[mg prod./L]	2 0 72 h 7 0 - 2 h 7 0 - 2 h 7	0 - 96 h
Control		0.0
0.00182	<u></u> 0 [∞] 96.6 [∞] [∞] 212.6 [∞] 4.5.9 * [∞]	5.6 *
0.00582	89.3 4 208,9 13.63	7.3 *
0.0186	\$7.7 C 329 *	30.0 *
0.0596	39.2° 2.7° 39.2° 31.8 *	58.8 *
QQ191	39 8 × × × × × × × × × × × × × × × × × ×	82.3 *
0.610	\$11.3 \$ \$\$ \$\$8.1 \$ \$9.0 *	92.0 *
1.95	7.7 92.5 *	94.5 *
6.25	2 × 9 × 9 × 9 × 92.2 *	95.6 *
20.0	0 011.5 × 0 × 9 88.8 *	97.4 *

#### Table 10.2.1-26:

Significantly ( $\alpha$ =0.05, me-sided maller reduced based or multiple sequentially-rejective Welch- t-test after Bonteroni-Holm for the 0-72 bour period and based on Williams multiple sequential t-test procedure for the 0-96 hour period

The study meets the validity criteria and the 0-72 and 0-96 hours endpoints based on nominal product concentrations were:





Results – 0 to 72 hou	rs
E Cro. 72 hours (05 % CD)	4.25 mg prod./L $Q^{\circ}$
$E_r C_{50} = 72$ nours (93 % C1):	(3.72 - 4.90 mg prod./L)
$E_{C_{12}}$ , 72 hours (05 % C L):	0.048 mg prod./L
$E_{r}C_{20} - 72$ flours (55 76 C.1.).	(0.040 - 🖉 56 mg prod. 🖉 🍈
$E_{C_{10}}$ -72 hours (95 % C I):	0.005 mg prod./L 🔧 👾
$E_{\rm r} C_{10} - 72$ hours (55 70 C.1.).	(0.003 - 0.006 mg prod./L)
E _v C ₅₀ - 72 hours (95 % CI):	<b>30.040 mg prod</b> /L
	(1)1038 - 0.041 mg prod (1)
E _y C ₂₀ -72 hours (95 % C.I.):	0.008 mg prod./L > (0.007 - 0.008 mg prod./L)
$E_yC_{10}$ -72 hours (95 % C.I.):	$Q^{\nu} \sim 0.003$ mg prod./L $C^{\nu} = Q^{0}$
ЕьС50 - 72 hours (95 % CI): 🔬 🔗 🐔	0.069 mg prod./L
O ^Y O ^Y	(0.060 - 0.02 IIIg prod./L)
E _b C ₂₀ -72 hours (95 % C.I. <u>)</u> :	(0.006 0.006 mg prod 1)
$E_bC_{10}$ -72 hours (95 % $(27)$ ):	0.002 mg/prod./L
	(0.002 - 0.002 mg.); od./L)
LOEC - 72 hours and a second s	
lowest concentration with an effect (based on growth rate,	$5^{\circ} \leq 0.09182$ mg prod/1.
biomass integral and yould) of or	
NOEC - 22 hours	
highest concentration without an effect (based on growth rate,	≪ 0.00182 mg⊈rod./L
biomass integral and yield of the second sec	
A Results of to 96 pour	rso ^y & ² y
EC	1.99 mg prod./L
	(1.67 - 2.41 mg prod./L)
E Co- 96 hours (95% C 10	🖉 💞 0.077 mg prod./L
	(0.058 - 0.099 mg prod./L)
⁶ F ₂ C ₁₀ -96 hours 95 % Γ.L.	∞ 0.014 mg prod./L
	(0.009 - 0.020 mg prod./L)
$\tilde{\mathcal{L}}^{\vee}$ Esc. $\tilde{\mathcal{L}}$ = 96 bours (95 % CD)? $\tilde{\mathcal{L}}^{\vee}$	0.044 mg prod./L
	(0.043 - 0.045 mg prod./L)
₩.C ₂₀ <b>4</b> 96 hou® (95 % C.L): 0 0 ∞	0.011 mg prod./L
	(0.011 - 0.012 mg prod./L)
Exel -96 60 urs (95% C 19: ~ ~ ~ ~	0.005 mg prod./L
	(0.005 - 0.006 mg prod./L)
A E _b C ₅₀ - 96 hotes (95% CD:	0.048 mg prod./L
	(0.046 - 0.049 mg prod./L)
$E_{\infty} = 96$ hours $O_{\infty} \ll C_{\infty} = 20^{\circ}$	0.009 mg prod./L
	(0.009 - 0.010 mg prod./L)
$\mathbb{F}_{\mathbf{k}} C_{10} \xrightarrow{\mathcal{V}} \mathbf{h}_{\mathbf{k}} \mathbf$	0.004 mg prod./L
	(0.004 - 0.004 mg prod./L)
lowest concentration with an effect (based on growth rate	< 0.00182 mg prod./L
white the second second second rates and vield)	ing prod. 12
NOECO96 hours	
highest compentration without an effect (based on growth rate	< 0.00182 mg prod /L
Anomas integral and vield)	

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC₁₀ values for *Pseudokirchneriella subcapitata*.



<mark>Biological</mark> endpoints	EC10 [mg a.s./L]	<mark>95% CL</mark>	NW	<mark>Relationship</mark> EC10/EC20/50	
Growth Rate	<mark>0.005</mark>	<mark>0.003 – 0.006</mark>	<mark>0.6 (fair)</mark>	$\frac{EC_{20}, 1007 < EC_{10}}{< EC_{50}, low}$	
Yield	0.003	<u>0.003 – 0.004</u>	0.333 (good)	$\begin{array}{c} \mathbf{EC}_{10} < \mathbf{EC}_{20}, \mathbf{Iow}^{(1)} \\ \mathbf{EC}_{10} < \mathbf{EC}_{20}, \mathbf{EC}_{20}$	
<b>Biomass</b>	0.002	0.002 - 0.002	0.0 (excellent)	EC ₁₀ < EC ₂₀ 46w (high)	
		A Q			
Assessment and	conclusion by ap	oplicant:			A
The study and its	data are consider	ed as acceptable	and reliable for	use in risk assessment	
The endpoint is: I	$E_r C_{50}$ (72 hours) =	= 4.25 mg prod./L			0
					*



Data Point:	KCP 10.2.1/08
Report Author:	
Report Year:	2018
Report Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Toxicity to
	Pseudokirchneriella subcapitata in an algal growth inhibition test
Report No:	134621210
Document No:	<u>M-636234-01-1</u>
Guideline(s) followed in	-OECD Guidelines for the Testing of Chemicals, Section 2, No. 201 Feshwater
study:	Alga and Cyanobacteria, Growth Rhibition Test" Adopted March 23, 2005,
	corrected July 28, 2011 $\bigtriangledown$
	- Commission Regulation (EC) No 761/2009, Omex, Part C, C.3: "Feshwater
	Alga and Cyanobacteria, Growth Inhibition Jest", Official Journal of the European
	Union (EN), dated August 24, 2009
	- EPA Guideline 712-C-606: OCSPP 850,4500, Algal Toxicity, January 2012,
	- Japanese MAFF, Gueidelines for preparation of Study Results, Algae growth
	Inhibition studies. Noification No. 12-Nousan-81470 JMAFF Test Guideline, 2-7-
	7, Algae growth Inhibition 2005 0 0 0 0
	- SANCO/3029/99 rev. 43/1/07/09: Residues: Guidanco for generating and
	reporting methods of Aralysis in Support of preregistation data requiremethosfor
	Annex II (part A; Section 4) and Annex III (part A; Section 3) of directive 91/414
Deviations from current	Current Gindeline, DECEA 201 (2006)
test guideline:	Deviations: None. All validity criteria vore met
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP/Officially recognised testing gacilities
recognised testing	
Tachitles:	
Acceptability/Reliability	

# Executive Summary

The green algo *Pseudokirch/Periella Subcapitata* sere exposed to FLU+TFS SC 500 (250 + 250) under static conditions for 96 hours. Algal cultures with an initial nominal cell count of approximately  $1.0 \times 10^4$  cells/mL were used to test the nominal concentrations of 0.0089, 0.0286, 0.0916, 0.293, 0.938 and 3.00 mg prod./L. The Gudy design included 4 replicates for each test concentration and 6 replicates for the control. At 24 hour-intervals, the cell@ensity (cells/mL) of each culture was counted.

Concentrations of fluopytam and trifloxystrobin were verified by LC – MS/MS on day 0, 1, 2, 3 and 4 for each concentration and control. For the pyram measured concentrations were in the 85.0 - 100 % range of nominal concentrations. For trifloxystrobin the recoveries showed a decrease over time with recoveries anging between 90.5 and 14.1 % of nominal concentrations on day 0, between 82 and 91 % on day 1 between 74 and 79 % on day 2, between 27.5 and 70.5 % on day 3 and between 1 and 69 % on day 4. No residues of fluopyram and thiloxystrobin were found in the control samples above their LOQ (LOQ for fluopyram as 0.2 wg a.s. 0.2 wg for trifloxystrobin: 0.75 wg a.s. 1.2 wg a.s. 1.2

The study fulfils all validity criteria of OECD 201 guideline.

After 72 hours the cells were smaller in the two highest test concentrations (0.938 and 3.00 mg prod./L) compared to the cells in the control. After 96 hours the three highest test concentrations (0.293, 0.938 and 3.00 mg prod./L) showed smaller and elongated instead of sickle-shaped cells compared to the cells in the control.



The 72 hour- endpoints based on nominal product concentrations were: 72 hour –  $E_rC_{50}$ : 0.419 mg prod./L (0.405 - 0.433 mg prod./L); 72 hour -  $E_bC_{50}$ : 0.0405 mg prod./L (0.0391 - 0.0420 mg mg prod./L ( $0.0391 - 0.0420 \text{ mg$ prod./L) and 72 hour  $-E_yC_{50}$ : 0.0432 mg prod./L (0.0412 - 0.0453 mg prod./L).

I. MATERIAL AND METHODS
Test material FLU + TFS SC 500 (250+250 g/L) $\swarrow$
Specification No.: 102000012886
Batch ID: PA1S0Q5 1 73 $\mathcal{A}$ $\mathcal{O}$ $\mathcal{A}$ $\mathcal{A}$
Content of a.s.: 21.3 % w/w fluopyram
21.4 % w/w trifforxystrobin
Density: 1.170 g/mL
Guidelines Not specified
adaptation
Test species Freshwater Green alog Restude tinchnetigella subcapitata
C the instant of the state of t
Culturing In-nouse 3 day old pre-culture neid under test conditions.
1 est solutions Nominal product concentrations $40.0089 \times 0.0286 - 0.0946 - 0.293 - 0.938 - 3.900 \text{ mg}$
prod./L & & & & & O & O & & ~
Corresponding measured concentrations, not prevanto
Control: untreated medium
Evidence of undersolved material. There were no remarkable observations of the test
medium during the exposure The test medium appeared clean
Replication No, of vessets per concentration (replicate of 4 (
No of versels per control (replicates): $6.7$ $0$
Exposure Static , By A D D
Total@xposyne duration: 96 høurs 🖓 🧳
Initial cell density 1 & 10 ⁴ cells/mL inteach test group a second seco
Test conditions Demperature: 22.5 - 23.8°C & C
Photoperiod: 24 hours light of a m
Light interstry 4550 - 5990 lux a 2
Two of kight: fluorescent hamps
$\mathcal{O}$ H of $\mathcal{O}$ training $\mathcal{O}$ and $\mathcal{O}$ and $\mathcal{O}$
Combestinite and a Shot of the
Conductivities not reported
Growth mealum same as culture mealum? Y es
Parameters Phe phowas measured in all test item concentrations and the control at the start, after 72 h
Measured and the end of the test Temporature was determined by a continuous measurement in one
Observations addational ocubated glass vessel
Cell density and prorphological examinations were determined by
spectrophotometric measurement daily. The algal cell densities were calculated by
subtracting the absorption of the blanks, from each of the measured absorption of the test
media (with algae)
Sampling for Supplicate samples of test solutions were taken at test initiation (0 hour) in all treatment
chemical analysis elevel and the control and then daily thereafter till test termination (96 hours).
Samples wat analysed by using liquid chromatograph (LC) – MS/MS.
Data analysis analysis wing linear max. likelihood regression was used for EC _x -value estimation
The stand where possible their 95 %-confidence limits.
LOE NOEC determinations were done using Welch t-test after Bonferroni-Holm.
$\sim \mathbb{O}^{\mathbb{V}}$ Calculations were done with Microsoft Excel sheets and the further statistical evaluations
with the commercial program ToxRat Professional (version 3.2.1).



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#### **II. RESULTS AND DISCUSSION**

rable rotati 27. Vallaty criteria	Table	10.2.1-27:	Validity	criteria
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		× "0"
Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained S
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	$\geq 16$	
The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3) in the control cultures must not exceed 35 %.	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
The coefficient of variation of average specific growth tates during the 72-hour test period in replicate control cultures must not exceed 7 %.		

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANTE/2020/12830, Rev.1.

Recoveries of fluopyram on day 0, 1, 2, 3 and 4 were between 85.0 and 100% of pominal concentrations (see table below). For triflavystrobin the measured concentrations showed a decrease over time: recoveries ranged between 90.5 and 111, 5 of nominal concentrations on day 0, between 82 and 91% on day 1, between 74 and 79% on day 2, between 27,5 and 70.5% on day 3 and between 1 and 69% on day 4.

The analytical results for both active substances showed a correct dosing at test start (day 0) with recoveries between 90.5 and 1114% of nominal concentrations. Therefore, and since the toxicity has to be attributed to the tested formulation as a whole, all calculated results were related to nominal concentrations of the formulation FDU + \$ S SC 500 (20+258 g/L).

No residues of fluopyram and trifloxystrobin were defected in the control samples above their limit of quantification (LOQ for fluopyram:  $Q^2 \mu g a_s./L$ , LOQ for trifloxystrobin: 0.75  $\mu g a.s./L$ ).

		. Qř			C)	407					
Non concen	nin: tration	Measured A.s. concentration & % of nom					of nomina	al ^A			
[mg prod./Lf	) β) [μg a.s./L]	Day 0	Day 1	Bay 2	Day 3	Day 4	Day 0	Day 1	Day 2	Day 3	Day 4
0.0089	1.892 🐇	J1.71	1.600	1,69	1 de la compañía de l	1.70	90.5	85.0	89.5	93.5	90.0
0.0286	6.079 💊	5.5P	5 <b>0</b> 0	5.50	<u></u> 5.49	5.39	90.5	87.5	90.5	90.5	88.5
0.0916	19 <b>.4</b> /1	<u>الالا</u>	Ø8.33~	≫18.17Q	19.2	19.0	95.5	94.0	93.5	98.5	97.5
0.293	£.281 ^	\$ 59.5	60.3	600	60.9	62.3	96.0	96.5	96.5	97.5	100
0.938	¥99.385	185	134	192	190	191	94.5	92.5	96.0	95.5	96.0
3.00	637,692	<u>,</u> 620 ∝	622	632	624	634	97.0	97.5	99.0	98.0	99.5

# Table 10.2.1-28: ⁽²⁾ Analytical results for fluopyram

A Not given in Report. Calculations based on measurements of 2 replicates

â



Non concen	ninal tration	М	easured	a.s. conce µg a.s./L	entratior ]	A		%	of nomin	al ^A		Å S
[mg prod./L]	[μg a.s./L]	Day 0	Day 1	Day 2	Day 3	Day 4	Day 0	Day 1	Dry 2	Day 3	Day 4	F
0.0089	1.907	2.11	1.74	1.41	0.53	0.04	111	91.0	° 74	27.5	2	
0.0286	6.128	6.06	5.28	4.64	2.79	0.06	99	86,5	75.5	<b>\$</b> €.5	6 ^{×1}	ÌQ
0.0916	19.627	19.1	17.21	14.98	12.8	6.70	97	87.5	76.5	65.5	¥ 34.5	C
0.293	62.782	60.1	55.7	48.4	42.8	38.9	96	Q89.0	77.0	68	62	Å
0.938	200.989	188	165	151	133	<b>1</b> 30	93.5	82.0	755	66.5	065	1
3.00	642.821	581	507	452	452	443	90.5	<b>\$</b> 3.5	<i>7</i> 9.0	<b>∲</b> 70.5	69	

#### Table 10.2.1- 29: Analytical results for trifloxystrobin

Not given in Report. Calculations based on measured

Biological results: Observations: After 72 hours there were no deviations of the shape of the algal colls in the 4 lowest test concentrations (0.0089, 0.0286, 0.0916 and 0.293 fragment to the algal colls in the 4 lowest test concentrations (0.0089, 0.0286, 0.0916 and 0.293 mg prod/L) compared to the cells of the control Flowever, the cells were smaller in the two highest test concentrations (0,938 and 3.00 mg prod/L).

After 96 hours the algae cells were not affected in the three lowest test concentration (0.0089, 0.0286 and 0.0916 mg prod./L). However, the three highest test concentrations (0293, 0,938 and 3.00 mg Table 10.2.1- 30: Cell density

Nominal S C S Mean cel	density	
concenteration 2 2 2 10 ⁴ co	ells/mL	
[mg p(rod./L] 24 h 21 48 h	∽ 72 h	96 h
Control 28 4 5 .5	183	335.0
0.0089	170.8	340.2
0.0286	111.2	275.6
$0.0916 @ 0^{5} 0.03 ~ 0^{7} ~ 0^{7} ~ 0^{7}$	52.6	155.3
0.293	14.4	39.8
	6.40	12.2
9.00 2.96 ~ Lo 18	3.37	4.80

### Table 10.2.1- 30: Cell density



Nominal concentration	Mean gro (based on co [1/0	wth rate ell density) d]	% Inhit	pition A
[ing prou./L]	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h 🔊
Control	1.735	1.453	- , °	~ · · · · · · · · · · · · · · · · · · ·
0.0089	1.712	1.457	1.3	, O [*] -0.3 Q [*]
0.0286	1.57	1.405 🖉	9,5*	3.3.7 5
0.0916	1.32	1.26	20,9 *	
0.293	0.889	0.92	48.8 * C	𝔅 6.6 №
0.938	0.618	0.625	64. <b>4</b> *	57.0*
3.00	0.404	0.391	76.7 * m	23.1 * ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
<ul> <li>^A -% inhibition</li> <li>* Mean value s</li> </ul>	means increase in growth r significantly different from t	elative to the control $\alpha = 0.05$ , one is	nded, Welch taget after Bo	onferrați-Holman, o
Table 10.2.1- 32:	Biomass			

#### Table 10.2.1-31: Algae growth rate

Table 10.2.1- 32:	Biomass	Å 4					Ő.	0
Nominal concentration	Cum	totive bio	mass A		Cur Cur	% Trihibi Mlative	tion of _K biomass ^B	
[mg prod./L]	0 - 72	Z Z	<b>0</b> - 96 ar		Q0 - 72 h		<b>&amp;_0 - 96</b>	h
Control	145.985	& Ê	403,791		~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	, Ôj	<u> </u>	
0.0089	126.562		281.054		₹\$.2 *		5.6	
0.0286	84.423	len i	276,8 <b>0</b> 9	. Ô	<u>د</u> 42.1 [€]	, , , , , , , , , , , , , , , , , , , ,	31.4	*
0.0916	41.485		144,445	Š,	0 [°] 71 [°] 74 [°] 74 [°]		64.2	*
0.293	JG 44.190	8 J.S	40.295	y w	90.3 *	7,	90.0	*
0.938	7.082		15.407	Ľ,	Ø 95.1%	1	96.2	*
3.00	6.231	<u> </u>	9:314	ð	95.7 *		97.7	*

А

Cumulative biomass is equal to the area under the growth curve. в

°TZ∂°, *

-% inhibition means increase in growth relative The control  $\mathcal{Q}$   $\mathcal{Q}$ Mean value significative different from the control (Weitch t-test after Bowferroni-Holm,  $\alpha = 0.05$ , one-sided) A A

Table 10.2.1- 33: Algae vield a contract of the second sec									
Nominal concentration	Ø Mean And 10 ⁴ ce	yield k/mLL	% Inhil	bition ^A					
[mg prød./L]	<b>∂</b> 0 - 72 🖓 🖉	`∕>`0 - 96∿h	0 - 72 h	0 - 96 h					
Control	181.995	<u>3</u> 3 <b>€</b> 017	-	-					
0.0089	LO9.804	~639.179	6.7	-1.5					
0.0286	\ 110.249 Q	274.611	39.4 *	17.8 *					
0.0916	S1_€28	154.291	71.6 *	53.8 *					
0.293	\$.388 A	<b>2</b> 38.822	92.6 *	88.4 *					
0.938	S 0 5.406	11.244	97.0 *	96.6 *					
2,90 2	2,568	3.798	98.7 *	98.8 *					

## Table 10.2.1- 33: 🖗 Alga

 $\frac{1}{2}$  inhibition means increase in growth relative to the control Mean value significantly different from the control ( $\alpha$ = 0.05, one-sided, Welch t-test after Bonferroni-Holm)



### **III.** CONCLUSION

icentrations after 72 and 96 hours were:	napoints based on nomir	nal product
	Š	
Results – 0 to 72 hours	Ĩ,	
ErC50 - 72 hours (95 % CI):	<b>4.419 mg prod</b> ð Ľ (0 * 405 – 0.433 mg prod	
E _r C ₂₀ -72 hours (95 % C.I.):	© 0.0624 mg prod./L © 0.0592 – 0.06 © mg pro	
E _r C ₁₀ -72 hours (95 % C.I.):	0.0230 mg produl 0.0214 - 0.0248 mg pro	d.Ĥ)
E _y C ₅₀ - 72 hours (95 % CI):	© <b>0.0432 mg prod./l</b> 4	d./L
E _y C ₂₀ -72 hours (95 % C.I.):	0.0147 mg prod./L (0.0136 – 0.0159 mg pro	(Te) (°
E _y C ₁₀ -72 hours (95 % C.1.):	0.0984 mg prod./k 0.0076 – 0.0093 mg pro	d./L.
E _b C ₅₀ - 72 hours (95% Clp	<b>9.0405</b> °mg prod./L (0.9391 – 9.0420 mg pro	0 ¢5/L)
$E_bC_{20}$ -72 hours (95% GL.):	0.0117 meprod/fs (0.010 – 0.024mg pro	// d./L)
EbC10 -72 hours (95 %C.I.)	0.006@mg prod./L 0.0056 0.0066 mg pro	d./L)
LOEC - 72 bours: 5 0' '0 lowest concentration with an effect (based on growth rate and 5 view) 6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	D.0286 mg prod./L	
ighest concentration without an effect (based on growth rate and g	0.0089 mg prod./L	
Iowest concentration with an effect (based on biomass)	$\leq 0.0286 \text{ mg prod./L}$	_
highest concentration without an effect (based on biomass)	<0.0089 mg prod./L	_



Results – 0 to 96 hour	s
E _r C ₅₀ - 96 hours (95 % CI):	<b>0.709 mg prod./L</b>
E _r C ₂₀ - 96 hours (95 % C.I.):	0.128 mg prod./L (0.122 – 0.135 mg prod./L)
E _r C ₁₀ -96 hours (95 % C.I.):	0.0025 mg prod./L (0.0488 – 0.0564 mg prod./L )
E _y C ₅₀ - 96 hours (95 % CI):	<b>40.0815 mg prod./L</b>
$E_yC_{20}$ - 96 hours (95 % C.I.):	Q 0.0320 mg/prod./4 (0.0300 – 0.0342 mg/prod./1 (0.0342 mg/
E _y C ₁₀ -96 hours (95 % C.I.):	© 0.0195/mg prod./L (200181 - 0.02140 ng prod./L)
EbC50 - 96 hours (95 % CI):	Ø <b>0.0563 mg.prod.7</b> ℓ
E _b C ₂₀ - 96 hours (95 % C.I.)	0.0178 0.0198 mg prod./L (0.0178 0.0198 mg prod./L)
$E_bC_{10}$ -96 hours (95 % $(2.5)$	(0.0699 - 0.0114  mg prod./C)
LOEC - 96 hours?	0.0286 mgprod./K
NOEC - So hours highest concentration without an effect (bassed on growth rate,	2 0.0089 mg prod./L
biomass and yield) y and yield	
Reliability assessment (EFSA 2015)	
The following table provides reliability indicators for $\mathbf{FC}_{10}$ val	Maes for <i>Pseudokirchneriella subcapitata</i> .
	Palationship
endpoints [mg a.s./L]	EC ₁₀ /EC _{20/50}
$\begin{array}{c c} Growthe Rate \\ \hline 0.029 \\ \hline 0.029 \\ \hline 0.0248 \\ $	$ \begin{cases}                                   $
Yield 3 0.0084 0 0.0095 0.2024	$\frac{\text{EC}_{10} < \text{EC}_{20}, \text{ low}}{(\text{high})}$
Biomass & 0.0061 0.0066 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 - 0 0.0086 -	$\frac{3}{(high)} = \frac{BC_{10} < EC_{20}, low}{(high)}$
Assessment and conclusion by applicant:	
The study and is data are considered as acceptable and reliab	ble for use in risk assessment.
The endpoint is: $E_{50}(72 \text{ hours}) = 0.469 \text{ mg prod./L}$	
<b>CP</b> [*] 10.2 [*] Additional long-term and chronic tox	cicity studies on fish, aquatic



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#### **CP 10.3 Effects on arthropods**

#### **CP 10.3.1** Effects on bees

The risk assessment has been performed according to the existing guidance for force at the time of the preparation and submission of this dossier namely the EU Guidance Document of Tegestrial Ecotoxicology (SANCO/10329/2002 rev 2) and EPPO Standard PP 3/10 Environmental Risk Assessment Scheme for Plant Protection Products – Chapter 10: Honeybees.

Where bees are likely to be exposed, Commission Regulations (EL9 283/2013 and 284 2013 Aquire testing of both acute (oral and contact) and chronic toxicity, including sub-lethal effects. Although the are no current testing requirements for any begin of the honey begin with Regulation EU 1107/2009, acute oral and contact bumble bee studies were conducted with fluopyram tech. and the representative formulation FLU + TFS SC 500 which is presented as additional information (see table below).

Consequently, in addition to the standard toxicity studies performed with addit honey bees (OECD 213 and 214) the following studies are also provided:

- 214) the following studies are also provided: (OECD 246/OECD 247; <u>M542447-01-1</u>, <u>M-516849-01-1</u> and <u>M-763123-001</u>)
- Acute and contact toxicity of FLU + TES SC 500 to adult bumble bees under laboratory conditions 4. Î
- (OECD 246/OECD 246, M-703910-04-1) Chronic 10-day toxicity test with FLU TFS SC 500 on adult Koney Sees under laboratory conditions (OECD/245; 1/537/9-02-19
- Toxicity to honey bee larvas under aboratory conditions following repeated exposure to the formulated product FAU + FFS SC500 (QECD, goidance document 239; M-738909-02-1)
- One semi-field study following EPPO 170 with the representative formulation FLU + TFS SC 500 using a more realistic spray scenario onto flowering Phaceia covering effects on brood development, adult and pupal mortality, for aging activity, behaviour and colony development and strength. This semicheld study is presented in MCP Section (0, Point 10.3.1.5/01, M-435338-01-1.

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	SC 500		a 🏻 🛸
Test substance	Test species/ study type	Endpoint	References
	Apis mellifera, acute test	LD ₅₀ oral (48 h) > <b>102.3 µg a.s./bee</b> LD ₅₀ contact (48 h) > <b>100 µg a.s./bee</b>	42005 <u>M-261594-01-1</u> KC45.3.1 40/01 K&A 8.3 12.2/01
Fluopyram	Bombus terrestris, acute test	LD ₅₀ oral (48 h) $>$ 92.5 µg a.s $\beta$ with the bee	2015) <u>M-542447-014</u> KCA8.3.1.01/06
tech.	Bombus terrestris, acute test	$LD_{50}$ contact (48 h) > 100 pg a.s./bumble bee	(2015) M-510549-04 KCA 8.3.1.f.2/04
	Bombus terrestris, acute test	LD ₅₀ oral (48 h) $\sim$ 0.5 µg a.s./bumble bee LD ₅₀ contact (48 h) $\sim$ 100 $\oplus$ g a.s./bumble bee	(2021) <u>M-763, 3-01-6</u> KCA 8.3.1.1(007 KCA 8.3.1(0.2/05
	Apis mellifera, acute test	LD goral (48 h) 208.8 vg prod./bee	(2007) <b>1-2881/3-01-1</b> <b>KCP[*]10.3.1.1.1/01</b> <b>KCP</b> 10.3.1.1.2/01
FLU+ TFS SC 500	Bombus terrestris, acute test	$I_{1}D_{50} \text{ oral}(48 \text{ h}) > 1216.23 \mu \text{g prod}/be $ LD ₅₀ contact (48 h) > 1285.12 $\mu$ g prod be $\lambda$	(2020) <u>M-738910-04-1</u> KCP 10.3.1.1.1/02 KCP 10.3.1.1.2/02
	Spis mellifera, C 10-day oral feeding test	1000  J $1000  J$ $100$	(2020) <u>M-753795-02-1</u> KCP 10.3.1.2/01
	Agris melkifera, kauya 22-day repeated feeding test	NOEC 240.48 mg prod./kg diet NOED 37.02 μg prod./larva EC ₀₀ > 691.21 arg prod./kg diet	(2020) <u>M-738909-02-1</u> KCP 10.3.1.3/01
Higher Tier	3. 4 6		
FLU + TFS SC 500	Honeybee Volony development – Semi Field	behaviour, nectar- and pollen storage, brood- abundance and development, colony strength as well as on queen survival after two applications at 0.56 Eprod./ba each (corresponding to 140 g fluory ram/ha and 140 g trifloxystrobin/ha). The first application to the bee-attractive crop <i>Phacelia</i> <i>tanacetfolia</i> was carried out at BBCH 59-61	(2012) <u>M-435338-01-1</u> KCP 10.3.1.5/01
Bold valtes used in	risk assessment	<ul> <li>Anthout dees present, while the second application</li> <li>Courred during full flowering (BBCH 64-65) with</li> <li>Courred during foraging on the crop during application.</li> </ul>	
a.s.: active substa	nce y Ly		

# Table 10.3.1-1: Ecotoxicological endpoints relevant for the risk assessment for bees for FLU+TFS SC 500 SC 500



orodinet/ha

wduct/wee

#### **Risk assessment for bees**

The risk assessment for bees for fluopyram is based on two foliar applications of the formulated product FLU + TFS SC 500 to grapes, each at a rate of 0.2 L product/ha (corresponding to an application rate) for fluopyram of 50 g a.s./ha). The acute toxicity endpoints (LD₅₀ values) for both the active substance fluopyram and the formulation FLU + TFS SC 500 are used as part of this risk assessment.

### Hazard Quotients

The risk assessment is based on Hazard Quotient approach  $(Q_H)$  by calculating the ratio between the application rate (expressed in g a.s. or product per ha) and the aboratory contact and oral LDs (expressed in  $\mu$ g a.s. or product per bee).

 $Q_H$  values are 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 honeybees.

maximum application rat

Hazard Quotient, oral:

Hazard Quotient, contact:

 $Q_{10} = \frac{maximum application rate}{\sum_{i=1}^{N} \frac{1}{2} \sum_{j=0}^{N} \frac{1}{2} \sum_{j=0}$ 

Table 10.3.1-2: Hazard quotients for bees for application in grapes, 2 x 50 g a.s. dra – oral exposure

Compound		Offal LD59	Max. application addre	Dazard quotient Qrig	Trigger	<i>A-priori</i> acceptable risk for adult bees
Fluopyram tech		> 102.3	10 50 m	<b>\$</b> 0.49 ×	50	yes
FLU + TFS SC 500	0 0	20868	234.8 ^ 2		50	yes
A Based on an	annlicat	ton rate of 200 m	prod /ha and a productor	ensity of 1 174	g/mI	

Based on an application rate of 200 rdf prod./ha and a product density of 1.174 g/mL

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

Table 10.3 7 - 3:	Hazard quotients	for bees for a	pplication in grape	s, 2 x 50 g a.s./ha –	- contact exposure
~°0'					-

Compound x	Contact ID50	ATax. application Fate [g/ha]	Hazard quotient Q _{HC}	Trigger	<i>A-priori</i> acceptable risk for adult bees
Fluopyram tech.	100 S	Q 50	< 0.50	50	yes
FLU + TFS SC 500		234.8 ^A	< 1.17	50	yes

A Based on an application rate 200 mL prod./ha and a product density of 1.174 g/mL

The bazard fuotients for contact exposure are below the validated trigger value for higher tier testing (i.e.  $Q_{HO} \leq 50$ ).



#### Further considerations regarding the risk to bees

The active substance fluopyram and the formulated product FLU + TFS SC 500 are both of low tox wity to bees. The technical material exhibits acute  $LD_{50}$  values for adult bees of > 100 µg a.s./bee (cortact) and > 102.3  $\mu$ g a.s./bee (oral). The formulated product FLU + TFS SC 500 is of low toxicity with acute oral and contact LD₅₀ values for adult bees in excess of 200 µg product/bee. HQ values based on the use in grapes for both the active substance and the formulated product are considerably lower than the avels. regarded to indicate a risk to bees. Acute contact and oral endpoints for bumble bees are similar and comparable to honeybee endpoints for both the active substance and the formulation (FAU + TAS SC 500). Hence, the findings indicate that bumble bees do not exhibit greater sensitivity to PLU +CTFS SC 500) or fluopyram tech. compared to the honey bee. The risk assessment for honey bees was therefore considered to be protective of other bees and to cover the exposure of pon-Apis bees such as Bombus terrestris.

In addition, a chronic oral toxicity test (10-day, feeding) as por OECD Guideline No. 245 as well as a chronic larvae laboratory study (repeated exposure) as per OECD Guidance Document No. 239 with the formulated product FLU + TFS SC 500 were carried out. Although the probability of Chronic Exposure to the formulated product for either honey bee adults of larvar is considered to be low, the applicant recognizes the new requirements for cheonic effects data as stipulated by Commission Regulation (EU) No. 284/2013. These studies address potential chronic toxicity to honey bees and effects on honey bee development and other honey bee the stages, respectively, in accordance with the data requirements as set out in Commission Regulation (EU) \$0. 28\$2013

### Chronic adult toxicity

A 10-day laboratory feeding study investigating the effects of FLU+ TES SC S00 was conducted to assess chronic toxicity to honey bees in accordance with QECD Guideline No. 245.

 $\bigcirc$ 

The study concluded that continuous addibitum feeding at 829.91 mg product/kg diet over a period of 10 days led to 44,68 % mortality. The LDD50 was determined as 16 \$3 µg product/bee/day. The NOEDD was determined to be 16.73 µg product/bee day. While the control and toxic reference item treatment groups fulfilled validity criteria, the results of the actual main test strongly deviated from the initial non-GLP range Ginding test used to determine the parge of doses that should be tested in the actual study to derive LG70/20/50 values As a result all but the lowest dest item dose yielded 10-day cumulative mortalities  $\geq$  90%. The regression analysis thus refied on only two data points to estimate the LC₅₀ and only a subset of treatments was selected for the Weibull regression as inclusion of all treatments did not show any dose-response telation ship. Details of the study are presented in KCP 10.3.1.2/01, M-753795-<u>02-1</u>.

# Chronic larval toxicity/effects of brood

A honey bee larval to vicity test assessing the effect of FLU + TFS SC 500 on adult emergence following repeated feeding exposure was conducted to address effects on immature honey bee life stages and their development. The 22-day labor dose-reporting test assessed larval and pupal survival as well as adult emergence, following exposure to nominal concentrations of 15.39, 38.48, 96.19, 240.48 and 601.21 mg product Ag diet The corresponding cumulative doses were 2.37, 5.93, 14.81, 37.03 and 92.59 µg product/harva. The 22 day NOED (emergence) was determined to be 37.03 µg product/larva (corresponding NOEC of 240,48 mg product/kg diet), indicating no risk to honey bee development. Details of the study are presented in KCP 10.3.1.3/01, M-738909-02-1.



#### Higher tier risk assessment for bees (tunnel tests, field studies)

Although the findings of the laboratory toxicity tests and the tier 1 risk assessment based on acute lests did not indicate a risk to bees due to the use of FLU+ TFS SC 500, further assessment of the pronic risk to adult bees and larvae is derived through findings from a higher tier study

A semi-field honey bee study (according to EPPO Guideline No. 170(4) (2000)) was conducted with the representative formulation FLU + TFS SC 500 under forced/confined exposure conditions with two applications at 0.56 L prod./ha each (corresponding to 140 g fluopyram/ha and 140 g triftoxystrobin/ha) (KCP 10.3.1.5; M-435338-01-1). The first application to the bee-attractive crop Phacelia taracetificia was carried out at BBCH 59-61 without bees present, while the second application during full of flowering (BBCH 64-65) with bees actively foraging on the crop during application. The application rate used exceeded the foliar spray rate in grapes as proposed in the GAP table by a factor of 2.8 and with applications made to a highly bee-attractive blooming crop under confined exposure conditions, the study represents a worst-case, field-realistic exposure situation for honey bee plonies

No short-term or long-term effects on mortality, colony strength and -development, brood development, food storage, honey bee behaviour, queen survival overall hive vitality and colony health, as well as on overwintering performance were detected at an application rate of 140 g a.s. ha onto flowering Placelia Ś tanacetifolia. L. Ŵ Ŧ

Furthermore, the results of the chemical residue analysis of bee relevant matrices (bowers, nectar and pollen) indicated maximum residues of floopyrain ranging from 1.1. mg a.s. (kg in peetar to 30 mg a.s./kg in pollen. These concentrations are well below the larval NOEC  $gR \ge 520$  mg a. G/kg (SCA 8.3.1.3/01; <u>M-617279-01-1</u>) or the chronic adult NOTC of 3333 mg a.s./kg (KCA 8.3  $\frac{1}{2}$  2/01; <u>M-540072-01-1</u>), indicating that adult bees and larvae would not be experiencing chronic exposure to concentrations of fluopyram exceeding the concentration at which no observable adverse effects would be expected even when foraging on a blooming frop treated with the formulated product FLU + TFS SC 500 at rates well above the proposed for a spray rate for groupes.

Therefore, it can be concluded that the representative formulation FLOU + TFS SC 500 does not adversely affect hone bees and honey be colonies when applied at a rate of 0.56 L/ha during honey

adversely affect hone obees and honey beercolonies when appled at a fate of 0.56 L/ha during honey bees actively foraging on a highly bee-attractive, flewering crop, addicating no risk to honey bee colonies.



- **CP 10.3.1.1** Acute toxicity to bees
- **CP 10.3.1.1.1** Acute oral toxicity to bees

## **Honeybees**

CP 10.3.1.1.1 Acuto	e oral toxicity to bees
Honeybees	
Data Point:	KCP 10.3.1.1.1/01
Report Author:	
Report Year:	
Report Title:	Effects of AE C656948+triftoxystrobin SC 250+250.g/L (acute contact and oral)
Report No:	34491035
Document No:	<u>M-288193-01-1</u>
Guideline(s) followed in	OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute
study:	Oral Toxicity Test, (adopted 21st September 1998); OECD 214: OECD Suidebre
	for the Testing of Chernicals, Honeybeer, Acute Contact Toxicity Test, (adopted
	21st Septemb@ 1998), Equivalent to VS EP OOPPTS Guideline No. 850.3620
	SUPP & & & & & & & & & & & & & & & & & &
Deviations from current	Current Guidelines: OECD 213 (1998) and OECD 214 (1998)
test guideline:	Deviations from OEClo Guidefine 21 3 The guideline specified relative humidity
	range of 50-70% was dot met in is deviation is not expected to have impacted the
	study results." All validity criteria were met
	Deviations from OECD outdeline 214. The guideline specified relative numbering
2	deviation to the guideline specified value of 1 uL to ansure blighte dispersion
K.	These deviations are not expected to have impacted the study results. All validity
- Sector - S	criteria were met
Previous evaluation	No. not previously submitted of the last
GLP/Officiall	Yes, conducted under GLPOfficially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability	Yest & Y

### **Executive Summary**

The purpose of this study was to determine the 20th contact and oral toxicity of FLU + TFS SC 500 (250+250 g/L) to the honey bee *tpis mallifered*L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 50 worker bees were exposed for 48 hours to a single dose of 200.0 µg product/bee by topical application (contact limit test) and 200.0 µg product/bee by feeding (oral limit test; actual dose based offintake of the test item was 208.8 µg product/bee).

The contact test comprised a water control group. In the oral test bees in the control group were exposed to 50 % aqueous sugar solution. In both tests a toxic reference item (dimethoate) was included.

The contact LD value (48 h) was determined to be > 200.0  $\mu$ g product/bee. The oral LD₅₀ value was determined to  $\dot{\Phi} = 208.8 \ \mu g \ product/bee.$ 

The study toffils all validity criteria of the current Guidelines OECD 213 (1998) and OECD 214 (1998).



#### I. MATERIAL AND METHODS

<u>Test item:</u> FLU + TFS SC 500 (250+250 g/L); Specification number: 102000012886; Batch identification: 2007-000441, Sample description: TOX07851-00; nominal content of a.s.: 250 g/L for both fluopyram and trifloxystrobin; analysed content: 21.4 % w/w fluopyram corresponding to 251.5g/L; 21.6 % w/w trifloxystrobin corresponding to 253.5 g/L; density: 1.174 g/mL;

<u>Test species:</u> Honey bee (*Apis mellifera* carnica L.); female worker bees from a healthy and queen-right colony.

<u>Test design</u>: Under laboratory conditions 50 worker bees were exposed for 48 hours to a single dose of  $(200.0 \ \mu g \ product/bee \ by teeding)$  (oral limit test; actual dose based on intake of the test item was 208.8 µg product/bee)

Tap water with 0.5 % Adhäsit (improves spreading of the test proplet on the water-opellent hairs on the thorax of bees) served as the water control group for the contact test. In the oral test bees in the control group were exposed to 50 % aqueous sugat solution. The toxic deference dimethoate Perfection  $\frac{1}{2}C$  400, 400.0 g/L nominal, 414.4 g/L analytical) was applied at nominal dose leads of 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee in the contact test and 0.30, 0.45, 0.08 and 0.05 µg dimethoate/bee in the oral test.

In the contact and oral toxicity tests each treatment group dest item, controls and reference item) comprised 5 replicates including 10 becs each

<u>Application in the contact test</u> A single 5  $\mu$  droplet of the control, test item and toxic standard (vehicle: 0.5 % v/v Adhäsit) was placed on the porsal bee thorax using a Burkard – Applicator following anaesthetization of bees with CQ₂.

Based on practical experiences à 5 ut droplet was chosen in deviation to the gitideline recommendation of a 1 uL droplet, since a higher volume ensured a more reliable dispersion of the test item.

<u>Application in the oral test:</u> The test item and reference item were applied in 50 % ready-to-use syrup (30 % Saccharose, 31 % Glucose, 39 % Fructose). The entreated sugar solution was offered to bees in the control group. The treated diet was offered in soringes, which were weighed before and after introduction into the cages (duration of freeding about 1 hour 20 minutes). After 1 hour and 20 minutes, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food (50 % sugar solution).

Dose levels: Nominal doses of the test item:

200 µg product/bee (oral limit test)

Actual dose of the test iten (oral test): 208.8 pg product/bee

Nominal doses of the reference tem: 0.30, 0.20, 0.15 and  $0.10 \ \mu g$  dimethoate/bee (contact test)

0.30, 0.15, 0.08 and  $0.05 \mu g$  dimethoate/bee (oral test);

Actual doses of the reference item (oral sest): 0.33, 0.17, 0.08 and 0.06 µg dimethoate/bee

<u>Test conditions</u> Temperature 25 °C; relative humidity: 26 - 46 %; photoperiod: 24 h darkness (except during observations)

<u>Statistics</u>: Results obtained with the bees treated with test item and the reference item were compared to those obtained with the control in both the contact and oral tests. The contact and oral LD₅₀ values of the reference item were estimated according to moving average computations (Thompson and Weil, 1952). The LD₅₀ calculation was carried out taking into account the mortality data corrected by control



mortality using Abbott's formula (1925). The software used to perform the statistical analysis was ToxRat Professional, Version 2.09 (®ToxRat Solutions GmbH, © 2005).

Dates of experimental work: April 16th to April 19th, 2007

#### **II. RESULTS AND DISCUSSION**

**Biological findings:** 

Contact test

At the end of the contact toxicity test (48 hours after application) othere was no mortality at 200.0 product/bee. No mortality occurred in the control (water + 0.5 % Adhägit). Ó

No behavioural abnormalities of the bees were observed during the partice trial at 200 µg productibee.

Table 10.3.1.1.1- 1: Mortal	lity and behavioura	al abnormalities	of the bees in	the contact to:	xicity test
			A		SK 4

	After h	After 24 h	After	48 h
Treatment group	Mortality Bebav.	Mortality Behay.	Mortanty ,	Behav. abnorm.
	Mean [%]	S Mean [%)	o ^o Mean	i [%]
Water control	₩ 0.0 % 0.0 Å		Ô 0.0	0.0
Test item [µg product/be				
200.0	0.0		\$0.0	0.0
Reference item [µg a.Qb	pee] $\mathcal{O}^{*}$ $\mathcal{O}^{*}$ $\mathcal{O}^{*}$		L	
0.10			6.0	0.0
0.15	4.9 2.0	10.0 12.0	20.0	4.0
0.20	Q.0 0 2.0 ž	200 \$ 8,0	34.0	0.0
030	14.00 10.0	<b>6.0</b>	72.0	8.0

Results are mean values based on 5 replicates (control, test item and reference item) containing 10 bees each Behav. aknorm. = behaviovral abnormalities

Test item= FLU + TFS SC 500 (250+250 g/L), reference item= dimethoate; water control = tap water

### Oral test

In the oral toxicity test the forminal dose of 200 pg FLUt+ TFS SC 500 (250+250 g/L)/bee corresponded to an actual intake of 208.8 µg product bee. This dose level led to 6.0 % mortality after 48 hours. 2.0 % mortality occurred in the control (50 % sugar solution).

No test item related behavioural abrormanties occurred.





	Afte	r 4 h	After	r 24 h	After	·48 h 🖉	~
Treatment group	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. aboorm.	ð
	Mear	ı [%]	Mean	n [%]	S Mean	n [%]	
Control	0.0	0.0	0.0	0.0	2.0	¢ 00	Ô
Test item [µg product/be	e]		Č.	×,		, 2° 2 %	Í
208.8	4.0	0.0	450	0.0	6.00		ĺ,
Reference item [µg a.s./b	ee]		4	Ő			0 [×]
0.06	0.0	0.0	0.0	Q 0.0 °		Ø.0 C	ř
0.08	4.0	2.0 🖉	Ø″14.0	2. <b>0</b>	~~18,00 [°]	\$ 0.0 ¢	
0.17	36.0	20.0	20.0	~ 20 x			
0.33	82.0	18.00°	98.0 [%]	2.0	Ø <b>8</b> .0	<u>2</u> .0 .	

#### Table 10.3.1.1.1-2: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Results are mean values of 5 replicates (control, test them and the ainiņg Behav. abnorm. = behavioural abnormalities; Test item= FLU + TFS SC 500 (250+250 g/L), perence item=

ĵ, K The endpoints for the contact and shal toxicity test are sho

Table 10.3.1.1.1- 3: Contact as	id ora	l toxicity of	FLU +	TFS SC	500 (250	+ <b>230 g</b> /	l) to	honey	bees
---------------------------------	--------	---------------	-------	--------	----------	------------------	-------	-------	------

Test item	FLU ⁴ TF <b>\$</b> SC 500 (250+250 g/L)
Test species	Honey bee Apis mellifer QL.
Exposure 🖉	Contact O Y O Oral Y
Test duration	
Dose rate [µg poduct/bee]	200.0 × × × × × × × × × × × × × × × × × ×
LD ₅₀ [µg product/bee]	208.8

Reference item

Keterence item The contact and oral BP₅₀ (2^Ah) values of the reference item (dimethoate) were calculated to be 0.30 and 0.14 µg as bee, respectively



Validity criteria:	
The contact and oral t the LD ₅₀ values obtain	tests were considered valid as the control mortality in each case was $\leq 10$ % and $\sim$ ned with the reference item (dimethoate) were within the required ranges.
Table 10.3.1.1.1- 4: Va	lidity criteria
Validity Criteria	Recommended O Obtrained
	Contact Test
Control mortality	Water control $0^{\circ} \leq 10^{\circ}$ $0^{\circ}$ $0^{\circ}$ $0^{\circ}$
	$\begin{array}{c c c c c c c c c c c c c c c c c c c $
	O Q Contact Test O D L
LD ₅₀ of reference	Dimethoate 0.00 - 0.30 µg a.s. Dee 0.30 Qg a.s. Dee
item (24 h)	$\frac{1}{\sqrt{2}} \sqrt{2} \sqrt{2} \sqrt{2} \sqrt{2} \sqrt{2} \sqrt{2} $
The toxicity of FLU + toxicity test on honey	bees 200 (250+250 g/L) was tested in both, an acute contact and an acute oral
The contact LD ₅₀ valu	ue (48 h) agas determined to be 200 \$ µg product/bee. The oral LD ₅₀ value was
determined to be $> 20$	\$ 8 μg product Bee.
Assessment and ass	<u> </u>
The study of its of	the adverse in rich account The
endpoints are:	ata and considered as acceptable and redable, for use in risk assessment. The
LD ₅₀ contact (48 hou	as) > 200.0 μg product/bee
LD ₅₀ oral (48 hours)	$> 208.8 \mu$ goroductbee
4	
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Bumble bees

Data Point:	KCP 10.3.1.1.1/02
Report Author:	
Report Year:	
Report Title:	Amendment no. 03: Fluopyram + trifloxystrobin SC 500 (250+250 g/L): Effects (acute contact and oral) on bumblebees (Bombus terrestris L.) in the aboratory
Report No:	144951105
Document No:	<u>M-738910-04-1</u>
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/P&IRA) US EPA OCSPP 850.3020 550.supp. OECD 246 and 247 (2010)
Deviations from current	Current Guidelines: QECD 246 and 245 (2013)
test guideline:	Deviations from OECD Guideline 246: No prformation on Sumble bee colonies
	concerning size, brood stages and number of adults are reported. The limit dose 4/
	was 250 µg a.s./bee instead of the recommended dose of 100 µg a.s./bee. An
	application volume of 4 µL was chosen in deviation to the guideline 2
	recommendation of \$41L. These deviations are not expected to have impacted the
	study results. All validity criteria of the current guideline were met.
	concerning size broad stages and number of bdults are reported. The limit dose
	1000000000000000000000000000000000000
	designing are not expected to have impacted the study results. All validity criteria
	at the convent subdeline were met
Previous evaluation:	No not previously submitted
GLP/Officially	Yes, conducted under GL9/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Rehability	Yes a francisco de la companya de la
g di	
Executive Summary	

Executive Summary

Ô The purpose of this study was to determine the acute contact and oral toxicity of FLU + TFS SC 500 (250 + 250) to the pumble bee (pomby's terrestits L.) Mortality of bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions 50 worker sumble bees were exposed for 48 hours to a single dose of 1185.12 µg product/bumble bee by topical application contact limit test) and to a single dose of 1185.12 µg product bumble beedby feeding (or limit test; actual dose based on the intake of the test item was 1216.23 jug product/bumble bee).

The contact test comprises a water control group (tap water with 0.1 % v/v Triton X-100). In the oral test bees in the control group were exposed to 50 % w/v aqueous sucrose solution. In both tests a toxic reference item (dimethoate) & as included.

The purpose of the analytical part of this study was to verify the concentrations of the active ingredients (fluop) am and triff systrobin) in the single contact application solution (limit test) and in the single oral feeding solution (limit test).

In the contact toxicity test the LD50 value (48 h) of FLU + TFS SC 500 (250+250 g/L) was estimated to be 1185 2 µg production bee. The contact NOED value (48 h) was calculated to be ≥ 1185.12 µg product/bumble bee.

The oral LD₅₀ value (48 h) of FLU + TFS SC 500 (250+250 g/L) was > 1216.23 μ g product/bumble bee. The oral NOED value (48 h) was calculated to be \geq 1216.23 µg product/bumble bee.



The study fulfils all validity criteria of the current OECD Guidelines 246 and 247 (2017).

I. MATERIAL AND METHODS

<u>Test item:</u> FLU + TFS SC 500 (250+250 g/L) (Origin Batch No.: EV57002709; Specification No.: 102000012886; TOX. No.: 21159-00; active substance fluopyram: nominal content: 250 g/L, analysed content: 21.1 % w/w (246.6 g/L); active substance trifloxystrobin: nominal content: 250 g/L, analysed content: of 21.3 % w/w (248.6 g/L); density: 1.169 g/mL (20 °C).

<u>Test species</u>: Adult bumble bees (*Bombus terrestris* L.); adult female worker bumble bees from healthy and queen-right bumble bee colonies obtained from a commercial bumble bee breeding compane After collection from the hive the bumble bees were kept, individually in cylindrical latticed plastic cages. Middle sized bumble bees were selected visually and randomly distributed to the treatment groups. Each bumble bee was weighed individually after anesthetisation with COC to prove a consistent distribution among the treatment groups. Bumblebees were acclematised to test conditions frontaet test: 24 hours; oral test: 45 hours) with *ad libitum* access to untreated 50% w/v sucrose solution.

<u>Test design</u>: Acute contact toxicity of FUU + TFS SC/500 (250+250 g/L) to addit bumble bees was assessed by exposing 50 worker bumble bees to 1185/12 µg product/bumble beed issolved in tap water containing 0.1 % v/v Triton X-100 Contact limit test). Additional groups of 50 and 50 addit bumble bees each were assigned to either water control (tap water containing 0.1% v/v Triton X-100) or a reference item (10 µg dimethoate/bumble bee) treatment group, respectively.

Acute oral toxicity of FLU + TES SC 500 (250+250 g/L) to adult bumble bees was assessed by exposing 50 worker bumble bees to 1185.12 ug product/bumble bee in 50 % w/x sucress solution (oral limit test). This nominal treatment dose corresponded to an actual mean oral dose of 12.23 µg product/bumble bee based on the actual mean intakes of the set item. In addition, 50 and 30 adult bumble bees each were assigned to either a water control (50 % w/y sucross solution) and reference item (mean oral dose of 4.3 µg dimethoate bumble bee) freatment group, respectively. Bumble bees which did not consume at least 80 % of the mean food uptake per treatment group were excluded from the evaluation (Test item: n= 20, Water control: n=45, Reference item; n=19/

<u>Application in the contact test</u>: In the contact to xicity test the test item was dissolved in tap water with 0.1 % v/v Priton X-100 and applied as one 4 μ L droplet onto the dorsal thorax of bumble bees using a calibrated pipette. The test end item was applied as one 4 μ L droplet of dimethoate, dissolved in tap water with 0.1 % v/v Priton X-100. For the control, one 4 μ L droplet of tap water containing 0.1 % v/v Triton X-100 was used. A 4 μ L droplet was chosen instead of 2 μ L due to a technical error during the preparation of the test item application solution.

<u>Application in the oral test</u> The test item and reference item were applied in 50 % w/v sucrose solution, which was used as carried food in the oral test. For the control pure 50 % w/v sucrose solution was offered to the bumble bees. Approximately 40 µL food solution per bumblebee was provided in syringes which were weighed before and after introduction into the cages in order to determine the exact consumption. After a maximum of 4 hour, at syringes containing remaining food were removed, weighed and afterwards replaced by fresh, unreated food. The calculation of the target dose was based on 40 mg food uptake. The ingester consumed oral doses were calculated based on the measured consumption.

In the acute contact and oral test mortality and sub-lethal effects were assessed at 4, 24 and 48 hours after treatment



Dose levels:

Nominal doses of the test item:

Actual dose of the test item (oral test):

Nominal doses of the reference item:

1185.12 μg product/bumble bee (oral limit test) actual 1216.23 µg product/bumble bee (based on the food intake) 10 µg dimethoate/bumble Bee (contact limit test)

1185.12 µg product/bumble bee (contact limit test)

4.0 µg dimethoate/bumble bee (oral light test)

Actual doses of the reference item (oral test): 4.3 µg/jimethoate/bumble bee

Test conditions: Temperature: 22.7 – 25.4 °C; repartive humidity: 44 ge 65 %; protoperiod: darkness (except during observation).

Statistics: Results obtained from the bumble bees treafed with the test item and the reference item were compared to those obtained from the control in both the contact and orabiest. For the evaluation of the results of the oral test, bumble bees which did not consume at least 80 % of the mean food aptak oper treatment group were excluded from the evaluation of prortality and behavioural abnormalities, as well as from the calculation of the final actual doses in the test item treatment group &cute contact and oral toxicity endpoints (e.g. LD50, LD20, QD10), yould not be determined from the limit modelling, as the mortality in the test item treatment groups did not reach or exceed 10 % at the end of the test. The contact and oral NOED of the test item was estimated using the multiple sequential sher best after Bonferroni-Holm (pairwise comparison, one-sided greater, $\alpha = 0.05$), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. The software used to perform the statistical analysis was To Rat Professional, Version 3.2.1 (® ToxRat Solutions GurbH).

Analytics: Freshly prepared application solution and feeding solution (20 m) per specimen) of the control and the test diem treatment group were sampled in duplicates on the day of application. The

ac ag the , α = 005 .ity prior to , al. Version 3.2.1 uon solution and feedin a group were sampled in du . (by using L C-MS/MS-method. . (b) using L C-MS/MS-method. . (b) using L C-MS/MS-method. . (c) August 23rd, 2019 (biological phase) . (c) August 23rd, 2019 (biological phase)



II. RESULTS AND DISCUSSION

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANTE/2020/12830, Rev.1.

In the oral test the mean recoveries of the active ingredients fluopyram and willoxystrobin in the est item spiked feeding solutions were 101 % and 104 %, respectively. In the contact test the recoveries of the active ingredients fluopyram and trifloxystrobin were 46,% and 50 %, respectively, ducto a technical error during the preparation of the test item application solution. With regard to the increased application of lume (4 µL instead of 2 µL) and recovery rates of approximately 50 % the required application dose was reached.

No residues of fluopyram and trifloxystrobin were found in the control solutions in the oral and contact test above the limit of detection (fluopyram-LOD: 0.04 μg as./L; μ loxy bobin-LOD: β9 μg as./L)

Table 10.3.1.1.1-	5:	Analytical	results	ć
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Test system	Nominal test concentration	- Covery
Contact Test	1185.12 125°g fluepyram/L application solution 1185.12 26 g trifloxystebin/L application solution	50 % A 46 % A
Oral Test	6.3 g sufloxystrobin/kg feeding solution	× 101 % 104 %

A technical error curred during the preparation of the test them application colution of the contact test. A 4 µL droplets was applied instead of 2 at to reach the required application dose in the contact test. With regard to the increased application volume and recovery rates of approximately 50 % the required application dose was reached.

Biological findi

Contact Test:

At the end of the contact toxicity teo (48 hours after application) there was 2.0 % mortality at 1185.12 µg product/bumble bee. No portality occurred in the water control group (water with 0.1 %





Table 10.3.1.1.1- 6: Mortality and behavioural abnormalities of the bumble bees in the contact toxicity

	lesi					Ű	×
	Afte	r 4 h	After	24 h	After	r 48 h 🔊 🖉	-S
Dose	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Morality	Beôav. abhorm. Ô	0°
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	M@an [%]	Mean [%]	
Water control	0.0	0.0	0.0	0.0	A 0.0	0 0 0 V	Ê,
Test item [µg pro	duct/bumble bee]	Ğ	Ő	í X		<i>.</i> C
1185.12	0.0	0.0	0.0	0.0	2.0	J 0.0	Ô
Reference item [µ	ıg a.s./bumble be	ee]	Ą	Â,			×
10	0.0	56.7	Q ⁷ 6.7	<u></u> ∧100.0 0 [°]	93.3 °	¢100.0	

The contact test was conducted at 250 µg fluopyram per sumble bee. The concentration was based of the active substance fluopyram, therefore, 1185.12 µg product/bumble bee was equivalent to 250 µg fluopyram@umble.

Application volume: 4 µL/bumble bee Results are mean values of 50 individuals per treatment group (control, test item) and 30 individuals for the reference item treatment group

Behav. Abnorm = Behavioural abnormalities

Test item = FLU + TFS SC 500 (250+250 g/By reference item; dimethodate; water control = tap vater contraining 0.1 % Triton X-100

Oral Test

At the end of the oral toxicity test 48 hours after application) 1216.23 µg product/fumble bee resulted in no mortality. In the water control treatment group (50% w/v sucress solution) 2.2% mortality occurred. No test item induced behavioural abnormalities were detected.

ð S		r4h 🖗 👸	After	§24 h 🕡	After	48 h	
Treatment group	Mortality	Behav y Dabnorm.	Mortality	Behav. ^apnorm.	Mortality	Behav. abnorm.	
	Mean	n [%] 🔊 🔬) Mean	a [%]	Mean	[%]	
Control	0.0	× 0.0 ×	00.0	0.0	2.2	0.0	
Test item [µg product/be	Test item [µg product/bg]						
1216,23	Č0.0 ~~	~0 <u>.0</u> ~	0.0	0.0	0.0	0.0	
Reference item [µg a.s./bed $\ \ \ \ \ \ \ \ \ \ \ \ \ $							
Q4.3	\$ <u>6</u> 9	Ø 1000	100.0	-	100.0	_	

Table 10.3.1.1.1- & Mortality and behavioural abnormalities of the bees in the oral toxicity test

^A The oral test was conducted at 250 up fluoptrain per Dumble bee. The concentration was based on the active substance fluoptrain, the effore, 1206.23 up product/bumble bee was equivalent to 256.56 fluopyram/bumble bee. Results are mean values of 50 individuals per treatment group (control, test item) and 30 individuals for the reference item treatment group

Mortality mean= thean of dead individuals for treatment group, considering only those bumble bees, which achieved at least 80 % of the mean food up take per treatment group (test item: n= 20, water control: n= 45, reference item: n=19)

Behav. Abnown = Behavioural abnormalities

Test item FLU FLU

The endpoints for the contact and oral toxicity test are shown in the table below.



Table 10.3.1.1.1-8: Contact and oral toxicity of FLU + TFS SC 500 (250+250 g/L) to bumble bees

Test item	FLU + TFS SC 500 (250 + 250)) 🖉 🗞
Test species	Bumble bee Bombus terrestris	L.
Exposure	Contact (tap water containing 0.1 % v/v Triton X-100)	Oral (50 % w/v succose solution) (based on recorded consumption considering bumble beer with food uptake of at least 80 % of the mean uptake per treatment group %
Target (nominal) dose rates [μg product/bumble bee*]	1185.12	0185.12
Actual dose rates [µg product/bumble bee*]	1185.12	
Test duration	24 h 4 h 5 h	
$LD_{50, 20, 10}$ [µg product/bumble bee] ^{2, 3}	> 11785.12 > 185.12	>4216.23
NOED [μ g product/bumble bee] ^{2,4}		≥ 1216.23
LOED [μ g product/bumble bee] ^{2,4}	>1185.12 1185.12 1185.12	0121626 C > 1216.23

The contact and oral tests were conducted at 250 µg flugyram per bumbQ bee. The conceptrations were based on the active substance fluopyram/therefore, 1185 Qug product/bumble beg was equivalent to 250 µg huopyram/bumble W bee)

bee). For the 1216.23 μg product/bumble bee tost item greatment group 20.bumble bees were considered for the evaluation. 1 2

Results obtained from test item treated groups were compared to those obtained from the water control treatment group. 45 bumble bees were considered for the evaluation for the water control treatment group.

3 As the test item treatment groups in the contact and shall test did not show more lity above 50.0, 20.0 and 10.0 %, no

statistical evaluation on the LDsc DD20 and LD10 was carried out. The NOED/LOED was estimated using Fisher's exact Fest after Bonferron Holm pairwise comparison, one-sided 4 greater, $\alpha = 0.05$).

Reference

The bumble bees of the reference item group were treated with 10 µg dimethoate/bumble bee in the contact test and 4.3 g dimethoate bumble bee in the oral test. The reference item mortality of 93.3 % and 100.0 % in the end of the contact and oral test (48 hours after application) was within the required range.

<u>• and the contact and oral posicity test were considered valid as the control mortality in each case was $\leq 10\%$ and $\geq 50\%$ in the reference item?</u>



	K	ecommended	Obtained
		Contact Test	
Control mortality	Control	$\leq 10 \%$	\$ 0.0 % K
		Oral Test	
	Control	$\leq 10\%$	
		Contact Test	
LD ₅₀ of reference	Dimethoate	≤ 50 %	93.3 4 5
item (24 h)		Oral Test	
	Dimethoate	$\geq 50\%$	
The toxicity of FLU - on bumble bees.	+ TFS SC 500 (250+2	U. CONCLUSION	contact and oral toxicity test
The contact NOED v value was $> 1185 12$	alue was cauculated	to be $\geq 1180, 12 \mu$ g product bu	impre des Ine contact LD ₅₀
The oral NOED value	e was calculated to be	≥1216.23 ug product@umbte	bee. The oraDLD ₅₀ value was
estimated to be > 121	6.23 µg product/bund	Ble been of the second	
	V A Q		
According to and ac	Sucio Shy of Sicon		s. 9
Assessment and co	NEIUNIOMEDV 2019011C214		S Y
Assessment and co	ata are condered a	Let: S S O	A in risk assessment The
The study and its c endpoints are: c	lata are considered as	s acceptable and reliable for u	the in risk assessment. The
The study and its contact and	lata are considered as 3 3 3 3 3 3 3 3 3 3	s acceptable and reliable for u	Se in risk assessment. The
Assessment and co The study and its c endpoints are: C LD ₅₀ contact (48 hg	lata are considered as ars) $\approx 185.12 \mu g$ pro	s acceptable and reliable for u duct/mamble bee	in risk assessment. The
Assessment and co The study and its c endpoints are: LD ₅₀ contact (48 hg LD ₅₀ oral (48 hours)	ata are considered as ars) $\approx 185.12 \mu\text{g}$ produce $> 1216.23 \mu\text{g}$ produce	t: S acceptable and reliable for u duct/mamble bee S C	See in risk assessment. The

Table 10.3.1.1.1-9: Validity criteria



CP 10.3.1.1.2 Acute contact toxicity to bees

Honeybees

<u>Honeybees</u>	
Data Point:	KCP 10.3.1.1.2/01
Report Author:	
Report Year:	
Report Title:	Effects of AE C656948+trifloxystrobin SC 250+250 g/L (acute contact and oral) on honey bees (Apis mellifera L) in the laboratory
Report No:	34491035
Document No:	M-288193-01-1
Guideline(s) followed in	OECD 213: OECD Guideline, for the Testing of Chemicals Honeybees, Acute
study:	Oral Toxicity Test, (adopted 21st September 1998); OECD 214 DECD Guidel Bre
	for the Testing of Chemicals, Honeybeer, Acute Contact Toxiery Test, (adopted
	21st September 1998; Equivalent to US EPA OPPTS GuideOne No. 850.3020
	SUPP
Deviations from current	Current Guidelines, OEC 213 (1998) and OECD 214 (1998)
test guideline:	Deviations from OECD Guideline 213. The guideline specified relative huminity
	range of 50-74% was not met, This deviation is not expected to have impaced the
	study results All validity esteria were more
	Deviations from OECD Guideline 214: The guideline-specified relative humidity
	range of \$0-70% was not met. On application colume of 5 uL was chosen in
	deviation to the guideline-specified value of uL to ensure reliable dispersion.
	These deviations are not expected to have impacted the study results. All validity
	criteria were met
Previous evaluation:	No. not previously submitted a start of the second se
,	
GLP/Officially	Yes conducted under GLAOOfficially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Rehability:	$Yes \sqrt{2}$

For study summary on acute contact and oral foxicity of ELC +TFS SC 500 to honey bees, please refer to CP 103.1.1.1/01.



Bumble bees

			° »
Data Point:	KCP 10.3.1.1.2/02	*	S S
Report Author:		\sim	
Report Year:	2020	Ş 4	
Report Title:	Amendment no. 03: Fluopyram + trifloxystrobin SC 500 (acute contact and oral) on bumblebees (Bombus terrestr	(250+250 g/L): Effe s L.) in the aborator	icts Ny Dia
Report No:	144951105		
Document No:	<u>M-738910-04-1</u>		
Guideline(s) followed in	Regulation (EC) No. 1107/2009		
study:	Directive 2003-01 (Canada/Po/RA)		
	US EPA OCSPP 850.3020 \$50.supp.		
Deviations from auront	OECD 246 and 247 (20kp ⁻)		, Ç
Deviations from current	Deviations from OECD Guideline 244: No statemation	on frimble bee colon	
test guidenne.	concerning size brood stages and symber of adult are re	ported The limit do	se d
	was 250 µg a s Abec instead of the recommended dose of	100 ug a.s./be@An	
	application voltime of 4 µL was chosen in deviation to th	e guideline (S ^o
	recommendation of 2 JL. These deviations are not expec	tetto have impacted	l the
	study result. All vandity conteria of the cuttent guideline	were met.	
	Deviations from OECD Guideline 247: No information	n bumble bee eoloni	es
	concerning size brood stages and number of adults are re	ported. The limit do	se
	was 250 µg a s./bee instead of the recommended dose of	100° µg a \$% bee. The	se
	deviations are not expected to have impacted the study re	sults. All validity cri	iteria
Previous evaluation:	No not previously submitted		
		65°	
GLP/Officially	Yes conducted under GL® Officially recognised testing	facilities	
recognised testing		, 	
facilities:			
Acceptability/Rehability	Yes y y y y		
O A			
For study summary on ac	ute contact and oral toxicity of FLU2+TFS SC 500 to l	oumble bees, pleas	e refer
to CP 100.1.1.1/02.		, r	
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Data Point:	KCP 10.3.1.2/01
Report Author:	
Report Year:	
Report Title:	Amendment no. 01 to final report - Fluopyram + trifloxystrobin SC 500 (250+250
	g/L): Chronic oral toxicity test (10-day feeding) to the honey bee, Apis mellitera
	L. under laboratory conditions
Report No:	S19-20249
Document No:	<u>M-753795-02-1</u>
Guideline(s) followed in	Regulation (EC) No. 1107/2009 (Oct 2009) 🖉 🖉 🖉
study:	OECD Guideline for the Testing of Chemicars Nos245 (2017).
	SANCO/3029/99, rev. 402000)
Deviations from current	Current Guideline: OECD 245 (2017) C C C C C C C C C C C C C C C C C C C
test guideline:	Deviations: None. Abvalidite criteria were thet.
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted ander GLP/Officially tecognised testing facilities 🔬 🖉
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q ^Y A A A A A A A A A A A A A A A A A A A

CP 10.3.1.2 Chronic toxicity to bees

Executive Summary

The objective of this study was to determine the chromo oral toxicity (LDDs/LCs, and NOEDD/NOEC) of (FLU+TFS SC 500 (250+250 g/L)) applied on reconsecutive days to young adults of the honey bee (*Apis mellifera* L.).

Worker honey bees (*Apis mellifera* L.) aged two days or less were stally exposed to a daily application of FLU + TFS SO 500 (250+250 g/L) diluted in the bee food (50 % w/waqueous sucrose solution with 0.1 % Xanthan) at nominal concentrations of 26557.26, 13278.63, 6639.32, 3319.66, 1659.83 and 829.91 mg product/kg feeding solution, corresponding to 343.64 446.90, 82.36, 51.77, 27.17 and 16.73 μ g product/bee/day (actual doses) for ten consecutive days. Two control groups, an untreated control (50 % w/v aqueous sucrose solution) and a thickener control (50 % w/v aqueous sucrose solution) and a thickener control (50 % w/v aqueous sucrose solution) with 0.1% Xanthan) as well a reference item (dimethorate) were included.

Mortality and behavioural abnormalities were assessed daily, except for the reference item group, as it can be assumed that moribund and affected been of that group will die by the end of the test. The concentration of the active substances theopy an and trifloxystrobin in the feeding solutions was verified analytically.

The LDL 5_0 and LC₅₀ was determined to be 5.95 up product/bee/day and 902.13 mg product/kg feeding solution, respectively. The DD_{1000} and $CC_{10/20}$ were not determined.

The NOEDD and NOEC were determined to be < 16.73 μ g product/bee/day and < 829.91 mg product/kg feeding solution, respectively $\sqrt{2}$

The study fulfils all validity criteria of the current Guideline OECD 245 (2017).

I. MATERIAL AND METHODS

<u>Test item </u>(FLU ⁽⁴⁾ TFS SC 500 (250+250 g/L); Specification No.: 102000012886; Batch No.: EV57005709; TOX No.: 21159-00, active substance fluopyram: nominal content: 250 g/L, analysed content. 21.1 % w/w (246.6 g/L), active substance trifloxystrobin: nominal content: 250 g/L, analysed content: 21.3 % w/w (248.6 g/L), density: 1.169 g/mL



<u>Test species:</u> Honey bees (*Apis mellifera* L.); freshly emerged young female worker bees (max. 2 days old); healthy, disease-free and queen-right honey bee colonies.

Test concentrations and dose levels:

Test item concentrations: 26557.26, 13278.63, 6639.32, 3319.66, 1659.83 and 829.91 mg product/sg feeding solution

Actual test item doses: 343.64, 146.90, 82.36, 51.77, 27.17 and 16.73 µg product/bee/day

Reference item concentration: 0.90 mg dimethoate/kg feeding solution

Actual reference item dose (calculated based on uptake of feeding solution); 0.0130 dimethoate/bee/day

An untreated control (C1, 50 % w/v aqueous success solution) and a thickener control (C2, 50 % w/v aqueous success solution with 0.1 % Xanthan) were also assessed.

Each group (test item, controls and reference item) comprised 5 replicate Containing to bees each. L.°

<u>Test design</u>: In a 10-day chronic toxicity feeding test max two days old worker honey bees (*Apis mellifera* L.) were orally exposed to a daily application of EL = TFS SC 500 (250+250 c/L) diffied in the bee food (50 % w/v aqueous sucress solution with 0.1. X anthan).

For the collection of honey bees, brood combs with capped cells were taken from outside hives and incubated under controlled environmental conditions in a climatic chamber in darkness. One day prior to test starting, the newly hatched worker bees were randomly collected directly from the frames, introduced into the test units and kept under test conditions until the start of the test. The acclimatisation period lasted from collection to the start of the test. During this period, bees were fed *ad libitum* with 50% w/v sucrose solution.

A fresh test item stock solution was obtained daily by mixing a defined arount of test item with a defined-amount of 50 % w/v aqueous sucrose solution with 0.1 % Xanthan Gum. That stock solution was used also as the highest test item concentration solution. The remaining test item concentrations were freshly prepared every day by mixing anquots of the stock solution with a defined amount of 50 % (w/v) aqueous sucrose solution with 0.1 % Xanthan Gum. The remaining test item concentrations were freshly prepared every day by mixing anquots of the stock solution with a defined amount of 50 % (w/v) aqueous sucrose solution with 0.1 % Xanthan Gum. The reference item stock solution was prepared daily and the respective feeding solutions were also prepared daily by mixing aliquots of the stock with a defined amount of 50% (w/v) aqueous sucrose solution. The respective feeding solutions (test item, control and reference item) were provided *ad libitum* in a plastic syringe, which had been weighed before application? The feeders remained in the cages for about 24 h. The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units.

Mortality for all the groups was assessed on a daily basis over the test period. Behavioural abnormalities were recorded at each observation interval, except for the reference item group, as it can be assumed that mortbund and affected bees of that group will the by the end of the test.

The daily food consumption was corrected by subtracting the mean evaporation figure of each day of application.

<u>Test conditions</u> Temperature: 32.9 - 33.7 °C; Relative humidity: 50.1 - 61.7 %; Photoperiod: 24 h darkness (diffuse artificial light only during handling and assessments).

<u>Statistics</u> Results obtained from bees treated with the test item were compared to those obtained from the thickness control group. A statistical significance of $\alpha = 0.05$ was considered for all tests, except where stated. To estimate the LC₅₀ and the LDD₅₀, a Weibull regression was carried out on a subset of four treatments (T1-T4). A subset of treatments was selected because including all the treatments did not show any dose-response relationship, and it was considered that the model did not reflect the general behaviour of the data. The results of this analysis were compared with the results of an estimation using the Spearman-Kärber method on the whole dataset. The LOEC and the NOEC were estimated by a step-down Cochran-Armitage test. The LOEDD/ NOEDD were calculated as the consumed dose in the group



with a concentration of test item equal to the LOEC/NOEC. The statistical calculations were performed with the computer program ToxRat Professional 3.2.1 and 3.3.0. \mathbb{Q}_{p}°

<u>Analytics:</u> All final diets of the control and test item treatment group were sampled in duplicate as analysis and retain samples directly from the prepared diet. The chemical analysis was performed by using LC-MS/MS detection.

II. RESULTS AND DISCUSSION

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANTE/2020/12830, Rev.1.

The analytical dose verification of the larval diet from day of until day 6 resulted for flyppyram in mean recoveries of 80 to 112 % considering combined results for main and retain samples. For trifloxystrobin the mean recoveries ranged between 89 - 98 % considering combined results for main and retain samples.

No residues were found in the control samples above the Limit of Quantification for fluorytam (LOQ: 0.01 mg a.s./kg) and above the Limit of Detection for torlloxy trobin (LOD 9.003 mg a.s./kg).

	· ~ ~ ~ ~			, ,
Treatment group [mg product/kg	Nominal 🖉	Mean measured concentration	Daily recovery range A	Mean recovery from target
diet]	[mg/a.s./kg/liet]	[mg a.s./kg diet]	[%] _@	[%]
Control				-
26557/26	5602,24 A	5879.90	68-144 (A) 193 - 154 (R)	105 (A) 137 (R) Combined: 112
13278.63	2801.12	2691.53	81 - 126 (A) 98 (R) ^B	96 (A)
6639.32	1409.56	1256.31	72 – 117 (A) 104 (R) ^C	90 (A)
3319.60		657.66	80 – 117 (A)	94 (A)
169.83	359.14 A	27#.52	54 - 90 (A) 81 - 108 (R)	78 (A) 95 (R) Combined: 87
829.91		125.35	54 - 87 (A) 80 - 101 (R)	72 (A) 88 (R) Combined: 80

able 10.3.1.2- 1:	Analytical results	for fluopyram
able 10.5.1.2- 1:	Anarytical resums	Internoham

LOD: Limit of Detection. fluops am = 0.003 mga.s./kg

LOQ: Limit Quantification Huopycam = 0.00 mg a.s./kg diet.

(A): Main samples

(R): Retain samples

Retain somples (R) were analysed for content confirmation when main samples (A) resulted in recoveries outside the 80 - 120% range of the recommended nominal concentration

^B Result based on the re-analysis of the only sample (D8-T5) which had to be re-analysed due to a recovery of 72% of nominal.

^C Result based on the re-analysis of the only sample (D1-T4) which had to be re-analysed due to a recovery of 72% of nominal.


Treatment group [mg product/kg	Nominal concentration	Mean measured concentration	Daily recovery range ^A	Mean recovery from targer	<i>S</i>
diet]	[mg a.s./kg diet]	[mg a.s./kg diet]	[%] 📎	[%]	P
Control	0	< LOD	- 4	4 . Q	
26557.26	5647.68	5264.57 Č3	71 - 1181A) 108 - 1⊈9 (R)	©3 (A) Q 13 (R) Combined: 98	? ?
13278.63	2823.84	2573.01	82,099 (A) 2002 (R) ^B ≼		ó
6639.32	1411.92	1261,58	$2^{-97}(A)$ $0^{-97}(A)$ $0^{-97}(A)$	89 (A)	/
3319.66	705.96	656.1 <u>8</u> °	80 ⁻⁷ 103 (0x)	× 3(A)	
1659.83	352.98	-308.22 	€ 65 - 96 (A) 85-109 (B)	 √ 87 (Å) 98 (R) ✓ 0 ✓	
829.91	176.49	1457.79 5 0 0 1457.79	×63 – 161 (A) © 88 – 136 (R) © 88 – 136 (R)	84 (A) 102 (R) Combined: 93	
LOD: Limit of Detection	on: 0.003 mg a.s./kg 🖉) V	
LOQ: Limit of Quantif	ication: triflox@strobin \$0.	01 m@a.s./kg 2		4	
(A): Main samples			JU ^V Q A	O.	
(R): Retain samples		O' AY . O		<i>م</i>	

 Table 10.3.1.2- 2:
 Analytical results for trifloxystrobin

Biological results Summary of mean mortality and toxicity OFFLU + TFS & 500 (250+250 g/L) to adult honey bees after 10 days of erronic exposure:



	Dees			a)		
Tuesday	Daily dose	Concentration	A	t day 10 🔊 🖉	Ş	
Ireatment	Consumed ^A	Concentration	Mean mortality	Corrected montality	y	
group	[µg prod./bee/day]	[mg prod./kg diet]	[%]			
Control	-	-	6.0		80	
Thickener control	-	- &	6.0		2	
	343.64	26557.26 💱	100		Å	
Test item	146.90	13278,53	× 98	Q7.87 0 \$	0 ≰	
(FLU+TFS	82.36	663 9 32	^Q 1005°	م م الم 100.00 م		
SC 500 (250+250	51.77	339.66	~ 100			
g/L))	27.17	×1659 \$ 3	کي 90 <u>کي</u>	\$9.36		
	16.73	4 829.91 €	480	[°] 44.6		
Reference	[µg a.s./bee/day]	[mg ax./kg diet]	A Ô			
Item	0.0130	6.90 ×	0100 ×	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		
	Endpoints 2 2 2 2 2 2 20 d b					
	LDD ₅₀ [µg prod./bee/dag	·] (95 % C.I.)		© 16:95 @13.66 – 19.47)		
	LDD20 [µg4prod./bee/day			Or No.		
Test item doses	LDD10 frig prod./bee/dag			n.d.		
	LOFDD [40 prod Dee/d			16.73		
	NOEDO [µg pîrsd./beed			< 16.73		
ð	LCA [mg pood./kg@eedin	golution (95 @C.I.		902.13 (684.03 - 1054.71)		
	LC20 mag prot kg feorin	g solution]		n.d.		
Test item concentrations	LCD [mg prod./kg/feedin	g solution]		n.d.		
_	LOEE Img ptod./kg.feed	ing solution]		829.91		
~	NØEC [10g prod]kg fee	hing solution]		< 829.91		

Table 10.3.1.2- 3:	10-day chronic oral toxicity test with FLU +TFS SC 500 (250+250 g/L) to young hone	зy
	haas		

Results are mean values of 5 replicates containing 10 bees each, Calculations are corrected for evaporation Corrected mortality (according to Arbort (1925), medified by Schneider-Orelli 1947) Ì

- Confidence interval C.I.:
- Ø n.d.: 4^{thot} determined 4^{thot} 5^{thot} 6^{thot} measured base on consumed feeding solution
- Estimated using Weibull analysis conditering asubset of four treatments (T1-T4) В

Ð

Bees were recorded as affected and moribund in all the test item treated groups, on different assessment days, starting of day 2 after the application (assessment 2) until the end of test period (assessment 10).

The daily food consumption was corrected by subtracting the mean evaporation figure of each day of application. Taking into account the actual food uptake, the bees consumed doses of 343.64, 146.90, 82.36, **6**.77, 27.17 and 16.73 μg product/bee/day, which caused mortalities of 100, 98, 100, 100, 90, and 48 % respectively, after 10 days.



In the test item group the food consumption ranged between 11.06 and 20.15 mg feeding solution per bee per day. A decreasing consumption of feeding solution was observed for increasing concentrations of test item. In the control and thickener control the food consumption was 22.04 and 22.20 mg feeding solution per bee per day, respectively.

As all test item concentrations showed recorded mortalities of greater than 20%, the L could not be reliably estimated.

Reference item

The reference item (dimethoate) was administered in one dosage of 0,0130 frg a.s. bee/day (actua increasing mortality leading average intake based on food consumption) which caused a continuously to 100 % mortality at day 10.

Validity criteria:

The in this study. uiderine All validity criteria of the current OEC

Table 10.3.1.2- 4: Validity criteria		W
Validity criteria according to OECD GD 243 (2017)	Recommended	Obtained
	Control ↓ 15.00	6.0 %
Mortanty after 10 days of exposure	Thickener Control <	6.0 %
Mortality after 10 days of seposure 2	Dimethoate 50 %	100 %

TFS SG 500 (250+250 g/L) was tested on young adult honey bees The chronic oral toxicity of PUU (Apis mellifera L.) in a 10-bay feeting stody under laboratory conditions.

The LDD₅₀ and LQ₅₀ were determined to be 16.95 µg product bee/day and 902.13 mg product/kg feeding solution, respectively The LDD_{10/20} and LO $_{0/20}$ could not be determined due to mathematical reasons.

The NOEDD and NOEC were determined to 7% µg product/bee/day and < 829.91 mg product/kg feeding soution, respectively

Assessment and conclusion by applicant

The study and its data are considered as acceptable and reliable for use in risk assessment. The ~O[®] endpoints (10 days) = 16.95 µg product/bee/day LDD₅₀ Ø davs 두 902 3 mg product/kg diet



CP 10.3.1.3	Effects on honey bee development and other honey bee life stages
CI 10.5.1.5	Effects on noney bee development and other noney bee me stages

Data Point:	KCP 10.3.1.3/01
Report Author:	
Report Year:	
Report Title:	Amendment no. 01: Fluopyram + trifloxystrobin SC 500 (250+250 g/L,g/L):
	Honey bee (Apis mellifera L.) larval toxicity test following repeated posure
	under laboratory conditions
Report No:	S19-21288
Document No:	<u>M-738909-02-1</u>
Guideline(s) followed in	Regulation (EC) No. 1107/2009 (Oct 2009)
study:	OECD Guidance Document 339 (2016)
	SANCO/3029/99, rev.4 (2000)
Deviations from current	Current Guidance Document: QECD 259 (2016)
test guideline:	Deviations: None. Abvalidite criterie were net.
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted ander GLP/Officially tecognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q A A A A A A A A A A A A A A A A A A

Executive Summary

The purpose of this study was to determine the thronic toxicity (ED_{50/20/10}, $EC_{50,20,10}$, NOED/NOEC for adult emergence at day 22) of EU + TFS So 500 (250+250 g/L) applied to honey bee, *Apis mellifera* L., larvae in an *in vitro* test after repeated exposure.

First instar honey bee larvae of *Apis mellifera* L. were transferred from brood combs of 3 hives to polystyrene grafting cells in 48 welk cell culture plates 2 days before the start of the exposure period (D1, grafting) carvae were exposed to 5 concentrations of FLU+ TFS SC 500 (250+250 g/L) (nominal 601.21, 240, 48, 96.19, 38.48 and 5.39 mg product/kg fiet corresponding to nominal cumulative doses of 92.59, 37.03, 14.81, 5 93 and 2.37 mg product/larva) via the larval diet on 4 consecutive days (day 3 to day 6). No additional feeding of the larvae took place after dap 6.

A reference item (denethoate tech at a cumulative dose of 7.39 μ g a.s./larva) and an untreated control were included in the experimental design.

The larval motality was recorded daily from day 4 to day 8. Additionally, other observations such as small body size or unconsumed diet on 100 were noted Pupal mortality was evaluated at day 15 and the adult emergence rate was assessed on day 22.

In the analytical phase of the study, the concentration of the active ingredients fluopyram and trifloxystrobin in the larvar diet of each day of the exposure period was determined. The mean recoveries ranged between 95 - 99% for fluopyram and 104 - 115% for trifloxystrobin in the final diets.

The NOED and LOED were determined to be 37.03 μ g and 92.59 μ g product/larva (based on adult emergence), respectively. The NOEC and LOEC were 240.48 mg and 601.21 mg product/kg diet, respectively. The C₅₀ and ED₅₀ values (based on adult emergence) were determined to be > 601.21 mg product/kg diet and >92.59 μ g product/larva, respectively. Since no clear dose-response relationship was found, noteliable EC₁₆₂₀ and ED_{10/20} for the emergence at 22 days could be determined.

The study falfils all valienty criteria of the current OECD Guidance Document 239 (2016).

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I. MATERIAL AND METHODS

<u>Test item:</u> FLU + TFS SC 500 (250+250 g/L); Specification No.: 102000012886; Batch, No.: EV57002709; TOX No.: 21159-00, active substance fluopyram: nominal content: 250 g/L, malysed content: 21.1 % w/w (246.6 g/L), active substance trifloxystrobin: nominal content: 250 g/L, analysed content: 21.3 % w/w (248.6 g/L), density: 1.169 g/mL

<u>Test species</u>: Honey bee (*Apis mellifera* L.), synchronized first instar ($\mathbb{E}\Psi$, ≤ 30 hours after grafting) larvae originating from three adequately fed, healthy tree of clinical symptoms, queen right bee colonies. The larvae were taken from hives that had not received treatments with chemical substances of for at least one month.

Test concentrations and dose levels:

5 test item groups; concentrations (nominal): 601.21,240.48 96.19, 38.48 and 15:39 mg product/kg diet.

Cumulative doses (nominal): 92.59, 37.03, 4.81, 5.93 and 2.37 g product/larva

One reference item group exposed to a dumulative dose of 7,39 µg dumethoate/larva (concentration of dimethoate: 48.0 mg a.s./kg diet.

One blank control group (untreated feeding diet) was also assessed

Each treatment group (test item, control, reference item) comprised replicates, with 16/larvae each (each colony represented a replicate).

<u>Test design</u>: First instar honey bee larvae of *Apis mellifera* L. were transferred from brood combs of at least 3 hives to polystyrene grafting cells in 48 well cell culture plates 2 days before the start of the exposure period (D1, grafting). From day 3 until day 6 of the test, 5 different concentrations of FLU + TFS SC 500 (250+250 g/L) mixed into the larval diet (aqueous yeast/sugar solution mixed with royal jelly 1:1 (w/w)) were fed to larvae of the test item groups. One single concentration of the reference item dimethoate mixed into the larval diet was fed to the larvae of the reference item group. A blank control (larval diet with water) was included in the experimental design. The volumes and contents of diets are presented in the table below.

		Y Y			
Table 10.3.1.3-1: Feeding scheme					
Test day		32	4 ²	5 ²	6 ²
Artificial diet 🔗 🦽 🖉		B B	С	С	С
Volume of diggper latora 🖉 🔬	2@µL 0°-0°	20 μL	30 µL	40 µL	50 µL
Composition of diets.					
Rôyal jelly 🔍 🎾		50 % w/w		50 % w/w	
Sugar solution 🖉 🖉	©50 % W/W	50 % w/w		50 % w/w	
Composition of sugar solution:					
Gucose 🗸 💭 💭	12% w/05	15 % w/v		18 % w/v	
Fructose \mathcal{O}^{Y}	12 % XOV -	15 % w/v		18 % w/v	
Yeast Oxtract	2 % v/v	3 % w/v		4 % w/v	
1 Day of grafting A	y y				
² Days of exposure 2	~0				
	¥				

The daily feeding volume increased from 20 μ L to 50 μ L diet per larva over the application period. The cuputative feeding volume from day 3 until day 6 of 140 μ L diet per larva was considered for the calculation of the cumulative doses per larva.

Assessment of larval mortality was performed during the larval phase from day 4 until day 8. Pupal mortality was assessed at day 15 and emergence of adults was evaluated at day 22. The presence of unconsumed food was qualitatively recorded on day 8. Other observations and any other adverse effects



were qualitatively recorded to aid in the interpretation of mortality in comparison to the control group.

Test conditions: Temperature: 31.0 - 35.2 °C; relative humidity: day 1 to 8: 54.6 - 100 %, day 8 to 25: 48.5 - 100 %. day 15 to 22: 47.9 - 72.7 %; photoperiod: 24 h darkness (except during handling and assessments). *Short-term deviations to temperature and relative humidity ranges as specification the guidleine lasted ≤ 30 mins per 24 hour period for temperature and $\langle 2 \rangle$ hours for relative humidate. Deviations to the temperature range of 23 - 40 °C did not occur.

Statistics: The NOEC and the LOEC for the emergence on day 22 were estimated by a stepdowing procedure based on the Cochran-Armitage test (one-sider greater). The NOED and the LOED wore considered the doses corresponding to the NOEC and the LOEC, respectively. Since no corrected emergence below 50 % was recorded, no statistical analysis was performed to estimate EC_{50}/ED^{50} , but it was estimated as higher than the highest concentration dose tested. To estimate the EC_{10/20} for emergence on day 22, a Probit analysis was performed. Where reliable values were obtained for these endpoints, $ED_{10/20}$ were considered as the doses corresponding to the estimated $EC_{10/20}$. The accepted significance level was $\alpha = 0.05$ (one-sided greater), except where stated. The statistical calculations were performed with the statistical program Tox Rat Professional version 3.2%.

Analytics: All final diets of the control and test item treatment group were sampled in duplicate as analysis and retain samples directly from the prepared diet. The chemical analysis was performed by using LC-MS/MS detection.

Dates of work: October 28th to November 18th 2019 (Piological phase)

2020 (analytical phase) February 20th May 1/3th

> IS AND DISCOSSION RESULT

Analytical results: document MOA 4 which comply with the EU regulatory requirements outlined within SANTE/2020/1283@Rev.1 Ľ

The analytical dose verification of the Tarval diet from da 23 until day 6 resulted in mean measured recoveries of 95 - 99 % for the opyram and 104 - 15 % for trifloxystrobin. In the control samples, the concentration was bolow the Limit of Detection for both active substances (LOD fluopyram:

concentration was below the Lipit of Detection for bot 0.003 mg a.s./kg, LOD trifloxystobin: 0.903 mg a.s./kg).



group [mg a.s./kg Time [mg a.s./kg diet] [%]	covery arget
D 3 <lod< td=""><td>\$~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~</td></lod<>	\$~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Central 0.00 D.4 <lod a-<="" td=""><td>S' 6</td></lod>	S' 6
Control $D.5$ $< LOD$ \sim \sim \sim	
D 6 < D	Û,U
D 3 2.94 90 0	
3.25 D4 2.73 84 2.73 97	
D5 3.71 3.71 114	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	
$\begin{array}{c c} D 3 & 7.94 & 98 & 7 \\ \hline \end{array}$	K) ^V
$8.12 \qquad \qquad D 4 \qquad 0^{\circ} \qquad 0^{\circ} 6.7 \\ \hline 0 \qquad 0^{\circ} \qquad 0^{\circ} 83 \qquad 0^{\circ} \qquad 0^{\circ} 83 \qquad 0^{\circ} \\ \hline 0 \qquad 0^{\circ} \qquad 0^{\circ} \qquad 0^{\circ} \\ \hline 0 \qquad 0^{\circ} \qquad 0^{\circ} \qquad 0^{\circ} \\ \hline 0 \qquad 0^{\circ} \qquad 0^{\circ} \qquad 0^{\circ} \\ \hline 0 \qquad 0 \qquad 0^{\circ} \\ \hline 0 \qquad 0 \qquad 0^{\circ} \\ \hline 0 \qquad 0 \qquad 0 \qquad 0^{\circ} \\ \hline 0 \qquad 0 \qquad 0^{\circ} \\ \hline 0 \qquad 0 \qquad 0^{\circ} \\ \hline 0 \qquad 0$	
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	
Test item 20.29 0.4 17.2 0.84 0.96	5
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	
50.73 30^{4} 10^{7} 10^{7} 95	5
\mathcal{D}	
$\psi_{1} = \sqrt{2} \frac{1}{\sqrt{2}} \frac{\sqrt{2}}{\sqrt{2}} \sqrt{$	
$\begin{array}{c ccccccccccccccccccccccccccccccccccc$,
\$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$ \$	
LOD: Limit of Dejection: 0.003 mg/fluopyram/kg	
TY D A C	

 Table 10.3.1.3- 2:
 Analytical results for fluopyram



Treatment group	Nominal conc. of trifloxystrobin [mg a.s./kg diet]	Sampling Time	Measured conc. of trifloxystrobin [mg a.s./kg diet]	Recovery from target [%] 👞	Mean recovery from target	
		D 3	< LOD	- 2		
Constant 1	0.00	D 4	< LOD	-07		
Control	0.00	D 5	< LOD	A-		Ô
		D 6	< LQD	""."-		Ę,
		D 3	3	101		, C
	2 27	D 4	3.52	S 108	Sin7 A	. 6
	5.27	D 5	3.54	108		
		D 6	3.55	ده [°] 109		Ø
		D 3	9.34 🕎 🔬	0° 114° 🕔		
	8.18	D 4	§ 9.62 5 x	, [∞] ,¶¶8 ,⊘		
		D 5	0 [×] 0 [×] 8.87× √	<u>کہ 108</u>		
		D 6 🛒	8.50	0 104	or an 4	0
	20.46	D 3 🔊	21×05 ~ ×	1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1, 1		
Test item		D 4	23.89 2	r´_`%¶7©	108	
i est item			× × 21.87× ×	2107 °		
		96 x	2130 ~	↓ 10 4	Ø. Ø	
		$\hat{Q}^{T} D 3_{A}$	\$6.66	ju p		
	51.14	DA	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	⊘103 _0	¢ 104	
	51.14	Ď Ś	⁶ 49.24	96 ^{Or}		
	Ψ.	& D 6	<i>√</i> 52,53 √	~ <u>1</u> 0\$		
	, Ô	O'D 3	147.51	× _{~~}		
	127.95	D 4	£ 67150.0 7	≪117 🛠	115	
		^۲ پر 19 <i>9</i> 5	0°148-89	& 11 6	115	
		~Д"D 6	N 124.11	0° 1193		

 Table 10.3.1.3-3:
 Analytical results for trifloxystrobin

LOD: Limit of Detection: 0.003/mg trifloxystrobin/kg

Biological results: On day 8 po individuals were observed with inconsumed Good of other affections. At the end of the test, in the final assessment of the emergence on day 22, no emerged bees were recorded as being affected (i.e. malformation). Ő 47 j, y \ll K,

On day 8, larval mortality was 00 % in the control group and 97.92 % in the reference item group. In the test item group label mortalities on day 8 were 500 %, 2.08 %, 4.17 %, 2.08 % and 4.17 % following a treatment with 601.21, 240.48 96.19, 38.48 and 15.39 mg product/kg diet, respectively.

In the final assessment on day 22 can address ence ate of 95.83% was determined for the honey bees in the comorol group. In the test item Greated group, the adult honey bees emerged at rates of 50.0 %, 95.83 %, 89.58 %, 9375 % and 87,50 % following exposure to test item concentrations of 601.21, 240,48, 96.19, 38.48 and 16.39 mg product/kg diet during the larval stages, corresponding to cumulative





			Day 8		Day 22	Ŵ	ð
Treatment	Cumulative dose (nominal) ^A	Concentration (nominal)	Larval mortality Day 3 - 8	Total mo Day 3	rtality -92	Ado)i Emergence Arate	ð,
group			abs.	abs. 🦿	corr. ^B	🔊 actual	Ĉo
	[μg product/larva]	[mg product/kg diet]	[%]				e) j
Control	-	-	0.00	₽ 17	_0	5 95.83	Ó
Tost itom	2.37	15.39	"ØÅ.17	مي 12.50	. 70	♀ 87,90	×
(FLU+TFS	5.93	38.48	2.08	6 23	Q ² 2.176	93.75	
SC 500	14.81	96.19	<u>4.17</u>	<u></u> 10.42 @	652	<i>‱</i> 89.58	
(250+250	37.03	240.48	2.08	4.17	9.00	95.83	
g/L))	92.59	601.21	50.000 A	50.00	47.830 [×]	50 .00 *	
Reference Item (Dimethoate)	7.39 ^c	52.8 × ×	97.92 A	0100.00 [°]	1400.00		

Table 10.3.1.3- 4:	Mortality of larvae and adul	t emergence in the re	epeated exposure	toxicity test
		9		2

A Based on the cumulative feeding forme from day 3/to day of 140 pt die Aarva В L

Corrected for control mortality according Abbott modified by Schreider-Ocelli ð

С µg dimethoate/larva D

Ø Statistically significant difference compare to control (Step-down Cochran Similage Test; $p \le 0.05$; one sided greater) ° mg Dimethoate/kg diet *

Table 10.3.1.3- 5: Calculated endpoints of the repeated exposure larvae toxicity test

Treatment DEndpoint: Adult emergence at day 22	
4D ₅₀ ¹	> 92.59
Test item cumulative	n.d.
doses by construction of the second s	n.d.
[µg product/larva]	92.59
P NOCO 3 CO 'NY CY CY	37.03
	> 601.21 mg prod./kg diet
Test item	n.d.
concentrations $EC_1 \delta^2 \sim C$	n.d.
[mg product/kg diet]	601.21 mg prod./kg diet
NOFC 3 C J	240.48 mg prod./kg diet

n.d.:

Not determined As no price ted mortaling above 50% was observed, the EC/ED50 values were empirically estimated to be greater than the highest concentration tested.

No clear dow response was observed during the definitive test, therefore, the EC/ED10 and EC/ED20 values could not be reliably calculated \Im Based \bigotimes the cubulative feeding volume from day 3 until day 6 of 140 µL diet/larva. 2

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Validity@riteria:

All validity criteria of the OECD Guidance Document 239 (2016) were met.



Table 10.3.1.3- 6: Validity criteria

Validity criteria acc. to OECD TG 239 (2016)	Recommen	nded	Obtained
Larval mortality between day 3 and day 8 in the control group (across all replicates)	Control	≤% ⊘	
Adult emergence rate until day 22 in the control group (across all replicates)	Control	j ≥ 70 %	^{595.8}
Larval mortality between day 3 and day 8 in the reference group (across all replicates)	The second secon	≥ 50 %Õ	92 % T
		, _O	

III. Conclusion

In a repeated exposure larval toxicity study performed in a dose-response design with FLU+TFS SC 500 (250+250 g/L), the NOED and LOED were determined to be \$7.03 and \$2.59 pg product/large (based on adult emergence), respectively. The NOEC and LOE were 240.48 mg and 601. D'mg product kg diet, respectively.

The EC₅₀ and ED₅₀ values (based on acult emergence) were determined to be >601.21 mg product/kg diet and >92.59 µg product/larva, respectively. Since no clear dose-response relationship was found, no reliable EC10/20 and ED10/20 for the mergence at 22 days, could be determined Ś

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Assessment and conclusion by applicant

Ô The study and its data are considered as acceptable and reliable for use in sisk assessment. The No No \$ 0 endpoints are:

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7.03 product/larxa 240.48 mg product/kg diet NOED - emergence davs) =

NOEC - emergence

CP 10,301.4

C.S.

Cr 10, 5C.4 Sub-lethal effects There is no particular study design test guideline to assess "sub-lethal effects, if occurring, are described and reported.



Data Point:	KCP 10.3.1.5/01
Report Author:	
Report Year:	2012
Report Title:	Toxicity testing of fluopyram + trifloxystrobin SC 500 (250+250) G on honey bees (Apis mellifera L.) under semi-field conditions Aunnel test -
Report No:	64841037
Document No:	<u>M-435338-01-1</u>
Guideline(s) followed in study:	OEPP/EPPO, No.170(4) (OEPP/EPPO, 2010)
Deviations from current test guideline:	Current Guideline: EPPO 179 (4) (2010) Deviations: A mortality and behaviour assessment was carried out on day 6 after the application, instead of day 7 as recommended by the guideline. These deviations are not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes v v v v v v v

CP 10.3.1.5 Cage and tunnel tests

Executive summary

This semi-field tunned study was designed to evaluate the acute, short term and long-term effects on honey bees (*Apis mellifera* L.) for wing repeated foliar applications of FLU + TFS SC 500 (250+250 g/L) onto bee-attractive *Phacelia tanacelifolia*

The test was conducted under confined exposure conditions (tannel) with each tunnel comprising a 40 m² plot of flowering *Phatelia tanacetifolia*. In total 12 tunnels were used: 6 for the test item treatment and 3 each for the control group and the reference item group. One honey bee colony was used per tunnel. The test item was applied twice to the crop the 1st test item application was conducted at BBCH 59 - 61, just at the beginning of the flowering period, without honey bees present. The 2nd test item application was conducted concurrently to a tap water (control group) and a reference item application (reference item group) during honey bees actively foraging on the full flowering *Phacelia* crop (BBCH 64 - 65). Both fest item applications were conducted at a rate of 560 mL of FLU + TFS SC 500 (250+250 g/L) in 400 L water/ha (corresponding to nominally 140 g fluopyram/ha + 140 g trifloxystrobin/ha).

Mortality and foraging activity (flight/density) and honey bee sub-lethal effects were assessed before and after the 2^{nd} application. Colony assessments (nectar stores, pollen stores, eggs, larvae, pupae, colony strength) were made 6 day before the 2^{nd} application and at days 7, 14, 21, 28 and 42 following the 2^{nd} test item application and the corresponding applications in the control group and in the reference item group, respectively.

No adverse effects on mortality, foraging activity, behaviour, nectar- and pollen storage, broodabundance and development, colony strength as well as on queen survival were observed.

Based on the results of this study, it can be concluded that FLU + TFS SC 500 (250+250 g/L) does not adversely affect honey bees and honey bee colonies when applied at a rate of 560 mL during honey bees actively for aging on a bee-attractive, flowering crop.

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I. MATERIAL AND METHODS

<u>Test item:</u> FLU + TFS SC 500 (250+250 g/L), Specification No.: 102000012866 - 03, Batch-code: 201° - 002701; Sample description: TOX No. 09384-00; Analysed content of active substances: fluopyram: 21.6 % w/w (252.4 g/L), trifloxystrobin: 21.6 % w/w (252.2 g/L), density: 1.16% g/mL (20°C)

<u>Test species</u>: Honey bees (*Apis mellifera carnica* L.); small bee colonies, maintained according to normal beekeeping practice. The bee colonies were apparently healthy, well fed and queen-right. No medical treatments were used in the colonies for at least 4 weeks prior to the experimental start. The colonies contained 5 combs with 2-3 brood combs containing all brood stages and at least 1 food comb with honey and pollen. The colonies contained about 2280 to 2625 honey bees on 5 to no some start.

Location of the field site: Germany

Test concentrations:

Test item treatment: 560 mL product in 400 L water/hav 1.64 g/L; corresponding to from inally 140 g a.s. fluopyram/ha + 140 g a.s. trifloxystrobin/ha).

Control: Tap water/ha (concurrently to the test item applications)

Reference item treatment: 1.5 L Perfektion EC in 400 L tap water ha (corresponding to 3.75 mL/L or 4.03 g/L) and a density of 1.074 g/mD?

A water volume of 400 L/ha was considered for all treatment groups

<u>Test design</u>: The test was conducted under confined exposure conditions (tunnel) in order to assess acute, short-term and long-term effects of epeated foliar applications of FLU+ TFS SC 500 (250+250 g/L) on honey bee colonies under semptield conditions. A plot of *Phatedia tanacetifolia* with an effective crop size of 40 m² was prepared for each twinel. Six tunnels were treated with the test item: once at the beginning of the flowering period (at BBCH 59 - 61) without honey bees present (= 1st test item application) and a second time during full powering of the crop (at BBCH 64 - 65), with honey bees actively foraging on the crop during application (= 2nd test item application).

Three tunnels were concurrently to the 2^{nd} st item application treated with tap water (controls) and three tunnels were treated with a reference stem (perfektion EC (BAS 152 11 I), 400 g/L dimethoate), respectively during honey bees actively for aging on the crop.

The horey bee colonies were introduced into their respective tunnels 11 days before the 2nd test item application (during full flowering) and the corresponding applications in the control group and in the reference item group, respectively. One boney bee colony was used per tunnel.

Three of the six tunned being treated with the test item were exclusively assigned for monitoring of residues collected by foraging honey bees (pollen and nectar) on the day of 2^{nd} test item application (Day 0) as well as on the day following the 2^{nd} gest item application (Day 1).

The contribution of the provided exposure phase of the honey bees inside the treated crop was 7 days following the 2nd test item application (during full flowering) and the corresponding applications in the control group and in the telerence item group respectively. The conditions of the colonies were examined until day 42 following the 2nd test item application.

<u>Endpoints:</u> Mortality and foraging activity (flight density) of the honey bees were assessed before and after the 2nd application. Sub-lethal effects, such as changes in behaviour (e.g. intensive cleaning, discoordinated movement, exaggerated motility, aggressiveness, lethargy, apathy, obvious symptoms of intoxication, etc.) were also pronitored. Colony assessments (nectar stores, pollen stores, eggs, larvae, pupae, colony strength) were made 6 days before the 2nd application and at days 7, 14, 21, 28 and 42 following the 2nd test item application and the corresponding applications in the control group and in the reference item group, respectively.

<u>Test conditions:</u> Natural field conditions. Weather conditions were good during both applications. The sky was a little cloudy but temperatures were warm with no precipitation. First rain occurred following



2 days after the 1st test item application and 6 days after the 2nd test item application. The weather was variable but warm for the remainder of the trial.

Statistics: Statistical evaluation was done for mortality and the brood termination rates using Shapiro-Wilk's test (check for normal distribution), Levene's test (check for homogeneity of variance), Sudent's t-test (pairwise). Software: TOX Rat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Dates of work: August 08th, 2013 to October 10th, 2011.

II. RESULTS AND DISCUSSION

Analytical results:

Full details and acceptable validation data to support the shalytical method acceptable validation data to support the shalytical method acceptable within document M-CA 4, which comply with the EU regulatory requirements outlined within SANTE/2020/12830, Rev.1.

The residues of fluopyram and its metabolites fluopyram-pyridyl-acetic acid (BCS-AA10189) and fluopyram-benzamide (AEF148815), as well as triflexystrobin and its metabolite CGA327113 in nectar, pollen and flowers were determined by High Performance Liquid Chromatography (HPLC, coupled with tandem mass spectrometry (MS/MS) detection. Due to high amounts of co-extracts, the fluopyram metabolite AEC656948-pyridyl-carboxync acid was not analysed. Nectar was extracted from the honey stomachs of collected forage bees). Pollen was collected from the pollen baskets of prepared forager bees. Residues of the above were determined in nectar, pollen and flowers on Day 0 and Day 1 after 2nd test item application.

The Limit of Quantitation (LQQ) was 0.01 nQ/kg ($\beta c.10 \mu Qa.s./kg$) for all compounds and the Limit of Detection (LOD) was estimated to be 3 times lower that the LOQ (i.e. approximately 3.5 μ g/kg).

For the fluopyram metabolite fluopyram pyridyl-acetic acid residues in pollen, the average recovery rate was unexpected volume (< 70%) with individual values between 52% and 76%. Therefore, the metabolite fluopyram pyrid@-acetic acid tesidue values of the reated samples were corrected for the mean recovery value of 59%.

C

No residues were found in the control samples above 30 % of the Limit of Quantitation.

Sample	Tunnal		Concentration range (min –)	max)
materia		Fluopyram	Viluopyram-pyridyl-acetic acid (BCS-AA10189)	fluopyram- benzamide (AEF148815)
Flowers	₩3	\$.3. 0 − 5.0	<lod< td=""><td><lod -="" <loq<="" td=""></lod></td></lod<>	<lod -="" <loq<="" td=""></lod>
Nectar		Q.025 _1.1	<pre>Cov <lod -="" <loq<="" pre=""></lod></pre>	<lod -="" <loq<="" td=""></lod>
Pollen	<i>1-3</i> ∖	0 3.0 30 Q	<loq 0.01="" <sup="" –="">A</loq>	<loq -="" 0.017<="" td=""></loq>

Table 10.3.1.5- 1: Summary: sidue of fluopyram and its metabolites in pollen, nectar and flowers

LOQ: Limit of Quantification) for for all compounds = 0.01 mg/kg (= $10 \mu g/kg = 10 ppb$)

Values was corrected for mean of LOQOE covery level of 59 %.



Sample	Tunnel	Concentration [r	range (min – max)
material		Trifloxystrobin	Metabolite CGA321113
Flowers	1 - 3	5.3 - 10	0.022-0.072
Nectar	1 - 3	0.015 - 0.74	LOD - 0.019
Pollen	1 - 3	2.4 - 25	₹0.013 - 0.054 ° « «

Table 10.3.1.5- 2:	Summary: residues of trifloxystrobin and its metabolite in nollen, nectar and flowers
1 abit 10.5.1.5- 2.	Summary, residues of trinoxystroom and its inclabolite in policit, nectar and nowers

 $\overline{\text{LOQ: Limit of Quantification for for all compounds}} = 0.01 \text{ mg/kg} (= 100 \mu \text{g/kg})$

LOD: Limit of Detection for for all compounds \approx LOQ/3 (i.e. \approx 3.5 µg/kg)

Value was corrected for mean of LOQ recovery level of \$9 %.

Biological results:

Mortality

rg similar natural m Starting conditions of the experiment were deal maintain natural mortality evels mongthe different treatment groups before the application during full flowering for statistically significant difference of the colonies, Student t-test, pairwise comparison to the control, two-sided $\omega = 0.05$).

Exposure phase in the tunnels (day @ after application to day 7)

On the day of the 2nd test item application and the corresponding applications in the control group and in the reference item group, respectively, mortality rates were slightly higher in the test item group (27.3) compared to the control (19.7), however, this difference was not statistically significant (Student t-test, pairwise comparison, $\alpha = 0.05$, one-sided greatery.

Over the course of the remaining exposure period mortality rates in test iten reatment and control groups were similar worhout any statistically significant differences (Student t-test, pairwise comparison, one-sided greater, q = 0.05) at any assessment day.

The overall daily mean number of dead bees (Day () to Day 7) also showed no statistically significant differences between treatment (35.4) 13.2 bees) and control (33.5) ± 10.8 bees) (Student t-test, pairwise Ś comparison, one-sid@d greater, $\alpha \gtrsim 0.05$). L

In contrast to the observations on the test item treatment group and the control group, application of the reference item (dimethoate at a rate of 600 g a.s. ba) resulted in a markedly increased number of dead bees found in the paps and on the gauze strips in the crop between day 0 and day 4, which was statistically significantly different from the control (StudenO-test, pairwise comparison, $\alpha = 0.05$, onesided greater). Mortality increased up to approximately 25 x the levels of the control values on day 1





to day 7)			o° 🗞
		Mean dead bees ± S.I	D. B
l îme A	Control	FLU + TFS SC 500 (250+250 g/L)	Reference item
Day -3	61.7 ± 35.5	94.7 ± 29.7 n.s.	$59.0 \pm 54\%7$ % n.s.
Day -2	17.7 ± 8.6	30.7 ± 3.8 n.s.	49.7 ± 35.1 ∞ n.s. √
Day -1	37.0 ± 21.5	37.3 9.1 p.s.	51 5 ± 8.5 n s
Day 0 b.a. ^A	31.0 ± 24.3	35.3 ± 14.6 Q.s.	4503 ± 14.0 m/s.
Daily mean day -3 to 0 b.a.	36.8 ± 18.4	49.5 ± 30.2 n.s.	51.4 ± ₽7 Ôn.s.
Day 0 a.a.	19.7 ± 5.0	27.3 ± 8.1 n_{\star} n s.°	∡407.3 <u></u> ∉ 78.7 ° * °
Day 1	35.5 ± 13.8	25.7 ± 7.5 🔨 🔞 s.	₩83 k. ®± 132@
Day 2	20.3 ± 5.5	$23.0 \pm 7.20^{\circ}$ / n.s.	$0^{-1} 20^{-1} 0 \pm 90^{-4}$
Day 3	49.7 ± 5.8	49.7 ± 35.0 Å n.s	\$63.3 ± 44.8 *
Day 4	39.7 ± 19.7	∞54.3 €7.2 n®.	^{"0} 112.9 ¥ 31.2 → *(,°
Day 5	43.3 ± 27.5 %	49:3≠11,0 ≤ _n.s.	41.7 ± 19.8 18.
Day 6	26.7 \$ 9.6 \$	13.7 ± 7.6 $n.s.$	45.7 ± 17.8 (n.s.
Day 7	33 0 ± 13.9	35.0 ± 22.6 $n.s^{-1}$	©61.3 ± 4.9 ○ *
Daily mean day 0 to day 7 a.a.	33.5 ± 10.8	√35.4 13.9 0 µs.	233.0 ± 270.0 *

Summarised mortality data for exposure phase in the tunnels (day 0 after application Table 10.3.1.5- 3:

Before application; b.a.: a..a.: After application;

S.D.:

Days -3 to -1 = days before 2^{-4} application; do 0 = day of 2^{nd} application; day 2^{nd} of 7 = days after 2^{nd} application Mean values (rounded) of three tumpels of cach treatment group; ss: Student t-test, pairwise two sided (before application); as a side of the state of В

Statistics: Student t-test, pairwise two sided (before application); pairwise, one-sided greater (after application), $\alpha = 0.05$ Not statistically significantly different from the control; n.s.:

Statistically significant difference to the control

Foraging activity

Two days before the and testitem application, the flight densities in all experimental groups were very low due to unfavourable weather conditions. No statistically significant differences in overall daily mean flight intensity were found between the colonies during the pre-exposure period (Student pairwise t-test, $\alpha = 0.0$ (two sided), is dicating ideal starting conditions.

Exposure phase in the tunnels (day 0 after application to day \hat{T})

After the 2^{nd} test item application of FDU + TFS SC 500 (250+250 g/L), the foraging activity of the bees was comparable or oven higher in the tesp item freatment group compared to the control group. An overall comparison of the mean fight activity and not show a statistical significant difference between the control and the test item treatment (Student t-test, pair-wise comparison to the control, one-sided smaller, $\mathbf{x} = 0.05$). In contrast, the application of the reference item (dimethoate) resulted in a clear decrease of flight intensity that il the end of the confined exposure period (day 7), which was statistically significantly lower compared to the control (Sordent t-test, pairwise comparison, one-sided smaller, $\alpha =$ 0.05).



Time A		Mean flight density [Bees/m ^{2 B}]	
1 ime	Water Control	FLU + TFS SC 500 (250+250 g/L)	Reference item
Day -3	25.0 ± 2.6	27.4 ± 0.7 - Ø	26.4 ± 2.1
Day -2	0.0 ± 0.0	0.2 ± 0.4	0.1±02 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Day -1	15.0 ± 4.2	12.7 ± 1.9	18.9* 1.8 -
Day 0 b.a.	12.2 ± 3.2	3.0 ± 0.9 0.9 -	9 3 ± 1.7
Daily mean day -3 to 0 b.a.	13.1 ± 10.3	13.3 ± 11.1 % n.s.	19.7 ± 10.4 m.s.
Day 0 a.a.	10.3 ± 1.1	② 11.8 ± 1.5 ∽ -	0 0.8 ± 1 0 -
Day 1	10.6 ± 1.1	v 17.6 ± 4.0 v ∞ -	0.0 ≠ 0.0
Day 2	23.0 ± 2.3	21.3 ± 1/3	0.0 ± 0.0
Day 3	19.6 ± 342	©°21.6; 2.2 ≤ 3	0.1±0,2 ~ -
Day 4	22.1 ± 0.0	232±1.4	0.0±0.0
Day 5	21.4 ± 2.5	22.3 ± 92	$0 \mathcal{Q} = 0.4 \mathcal{Q}^{\vee}$
Day 6	13%4 ± 1,0°%	15.7%1.5 - 0	0.1 ± 0.2 0.7
Day 7	2.7 ± 1.55	Ø ⁷ 3,9⊊1.3 6 [∞] ,- [∞]	3 ±≪0.3
Daily mean day 0 to day 7 a.a.	Q15.5 # 7.0	17.2 ± 6.6 (p.s.	0.2 0.3 *
a Before application			ř. Č. <u>Q</u>

 Table 10.3.1.5-4:
 Summary of foraging activity findings

After application;

a.a.: After application; ^A Days -3 to -1 = days before 2nd application; day f^{\neq} day of 2nd application day 1 to $f^{=}$ days after 2nd application ^B Mean values (rounded) of three tunnels of each treatment group; Statistic: Student t-test, pairwise, two sided (before application); pairwise, one sided smaller (after application), $\alpha = 0.05$

Not statistically significantly different from the control n.s.:

Statistically significant difference to the control

Behavioural abnormalities

TES S SC 500 (250+250 g/L) and in the control No behaviourababnormalities occurred in the FLC Ô group at any assessment day, respectively. Ŵ

caused behavioural abnormalities (moving coordination problems, The reference item treatment abnormal cleaning) at deast through the first day following application.

Brood assessment

Over the entire assessment period of 42 days de. over a period comprising two complete honey bee brood cycles) following the 2nd test item application and the corresponding applications in the control and the reference item groups, respectively, the proportions of the different brood stages (eggs, larvae, pupae) fluctuated according to a normal development pattern in the control and in test item treated group, respectively. The observed variability of different brood stages was typical and followed a natural pattern. The total number of blood cells (i.e. sum of eggs + larvae + pupae) in the item treatment group was not statistically significantly different to the control group at any assessment date.

Overall, no adverse effects of the test item on honey bee brood have been observed throughout the study. All queers in the respective colonies of the three experimental groups were either directly observed during all colony assessments or at least a sufficient amount of freshly laid eggs was observed during the assessments, as clear sign of the presence of a healthy queen.



Time ^A	Water	Control	FLU + TFS	S SC 500 (g/L)	250+250	Re	ference iter	n	Ç,
Mean ^B SD ^C			Mean ^B	SD ^C	Statistic	Mean ^B	≽ SD ^C	Statistic	\$
Day -6	8820	1487	7560	3541	n.s.	9540 🔊	2509	ns	
Day +7	5940	2210	6750	3274	n.s	41 <u>40</u>	1535	(C))
Day +14	6120	1953	6660	1091	n.s	6\$7Ŏ	1582	n.s ∞	
Day +21	8280	1535	8280	1217 🔊	n.s	6 660	10,91	y n.s 🔊	, C
Day +28	4320	1429	4860	810	n.s	<i>Q</i> 4050	_@/177 🔊	ns	Å.
Day +42	2700	270	2880	562	n.s 🗸	3870	[∞] 412 √	Ô.S	,
^A Time in relation to the 2^{nd} test item application A Q Q A L U									
^B Mean nun	nber of cells	with all broc	d stages per col	lony@mean v	value of 3 col	lonies) 🖓	<u>`</u> 0′	ð Ú	
C SD: Stand	lard Deviatio	n		· · ·	, O	N W			
n.s.: Not statist	tically signif	icantly differ	ent from the ou	ptrol; 🧔	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~			×Q	
Statistic: Student	t-test, pairwi	se compariso	on to the control	, one-sided	sphatler, α	ð.05 🛫 🖉) V	A	
Strength of co	olonies				Ŭ Ĉ	A .08			

Table 10.3.1.5- 5: M	Aean number of all brood stages	(eggs+larvae+pupae)	in the colonies
----------------------	---------------------------------	---------------------	-----------------

I.S.. Not statistically significantly different from the centrol; Statistic: Student t-test, pairwise comparison to the control, one sided smaller, $\alpha = 0.05$ Strength of colonies The mean number of honey bees per colony to bill tracting The mean number of honey bees per colony in all test item proupsincluding the colonies to be used for residue analysis was very similar siedays pefore application. Population development was similar in the control and test item treatmenogroup. There was no statistical significant difference to the control group at any assessment date (Student t-test, pairwise comparison, or = 0.05; one oded smaller). Mean colony sizes increased or were on a comparable level during the exposure phase in the tunnels. In contrast, colonies size in the reference item treatment group decreased down to 64 % during the exposure phase in the tunnels, when compared to the corresponding pre-treatment value.

	`	<u> </u>		<u> </u>	Y AN	<u> </u>	\sim		
Timo A	W	ger Cont	rol 🖉 🧯	FLC See	+ TF\$\$0 50+ 25 0 g/	C 500 s Las	Ref	erence ite	m
1 mie	©Mean ^{BO}	SD ^C	Å[%] ^D Å	Mean ^B	SD C	[%] ^{\$\$}	Mean ^B	SD ^C	[%] ^D
Day -	2430	Q412	166	2625	689	Q 00	2235	264	100
Day ∔7∕	2400 🔌	423	~9 9 <i>č</i>	S 3096	ر» 579	¢¥118	1365	213	61 *
Day +14	2385	392	\$ 98 \$	3090	901	118	2265	506	101
Day +21	2000	£\$94 <i>k</i>	83	ু ∜2⁄820	927©	107	1710	315	77
Day +28	1530	°¥707,∾	, C	³ 1725	767	66	1485	429	66
Day +42	~QI560 O	1.58	$\sqrt{64}$	1935	~585	74	1440	119	64

Table 10.3.1.5- 6: Summary of the strength of the colonies

Time in relation to the D test in application А

в Mean value of 3 colonies С

SIQ Standard Deviation D

Percentage in relation to first assessment on day -6 Statistically significantly different from the control, *

Statistic: Student t-test, pairwise comparison to the control, one-sided smaller, $\alpha = 0.05$

All validity criteria were met on this study.



Foraging activity shortly before application All groups ≥ 5 bees/m ² Mean flight densities before application: 12.2 bees per m ² in the control timnels, 13.0 bees per m ² in the reference item tunnels, 9.3 bees per m ² in the reference item tunnels, sufficient to establish a high exposure level of the bees sufficient to establish a high exposure level of the bees sufficient to establish a high exposure level of the bees sufficient to establish a high exposure level of the bees motality can be considerable. Control Should not have been considerable. As the facan mortality filer application (30.5 dead bees) resembled the level before the application (30.5 dead bees) resembled the level before the application (30.5 dead bees) resembled the level before the application (30.5 dead bees) motality can be considered to be within the range of typical mortality levels under confined semi-field conditions. Reference Item Mortality Reference Item (dimethoate) There should be a high moner of impacted bees Mortality Reference Item (dimethoate) There should be a high moner of impacted bees Mortality Reference Item There should be a high moner of impacted bees There should be a high moner of impacted bees Mortality Reference Item There should be a high moner of impacted bees There application reference infortality semication (accept of days 5 and 6). Therefore, the post application (accept of days 5 and 6). Therefore, the post application mortality demonstrated the	Validity Criteria	Treatment Group	Recommended	Obtained
Control MortalityControlShould not have been considerable.As the facan mortality after application (35.8 dead bees) resembed the level before the application (36.8 dead bees) mortality levels under confined semi-field conditions.Reference Item MortalityReference Item (dimethoate)Should not have been considerable.As the facan mortality after application (36.8 dead bees) mortality levels under confined semi-field conditions.Reference Item MortalityReference Item (dimethoate)There should be high mpaced beesThere should be high mpaced beesThere should be offer the application (application (app	Foraging activity shortly before application	All groups	\geq 5 bees/m ²	Mean flight densities before application: 12.2 bees per m ² in the control prinels, 13.0 bees per m ² in the test item tunnels, 9.3 bees per m ² in the reference item tunnels. The considerable effects observed in the reference item treatment demonstrated that the foraging activities were sufficient to establish a high exposure level of the bees.
Reference Item Mortality Reference Item (dimethoate) Reference Item (dimethoate) The chould be high mpacted bees The cost application reference inortality (mean number of dead bees on day 1 after application) accounted for 831.7 and exceeded the control mortality value (mean number of dead bees on day Dafter application: 35.8) by a factor of dead bees on day Dafter application: 35.8) by a factor of dead bees on day Dafter application inoreased mortality rates compared to the control occurred on all days following the application (except of days 5 and 6). Therefore, the post application mortality demonstrated the	Control Mortality	Control	Should not have been considerable.	As the mean mortality, ther application (33.5 dead bees) resembled the level before the application (36.8 dead bees) mortality can be considered to be within the range of typical mortality levels inder confined semi-field conditions. Mortality at the colony (in the traps) was consistently low, indicating that colonies were healthy and acopted to the tumnel conditions.
substances and the validity of the test system.	Reference Item Mortality	Reference Item (dimethoate)	Thereshould be high of momber of impacted bees	The post application reference mortality (mean number of dead bees on day 1 after application) accounted for 831.7 and exceeded the control mortality value (mean number of dead bees on day Dafter application: 35.3) by a dactor of approximately 24. Statistically granificant increased mortality rates compared to the control occurred on all days following the application (except of days 5 and 6). Therefore, the post application mortality demonstrated the exposure of the bees, their sensitivity to potentially bee-toxic substances and the validity of the test system.

Table 10.3.1.5-7: Validity criteria

In order to assess the risk of FLU + TFS SC 5007250+250 g/lb to howey bees and honey bee colonies, honey bees were exposed under the realistic bit severe (forced) exposure conditions of a semi-field test (confinement in gauze tonnels) The test item was applied two times to the highly bee attractive surrogate crop Phacelia tanacettolia, the 1st test item application was conducted at BBCH 59 - 61, just at the beginning of the Dewering period, without honey bees present. The 2nd test item application was conducted concurrently to a tap water (control group) and the ference item application (reference item group) during honey bees actively for aging on the full flowering Phacelia crop (BBCH 64 - 65). Both test item appleations were conducted at a rate of \$60 mL of FLU + TFS SC 500 (250+250 g/L) in 400 L water/ha_corresponding to nopinally 140 gas. fly@pyram/ha + 140 g a.s. trifloxystrobin/ha).

No adverse effects og mortality, foraging activity, behaviour, nectar- and pollen storage, broodabundance and development, colony strength as well as on queen survival were observed. Based on the results of this study, it can be concluded that TLU + TFS SC 500 (250+250 g/L) does not adversely affect honey bees and honey bee colonies when applied at a rate of 560 mL product/ha during honey

anect noney bees and noney bee colonies when applied a bees actively foraging on a bee-attractive. flowering crop.



Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

FLU + TFS SC 500 (250+250 g/L) does not adversely affect honey bees and honey bee colonies when applied at a rate of 560 mL product/ha (corresponding to nominally 140 g fluopyram/ha + 140 g trifloxystrobin/ha) during honey bees actively foraging on a bee-attractive, flowering crop

Ô

Data Point:	KCP 10.3.1.5/02
Report Author:	
Report Year:	
Report Title:	Determination of residue levels of thiopyration + trifloxystrobin in nectar and
_	pollen, collected by honey bees under confined semi-field conditions
Report No:	E 319 4290-8 2 ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~
Document No:	$\underline{M-433544-010}^{\prime}$
Guideline(s) followed in	US EPA OG SPP Guideling No. 850 SUPP 2 2 2
study:	
Deviations from current	Current Guidelone: not applicable of a construction of a construct
test guideline:	
Previous evaluation:	No rot previously submitted \mathcal{O} \mathcal{O}
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliabi	Yes a a a a
S, S	

Summary *©*

This study aimed to examine the honey bee exposure levels of fluopyram- and trifloxystrobin in nectar, pollen and flowers of *Phaceful tanacetifolia* after foliar applications of FLU+TFS SC 500 (250+250 g/L) at a rate of 560 mL product in 400 L water ba (corresponding to nominally 140 g a.s. fluopyram/ha + 140 g a.s. trifloxystrobin/ha)

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the 100 regulatory requirements outlined within SANTE/2020/12830, Rev.1.

The residues of fluopyram and its metabolites fluopyram-pyridyl-acetic acid (BCS-AA10189) and fluopyram-benzamide (AFF148815), as well as trifloxystrobin and its metabolite CGA321113 in nectar, pollen and flowers were determined by High Performance Liquid Chromatography (HPLC, coupled with tandem mass spectrometry (MSMS) detection. Due to high amounts of co-extracts, the fluopyram metabolite ACC656948-pyridyl-carboxylic acid was not analysed. Nectar was extracted from the honey stomachs of collected forager bees). Pollen was collected from the pollen baskets of prepared forager bees. Residues of the above were determined in nectar, pollen and flowers on Day 0 and Day 1 after 2nd test item application.

The Eimit & Quantitation (LOQ) was 0.01 mg/kg (i.e.10 μ g a.s./kg) for all compounds and the Limit of Detection (LOD) was estimated to be 3 times lower than the LOQ (i.e. approximately 3.5 μ g/kg).

For the fluopyram metabolite fluopyram-pyridyl-acetic acid residues in pollen, the average recovery rate was unexpectedly low (< 70 %) with individual values between 52 % and 76 %. Therefore, the



metabolite fluopyram-pyridyl-acetic acid residue values of the treated samples were corrected for the mean recovery value of 59 %.

No residues were found in the control samples above 30 % of the Limit of Quantitation.

Table 10.3.1.5- 8. Summary. residues of huopyram and its metabolites in ponen, nectar and nov	Table 10.3.1.5- 8:	Summary: residues of fluopyram	and its metabolites	in pollen, nectar	[.] and flow
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A summary of and pollen of Table 10.3.1.5	of the residues <i>Phacelia tana</i> - 8: Summar	of fluopyram and trifloxystrobin (and their metabolites) in flowers, rectar cetifolia is presented in the tables below.	Ş
Sample	Tunnal	Concentration range (man – max)	
material	i unnei	Fluopyram BCSAA10189) (AEF148815)	
Flowers	1 - 3	$3.1-5.0$ \cancel{A} \cancel{C}	
Nectar	1 - 3	0.025 - 14 $200 LOQ - LOQ -$	
Pollen	1 - 3	3.0 - 30	

LOQ: Limit of Quantification) for for all compounds = 0.01 mg/kg (= 10 µg/kg Value was corrected for mean of LOQ recovery level of 59 %

	st n° .		·	0	
Table 10 3 1 5- 9	Summary residues	of trifloxysteph	in and its metal	hotite in nøllen.	nectar and flowers
1 4010 10.0.11.0 7.	Summary. restaucs	of thatoxysty ob	in agra its incta	Source mopulation	, accual and notices

Sample	Tunnek	Conceptration	m range (min, max) [mg/kg]
material		Trifloxystrobin &	O Metabolite CGA321113
Flowers	153 d ×	9 <u>5</u> 3-10 <u>5</u>	Ø.022 – 0.072
Nectar	ČĨ - 3	«0.015 _ 9 .74	C = 200 - 0.019
Pollen	j 1 - 20 , O	0 2.4 25	0.013 - 0.051

LOQ: Limit of Quantification for for all compounds = $(10^{10} \text{ mg/kg}) = 10 \text{ µg/kg} = 10 \text$

LOD: Limit of Detection for for all compounds \approx LOOD (i.e. $\approx 3.5 \,\mu g/M_{\odot}$)

Value was corrected for mean of LOG ecovery level of

Assessment and conclusion by applieant:

The study and its date are considered as acceptable and feliable for use in risk assessment.

The residue levels of fluopyrang were within the range of 3.1 to 5.0 mg/kg in phacelia flowers, within the range of 0.025 to 1.1 mg/kg is nector and within the range of 3.0 to 30 mg/kg in pollen, respectively. Ø

The residue levels of transformed in where within the range of 5.3 to 10 mg/kg in phacelia flowers, within the range of 0.015 to 0.74 mg/kg in brectar and within the range of 2.4 to 25 mg/kg in pollen, respectivel

CP 1Ø **Field tests** with honeybees

Further testing was not necessary when considering the outcome of the risk assessment and the results of the lower-tier studies.



CP 10.3.2 Effects on non-target arthropods other than bees

For the formulation FLU + TFS SC 500 Tier 1 (laboratory studies on glass plates) with *Aphidius rhopalosiphi* and *Typhlodromus pyri* and Tier 2 studies (extended laboratory studies) with *Orbus laevigatus* and *Coccinella septempunctata* were conducted to determine potential effects on non-target arthropods.

		T T	•••		
ible 10.3.2- 1: Eco for]	toxicological endpoints rele FLU + TFS SC 500	evant for the	e risk asses	ment for non	arget arthropods
Fest species, Reference	Tested Formulation, study type, exposure	Ecotoxi	cological En	appoint Q	
Aphidius rhopalosiphi	FLU + TFS SC 500 O	$\mathbb{R}_{50} > \mathbb{R}_{50}$	200 mJ pro	du@ha 🔗	A A A
(2007) M-283599-01-1	200 mL product tha	Corr. M	′[%] ortalio	Effects on re	moduction ² %
KCP 10.3.2.1/01	400 mL product/ha 800 mL product/ha		× 0.0 × 3.3		
	1600 mL product/ha	S, U			
Typhlodromus pyri	FLU + TFS SC 300	$L_{\rm Ne50} > 3$	200 mL pro	duce na	2.0 v
(2007) M-283552-01-1	200 mL product/ba	NCOIL DA	20 ×		.8
KCP 10.3.2.1/02	400 mL productora		1.0 °° 3.1 °°		0.8 6.2
, S	1600 mL product/ha		6.2% 19.4		.3 .0
Orius laevigatus (2007)	ELU + DYS SC 500 Extended laboratory,		3@mL prod	uct/ha	
KCP 10.3.2.201	grape-vine leaf discs	Core M	lortanty] ^A	Effect on ecundity [%] ^B	Effects on fertility [%] ^B
Ê.	Control 25 0 product/ha	8° 2		- 4.6	- 3.7
	50 mL product/ha		.6	10.7 17.6	11.0 16.8
	200 mL product/ha		8	-	-
		× ~			
		4Q° Y			
	~				





Off-field: Max single application file \times MAF \times drift factor/VDF \times correction factor

0.2 D product/ha m grapes Application rate: x (2 application factor) = 1.7 (2 applications) MAF

¹⁵ Candoffi *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



Drift factor = 82nd percentile for 2 applications; 0.0723 (late) (according to Ganzelmeier)

VDF = vegetation distribution factor = 5 (Tier 1 and 2; studies with 2D exposure system) and 1 (Tige 2) studies with 3D exposure system)

Correction factor = 10 (Tier 1) and 5 (Tier 2)

The risk at Tier 1 is considered acceptable if the calculated HQ is < 2. The fisk at Tier 2 is considered acceptable if the PER is below the application rate causing 50 % effect $\frac{1}{2}$ $\frac{1}{2$

Table 10.3.2- 2:	Exposure calculation fo	r in-field	(Tier 1	an@2
14010 101012 21	Enposare carcananon ro		W	

		~()	¥ . •	
Crop	No. of appl.	Appl. rate	MAF	PER in-field
		🗗 prod2/ha] 🕺		[L prod/ha]
Grapes	2	A 0 ²	Q1.7	
MAF: Multiple application	on factor: PER: Predicted	environmental rate	\rightarrow \rightarrow .0	

Table 10.3.2- 3:	Exposure c	alculation	for the	off-field	scenai	in Tie	rÂ	ř
				A	~		7(1)	

Сгор	No. of appl	ØAppl, rate [L prod./ha]	MAF	Drift [%]	VDF	Correction factor	≪ PER _{off-field} [©] [L prod./ha]
Grapes, late	2 🔊	0°0.2 ~~	1	7.23	5		0.049

MAF: Multiple application factor; VDF: Vegetation distribution factor; PER: Predicted environmental rate

Table 10.3.2- 4:	Exposore	caleulation	the off-fiel	scenação	(Tiey 2)	
	A - N	-	A Y 1/		W// //	· · ·

Crop	N©of appl.	Oppl. rate [L pred./ha]	YAF ODr	ift Test	V DF	Correction factor	PER _{off-field} [L prod./ha]
Grapes, 🔌 late	¢ ¥ 2		10 T.	23 2D-Cy		5	0.025

MAF: Multiple application factor, VDF: Vegetation distribution factor; PER Predicted environmental rate

Jaevigatus (M-297476-01-1) and Chrysoperla carnea (M-482453-Relevant for the extended lab styles with Orius 01-1)

Risk assessment for non arge orthropods

Ľ The risk assessment was performed according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev. 2 final 2002) and to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi et al. 200016).

¹⁶ Candoffi 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



Гier 1	in-field	risk	assessment for	non-target :	arthropods
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Crop and application rateSpeciesPERim-field [L prod./ha]LRso [L prod./ha]HQTriggerGrapes 2 $\times 0.2 L$ prod./haAphidius rhopalosiphi Typhlodromus pyri0.340>3.20.1062SeciesTyphlodromus pyri0.340>3.2<0.1062PER: Predicted environmental rate; HQ: HQ: Hazard quotientHQvalues are below, the trigger of concern, indicating acceptable risk for non-target arthropods.Image: Concern, indicating acceptable risk for non-target arthropods.Tier 1 off-field risk assessment for non-target arthropodsPER prod./haLRso HQHQFrigger FriggerGrapes, late 2 $\times 0.2 L$ prod./haAphidius rhopalosiphi Typhlodromis pyri0.040>3.2<0.0052PER: Predicted environmental rate; HQ: HQ: HIPER prod./haLRso HQHQFrigger FriggerGrapes, late 2 $\times 0.2 L$ prod./haAphidius rhopalosiphi Typhlodromis pyri0.040>3.2<0.0052PER: Predicted environmental rate; HQ: HQ: Hazard quotientHQFrigger For the standard species, the off-field HQ values are below, the trigger of concern, indicating acceptable risk for non-target arthropods2For the standard species, the off-field HQ values are below, the trigger of concern, indicating acceptable risk for non-target arthropodsPER For the standard species, the off-field HQ values are below, the trigger of concern, indicating acceptable risk for non-target arthropodsPER For non-target arthropodsTier 2 in-field risk assessment for non-target arthropods<	Û,			
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IQ: Hazard quotientFor the standard species, the off-field HQ values are below the the trigger of concern, indicating acceptable risk for non-target arthropodsFier 2 in-field risk assessment for non-target arthropodsFier 2 in-field risk assessment for non-target arthropodsFable 10.3.2-7:Tier 2 in-field risk assessment for non-target arthropodsCrop and application ratePecies $PERin-field$ $LRso/ERso$ $[L prod./ha]$ PERin-field Δ $[L prod./ha]$	PER: Predicted environmental rate;			
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Tier 2 in-field risk assessment for non-target arthropodsTable 10.3.2-7:Tier 2 in-field risk assessment for non-target arthropodsCrop and application rateSpeciesPerciesP	σan			
Fier 2 in-field risk assessment for non-target arthropods Cable 10.3.2-7: Tier in-field risk assessment for non-target arthropods Crop and application rate Species PERin-field LR50/ER50 PERin-field LR50/ER50 With ≤ 50% effect	g an			
Fier 2 in-field risk assessment for non-target arthropods Fable 10.3.2-7: Tier in-field risk assessment for non-target arthropods Crop and PERin-field Application rate Percies				
Table 10.3.2-7: Tier in-field risk assessment for non-target arthropods Crop and application rate $PER_{in-field} = LR_{50}/ER_{50}$ PER $in-field$ below rate $PER_{in-field} = LR_{50}/ER_{50}/ER_{50}$ PER $in-field$ below rate $PER_{in-field} = LR_{50}/E$				
Fable 10.3.2- 7: Tier in-field risk assessment for fron-target arthropods Crop and application rate PERip-field A LR50/ER50 PERin-field below rate With ≤ 50% effect				
Crop and application rate pecies y y [L ptod./ha] [L prod./ha] PERin-field below rate with ≤ 50% effect				
crop and species β				
	te t			
Grapes, late Orther laevigatus 0 0.139 No				
2 × 0.2 L prosona Chrysoperla caurlea V S >2.34 Yes				
PER: Predicted environmental rate of the second sec				
The \vec{P} R _{in fold} is before the value with < 50% effect for Chrysoperla carned but not for Orius laevia.				

The PER_{in-field} is below the rate with \leq 50% effect for *Chrysoperla carnea*, but not for *Orius laevigatus* for the application of 2 \sim 0.2 L Grod. ha in grapes. Hence, an aged residue study with *Orius laevigatus* is presented to demonstrate the potential for recovery of in-field non-target arthropod populations and thus no unacceptable risk.



Refinement

Based on the provisions of the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002final) "it has to be demonstrated that there is a potential for re-colonisation / recovery at least within one year but preferably in a shorter period depending on the biology (seasonal) patter for the species... The assessment may be based on field studies or other evidence (e.g. Fesults of aged residues studies, environmental fate information)."

For this purpose, an extended laboratory test with Orius Juevigatus and aged residues of EMU SC 500 on grapevine leaves, sprayed under semi-field conditions, was conducted for an apprcation rate of 2 x 0.8 L product/ha and a 7 days interval (KCP #0.3.2.2/02, M297476-01-1) The results Drowed that potential for recovery was given 21 days after the last application.

The application pattern of FLU + TFS SC 500 in grapes is 2 x 200 mL product/ha und thus covered by the tested application pattern of 2 x 0.8 L product/ha and a 7 days interval. Thus, for the application of FLU + TFS SC 500 in grapes a recovery within 21 days car be expected

Tier 2 off-field risk assessment for non-target arthropods

Table 10.3.2- 8:	Tier 2 off-field	risQăs	sessment	stôr no	ntarget	arthro	pôe	Ĭ
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Crop and application rate	Species	Øff-fiel@PER [Lprod./ha	LAR%/ER%	PERoff-field Below rate with \$50% effect
Grapes, late	Orius laevigatus	0.025	0,139	Yes
2×0.2 L prod./ha	Chrysopperla cadhea 🔊	0,025	2.340	Yes
PER: Predicted enviro	onmental rate	8 67 5		ŝ

The PER off-field is below the rate ffect for all species and uses indicating an acceptable risk for non-target arthropody

Conclusion

Ø From the data and risk assessments presented above, it is concluded that unacceptable effects of





CP 10.3.2.1 Standard laboratory testing for non-ta	rget arthropods
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Data Point:	KCP 10.3.2.1/01
Report Author:	
Report Year:	
Report Title:	Dose-response toxicity (LR50) of AE C656948 & trifloxystrobin SC 250 + 250
	g/L to the parasitic wasp Aphidius rhopalosiphi (Destefani-Perez) under laboratory
	conditions
Report No:	
Document No:	<u>M-283599-01-1</u>
Guideline(s) followed in	IOBC (Mead-Briggs et al. 2000); Equivalent to US EPA OPOTS Guideline Do.
study:	850.SUPP A 6 6 4 4
Deviations from current	Current Guideline: MEAQ-BRIGGS ET XL. (2000)
test guideline:	Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously something of the second sec
GLP/Officially	Yes, conducted under GDP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$ Yes \qquad \bigcirc \qquad \checkmark \qquad \checkmark$

Executive Summary

In a laboratory study the lethal and subterhal toxicity of FLU + TFS SC 500 (250+250 g/L) on the parasitoid wasp, *Aphidit rhopalosiphi* was investigated. Five test item rates from 200 - 3200 mL product/ha were tested. Per test item rate 3 replicates of 10 wasps were exposed to FLU + TFS SC 500 (250+250 g/L) on treated glass surface.

All of the validity sriteric according to Mead Briggs Fal. (2000) were mel

After 2, 24 and 8 hours mortality of the wasps was assessed. After 48 hours no statistically significant differences compared to the control occurred and a corrected nortality up to 3.3 % was found in the treatment rates. The LR₅₀ was estimated to be \$3200 mL product/ke.

At 48 hours, 15 surviving females per treatment were confined individually for 24 hours over untreated aphid-infested wheap plants with the host cereal aphids *Rhopatosiphum padi*. After removal of the adult wasps, the aphid-infested plant over left for 1 days, before the reproductive capacity was assessed. No effects on the reproductive performance of the surviving wasps were found in any of the test item rates.

I. MATERIAL AND METHODS

<u>Test item</u>? FLU + TFS SC 500 (250+250 g/L); specification number 102000012886; batch ID 2006-004983; TOX07762 00; analyse content of a set = 246.1 g/L fluopyram, 245.8 g/L trifloxystrobin; density 1.165 g/mL.

<u>Test design</u>: Adults of the parasitoir wasp, *Pphidius rhopalosiphi* (less than 48 h old) were exposed to dried spray residues of the product applied on glass plates at rates of 200, 400, 800, 1600 and 3200 mL product/ha and the effect were compared to those of a purified water control. All treatments were applied at a nonunal volume rate of 200 L spray solution/ha using a calibrated laboratory track sprayer. A toxic reference (Dtmethodre EC 400) applied at a rate equivalent to 0.3 mL product/200 L water/ha was included to indicate the relative susceptibility of the test organisms and the test system. For feeding 25% w/w solution of aqueous fructose was provided on a cotton wool pad.

Mortality of 30 adult wasps (3 replicates of 10 wasps per test group) was assessed 2, 24 and 48 h after exposure.



At 48 h, surviving wasps (n = 15 females per treatment) were removed and their reproductive capacity assessed by confining them individually over untreated wheat plants infested with the host cereal aphids, Rhopalosiphum padi.

The adult wasps were removed after 24 h and the aphid-infested plants left for a further 11 days before the numbers of aphid 'mummies' (the pupal stage of the wasp) that had developed was recorded.

<u>Climatic conditions</u>: The climatic test conditions of the mortality assessment phase were 19 temperature and 65 - 73 % relative humidity with a photoperiod of 16 hours light and a fight intensity of 2100 lux. In the reproduction assessment phase (Aphid parasitisation) the temperature ranged between 19 - 22 °C with a photoperiod of 16 hours light and a light incensity of 440@Iux.

<u>Statistics</u>: Fisher's Exact Binominal-test ($p \le 0.05$) was applied to portality data to compare individual test-item treatments with control. For reproduction data, treatments were compared to control by Dunnett's multiple t-test ($p \le 0.05$).

Dates of work: November 27, 2006 – December 11, 2006

II. RESULTS AND DISCUSSIO

In a worst-case laboratory study, the effects of exposing, be parapitoid wasp Aphidius hopglosiphi to fresh dry residues of FLU + TFS \$\$ 500 (250+250 g/L) on glass plates were determined.

Corrected mortalities of 0, 0, 3 & 0 and 3.3 Wivere Diserved after 18 hours in the 200, 400, 800, 1600 and 3200 mL product/ha treatment rates of the product. There were no statistically significant effects on wasp survival. The median lethal rate (LRG) was estimated to be > 3200 mL product/ha.

At 200, 400, 800, 1600 and 3200 mL product/ba, the reproductive performance of the mites was reduced by 0, 5.2, 9.0, 14.2 and 22.6 % relative to the control. There was no statistically significant effect of the product on reproduction (mean number of munimies per female) and all tested rates (DUNNETT's multiple t-test, 1-sided, p (0.05) compared to the confool group.

The results are summarised in the table below

Table 10.3.2.1- 1:	Effects of dried spray residues on the parasitic wasp Aphidius rhopalosin	hi
K.V	(Homenootora, Braconidae) in a Jaboratory study	
* 7		

r				
Test item:	¥LU + ¥FS S© 500 (2	250+250g/L)	~	
Test organism: 👸	Aphidius rhopalosiph	4	Č,	
Exposure on:	Glass plates 6			
- A		Corrected	Reproduction ³⁾	Effects on
Treatment		C mortality 2	[mummies/	reproduction ⁴⁾
		× × %	female	[%]
Control			15.5	-
<i>"</i> ¢	290 0		15.5	0
Taat itaan	~400 ×	× 00	14.7	5.2
	806	3.3	14.1	9.0
A A	A 1600 V	<i>™</i> 0	13.3	14.2
	\$200	3.3	12.0	22.6
Toxis ref. S	C 0.3 S	¥ 100	-	-
402 5	>3200 ml nroduct/	ha		

<u>200 mas product/ha</u>

Application rate for test item in terms of mL product per 200 L water/ha. 1)

Mortality corrected for any control treatment deaths using Abbott's formula. 2)

- For effects on reproduction, a negative value indicates an increase relative to the control. 4)
- 5) Median lethal rate estimated empirically.

³⁾ The mainbers of parasitized aphids per female in each test-item treatment were compared to the numbers in the control by Dunnett's multiple t-test ($p \le 0.05$).



Validity criteria:

Table 10.3.2.1- 2: Validity criteria

All of the validity criteria were met (accordin	g to Mead-Briggs et al., 2000).	
Table 10.3.2.1- 2: Validity criteria		
Validity criteria	Required	Obtained S
Mortality within the control treatment at 48 h	$\leq 13\%$ (i.e. 5 wasps from 40)	
Mortality within the toxic reference treatment at 48 h	> 50 %	
In the reproduction assessments the mean number of mummies in the control	and be more than two zero values	15.5 muthimies per female, no zero values
	Conclusion	
The LR ₅₀ was estimated to be $> 3200 \text{ m}$ pro	disct/ha	
The figures obtained fulfil the validity critera Mead-Briggs <i>et al.</i> (2000).	a of the laboratory frethodorsin	g grass plates according to
Assessment and conclusion by applicant:		
The study and its data are considered as acce	plable and reliable for use in r	isk assessment.
The endpoint is: LR 3200 mL product/ha		Ĵ. Ĵ.
		<i>7</i>



Data Point:	KCP 10.3.2.1/02
Report Author:	
Report Year:	2007
Report Title:	Dose-response toxicity (LR50) of AE C656948 & trifloxystrobin SC 250 + 250
	g/L to the predatory mite Typhlodromus pyri (Scheuten) under laboratory
	conditions
Report No:	06 10 48 190
Document No:	M-283552-01-1
Guideline(s) followed in	IOBC (Bluemel et al. 2000); Equivalent to US EPA OPPTS Guideline No.
study:	850.SUPP
Deviations from current	Current Guideline: BLÜMEL ET AL. (2000), O ^S
test guideline:	Deviations: The cumulative reproduction perfemales are counted from day to
	day 14. Any eggs found on day 7 were removed and not counted in the fecundity
	assessment. All validity of teria were met
Previous evaluation:	No, not previously submitted 3 2 2 2 2 2
GLP/Officially	Yes, conducted under GLAOfficially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q Q

Executive Summary

In a laboratory study the lethal and subledial toxicity of FLU + TFS SC 500 (250+250 g/L) on the predatory mite, *Typhlodromus pyr*OScheuten (Acari: Phytoseiidae), was investigated Five test item rates from 200 - 3200 mL product/ha were tested. Ber test item rate 5 replicates of 20 mites were exposed to FLU + TFS SC 500 (250+250 g/L) on treated glass surface.

All of the validity offeria according to Boumel et al. (2000) were met.

After 3, 7, 9, 11 and 14 days mortality of the mites was assessed. After 7, days no statistically significant differences compared to the control occurred and a corrected mortality up to 13.4 % was found in the treatment rates. The \mathbb{CR}_{50} was estimated to be 3200 pc product/has

Assessments of mite reproduction were made at 9,11 and 14 days after treatment. After 14 days no statistical significant effects of reproduction were found.

S S & Material and Methods

<u>Test item</u>: FLO + TFS SC 500 (250+250 FL); specification No.: 102000012886, batch ID 2006-004983; sample description: TOX No. 97762-00; analysed content of a.s.: 246.1 g/L fluopyram, 245.8 g/L trifloxystrobin; density 1.165 g/mL.

<u>Test design</u>: Protonymphs of the predatory mite *Pyphlodromus pyri* Scheuten (Acari: Phytoseiidae; less than 24 hours old, from a synchronised cohort) were exposed to dried spray residues of the product applied on glass plates at rates of 200, 406 800, 1600 and 3200 mL product/ha in 200 L deionised water/ha. The effects were compared to those of a deionised water control. Dimethoate EC 400 (10 mL product/ha in 200 L water ha) was used as a toxic reference item. During the assessments the predatory mites were fed with polien (*Pipus nigra* and *Betula pendula*) at each assessment day.

On day 3, 7, 5, 11 and 14 after the application, the number of surviving predatory mites was counted, dead mites were recorded and removed. The number of laid eggs was determined on days 9, 11 and 14. Any eggs found on day 7 were removed and not counted in the fecundity assessment. The final assessment for mortality was performed on day 7 after treatment and the final assessment for reproduction was made on day 14 after treatment.



Climatic conditions: The climatic test conditions were 23 - 26 °C temperature and 72 - 79 % relative humidity with a photoperiod of 16 h and a light intensity of 2200 lux.

<u>Statistics</u>: Fisher's Exact Binominal-test ($p \le 0.05$) was applied to mortality data, to compare individual treatments with the control. For comparison of reproduction data from individual treatments with control DUNNETT's multiple t-test (1-sided, $p \le 0.05$) was used.

Dates of work: November 27, 2006 - December 12, 2006

II. RESULTS AND DISCUSSION

The effects of exposing the predatory mite Typhlo Fromus pyri to dried spray of SC 500 (250+250 g/L) on glass plates were determined.

Corrected mortalities of 2.1, 1.0, 3.1, 6.2 and 13.4 & were observed in the 200 400, 800, 1600 and 3200 mL product/ha treatment rates of the product. None of the treatment rates differed significantly from the control (Fisher's Exact Test, p \$0.05) and the median lethal rate (BR₅₀) was estimated to be > 3200 mL product/ha, the highest rate is sted. Ø

At 200, 400, 800, 1600 and 3200 mL product/ha, the reproductive performance of the portes was reduced by 0.8, 0, 0, 0.3 and 1.0 % relative to the control. There was no statistically significant effect of the product on reproduction at all tested rates (DUSNETT's multiple t-test, 1 sided, p 0.05) compared to the control group.

The results are summarised in the table beto

Table 10.3.2.1- 3: Effects of dried Spray residues on the predatory mit Typhlodromus pyri (Acari: Phytoseridae) in a laboratory study Q Y , Ø Ô

Test item:	FLUD TFS SC 500	(250+250 g/L)		
Test organism:	Typfilodromus pyri		çı dı .	
Exposure on:	Glass plates 🔌	<u> </u>	<u> </u>	
	Dete 1	Corrected	Mean number eggs	Effects on
Treatment		mortality 🕅	v per Gemale 3)	reproduction ⁴⁾
	[Mail product/naj	. Õ [%] × &	(8-14 DAT)	[%]
Control		X Q O	> 7.27	
	<u>200</u>	o <u>~</u> 1.0	7.21	0.8
<u>v</u>	2 ⁰ 400 ~	°1.0,0°	7.33	- 0.8
Test item	×90 ×		7.72	- 6.2
4	1600		7.25	0.3
	∿~ 3200Q	\$3.4 ×	7.20	1.0
Toxic ref.	× 10 ~	^{79.4}	-	-
LR30 ⁵⁾	> 3200 mL produc	t/ha		

1) Application rate for test item in terms of ml product per 200 L water/ha.

- Mortality corrected for any congol treatment dealls using Abbott's formula. Negative values indicate an increase in 2) survival, relaive to the control
- 3) Results for reproduction in adividual test-item treatments compared to the control by Dunnett's multiple t-test, 1-sided (p ≤ 0.05) Ĉ
- 4) For effects on eproduction, a positive value indicates a decrease, relative to the control.
- Median lether ate estimated empirically.

Validito criteria:

All of the validity criteria were met (according to Blümel et al., 2000).



Table 10.3.2.1- 4: Validity criteria

Validity criteria	Required	Obtained 🖉 🖉
Mortality within the control treatment during the 7 day test	≤ 20 %	3%
Toxic reference mean mortality of protonymphs at day 7 (control corrected)	50 -100 %	79.4%
Mean cumulative number of eggs produced per female from 7 to 14 days in the control	\geq 4.0 per female	7.27 per female
	Conceusion	
The LR ₅₀ was estimated to be > 3200 mL prod	luctona.	
Blümel <i>et al.</i> (2000).	of the aboratory method us	ng glass plates according to
Assessment and conclusion by applicants		
The study and its data are considered as accept	ptable and reliable for use in	risk assessment.
The endpoint is: LR ₅₀ > 3200 gal product/ha		× ~ ~



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

	n an
Data Point:	KCP 10.3.2.2/01
Report Author:	
Report Year:	2007
Report Title:	Dose-response toxicity of AE C656948 & Trifloxystrobin SC 250 + 050 to the
	predatory bug Orius laevigatus (FIEBER) (Heterostera: Anthocoridae) under
	extended laboratory conditions 😵
Report No:	07 10 48 049 A
Document No:	<u>M-297476-01-1</u>
Guideline(s) followed in	IOBC (BAKKER et al. 2000) modified for the exposure on natural substrate
study:	(excised leaf discs); Equivalent to US EPA OPP S Guideline No. 850 SUPP
Deviations from current	Current Guideline: BAKKER of AL (2000) brodified
test guideline:	Deviations: None. A Dvalidity criteria were pret. Deviations: None. A Dvalidity criteria were pret.
Previous evaluation:	No, not previously submitted Q A A A A A
GLP/Officially	Yes, conducted under GLP/Officially recognized testing facilities 🖉 🖉
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q' a a a a a a a a a a a a a a a a a a

Executive Summary

In an extended laboratory test the effects on the survival and reproduction of FLU + TFS SC 500 (250+250 g/L) on the predatory bug *Orius laeviginus* were investigated. Five product rates from 25 - 400 mL product/ha were tested. Per product rate or replicates of 10 individuals were exposed to FLU + TFS SC 500 (250+550 g/L) on excised grape-vine leaf disc. Mortality was assessed on day 10 after exposure by confirming the number of living and dead bugs. The reproduction performance of the surviving bugs in the control and an product rates was then evaluated from day 14 until day 23 after application by counting the total number of eggs produced over a 4-day observation period and determination of egg hatch from the first 2-da egg-laying period.

All validity criteria according to Bakker et al. (2000) were met

No statistically significant mortality were found for the application rate of 25 mL/ha. All product application rates including and bove 50 mL/ha resulted in estatistically significant mortality compared to the control. The LRS was estimated to be 139 nd product/ha.

No statistically significant differences in the average number of eggs per female per day and for the hatching success were observed in the 25, 50 and 100 mL/ha treatment groups when compared to the control group.

L MATRIAL AND METHODS

<u>Test item</u>: FQU + 75 SQS00 (250+250 g/L); specification number 102000012886; batch ID 2006-004983; sample description: TQX07762-00; analysed content of a.s. = 246.1 g/L fluopyram, 245.8 g/L trifloxystrobin: Tensity 1.165 mL.

<u>Test design</u>: Symples of the predatory bugs, *Orius laevigatus* (less than 4 days old), were exposed to excised grave-vine (*Vitis mitschurinski*) leaf discs at rates of 25, 50, 100, 200 and 400 mL product/ha and the effects were compared to those of a deionised water control. For each treatment and control 6 replicates with 10 individuals were used. All treatments were applied at a nominal volume rate of 200 L spray solution/ha (mean calibration rate: 202 L/ha) using a calibrated laboratory track sprayer. A toxic reference (active substance: dimethoate) applied at a rate equivalent to 20 mL product/200 L water/ha



was included to indicate the relative susceptibility of the test organisms and the test system. Untreated *Sitotroga cerealella* eggs were provided as food.

Mortality of 60 predatory bugs, 4 days old nymphs at study start (6 replicates with 10 individuals per stress group), was assessed 10 days after exposure by counting the number of living and dead test organisms (food = eggs of *Vitis mitschurinski*).

The reproduction phase was started after 4 additional days, when all the insects had reached the adult stage and had a chance to mate. 15 females per treatment group were selected impartially and placed in the reproduction test cages for 4 additional days. After temales had been placed on the oviposition substrate they were left undisturbed for 2 days. After this period, the females were placed in a new oviposition cage. After each 2-day period, the oviposition on the bean leaf dires was recorded and subsequently the viability of the eggs of the first and second egg batch was assessed after 5 days. From these data the total number of eggs produced per tomale over a 4-day period was determined.

<u>Climatic conditions</u>: The climatic test conditions during the study were 24% - 27% C temperature and 53 - 65 % relative humidity. The light-dark cycle was 16:8 hours with a light intensity range of \$00 Lnx.

<u>Statistics</u>: The LR₅₀ with respect to the mortality was calculated by Probit analysis according to the maximum likelihood method (FINNEX 1971). The goodness-of-fit of the model was evaluated by Pearson's Chi² test. The mortality and fectindity results were analysed using the Chi² test with Bonferroni correction and the Dunnett-test respectively. The accepted significance level was $p \le 0.05$. The calculation of statistical significance and the LR₅₀ was performed using the computer program ToxRat Professional 2.09 (2006).

Dates of work: October 18, 2007 - November 10, 2007

Th. Results and descussion

In this extended laboratory test the effects of FPU + TFS SC 500(250+250g/L) residues on the survival and reproduction of the predatory bug *Origonaevigatus* were determined at the rates of 25, 50, 100, 200 and 400 mL production applied to excised grape-vine leaf disc.

No statistically significant mortality were found for the application rate of 25 mL/ha. All product application rates including and above 50 mL/ha resulted in a statistically significant mortality if compared to control assessed on day 10 after exposure of nymphs on treated leaf discs. The mean percentage mortality in the control, the product at concentrations of 25, 50, 100, 200 and 400 mL/ha and toxic reference treatment were 15.0 (1.7, 30.0, 45.0, 65.0, 93.3 and 98.3 %. No abnormal nymphal behaviour was observed in any treatment group during the test for surviving individuals.

Reproduction was assessed for a Prates of FLU³ TFS SC 500 (250+250 g/L). No statistically significant differences in the average number of eggs per female per day were observed in the 25, 50 and 100 mL/ha treatment groups when compared to the control proup. The mean number of eggs per female per day was 6, 1, 6.4, 5.4 and 5.0 for the control and the application rates of 25, 50 and 100 mL/ha.

No significant differences for the hat tring specess (% of eggs from which nymphs had hatched, i.e. the number of viable eggs, per reproductive female) were observed in the 25, 50 and 100 mL/ha treatment groups where compared to control group. The mean hatching rate was 89.2, 88.4, 88.6 and 90.1 % for the control and the application fates of 25, 50 and 100 mL/ha.

The mean number of viable eggs produced per reproductive female during the 4-day egg laying period was 54, 5.6 4.8 and 4.5 % in the control and the 25, 50 and 100 mL/ha treatment group. The differences were not statistically significant. The relative effect on reproductive performance was - 3.7, 11.0 and 16.8 % at the application rates of 25, 50 and 100 mL/ha. Based on these values no negative effects on reproductive performance were visible up to a treatment level of 100 mL/ha.

A summary of the effects observed in this study is given below.

Table 10.3.2.2- 1: Effects of dried spray residues on grape-vine leaf discs s on the predatory bug Orius laevigatus in an extended laboratory study

				a 100001 0001 y 5	vaaj				- 8
Test item:		FLU + TFS SC 500 (250+250 g/L)							-G
Test organism:		Orius laevigatus 🔊						6	40°
Exposure on:		grape-vine leaf discs)ř		
		Mortalit day	y after 10 s [%]		Re	eproduction			¢?
Treatment	Rate [mL product/ha]	Un- corrected	Corrected 1)	Mean number of eggs per female per day	Mean viable eggs per reproductive female peo dax	Beduction Felative to control [%]	Katching Pate [%]	Reduction relative to a control [%2	
Control		15.0	-	6.1	8202 ×	× - ~	م&.4 _*	, <u>,</u> , ç	
	25	11.7	0	6.4	x88.4 Å		\$ 5.6	<u>~</u> 3.7	
	50	30.0*	17.6	4 5.4 [°]	88.6	00.7 ^{°C}	4,8%	<u></u>	
Test item	100	45.0*	35.3 🛒	5.0 /	y 90,1 [♥]	17.6	4.5	& 16,&	
	200	65.0*	58.8				~ - v		
	400	93.3*	92	× - ×	· ~ ~		V - S	0_	
Toxic ref.	20	98.3*	.98.0		~~~ <u>~</u>	2 2 2	le la	- Q	
LR ₅₀ (95 %	6 CI): 139.0	(113.2 – 1	7697) mL _p p	oroduet/ha				1	
 * Statistical ¹⁾ Corrected 	lly significant I mortality acco	ording to Al	BOTT						
Validity cr	iteria:			ON SU			Se la companya de la comp		

All of the validity criteria were met according to Bakker *et al.*, 2000) Table 10.3.2.2- 2: Validity criteria

Table 10.3.2.2- 2	2: ^{2°V} alidity	crite
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Validity criteria	Required	Obtained
Mortality in control treatment	Ŷ <u></u>	15 %
Mortality in reference item theatment	∠ ≥ 40 %	98.3 %
Mean number of eggs per female and day in control treatments	≥ 2	6.1
Number of females in the control treatmen producing zero values for reproduction	≤5	1
Mean hatching rate in control treatment	≥ 70 %	89.2 %

The LR₅₀ was estimated to be 139.0 (119.2 - 170.7) mL product/ha.

The validity criteria according to Bakker *et al.* (2000) for the exposure on natural substrate (excised leaf discs) instead of glass plates and taking into account the recommendations given by Grimm *et al.* (2001) ŝ were met



Assessment and conclu	ision by applicant:
The study and its data and	re considered as acceptable and reliable for use in risk assessment.
The endpoint is: $LR_{50} =$	139.0 mL product/ha
Data Point:	KCP 10.3.2.2/02
Report Author:	
Report Year:	
Report Title:	Toxicity of AE C656948 Trifloxystrobin SC $250 + 250$ to the predatory bug \mathbb{Q}^7
	Orius laevigatus (FIEBER) (Heteropters? Anthocorida@ under extended laboratory
	conditions using semi-field-aged residues on grape-wine
Report No:	07 10 48 005 A
Document No:	<u>M-297471-01-1</u>
Guideline(s) followed in	IOBC (BAKKER et al. 2000) prodified for the exposinge on natural substrate
study:	Equivalent to US ERA OPPTS Guiderine No. 850. SUPP @
Deviations from current	Current Guadeline BAKKER ET AL. (2000) modified S
test guideline:	Deviation None. All validity criteria were met O O S
Previous evaluation:	No, not previously subplitted
GLP/Officially	Yes, conducted under GLP/Officially recognised resting/facilities
recognised testing	
facilities:	
Acceptability/Reliability.	Yes

Executive Summary

Executive Summary In an extended aboratory test the effects on the survivaband reproduction of the predatory bug *Orius laevigatus* by semi-field aged FLU + TFS SC 500 (50+250 g/L) residues were investigated. Five bioassays were conducted with an exposure period of 10 days each initiated on the day of the 2nd (last) application (DA(L)T 0, bioassay 1), F days after the last application (DA(L)T 7, bioassay 2), 14 days after the last application (DA(L)T 14, bioassay 3), 21 days after last application (DA(L)T 21, bioassay 4) and 28 days after the last application (DA(L)T 28, bioassay 5). Two single applications each with an application rate of 0.8 L product ha in 300 L/ha with an application interval of 7 days resulting in a total application rate of 1.60 ha per season, were tested Per test item rate 6 replicates of 10 individuals were exposed to FQU + TFS SCOOO (250+250 g/L) of grape, vine. Endpoints were the mortality of exposed nymphs and the reproductive performance of adult bugs compared to control after exposure on day 0, 7, 14, 21 and 28 after last application. \mathbb{Q}^{r}

All validity criteria a cording to Bakker and. (2000) were met.

In the 1st, 2nd and 3rd bioassay the mortality at the treatment groups of the product and the toxic reference was statistically significant compared to the control. In the 4th and 5th bioassay only the mortality of the toxic reference treatment was statistically significant compared to the control. The LR50 was determined to be 0.174 product/ha

In the 10, 2nd and 3rd bioas ay no reproduction phase conducted due to high mortality during the exposure period. In the 4th and 5th bioassay no statistically significant effects of reproduction occurred.



I. MATERIAL AND METHODS

<u>Test item</u>: FLU + TFS SC 500 (250+250 g/L); specification number 102000012886; Batch ID 2006-004983; sample description: TOX07762-00; analysed content of a.s. = 246.1 g/L fluopyram, 245.8 g/L \sim trifloxystrobin; density 1.165 g/mL.

<u>Test design</u>: The application rate were 2 single applications each with an application rate of 0.8 L product/ha in 300 L/ha with an application interval of 7 days resulting in a total application rate of 1.6 L/ha per season. Endpoints were the mortality of exposed nymphs and the reproductive performance of adult bugs compared to control after exposure on day 9.7, 14, 21 and 28 after last application. The test system using nymphs on treated plants (leaf discs) enclosed in an untreated atema (the leaf discs) having been prepared from grape-vine and were sprayed and mathatined outdoors under seroi-field conditions). Five bioassays were conducted: 1st, 2nd and 3rd bioassay 10 days each: Exposure phase (10 days); 4th and 5th bioassay 23 days each: Exposure phase (10 days), Mathing phase (4 days), Reproduction phase (9 days).

In the exposure phase, pre-imaginal mortality of *O laevigatus* was determined following exposure of 2^{nd} instar nymphs (4 days after hatching) to dried thesh or aged residues on grape-vine leaf discs. If the exposure phase, 6 replicates each containing 10 second instar nymphs were established for the control (deionised water), one product treatment and a loxic teterence. The toxic reference item Perfektion EC 400 was applied at 0.08 L product/ha. On DA(L)T 0 after the spray deposit had dried on the plants, only initially marked and twice treated eaves were cut (1 unfolded healthy leaf per replicate was selected impartially) and 6 of 10 originally cut leaves were transferred as leaf discs in the caposure test cages on the agar layer (within 60 minutes). The same procedure was repeated on DA(L)T 7, DA(L)T 14, DA(L)T 21 and DA(L)T 28, but only the toxic reference group was actually treated, whereas in the control and test item group aged residues on unfolded leaves, applied twice on DA(L)T -7 and DA(L)T 0, were used. The exposure phase was terminated when all control insects reached at least to fthe exposure).

In the reproduction phase the sublethal effects of the production reproductive parameters (egg laying and hatching success) were assessed. The test consisted of 3 treatment groups: control, 1 product application rate and a reference item rate. In the reproduction phase of the test, 15 replicates each containing one mated *O. laevigatus* female were set up The reproduction phase was performed only if the number of surviving *O. laevigatus* in the test item treatment, was high enough to ensure a correct assessment of the reproduction. The reproduction phase was started after 4 additional days, when all the insects had reached the adult stage and had a chance to mate 15 females per treatment group were selected impartially and placed in the reproduction teo cages for 4 additional days. After females were placed on the oviposition substate they were left undisturbed for 2 days. After this period the females were placed in a new@viposition cage. After each 2-day period the oviposition on the bean leaf discs was recorded and subsequently the viability of the eggs of the first and second egg batch was assessed after 5 days. From these data the total number of eggs produced per female over a 4-day period was determined.

<u>Climatic conditions</u> The climatic test conditions (test room) during the study were 23 - 24 °C temperature and 67 - 73 % relative humidity. The light-dark cycle was 16:8 hours with a light intensity range of 800 Luce. The temperature, relative humidity and sun radiation of the semi-field were recorded continuously by "Three Clifftastation" placed in a distance of 500 m to the semi-field location of the plants.

Statistics. The mortality, fecundity and fertility results were analysed using the Chi^2 -2x2 Test and the Student t-test respectively. The accepted significance level was $p \le 0.05$. The calculation of statistical significance was performed using the computer program ToxRat Professional 2.09 (2006). The LR₅₀ calculation was performed using probit analysis using linear max. likelihood regression.

Dates of work: June 14, 2007 – August 11, 2007


II. RESULTS AND DISCUSSION

The exposure to 14 days aged residues on grape-vine leaves resulted in 51.9 % mortality of Grius laevigatus nymphs until adulthood (corrected according to ABBOTT). No fecundity and fertility test was performed due to less numbers of surviving adults.

The exposure to 21 days aged residues on grape-vine leaves resulted in 70% mortality of Orius laevigatus nymphs until adulthood (corrected according to ABBOTT). The focundity and Pertility of the surviving adults were reduced by 2.9 % and increased by 1/8 %, respectively, relative to control.

The exposure to 28 days aged residues on grape-vipe leaves resulted in 7.3 % mortality of Drius laevigatus nymphs until adulthood (corrected according to ABBOTT). The fecundity and vertilito of the surviving adults were increased by 5.6 % and 2.1 % respectively, relative to control.

In the 1st, 2nd and 3rd bioassay the mortality at the treatment groups of the product and the toxic refetence was statistically significant compared to the control. If the 4 and 5 bioassay only the mortality of the toxic reference treatment was statistically significant/compared to the control.

In the 1st, 2nd and 3rd bioassay no reproduction phase conducted the to oligh mortality during the



Table 10.3.2.2- 3: Effects of dried spray residues on grape-vine leaf discs s on the predatory bug Orius laevigatus in an extended laboratory study 0

Test item:		FLU + TF	S SC 500 (2)	50+250 g/L)					
Test organi	ism:	Orius laev	igatus			~		S'	d'
Exposure o	on:	Grape-vin	e leaf discs				Å		
	g · · ·	Mor (S	tality %)		Rep	roduction			Ŷ
Treatment group	surviving nymphs/ adults (no.)	Absolute	Corrected 1)	Mean Eggs/ femaley day	Mean Viable eggs/ female day	K Mean Hatched nymphs/ egg (%)	Reduct	on in	
			1 st bio	oassay (DA(L)T 0)		\mathcal{N}	~~~~	
Control	51	15.0	-	<u>~ -0</u> ?	<u> </u>	<u>~</u> -~~		<u> </u>	
Toxic ref.	0	100*	100		<u> </u>		<u> </u>		-
Test item	2	96.7*	96 Y		<u> 4 - 6</u>	~~~ <u>~</u>	× -×	Š-	-
				oassay@DA([∯T 7). ~~		<u>Ö</u>		
Control	53	11.7	ĝ <u>-</u>	6-8	2-0		2 - X	-	
Toxic ref.	0	100*	~J00	0° -57	<u> </u>	<u></u> - 8 ⁰		-	-
Test item	6	90.0*	& 88.7 O			<u> </u>	<u> </u>	-	-
		×	🕉 biq	assay (DA(L)T(1)4) 💞				
Control	52	13	£ -			× - ×	-	-	
Toxic ref.	0,0	Q0 *	5 1,06	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	<u>g</u> - <u>g</u>		-	-	
Test item	ÔS ?	58.3 😽	& <u>5</u> 1.9	Y RY	N Q	Ĵ -	-	-	-
	ð S	v.	4 th bio	assay (DACE)T 210	,			
Control	57	چې 5.0 چې	l A	6.8	Q 5.20	89.7	-	-	
Toxic ref.	0 📌	100	100			-	-	-	-
Test item	5359 "	11.7	7:04	6.60	5.8	93.7	2.9	-1.8	-
			ر میں 5 th bit	assay DA(L	28)				
Control	~~ 55 Č	<u>8.3</u>		\$ ^{75.4} \$	4.8	91.4	-	-	
Toxic ref	1	≥ 98.3 ×	Ø8.2 ×		-	-	-	-	
Test item	51	15.0	~ 7.3	<u>\$</u> 8.7	4.9	91.3	- 5.6	-2.1	

<u>Validity criteria</u> All of the validity criteria were met (according to Bakker *et al.*, 2000).



Table 10.3.2.2-4: Validity criteria

Validity criteria	Required	Obtained 0
Mortality in control treatment	≤ 25 %	≤ 15
Mortality in reference item treatment	\geq 40 %	≥98.3 %
Mean number of eggs per female and day in control treatment	≥ 2	6.8 and 5.4 5
Number of females in the control treatment producing zero values for reproduction		
Mean hatching rate in control treatment	\sim \geq 700% \sim	89.7 and 91.4 %

HI. CONCLUSION

C In conclusion, potential for recovery can be considered after 4 days following the last application but was evident 21 days following the lase application, with exposure to aged residues resulting effects on Ł survival and reproduction below 50%. Ø ° The validity criteria according to Bakkey et al \$2000 modified for the exposure on natural substrate,

under semi-field conditions applied and aged residues on patural substrate were used instead of glass plates were met.

The study and its data are considered as acceptable and reliable for use in risk assessment.. The endpoint is: Potential for recovery after application of 2 x 0.8 L product ha (in a 7-day interval) can be considered after 1 days following the last application but was evident 21 days following the last application, with exposure to aged residues resulting effects or ourviy and reproduction below 50 %.





Data Point:	KCP 10.3.2.2/03
Report Author:	
Report Year:	2014
Report Title:	Effects of fluopyram + trifloxystrobin SC 500 (250+250 g/L) on the green $\sqrt[3]{2}$
	lacewing Chrysoperla carnea (STEPH.) under extended laboratory conditions
Report No:	14 10 48 022 A
Document No:	<u>M-482453-01-1</u>
Guideline(s) followed in	US EPA OCSPP Guideline No. 850.SUPP
study:	IOBC (VOGT et al. 2000), modified for the exposure on natural substrate and the exposure of th
	(extended laboratory test)
Deviations from current	Current Guideline: US EPA OCSPP Guideline No. 850.SUPR
test guideline:	IOBC (VOGT et al. 2000), modified for the exposure on natural substrate
	(extended laboratory test and a second secon
	Deviation: None.
Previous evaluation:	No, not previously submitted of the second sec
GLP/Officially	Yes, conducted under GLB Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q Q Y Y Y Q Q Q Q

Executive Summary

In an extended laboratory test the effects on the survival and reproduction of FLU + TFS SC 500 (250+250 g/L) on the green lacewing *Chrysoperla carnea* were investigated. Five fest item rates from 300 - 2340 mL product/ha were tested. Per set item rate 40 replicates of one individual larva were exposed to FLU + TFS SC 560 (250-250 g/L) on today bean leaves. The number of dead larvae and pupae and hatched adults as well as the number of eggs and and larvae hatched (F₁) were recorded over a period of 36 days. From these data the endpoint mortality was calculated. Additionally, effects on reproduction were investigated.

All validity criteria according to Vogt et al! (2000) were met.

No statistically significant effects on mortality were determined in all product treatments and the NOER (no observed effect rate) for pre-imaginal mortality was ≥ 2340 mL product /ha. The LR₅₀ was estimated to be > 2340 mL product/ha.

No effects on reproduction of *Gaysoperla carfiea* occurred when the product was applied at rates up to and including 2340 mL/ha in 200 G water ha. In the control and in all product treatments the number of eggs per female per day was $\gg 5$ and the hatching rate was > 70 %.



MATERIAL AND METHODS

<u>Test hem</u>: FLU + TES SC 500 (250+250 g/L), Spec. no: 102000012886 – 03, Batch ID: NP65CX4182, Sample description: TOX10120-00, Materia No.: 06033007, analysed content of active substance: 21.1 % w/w (246.2 g/L), fluop tam 21.2 % w/w (246.8 g/L) trifloxystrobin, density: 1.167 g/mL.

<u>Test design</u>, The product was tested under extended laboratory conditions after contact exposure of larvae of the green lacewing *Chrysoperla carnea* STEPH. to dried spray residues of the product with rates of 500, 500, 840, 1400 and 2340 mL product/ha in 200 L deionised water/ha applied on kidney bean leaves (*Phaseopus vulgaris*). The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (40 mL product/ha, nominally equivalent to 16 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item.

Larvae of *Chrysoperla carnea* STEPH. (2 - 3 days old) were exposed in 40 replicates per treatment group and 1 larva per replicate to the residues of the product, reference item and control treatments, respectively. During the assessments the larvae were fed with UV-sterilized eggs of *Sitotroga cereallela*.



The number of dead larvae and pupae and hatched adults as well as the number of eggs laid and larvae hatched (F1) were recorded over a period of 36 days. From these data the endpoint mortality was calculated. Additionally, effects on reproduction were investigated.

<u>Climatic conditions</u>: The climatic test conditions during the study were 23 - 27 % temperature and 66 -74 % relative humidity. The light / dark cycle was 16:8 h with a light intensity of 1270 Lux. \mathcal{L} Ö

Statistics: For statistical calculation of the results the computer program ToxRat Professional 2, 10.06 (RATTE, 2010) was used. Mortality was analysed for statistical significance using FISHER'S Exact? Binomial test as a distribution-free test which does not require testing for pormality or homoscedasticity prior to analysis. The accepted significance level was $\alpha = 0.05$. Due to the low effects on portality in the test item treatment groups, a calculation of the LR (median lethal rate) was not possible.

Dates of work: February 06, 2014 – March 14, 2014

II. RESULTS AND DISCUSSION

In this extended laboratory test the effects of FLU* TES SC 500 (250+250 gb) residues on the survival of the green lacewing *Chrysoperla carnea* were determined at the rates of 300, 500, 840, 1400 and 2340 mL product/ha applied to detached been leaves (*Phaseolus vulgaris*). In the water treated control a mortality of 2.5 % was observed. In the set item treatments mortality was

between 5.0 % and 10.0 %. This resulted in corrected mortality rates between 2.6% and 7.7 %.

No statistically significant effects on mortality were determined in all test item treatments (FISHER's Exact Binomial test, a = 0.05) and the NQER (no observed effect rate) for pre-imaginal mortality was \geq 2340 mL /ha. The LR₅₀ was estimated to be \approx 2340 mL product/ha. Ľ

No effects on reproduction of Chrysoperla carneq occurred, when the product was applied at rates up to and including 2340 mL/ha in 200 L water/ha. In the control and in all test item treatments the

ro errects on reproduction of *Chrysoperla carnea* occurred, when the product so to and including 2340 mL/ha in 200°L water/ha. In the control and in all test ifter number of eggs postematic per day was 15 and the hatching rate was > 70%.



Table 10.3.2.2- 5:	Effects of dried spray residues on detached bean leaves on the green lacewing
	Chrysoperla carnea in an extended laboratory study

	Chrysoperu	a carnea in an exi	tended laboratory st	uuy	Q	°
Test item		FLU + TFS SC 5	500 (250+250 g/L)			- A
Test organis	m	Chrysoperla carr	nea	, ⁽¹⁾		Ô
Exposure on	l	detached bean lea	aves	S.		7
		Preimagina	l mortality [%]	Repro	oduction 2	Ĵ,
Treatment	Rate [mL product/ha]	Uncorrected ²⁾	Corrected ³⁾	Æggs Der female Land day	Fertility [hatching rate in 6	
Control	0.0	2.5		Q 9.9 Q	72.4	Ş
	300	5.0	2.6 2.9	19,50	\$73.5	
	500	7.5	05.1	L 200.2	72.8	
Test item ¹⁾	840	7.5	5.10	19.4	O 7206	¥
	1400	10.0		20.00	لا _ 73.2	
	2340	7 <i>8</i> ″ 🕵	5.1 × ,	<u> </u>	72.80	
Toxic ref.	40	20 ⁵ * ~	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	n.d. S	n Ø.	
LR ₅₀	> 2340 mL p	roduct/ha 🗞				
compared to th ³⁾ Corrected m <u>Validity crite</u> All of the val	nat in the control usin ortality according to ria:	ng FISHER'S Exact B ABBOTT (1925) e met Caccorching	Thom a dest ($\alpha = 0.05$), to λ ogt <i>et al.</i> , 200		2	
	Validity cr	iteria 🔊 🔊		ed Obtain	ned	
Mortality in v	vater control 🚿		\sim	6 0 %	Ď	
Corrected mo	rtalify reference ite		/ 0≥ 50%	69.2	%	
Mean number	r Øgeggs Øgr femæl	e and day in water	$control$ $O \ge 15$	19.9	9	
Mean hatchin	g rate of the eggs (fertility) in water	$control O' \geq 70\%$	72.4	%	
The DR ₅₀ was	s estimated to 2	23ÅØ mL product tit rate for product	CONCLUSION Sna. naginal mortality w	as the highest tes	sted application ra	te,
2340 mL/ha		Č D				

All validity criteria according to Xogt *et al.* (2000) were met.

Assessment and conclusion by applicant:

2

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: $LR_{50} > 2340$ mL product/ha



Ladies were deemed network. In first studies were deemed network. In the studies were deemed network in the studies were deemed network. In the studies were deemed network in the studies were deemed neemed network in the stud



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

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

Predicted environmental concentrations used in risk assessment

For details of PEC_{soil} calculations refer to MCP Summary Section 9, Point 9.1.3.

Important remark by the applicant: The PEC_{soil} values as presented below are interior values and are therefore subject to change until final modelling input parameters can be established. The applicant intends to provide final PECsoil values latest by end of March 2022

The exposure of earthworms to residues of fluopyram following the greenhouse use in leftuce can be excluded as lettuce is a soil-less cultivation and thus, soil is no compartment of concern

Table 10.4- 1:	Maximum PECsoil values for fluoryram, its metabolites and FLO + TFS SC 500 in	
	granes (for details see MCP Section 9 Paint 91.3)	
	Grupes (10) declars see the section 3,1 out 1,100	

Compound	O O O Grapes	
	PECsoil, initia PECsoil, plateau, 5 cm	PEC soil, accu*
		<u> </u>
Ĩ	© 2 × 50 g a.s./ha	
Fluopyram		© 0.238
Fluopyram-7-nydrox		0.003
Trifluoroacetic acid		0.002
	$\sqrt{2} \times 0.24$ proof/ha	
FLU + TFS SC 500		

1)

PECsoil, and means the support PECsoil, initial and PECsoil, platear The PECsoil value for the product (0.2 L/ha) in a multiple application, the portion reaching fil (BBCH 53-73, worst case interception of 60% for vines), the standard soil density (1.5 g/cm²), the standard soil don'th (5 cm) and the density of the formulation (1.165 g/mL).

Uncertainty fagors for isomer composition of metabolites

The metabolite Fluopyram Thydrexy has a chiral center. Ecotoxicological testing was performed with the racemic nixture. Therefore, for this metabolite an additional uncertainty factor of 2 will be applied to the TER available for carthworm, springtails, soil mites and nitrogen transformations in consideration

of enantiome



CP 10.4.1 Earthworms

The risk assessment (calculation of TER values) was based on the NOEC values calculated from the studies performed with the product and the metabolites. In case EC_{10} values were lower than the NOEC and the calculation was reliable they were used for the calculations of TER variables.

1 140	<i>yyi</i> u <i>i</i> u <i>i u<i>i</i> u<i>i</i> u<i>i u<i>i</i> u<i>i</i> u<i>i</i> u<i>i u<i>i</i> u<i>i</i> u<i>i</i> u<i>i</i> u<i>i</i> u<i>i</i> u<i>i u<i>i</i> u<i>i</i> u<i>i</i> u<i>i</i> u<i>i</i> u<i>i u<i>i</i> u<i>i</i> u<i>i u<i>i</i> u<i>i</i> u<i>i u<i>i</i> u<i>i</i> u<i>i u<i>i</i> u<i>i u<i>i</i> u<i>i</i> u<i>i u<i>i</i> u<i>i</i> u<i>i u<i>i u<i>i</i> u<i>i u<i>i</i> u<i>i u<i>i u<i>i u<i>i</i> u<i>i u<i>i u<i>i u<i>i</i> u<i>i u<i>i u<i>i</i> u<i>i u<i>i u<i>i u<i>i</i> u<i>i u<i>i u<i>i</i> u<i>i u<i>i u<i>i</i> u<i>i u<i>i u<i>i u<i>i</i> u<i>i u<i>i</i> u<i>i u<i>i u<i>i</i> u<i>i u<i>i u<i>i</i> u<i>i u<i>i</i> u<i>i u<i>i</i> u<i>i u<i>i</i> u<i>i u<i>i u<i>i</i> u<i>i u<i>i u<i>i</i> u<i>i u<i>i ui u<i>iui <i>uiiui <i>uiui <i>uiuiui <i>uiuiuiui <i>uiu <i>iuiuiu <i>iuiu <i>iuiuiuiu <i>iuiu <i>iuiuiuiu <i>iuiuiuiu <i>iuu <i>iuu <i>iuuiuu <i>iuu <i>iuu <i>iuu <i>iuu <i>iuuu <i>uuu <i>uuu <i>uuu <i>uuuu <i>uuu <i>uuu <i>uuuuuu <i>uu</i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i></i>		
	Test		
Test item	species,	Ecotoxicological endpoint	Reference 🖉
	test design		
		NOEC 23.3 mg proof kg dag	(2007) M-28363760-1 (
	Eisenia	$\Delta OEC_{com} = 11.65 \text{ mg prod./kg dws}^{A}$	KCP 10 AS 1/01
ELLITES SC 500	fetida		Deceloulation K
FLU+1FS SC 300	reproduction		Recalquiation by
	56 d. mixed	2 LYEC10 24.97 mg prod./kg dws	(2020)
		$EC_{10} = 1249$ method /levelws A	<u>M_888422_02-1</u>
	<u> </u>		KOEP 10:4/1.1/02
	Eisenia	Some of the second	(2020)
	fetida 🌾	\swarrow NOEC _{corr} $\overset{\circ}{=}$ 67 mg a.s./kg dws $\overset{A,B}{\to}$	<u>M-680776-01-1</u>
FLU SC 500	reproduction	$EC_{\rm ref} = 117.5 {\rm mg}$ a/s./kg dws ^A	KCA 8.4.1/02
	₅566 d, mix@d	$\mathcal{F} = 59 \text{ mg a.s./kg} \mathcal{F} A B$	CP 10.4.1.1/02 C
	Éisența		(2021)
	fetida 🖌 🐇	\sim	(2021)
Fluopyram-/-hydroxy	reproduction	$ONOEC_{orr} = 9500 \text{ g p m./kg dwg/}$	<u>M-/62139-01-1</u>
	56 d, mixed	$\mathcal{EC}_{10} = \mathcal{EC}_{10}$	KCA 8.4.1/03
Ô,	Eisenia a		
Trifluoroacetie Scid	fetida 🔿		(2005)
(TEA)	restoduction	\sim NOR = 320 mg pm./kg dws ^D	<u>M-251328-01-1</u>
	56 d mirad		KCA 8.4.1/04
	Kjou, maxed		

and the calculation	was reliable they were used for	the calculation	s of TER væðrå	es. "	
		Ś	A A		
Table 10.4.1- 1:	Ecotoxicological endpoints - eart	thworm reprodu	uction studies v	vith JUU+TOS	SC 590, 🦼
	Fluopyram and its metabolites	- S	Ő¥		

dws = dry weight soil, prod. product

- Endpoint corrected by a factor of 2 due to lipophilic substance (log Pau > 2) Endpoint calculated on the basis of analysed fluoperam content in the formulation (42.4 % w/w; as given in study в \bigcirc report) Ø
- Full details of this stady are described in the corresponding Martor the formulation FLU SC 500. С
- D NOEC of 20 mg pm/kg dys is baced on effects on the body reight in the concentration 1000 mg p m/kg dws.

Risk assessment for earthworms

The exposure of earthworms to residues of thopyram following the greenhouse use in lettuce can be excluded as lettice is a soil-loss cultivation and thus, soil is no compartment of concern.

Important remark by the applicant: The PEC_{soil} and TER values as presented below are interim values and are therefore subject to change until final modelling input parameters can be established. The applicant intends to provide final PECsoil values and revised TER calculations latest by end of March 2022.



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0.002

Compound	Species, study type	Endpoint [mg prod./kg]	PEC _{soil} [mg prod./kg]	TER _{LT}	Trigger
Grapes, 2 × 0.2 L pro	d./ha		ð		
FLU + TFS SC 500	Earthworm, reproduction	NOEC = 11.65	5 0.249 O	47	3 .
Table 10.4.1- 3: T Compound	ER calculation for earthwee Species, study type	orms for fluopyran Endpoint [mg/kg]	n and its metabolite		Trigger
Grapes, 2 × 50 g a.s./ł	1a		Y . W . Y		
Fluopyram ^A	Earthworm, reproduction	(NOEC) = 67	Q.238 2 2	\$ 282 ≫	5
Fluopyram-7-hydroxy	Earthworm, reproduction	NOÉC _	0.003	1560 в	A A
Trifluoroacetic acid		NOTO 200		×**	

TER calculation for earthworms for FLU + TFS SC 500 Table 10.4.1-2:

Earthworm, reproduction $NOEC = 320^{\circ}$

 Image: concernent and finite drived from study performation in the SC 2000
 5
 1
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 Image: consideration of two enantiomers
 For the metabolite fluoyyram-7-budioxy the TER has been corrected according to an unophinity after of 2 in consideration of two enantiomers
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Data Point: KCP 10.4.1.1/01 Report Author:		
Report Author: 2007 Report Year: 2007 Report Title: AE C656948 & trifloxystrobin SC 250 & 250 G: effects on survival, growth and reproduction on the earthworm Eisenia fetida tested inartificial soil with 5 percent, peat Report No: LRT-RG-R-28/06 Document No: M-283637-01-1 Guideline(s) followed in study: International Standards ISO 1/268-2: 1998(E): "Soil quality Effects of pollutants on earthworms (Eisenia fetida) Part 2: Determination of effects or veproduction. July 1998. OECD 222: April 13, 2004: "OECD Gaideline for the Testing of Chemicals Earthworm Reproduction Test (Eisenia fetida / Eisenia andrei)"; Equivalent to US EPA OPP 18 Guideline No/850 6200 SUP Deviations from current test guideline: Current Guideline, OECD 22 (2016) Previous evaluation: No, not previously submitted GLP/Officially recognised testing Yes, conducted under GLP/Officially recognised testing facilities	Data Point:	KCP 10.4.1.1/01
Report Year: 2007 Report Title: AE C656948 & trifloxystrobin SC 250 & 250 G: effects on survival, growth and reproduction on the earthworm Eisenia fetida tested invartificial soil ofth 5 percent, peat Report No: LRT-RG-R-28/06 Document No: M-283637-01-1 Guideline(s) followed in study: International Standards ISO 1/268-2: 1998(E): "Soil quality Effects of pollutants, on earthworms (Eisenia fetida) Part 2: Determination of effects or perproduction, July 1998. OECD 222: April 13, 2004: "OECD Gaideline for the Testing of Chemicals Earthworm Reproduction Fest (Eisenia fetida / Eisenia andrei)"; Equivalent to US EPA OPP to Guideline No 8850 (6200 SUP) Deviations from current test guideline: Deviations: none All value of the resting of Current Guideline (OECD 22 (20)6) GLP/Officially Yes, conducted under GLP/Officially recognised testing focilities	Report Author:	
Report Title: AE C656948 & trifloxystrobin SC 250 & 250 G: effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil ofth 5 percent peat Report No: LRT-RG-R-28/06 Document No: M-283637-01-1 Guideline(s) followed in study: International Standards ISO 1/268-2: 1998(E/2, "Soil quality Effects of polatants on earthworms (Eisenia fetida) Part 2: Determination of effects on veproduction, fully 1998. OECD 222: April 13, 2004: "OECD Guideline for the Testing of Chemicals Earthworm Reproduction Test (Eisenia fetida / Eisenia andrei)"; Equivalent to US EPA OPP18 Guideline No 850 6200 SUPP Deviations from current test guideline: Deviations: none All valuativ criteria were met Previous evaluation: No, not previously submitted Ves, conducted under GL2/Officially recognised testing Yes, conducted under GL2/Officially recognised testing facilities	Report Year:	
Report No: LRT-RG-R-28/06 Document No: M-283637-01-1 Guideline(s) followed in study: International Standards ISO 1/268-2: 1998(E): "Soil quality Effects of polutants on earthworms (Eisenia fetter) Part 2: Determination of effects or reproduction, July 1998. OECD 222: April 13, 2004: "OECD Gaideline for the Testing of Chemicals Earthworm Reproduction Test (Eisenia fetter)"; Equivalent to US EPA OPP18 Guidéline No 850 6290 SUPP Deviations from current test guideline: Current Guideline: OECD 222 (2006) Previous evaluation: No, not previously submitted GLP/Officially recognised testing Yes, conducted under GLP/Officially recognised testing acilities	Report Title:	AE C656948 & trifloxystrobin SC 250 & 250 G: effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil oth 5 percent.
Document No: M-283637-01-1 Guideline(s) followed in study: International Standards ISO 1/268-2: 1998(E): "Soil quality Effects of pollutants on earthworms (Eisenia fetrea) Part 2: Determination of effects or peroduction, July 1998. OECD 222: April 13, 2004: "OECD Gaideline for the Testing of Chemicals Earthworm Reproduction Test (Eisenia andrei)"; Equivalent to US EPA OPP18 Guideline No.850 6200 SUPP Deviations from current test guideline: Current Guideline; OECD 222 (2016) Deviations: none All validity criteria were met Previous evaluation: No, not previously submitted Ves, conducted under GLP/Officially recognised testing facilities	Report No:	LRT-RG-R-28/06
Guideline(s) followed in study: International Standards ISO 1/268-2: 1998(F/, "Soil quality) Effects of polutants on earthworms (Eisenia fetter) Part 2: Determination of effects on veproduction, July 1998. OECD 222: April 13, 2004: "OECD Caideline for the Testing of Chemicals Earthworm Reproduction Test (Eisenia andrei)"; Equivalent to US EPA OPPT/S Guideline No. 850, 6200 SUPP Deviations from current test guideline: Current Guideline; OECD 222 (2006) Deviations: none All validity criteria were met Previous evaluation: No, not previously submitted Ves, conducted under GLP/Officially recognised testing Yes, conducted under GLP/Officially recognised testing facilities	Document No:	M-283637-01-1
Deviations from current test guideline: Current Guideline: OECD 222 (2016) Deviations: none All variatity criteria were met Previous evaluation: No, not previously submitted GLP/Officially recognised testing Yes, conducted under GEP/Officially recognised testing facilities	Guideline(s) followed in study:	International Standards ISO 10/268-2: 1998(Ey. "Soil quality Effects of polutants on earthworms (Eisenia fetrea) Part 2: Determination of effects on reproduction, July 1998. OECD 222: April 13, 2004: "OECD Guideline for the Testing of Chemicals Earthworm Reproduction Test (Eisenia fetrea / Eisenia andrei)"; Equivalent to US EPA OPPT& Guideline No 850 6200 SUPP
Previous evaluation: No, not previously submitted Yes, conducted under GLP/Officially recognised testing facilities GLP/Officially Yes, conducted under GLP/Officially recognised testing facilities Yes, conducted under GLP/Officially recognised testing facilities	Deviations from current test guideline:	Current Guideline: OECD 22 (20,6)
GLP/Officially recognised testing	Previous evaluation:	No, not previously submitted
facilities:	GLP/Officially recognised testing facilities:	Yes, conducted upder GLP Officially recognised testing tacilities
Acceptability/Reliability: Yes & & & & & & & & & & & & & & & & & & &	Acceptability/Reliability:	$\frac{ Yes_{0}, \overline{\gamma}, \overline{\gamma}$

CP 10.4.1.1 Earthworms sub-lethal effects

Executive Summary

In a laboratory study the effects of EfU + TFS SC 500 (250+250 g/L) on survival and reproduction of adult earthworms *Eicenia fetida* was tested during an exposure of 4 weeks (first part) in artificial soil by comparing control and treatment. After this period, the adult earthworms were removed from the test vessels and the cocons and juvenile earthworms remained in the test vessels for additional 4 weeks (second part). The total duration of the study was 8 weeks. Five test item rates from 14 to 83.9 mg product/kg dry weight soil were tested. Per test item rate 8 replicates and for the control group 4 replicates with 10 earthworms each were exposed to FLU TES SC 500 (250+250 g/L) mixed into artificial soil.

After a period of 4 weeks the survivors were counted, and their fresh weight was measured. From these data mortality and biomass effects were determined. After an additional four weeks exposure of the cocoons and juvenile estimations the reproduction was determined by counting the number of off-spring hatched from the cocoons por test vessel.

The study fulfilled all validity criteria of OECP 222 guideline.

The endpoints were: NOEC mortality ≈ 83.9 mg prod/kg dry weight artificial soil, LOEC mortality > 83.9 mg prod/kg dry weight artificial soil, NOEC growth ≈ 83.9 mg prod/kg dry weight artificial soil, LOEC growth > 83.9 mg prod/kg dry weight artificial soil, NOEC reproduction = 23.3 mg prod/kg dry weight artificial soil, LOEC reproduction = 37.3 mg prod/kg dry weight artificial soil.

The EC₁₀ and EC₂₀₇ values chated to reproduction were calculated being 24.97 mg prod./kg and 46.24 mg prod./kg, respectively.

I. MATERIALS AND METHODS

<u>Test item</u> FLU + TFS SC 500 (250+250 g/L), specification no.: 102000012886; Batch No.: 2006-004985; TOX07762-00, analytical findings: 21.1 % w/w fluopyram equivalent to 246.1 g/L, 21.1 % w/w trifloxystrobin equivalent to 245.8 g/L; density: 1.165 g/mL (20°C).



Test design: Ten adult earthworms (*Eisenia fetida*) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments in an artificial soil (with 5 % peat content). The study consisted of 2 parts: Adult earthworms were exposed to the test item for a \mathcal{A} period of 4 weeks (first part). After this period, the adult earthworms were removed from the test sets? and the cocoons and juvenile earthworms remained in the test vessels for additional 4 week@(second part). The total duration of the study was 8 weeks.

During the test the adult earthworms were fed once per week with approximately 5 g food Dessel minal manure). The offspring were fed only once at the start of the second 4 weeks exposure period by mixing the food into the soil. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 74 % industrial quartz sand, 5% Sphagnung peat (air dried and finely ground), 20 % Kaolinite clay, 1 % food.

After a period of 4 weeks the adult earthworms were removed from the test vessels and the survivors were counted and their fresh weight was measured. From these data portality and bomass effects were determined. The cocoons and juvenile earthworms remained in the vessels for additional 4 weeks. From these the reproduction was determined by counting the number of offspring batched from the cocoons after this additional test period per test vessel.

<u>Climatic conditions</u>: The climatic conditions were in the temperature range 18 with a photoperiod of 16 hours light and a light intensity of 400 to 800 tux. Ø

The reproduction of the surviving test organisms was statistically evaluated by means and Dundett in the reproduction of the surviving test organisms was statistically evaluated by mean multiple sequential t-test. Dates of work: October 16,2006 December 18, 2006 Statistics: Changes in body weight were statistically evaluated by means at a Dunnett's multiple t-test. The reproduction of the surviving test organisms was statisticall devaluated by means of a Williams multiple sequential t-test.



II. RESULTS AND DISCUSSION

Parameter		[mg prod	Trea luct/kg dry	tment 🧳 🦉	(ial soil]*	
	Control	14	23.3	37.3	55.9 👡	8329
Mortality of adult earthworms [%] after 28 days	0	0	$\sqrt[\infty]{0}$			
Significance (Mortality)	-	-	Ş -			
Mean change of body fresh weight of the adults from day 0 to day 28 [%]	+ 50.4	+ 54.0	+ 52.8	÷ + A.8	Q+45	+ 52.7
Standard deviation	± 7.6	4.0) ±2,2	± 5.0	£2.8 ²	¥ ± 3.6
Significance (body fresh weight)**	-	n.s	n.s. Q	n.s.	n.s.of	n.s.
Mean number of offspring per test vessel after 56 days	234.8	230.0	2175	0 ^{190.0} 2	174.8 ×	1595
Standard deviation	± 23.5	&_± 18.€>	<u></u> ≤19.3 @	±29,1	_©± 34,@″	± 10.5
Statistical comparison to the control ***	Q- 0	n.s.	n.s	S.		s.
Significance (reproduction)***		<u> </u>		Q' + U	<u> </u>	+
Ŵ	<u> </u>	dalt Mortali	ty ×	Growth	Repro	duction
NOEC [mg test item/kg dry werght soil]		\$83.9		× ≥ 83,0 ×	2:	3.3
LOEC S ([mg test item/kg des weight soil]		> 83.9		≥ 83.9	3	7.3
EC10 (mg prod.kg dry voight arti	ficial soil)	O L	s. s	y V	24	.97
95% confiden@limits	U %	4 <u> </u>		, O	(11.09	- 34.41)
EC ₂₀ (mg prod./kg dry weight art	iticial soil	~~~ (d'a,	No.	46	.24
95% controlence limits	<u>```</u> ``	Q~	~~~~	¥ —	(33.10	- 58.04)

n.d.: not decrimined due to matteral passons (mappropriate data Or value 15 beyond We tested concentrations

Conversion according to the condition of test 500 g dty weight artificial soil and 200 cm2 surface area per test vessel; the density of the test it on was taken into account

- vessel; the density of the test item was taken into account) statistical significance compared to the control Dunnett's Multiple t-test, two-sided, $\alpha = 0.05$) **
- statistical Egnificance compared to the control Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$) ***
- **** recalculations were performed with the software Toy RatPro 3.2 (M-688422-02-1)
- mean value not statistically significantly different compared to the control ($p \ge 0.05$) n.s.:
- mean value statistically significantly different compared to the control (p < 0.05) s.: all'

Mortality: No mortality of addit carthororms was observed after 28 days test duration in the control group and at any application rate, including the highest rate of 83.9 mg prod./kg dry weight artificial soil (corresponding to 18.0 L prod.ha).



Effects on growth:

No statistically significant different values for the growth relative to the control were observed *a* all application rates. Therefore, the endpoints related to growth were: NOEC: ≥ 83.9 mg prod. 300 dry weight artificial soil (corresponding to \geq 18.0 L prod./ha) and LOEC: \geq 83.9 mg prod./kg dry weight artificial soil (corresponding to ≥ 18.0 L prod./ha)

Effects on reproduction:

No statistically significant different values for the number of juveniles per test vessel relative to the control were observed at the two lowest application rates of 14 and 23,3 mg prod./kg dry weight application soil (corresponding to 3.0 and 5.0 L prod./ha). Statistically significant different values for the humber of juveniles per test vessel relative to the control were observed at the three highest application rates of 37.3, 55.9, 83.9 mg prod./kg dry weight artificial soil (corresponding to 8.0, 92.0 and 18.0) prod/ha)

Therefore, the endpoints related to reproduction were: NOEC: 23.3 mg prod./kg dry weight artificial soil and LOEC: 37.3 mg prod./kg dry weight article al sout. The EC10 (C.I.) and EC20 (C.I.) values were calculated to be 24.97 (11.09 – 34.41) mg prod./kg soil dry weight and 46.24 (33.10 58.04) mg prod./kg

calculated to be 24.97 (11.0) 94.41) hig producting solution weight and #0.24	\$2.10 20.0 mg mg pagad./ K
soil dry weight, respectively.	
Validity criteria:	
All validity criteria of the OECD 222 guide one were met.	ő Ó
	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
	× Q
Table 10.4.1.1- 2: Validity criteria	
Validity criteria acc. to OECD 222 (adopted 2016)	> Obtained
Validity criteria acc. to OFCD 222 (adepted 2016) Mortality of the adults on the ontrol O 40 50 50 50 50 50 50 50 50 50 50 50 50 50	<b>Obtained</b>
Validity criteria a.e. to OFCD 222 (adopted 2016)Mortality of the adults in the ontrol $\bigcirc$ $\bigcirc$ Mortality of the adults in the ontrol $\bigcirc$ $\bigcirc$ Number of juveniles (earth-worms per control vessel) $\bigcirc$ $\bigcirc$ $\bigcirc$	© Obtained 0 % 234.8
Validity criteria ace. to OFCD 222 (adapted 2016)Mortality of the adults in the ontrol $\checkmark$ Mortality of the adults in the ontrol $\checkmark$ Number of juveniles (earthworms per control vessel) $\checkmark$ Coefficient of variance of reproduction in the control $\checkmark$	© Obtained 0 % 234.8 10.4 %

# Reference test?

The toxic standard reference test, with the test item sprayed onto the artificial soil, was performed last from January to March 2006 (Study No.: Rg 02/06, Report No. LKC-Rg-R-Ref-6/06; NON-GLP).

No mortality of the adult earthwords was observed 28 days after application. No statistically significant different values for the blomas felative to the control were observed at any application rate, including the highest rate of 0.50 kg teg item ba (resQts of a Dunnett's Multiple t-test, two-sided,  $\alpha = 0.05$ ). The number of juveniles per test vessel of the application rate of 0.50 kg reference test item/ha was statistically significant reduced to the control (results of a Williams multiple sequential t-test, one sided smaller, a = 0.0

The results of the reference test item indicated that the test system was sensitive to the reference test item?



# **III. CONCLUSION**

All validity criteria were met. The endpoints were:

- $\geq$  83.9 mg test item/kg dry weight artificial soil (corresponding)  $\delta \geq$  18.0 L ptod NOEC_{mortality}:
- > 83.9 mg test item/kg dry weight artificial soil (corresponding to  $\geq$  18.0 C prod that LOEC_{mortality}:
- ≥ 83.9 mg test item/kg dry weight artificial soil (corresponding to ≥Ø8 NOEC growth: > 83.9 mg test item/kg dry weight ar fuicial soil (corresponding to 2 18.0 E prod./ha) LOEC_{growth}: e e
- L. NOEC_{reproduction}: = 23.3 mg prod./kg dry weight artificial soil teorresponding to = 52 prod./ha)
- LOEC_{reproduction}: = 37.3 mg prod./kg dry weight artificial soil (corresponding to 8.0 L prod./ha)  $EC_{10reporduction}$ : = 24.97 mg prod./kg dry weight artificial soil  $EC_{20reproduction}$ : = 46.24 mg prod./kg dry weight artificial soil

vifico as "Y ی میں ۱". The normalised is classif According to EFSA (2015) the level of protection for the E width of confidence interval (NW) rating for the EC₁₀ is 'fair

Assessment and conclusion by applicant: The study and its data are considered as acceptable and rehable for use in risk assessment.

Licati <u>is "far"</u> <u>is contained on the formula of the form</u>



Data Point:	KCP 10.4.1.1/02
Report Author:	
Report Year:	2020
Report Title:	Statistical re-evaluation (non-glp) of the Eisenia fetida reproduction study with AE
	C656948 + trifloxystrobine SC 250 & 250 G (Leicher, 2000; <u>M-283637-07</u> )
	using the probit analysis
Report No:	<u>M-688422-02-1</u>
Document No:	<u>M-688422-02-1</u>
Guideline(s) followed in	None & Ly Ly Ly Ly
study:	
Deviations from current	Current Guideline: not applicable
test guideline:	
Previous evaluation:	No, not previously submitted
GLP/Officially	not applicable & @ ~ ~ ~ ~ ~ ~
recognised testing	
facilities:	
Acceptability/Reliability:	Yes $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$ $\sqrt[4]{\gamma}$

# Summary

The *Eisenia fetida* reproduction study with FLC + TFS SC 90 (M28369-01-1) was statistically reevaluated based on the number of juveniles of *Eisenia fetida* provided in the study report Probit analysis was performed in order to derive EC₄₀, EC and EC₅₀ -values for the % effect on the number of juveniles of *E. fetida* compared to the control. This re-analysis was performed using the software ToxRatPro 3.2.

Table 10.4.1.1- 3: Rezeniculated EC1 and EC20 values (with confidence limits) for % effect on number of joreniles compared to the control

			EC ₅₀
Value [mg prod./kg]	29.97 0 6	46,24 0 0	n.d.
Lower 95% cl	11.0% 2 0	33.10 0 0	n.d.
Upper 🕉 -cl	34901	0 ⁹ 58.04 ⁹ . 0 ⁹	n.d.

n.d.: not determined due to mathematical Geasons (mappropriate data) or value is beyond the tested concentrations

# Conclusion:

The EC₁₀ and EC₂₀-values were 24.97 mg profe/kg df weight artificial soil and 46.24 mg prod./kg dry weight artificial soil, respectively.

According to EFSA (2015) the level of protection for the  $EC_{10}$  is classified as "high". The normalised width of confidence interval (NÅY) rating for the  $EC_{10}$  is "fair".

# Assessment and conclusion by applicant:

The data is considered as supplementary data with no use in risk assessment. The endpoints are:  $EC_{10} = 24.9$  mg prod./kg dws (based on reproduction) and  $EC_{20} = 46.24$  mg prod./kg dws (based on reproduction)



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## **CP 10.4.1.2 Earthworms field studies**

In view of the results presented above, no field studies were necessary. However, further information on the formulation FLU SC 400 is presented in the active substance dossier MCA 8.4.1.

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

The risk assessment (calculation of TER values) was based on the NOEC values calculated from the studies performed with the product and the studies beformed with the product and the studies beform the studies beform the studies beform the studies beform the studies before the studies performed with the product and the metabolites. In case EC values were lower than the N and the calculation was reliable they were used for the calculations of JER values Ĩ,

Table 10.4.2- 1:	Springtail and soil miter	eproduc	tion stu	dies with	ı p <u>ro</u> du	ict 🕰	J+TFS	SC 500	and
	fluopyram metabolites		Ì	, K	Å,		Å.	Ø	A CONTRACTOR

Test substance	Test species, test design	E E	cotoxicological Endpoint		Reference
Springtails, repro	duction 🔗	à à		Ŭ,	Ö 😽
FLU+TFS SC 500	Folsomia cardida reproduction & 28 d, maxed O	NOPC NOPC EC10 EC10	$= \frac{3}{62} \text{ mg/prod./kg/dws}^{2}$ $= 281 \text{ mg/prod./kg/dws}^{2}$ $= 302.5 \text{ mg/prod./kg/dws}^{2}$		(2017) M-576685-01-1 CP 10.4.1.2/01
FLU SC 500	Folsomia condida reproduction 88 d, nyixed	NOEC NOECcorr EC16 EC16	$= \frac{9}{5.5} \text{ mg a.s./kg dws}^{B}$ $= \frac{37.8 \text{ mg a.s./kg dws}^{B}}{37.8 \text{ mg a.s./kg dws}^{B}}$ $= \frac{102 \text{ mg a.s./kg dws}^{B}}{39 \text{ mg a.s./kg dws}^{A,B}}$		(2019) <u>M-675002-01-1</u> KCA 8.4.2.1/01 KCP 10.4.2.1/03 ^C
Fluopyram-7-	Folsomia cândida reproduction O 28 d, mixed	NOBĆ NQEC _{corr}	$\neq$ 562 mg p.m./kg dws/ $\neq$ 281 ong p.m./kg dws ^A = 61 mg p.m./kg dws	,	(2020) <u>M-755397-01-1</u> KCA 8.4.2.1/03
Trifluoroacetic acid (TEA)	Folsemia cardida reproduction 28 d, mixed		$\geq 100 \text{ mgG/m./kg dws}$ (Na $\gg$ 84 mg p.m./kg dws (TF. calculation not possible	a-TFA) A) ^d	(2012) <u>M-436127-01-1</u> KCA 8.4.2.1/05
Soil mites, reprod	uction 🔗 🔗	<u>N R</u>			
FLU+TFS SC 500	Hyprograms active feproduction 14 d, faixed ~		= 316 mg/prod./kg dws $\sim$ 158 mg/prod./kg dws ^A = 457 mg prod./kg dws = $\sim$ 8.5 mg prod./kg dws	A	(2016) <u>M-548820-01-1</u> KCP 10.4.1.2/02
FLU SC 500	Hypoaspis aculeifer peproduction 14 domixed		$224 \text{ mg a.s./kg dws}^{B}$ $2212 \text{ mg a.s./kg dws}^{A, B}$ calculation not possible		(2020) <u>M-678468-01-1</u> KCA 8.4.2.1/02 KCP 10.4.2.1/04 ^C
Fluopyram-7-57 hydroxy	Hypoaspis aculeifer reproduction 14 demixed	XOEC NOEC _{corr} EC ₁₀	$\geq$ 1000 mg p.m./kg dws $\geq$ 500 mg p.m./kg dws ^A calculation not possible		(2020) <u>M-754291-01-1</u> KCA 8.4.2.1/04



Test substance	Test species, test design	Ecotoxicological Endpoint	Reference	~
Trifluoroacetic acid (TFA)	<i>Hypoaspis</i> <i>aculeifer</i> reproduction 14 d, mixed	NOEC $\geq$ 100 mg p.m./kg dws (Na-TFA) $\geq$ 84 mg p m./kg dws (TFA) ^D $\bigcirc$ EC ₁₀ calculation not possible	(2012) M-436326-06-1 KCA 8.4.2405	

dws = dry weight soil, a.s. = active substance; p m. = pure metabolite, prod. = product

^A Endpoint corrected by a factor of 2 due to lipophilic substance (log  $P_{OW} > 2$ ) ^B Endpoint calculated on the basis of analyzed fluorurum content in the formula

- Endpoint calculated on the basis of analysed fluopyram content in the formulation
- (42.4 % w/w; as given in study report) C Eull details on this study are described

Full details on this study are described in the corresponding MCP for the formulation FLU SCODO.
 As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the order was converted to trifluoroacetic acid with factor 0.84.

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

The exposure of soil meso- and macrofauna to residues of fluopyrand following the greenhouse use in lettuce can be excluded as lettuce is a soil-less cultivation and thus soil is no compartment of concern.

**Important remark by the applicant:** The PEC  $_{\text{oil}}$  and PER values as presented below are interim values and are therefore subject to change until final modelling input parameters can be established. The applicant intends to provide final PEC_{soil} values and revised TER calculations latest by end of March 2022.

Table 10.4.2-2: CR calculation for other non-target soil meso- and macrofauna for

Compound	Species study type &	Endpoint	PEC _{soil} [mg prod./kg]	TERLT	Trigger
Grapes, 2 🛪 0.2 L	prod./ha		Y Y		•
FLU + TFS SC 50	0 Holsomia candida	NOEC $= 281$	0.249	1129	5
FLU + TFS SC 50	0 Hypoaspis of uleifer	ONOECO = 158	0.249	635	5

Table 10.4.2- 3:	TER calculation for other non-target soil meso- and macrofauna for fluopyra	m and
	its metabolites	<i>°</i>

Compound	Species, study type	Endpoint [mg/kg]	PEC _{soil} [mg/kg]	TER _{LT}	Trigger
Grapes, 2 × 50 g a.s./ł	18		Ĩ	Ş	~ <u>~</u>
Fluopyram ^A	Folsomia candida	NOEC = 37.8	0.238	159	5
Fluopyram ^A	Hypoaspis aculeifer	NOEC 2212	0.238	≥ 891	27 .S
Fluopyram-7-hydroxy	Folsomia candida	NOEC 281	0.003	46838 ^B	P5 ×
Fluopyram-7-hydroxy	Hypoaspis aculeifer	NOE $\geq 500$	Ø:003	≥©33333 ^B	5.0
Trifluoroacetic acid (TFA)	Folsomia candida	NOTEC $\geq 84$	0.000	² 4200 ×	
Trifluoroacetic acid (TFA)	Hypoaspis aculeifer	NOEC 284	Ø.002~0	200 ×	5 <u>4</u> ~ ~





CP 10.4.2.1	Species level testing
	species it is in testing

Data Point:	KCP 10.4.2.1/01
Report Author:	
Report Year:	
Report Title:	Fluopyram + trifloxystrobin SC 500 (250+250) G: Effects on mortality and reproduction of the collembolan species Folsomia candida tested in orificial soil
Report No:	16 10 48 273 S
Document No:	<u>M-576685-01-1</u>
Guideline(s) followed in	EU Directive 91/414/EEC; Regulation (EC) No 107/2009 (2099); USEPA
study:	OCSPP Not Applicable; OE $232$ (2009) $\sqrt{2}$
Deviations from current	Current Guideline: OECD $232$ (2016) $\sqrt{2}$ $\sqrt{2}$
test guideline:	Deviations: none. All valuatity criteria webe met
Previous evaluation:	No, not previously submitted $\mathcal{A}^{\circ}$ $\mathcal{A}^{\circ}$ $\mathcal{A}^{\circ}$ $\mathcal{A}^{\circ}$ $\mathcal{A}^{\circ}$
GLP/Officially	Yes, conducted under GLPOfficially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q V V V V V V V V

# **Executive Summary**

In a laboratory study the effects of FLU + TFS SC 500 (250+250.9 L) on survival and reproduction of the collembolan species *Folsomia candida* was tested during an exposure of 28 days in artificial soil by comparing control and treatment. Fight test itervates from 18 to 1000 mg product kg dry weight soil were tested. Per test itervates and for the control group 8 replicates with 10 collembolans each were exposed to FLU + TFS SC 500 (250-250 gC) mixed into artificial soil. Mortality and reproduction of the collembolans was assessed after 28 days.

The study fulfilled all validity criteria of OECD 232 guideline.

The test item showed statistically ognificantly adverse effects on adult mortality of the collembolan *Folsomia candida* invartificial soil at the highest concentration of 1000 mg prod./kg d.w.

The test item caused a significant reduction of reproduction of the collembolan Folsomia candida in artificial soil at the highest concentration of 1000 mg prod./kg d .

Therefore, the No-Observed-Effect-Concentration (NOEC) for mortality and reproduction was determined to be \$62 mg prod to go soil it.w. and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be 1900 mo prod to go soil d.w.



<u>Test stem</u>: FLU  $\pm$  FFS SC 500 (250 250 g/L); specification no.: 102000012886; Batch No.: PAIS005173; REG4003250; analytical indiges: 246.2 g/L (21.1 % w/w) fluopyram, 250.8 g/L (21.5 % w/w) trifloxystrobin; density:  $\frac{1}{2}$  165 g/mL ( $\frac{2}{3}$ °C).

<u>Test design</u>: Pen collembolaris (9 - 12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatment. Additionally two replicates each treatment group and the control without collembolans were used for measurement purposes. Concentrations of 18 32, 50 100, 178, 316, 562 and 1000 mg prod./kg dry weight artificial soil were mixed into the artificial soil. During the study the collembolans were fed with granulated dry yeast. The artificial soil was prepared according to the guideline with the following constituents (percentage distribution on dry weight basis): 75 % fine quartz sand, 5 % sphagnum peat, air dried and finely ground and 20 % Kaolin clay. Mortality and reproduction were determined after 28 days.



Climatic conditions: The climatic conditions were in the temperature range 18.6 - 19.9 °C with a photoperiod of 16 hours light and a light intensity of 580 lux.

Statistics: For statistical analysis Multiple Sequentially-rejective Fisher Test (After Bonferron Folm one-sided greater,  $\alpha = 0.05$ ) was applied to mortality data and William's t-test (one-sided smaller, 0.05) was applied to reproduction data. The ECx values were calculated with Probit analysis.

# II. RESULTS AND DISCUSSION

ý <b>11</b>	*	6	
Dates of work: Oc	tober 10, 2016 – November 07, 2016		5 ⁴ 5 ⁴ 9
	II. RESULTS AND DISCUSSION	N N N N	
Table 10.4.2.1- 1:	Effects on mortality and reproduction of Folso	mia candida after 1	reatment with FLL
	+ TFS SC 500 (250+250 g/L) $Q_0^{40^{\circ}}$	/ . O [*] ~~	

Test concentration [mg prod./dry weight	Adult mortality	Significance (*)	Mean number of	Reproduction	Significance		
artificial soli	70	À,	$0^{\circ}$ Vessel $\pm 5.6\%$				
Control	2.5		686 ⁷ ±0, 57 (	× ,0 [×] - ,			
18	2.5	, ⁽¹⁾ - ⁽¹⁾	04 ~≠ 50	× 103	Ŷ Ô		
32	2.5		\$685\$ ± \$83	27 1 <b>6</b> (	<u></u> \$-		
56	2.5	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	6 <b>85</b> ±21440	َنْ 100 <u>مَنْ 100 مَنْ 100 مَنْ</u>	°∼ -		
100	2.5	Ž- Ö	<b>6</b> 7 <b>⊈</b> 53	° 97₀°	<ul> <li>-</li> </ul>		
178	25	, ² S	681 ± 54 %		-		
316	<i>_ ©</i> 5.0		$686^{\circ} \pm 33^{\circ}$		-		
562	≫15.0∆	- 4	<b>6</b> 16	≪ 90%	-		
1000	325		$521^{5} \pm 063$	76	+		
	L End	oints 🖓 🕺 ^		Mortality	Reproduction		
NOEC [mg prod Ag dry	weight artific	cial soil] 🤿		<u>م</u> کې 562	562		
LOEC [mg prod/kg dr	veightartifici	soil]		1000	1000		
$LC_{50}$ [mg prod./kg dr/weight artificial soil] ¹⁾							
EC10 [mg prod./kg dry we	aght artificial	l solo ⁱ (95 % C			605 (511 - 715)		
EC20 [mg prod./kg dry ve	ght antificial	soil] ¹⁾ (05 [%] % C			887 (808 - 973)		
Calculations were donewrith	inrogunded wash						

Not determined n.d.:

Standard deviation SD:

Confidence IntervaC C.I.: 1)  $EC_x = Probit analysis$ 

Test After Bonferrood Holm, one-sided greater,  $\alpha = 0.05$ , "-": non-significant; Multiple Sequentially-rejectiv * "+" rignificant

non-significant; "+": significant ** Wohiam's t-test, one

# ~ Mortality:

the adult Folsomia candida died which is below the allowed maximum of In the controlorou  $\leq 20 \%$  mortalit

Statistically significant effects on mortality compared to the control were observed at a concentration of 1000 mg test from/kg soil dry weight. No effects on behaviour of the collembolans were observed during the test.

The NOPC for mortality was determined to be 562 mg prod./kg soil d.w., a LOEC with 1000 mg prod./kg soil d.w. and a  $LC_{50}$  with > 1000 mg prod./kg soil d.w.



# Reproduction:

Statistically significant effects (Williams-t-test,  $\alpha = 0.05$ , one-sided smaller) on the number of juve files compared to the control group were recorded at a concentration of 1000 mg test item/kg soil d.ws Ô The NOEC for reproduction was determined to be 562 mg prod./kg soil d.w. and the LOEC 4000 mg prod./kg soil d.w. The EC₁₀ and EC₂₀ values for reproduction were calculated to be 605 and 887 mg prod./kg soil d.w., respectively. Validity criteria: from J Validity criteria for the untreated control of the study according **Q** used. Table 10.4.2.1- 2: Validity criteria Required Øbtained Validity criteria acc. to OECD 232 (adopted 2016) 2,3% Mean adult mortality in control Mean number of juveniles per replicate (with 686 collembolans introduced) Coefficient of variation calculated for the number of 8.4 % juveniles per replicate All validity criteria ideline Reference te To verify the sensitivity of the test system the reference item boric acid is routinely tested at concentrations of 44, 60, 100, 950 and 225 mg/kg soil dry weight. The collembolans of the reference test were from the same source culture as those used in the definitive test. In the most recent study (BioChem project No. R 1010 48,003 S, dated 2016-08-08) the EC₅₀ was determined to be 104 mg/kg soft dry weight. The LCs0 was determined to be 165 mg/kg soil dry weight. The NOEC for mortality and for reproduction was determined to be 67 and 44 mg/kg soil dry weight, respectively. The EC₅₆ value for the peproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2009). The EC 30 therefore showed that the test system was sensitive. K CONCLUSION were met. The endpoints were: All validity criteria 562 mg prod kg dry weight artificial soil 1000 mg prod./kg dry weight artificial soil LOECmortality

- NOECreptoduction: 562 mg prod./kg dry weight artificial soil
- LOEC reproduction: 1000 mg prod./kg dry weight artificial soil
- EC10 reproduction: 605 mg prod./kg dry weight artificial soil



EC_{20 reproduction}: 887 mg prod./kg dry weight artificial soil

According to EFSA (201	5) the level of protection for the $EC_{10}$ is classified as "high". The normalised
Assessment and conclu	sion by applicant:
The study and its data an	re considered as acceptable and reliable for use in risk assessment.
The endpoint is: NOEC	= 562 mg prod./kg dws $\sqrt{2}$
	O'*****
Data Point:	KCP 10.4.2.10 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2
Report Author:	
Report Year:	
Report Title:	Fluopyram + trifloxystepbin S6500 (250+2500G: Influence of mortality and
	reproduction of the soft mite species Bypoasons aculater tested in artificial soil
Report No:	E 428' 4844' - 4' - 2' - 4' - 4' - 2' - 2' - 2' -
Document No:	<u>M-548820+01-1</u> C
Guideline(s) followed in	GECD 226 from October 03, 2008: QECD guideline for the festing of Chemicals
study:	- Predatory mite (Hypoaspis@GeolaeJaps) aculeifer) reproduction test in soil; US
<u> </u>	EPA OCSPF: not applicable
Deviations from current	Current Gaideline. OECD 226 (2016)
test guideline:	Deviations: none. All validits Griterias vere met.
Previous evaluation:	No, that previously submitted
GLP/Officially	Kes conducted under GCP/Officially recognised testing facilities
recognised testing	
facilities	
Acceptability/Reliability:	Yes 2 2 2 2 2
Executive Summary 🔿	

In a laboratory study the effects of FLU  $\rightarrow$  TFS SC 500 (250+250 g/L) on survival and reproduction of the soil mite species *Hypolaspis oculeiter* was rested than an exposure of 14 days in artificial soil by comparing control and treatment. Five test item rates from 100 to 1000 mg product/kg dry weight soil were tested. Per test item rate 4 replicates and for the control group 8 replicates with 10 soil mites each were exposed to FLU + TFS SC 500 (250+250 g/L) mixed into artificial soil. Mortality of the soil mites was assessed after 14 days.

The study fulfilled allyalidity criteria of OECD 226 guideline.

Concerning the mortality of the adult test organisms no statistically significant differences compared to the control occurred. Therefore, the No-Observed-Effect-Concentration (NOEC) for mortality is  $\geq$ 1000 mg prod/kg dys. The Lowest-Observed-Effect-Concentration (LOEC) for mortality is  $\geq$ 1000 mg prod/kg dys.

The reproduction rate of the soil mites was assessed after 14 days. Concerning the number of juveniles statistical analysis (William's t-test, one-sided smaller,  $\alpha = 0.05$ ) revealed a significant difference between control and treatment groups with 562 and 1000 mg prod./kg dry weight artificial soil. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is 316 mg prod./kg dry



weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 562 mg prod./kg dry weight artificial soil.

# I. MATERIALS AND METHODS

<u>Test item</u>: FLU + TFS SC 500 (250+250 g/L); specification no.: 102000012886; Batch No.: PAIS005173; REG40032-00; analytical findings: 21.1 % w/w fluopyram equivalent  $2462^{\circ}$  g/L. Ś 21.5 % w/w trifloxystrobin equivalent to 250.8 g/L; density: 1.165 g/mL/20 °C).

Test design: Ten adult, fertilized female Hypoaspis aculeifer per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatments synchronised culture at an age of 28 days after start of egg laying) Concentrations of 100, 178 316, 562 and 1000 mg prod./kg dry weight artificial soil were mixed into the artificial soil During the test, the Hypolepis aculeifer were fed with nematodes bred on watered oat flakes. The artificial spil was prepared according to the guideline with the following constituents (percentage distribution and ry worght basis): 75 % fine quartz sand, 5 % Sphagnum peat, air dried and fingly ground, 20% Kaolin clay,

After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus Extracted mites were collected in Arising Solution (20 % ethylene glycol, 80 % deionise@ water 2 g depergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a birocular. °~~

Climatic conditions: The climatic conditions were in the temperature ange 20.0 \$2 °C with a photoperiod of 16 hours lightand a light intensity of 400 - 800 lpx. W

Statistics: For statistical analysis Fisher's exact Binominal Test Bonferroni Correction, one-sided greater,  $\alpha = 0.05$ ) was applied to mortality data and Williams t-test (one-sided smaller,  $\alpha = 0.05$ ) was

work: November 25, 2015 - Detember 21, 2015



# **II. RESULTS AND DISCUSSION**

Table 10.4.2.1- 3:	Effects on mortality and reproduction of Hypoaspis aculeifer after treatment	t with	Ő
	FLU + TFS SC 500 (250+250 g/L)	Ň	The second secon

		0	,			$\circ$		
Test concentration [mg prod./dry weight artificial soil]	Adult mortality [%]	Significance (*)	Mean nu juveniles vessel	Reproduction [% of control]	Significance	þ		
Control	1.3	-	265.6+	21.2	Ž - ,			
100	2.5	-	320.8 生	27.5	0 120.8 Õ	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	L.	
178	2.5	-	349.5±	13.1	[♥] 120.3≪		) (	
316	2.5	-	267.8±	14.	° 100(8 "	, °O- "Oʻ		
562	7.5	- Ø	°214.8±	22.0	Ø 80 <u>%</u> 8 0			
1000	2.5	- &	186°.0±	چ 30.1%	70.0			
Endpoints								
NOEC [mg prod./kg dry	NOEC [mg prod./kg dry weight artificial sold]							
LOEC [mg prod./kg dry weight artificia@coil]								
$EC_{10}$ [mg prod./kg dry weight artificial soil] (95% confidence limits) $(25 - 645)$								
EC ₂₀ [mg prod./kg dry weight attrificial soil] ¹⁾ (95% confidence limits) (275 - 998)								
alculations were done with unrounded values 2 a								

SD: Standard deviation

1)  $EC_x = Probit analysis$ 

Ĉ * Fisher's exact Binegrinal Tetr with Bohferroni Correction, one sided greater,  $\alpha = 1$ ; non-significant; "+": significant

** Williams t gnificant

Ô

Mortality: Ô

dult Hypoaspis acuteifer ded which is below the allowed maximum of In the control group 1 Cof the  $\leq 20$  % mortality. Š L)

Concerning the morality of the adult test organisms Statistical analysis (Fisher's Exact Binomial Test with Bonferroni Correction, see-sided greater,  $\alpha \approx 0.05$ ) vevealed no significant difference between control and any treatment group.

Therefore the NOEC for mortanty is 2000 mg prod./kg dry weight artificial soil. The LOEC for mortality or >1000 mg prod./kg Wight attrificial soil.

# Repróduction:

Concerning the number of identity statistical analysis (William's t-test, one-sided smaller,  $\alpha = 0.05$ ) revealed a significant difference between control and treatment groups with 562 and 1000 mg test item/kg dry weight artificial soil

Therefore, the NOEC for reproduction is 316 mg prod./kg dry weight artificial soil. The LOEC for reproduction is 562 mg prod./kg dry weight artificial soil.



Obtained

1.3 %

265

# Validity criteria:

Validity criteria for the untreated control of the study according OECD 226 from July 29, 2016 vere wield.

Require

All validity criteria of the OECD 226 guideline were fulfilled.

# Table 10.4.2.1- 4: Validity criteria

Validity criteria acc. to OECD 226 (adopted 2016)

Mean adult mortality

Mean number of juveniles per replicate (with 10 mites introduced) Coefficient of variation calculated for the number of juveniles per replicate

# Reference test:

The most recent non-GLP-test (LAR/HR-O-21/15) with the ofference item dimethoate was performed at test concentrations of 1.0, 1, 5, 3.2, 5, 6 and 10.0 nor dimethoate kg dry weight artificial soil.

Dimethoate showed a  $LC_{50}$  of 1.9 mg a.s. Arg for mortality of the adult mites according Probit analysis using maximum likelihood regression (confidence limits could not be determined due to mathematical reasons).

The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight affificial soil. Therefore, the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous, Williams t-test  $\alpha = 0.05$ , one stilled smaller was used. Diracthoatt EC 400 G showed an EC₅₀ of 5.36 mg a.s./kg (99 % confidence limits from 4.75 mg a.s./kg to 5.68 mg a.s./kg) for reproduction according Probit analysis using maximum likelihood regression  $\alpha$ .

This is in the recombended large of the guideline, indicating that an EC₅₀ based on the number of juveniles of 3.0 - 70 mg as /kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

# All validity criteria were met. The entroints were: NOEC_{adult mortality}: $\geq 1000$ mg prod./kg dry weight artificial soil LOEC_{adult mortality}: $\geq 1000$ mg prod./kg dry weight artificial soil NOEC_{reproduction}: $\geq 1000$ mg prod./kg dry weight artificial soil LOEC_{reproduction}: $\geq 552$ mg prod./kg dry weight artificial soil EC₁₀ reproduction: $\geq 552$ mg prod./kg dry weight artificial soil EC₁₀ reproduction: $\geq 552$ mg prod./kg dry weight artificial soil EC₂₀ throduction $\geq 702$ mg prod./kg dry weight artificial soil

According to EFSA (2015) the level of protection for the  $EC_{10}$  is classified as "medium". The normalised width of confidence interval (NW) rating for the  $EC_{10}$  is "poor".







O

## **CP 10.5** Effects on soil nitrogen transformation

## Table 10.5- 1: Studies on nitrogen transformation with FLU+TFS SC 500, Flpopyram and i metabolites

Test substance	Test species, test design	Ecotoxicological endpoint
N-transformation		
FLU+TFS SC 500	Study duration 56 d	No unacceptable effects at an appl. 12.53 mg prod./kg dws rate of:
Fluopyram	Study duration 28 d	No unaccop able effects at an apple <b>3.3 mg as kg d</b> $\sim$ $\frac{M-281177-91}{KCA 8.5/01}$
Fluopyram-7- hydroxy	Study duration 28 d	No unacceptable effects at an appl. 10 mg p.m./kg dws rate of: KCA_8.5/03
Trifluoroacetic acid (TFA)	Study duration 0 28 d	No onacceptable 100 mg p m./kg dws (2003) efforts at anyappl. 1.344 mg p m./kg dws KCA \$5/04 (10FA) B

**Bold** values used in risk assessment dws = dry weight soil, prod. = product

А

Based on the endpoint 10.67  $\mu L$  prod./kg dys and aproduct density of 1.174 g/mL

As the study was conducted with sodium arifluor acetate which is the sodium salt of rifluor acetic acid, the endpoint В was converted to trifluoroacetic acid with factor 9.8

Risk assessment for itrogen Transformation s. S

0

The exposure of soil microorganisms to residues of fluopyram following the greenhouse use in lettuce can be excluded as lettice is a soil-less cultivation and thus, soil is no compartment of concern.

K,

S

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Ô Important remark by the applicant The PEC soil values appresented below are interim values and are therefore subject to change with final modelling onput parameters can be established. The applicant intends to provide final PEO_{soil} values and a revised risk assessment latest by end of March 2022. ÔŇ .4

E.					
Table 10.5- 2:	Rûsk Assess	sment for FLV + TFS SC	500 for nitrog	en transformation	1
Compound		Species	Endpoint [mg prod./kg]	PEC _{soil, max} [mg prod./kg]	Refinement required
Grapes, 2 × 02	L prod/ha				
FLU +TFS SC :	500 0	Soil micro-organisms	12.53	0.249	No
		<u>)</u>			



Table 10.5- 5: Kisk Assessment for Fluopyram and its metabolites for introgen transformation									
Compound	Species	Endpoint [mg/kg]	PEC _{soil, max} [mg/kg]	Refinement C					
Grapes, 2 × 50 g a.s./ha			ð						
Fluopyram	Soil micro-organisms	3.33	0.238	No					
Fluopyram-7-hydroxy	Soil micro-organisms	10	0.003						
Trifluoroacetic acid (TFA)	Soil micro-organisms	1.244	0.002	No No O					
A For the metabolite fluonyra	m-7-hydroxy the assessment of	onclusion relies	on appuncertainty fa	consideration					

Table 10 5 2.

Unda. of two enantiomers. Ø

According to regulatory requirements, the risk is acceptable if the effect on nitrogen transformation at According to regulatory requirements, the risk is acceptable if the effect of hitrogen transformation at the maximum PECas values is < 25% after 28 days/2m notese, deviations from the control exceeded 25% at concentrations which are clearly higher at an the PECa, indicating low risk to soft meto-organisms.



## ******

C ( 1	•
Study	summaries
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U	
Data Point:	KCP 10.5/01
Report Author:	
Report Year:	2007
Report Title:	Fluopyram + trifloxystrobin SC 500 (250+250) G: Determination of effects on nitrogen transformation in soil
Report No:	LRT-N-91/07 V Q. Q V A
Document No:	<u>M-295282-01-1</u>
Guideline(s) followed in	OECD 216; adopted January 21, 2000, OE Guideline for the Testing of
study:	Chemicals, Soil Microorganisms: Nitrogen Transformation Test Provident to 158
Deviations from current	Current Guideline: OECD 21622000
test guideline:	Deviations: At day 42 the soft moisture was below the demanded minimum of
-	40 % of the maximum water holding capacity (36.1 %). The deviation listed above
	had no influence on the reliability of the study All validity criteria were met
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLD/Officially recognised testing facilities
recognised testing	
A second shift of Datish it is a	
Acceptability/Reliability:	
•	

Executive Summary

In a laboratory study the effect of FLU + TFS SC 500 (250+250 g/L) of the activity of soil microflora with regard to nitrogen transformation was tested during an exposure of 56 days in a loamy sand soil by comparing control and treatment. Two test item rates of 1.02 and 10.67 µL test item/kg dry weight soil (equivalent to 0.8 and 8 L test item/kg) were tested. Per treatment there were 3 replicates. The soil was enriched by 0.8 % laterne meal and a water content of 40 - 50 % of the water holding capacity was maintained during the test.

The study fulfilled all validit periteria of OECD 2 b guideline.

Nitrogen transformation was determined after 3 hours 7, 14, 28, 42 and 56 days. The 10-fold dose of FLU + TFS SC 500 (250+250 gL) (10.67 μ L fest item/kg) caused a temporary stimulation of the daily nitrate rates at the time interval 0 - 7 days, 14 - 28 days, and 28 - 42 days after treatment. Effects at the time interval 0 - 7 days and 14 - 28 days were statistically significant and with 44 and 26 % difference to control above the recommended < 25%.

%I. Meterials and methods

<u>Test item</u>: FLU + TFS SC 500 (250+250 g/K), Specification No.: 102000012886; Sample description: TOX07851-00; batch no.: 2007-00041; analytical content: 21.4 % w/w (251.5 g/L) fluopyram, 21.6 % w/w (253.5 g/L) trifloxystebin; density @174 g/mL.

<u>Test design</u>: A beamy and soil with pH 6.7, 0.94 % C_{org} and with the water holding capacity of 43.27 g 700 g dry soil was exposed for 56 days to 1.07 μ L and 10.67 μ L prod./kg dry weight soil. Application rates were equivalent to 0.8 L and 8 L prod./ha. Lucerne-grass-green meal was added to the soil 5 g/kg dry weight soil) to stimulate nitrogen transformation. For calculation of the test concentrations (mg/kg soil d.w.) a soil depth of 5 cm and a soil bulk density of 1.5 g dry weight/cm³ were assumed for conversion of soil volume to soil dry weight



The soil of each treatment was tested as a series of 3 replicates. 300 g soil dry weight per test vessel was weighted. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %).

Soil samples (10 g soil d.w. per replicate) were taken at intervals of 0, 7, 14, 28, 42 and 56 days after application and the NH₄-N-, NO₃-N- and NO₂-N-contents were determined. NH₄-nitrogen NO₃and NO₂-nitrogen were determined by using the Autoanalyzer III (BRAN+LUEBBE).

Climatic conditions: The test vessels were kept in darkness in a climatic room and the temperature ranged between 18 - 22 °C during the test. The water content of the soft ranged between 40 × 60 % 8 WHC. The pH value of the soil ranged between 5.83 and 6.14.

<u>Statistics</u>: Homogeneity of variances was determined by Cochrands Test, $\alpha = 0.95$. Depending on the results the appropriate T-tests were performed. In the T-test, the values of htrate N/kg dry weight soil/time interval/day from control soils and treated soils were compared. The statistica Kralculations were carried out using ToxRatPro 2.09.

Dates of work: August 21, 2007 - October

Validity criteria: According to OECD guideline 216 (2000), the variation of the nitrate-concentrations between control replicates should be less than \$15 % In this stud, a maximum coefficient of variation of 7 % was obtained. Therefore, the results of the study are considered validy

Observations:

Effects on nitrogen transformation Time interval/day in wil after treatment with Table 10.5- 4: FLU+ TFS SC 509 (250+250 g/L)

Time . 4		ontrol	ð. [¢] 0	ο 1.07 μ ο eqni	L p ival	rod Akg en tro 0	soil dry weight .8 L prod@ha	д 10.67 р У еді	uL uiva	prod./kg s llent to 8]	oil dry weight L prod./ha
[days]	Nit	sate-N	ц ц х х х	~Nitr	ate-	N ¹	% difference to	Nit	rat	e-N ¹⁾	% difference to control
0 - 7	- 2.06	±	0.11	- 1. 64	′ ± 。	×9.29		- 1.15	±	0.22	44*
7 - 14	9 .63	¢,	0.64	0.55	×↓ C	0.48	12 ^{n w}	0.60	±	0.14	4 ^{n w}
14 - 28 🕰	1.04	±	0.03	91.14 <i>A</i>	, A	<u></u> 024	9 ^{n s}	1.32	±	0.13	26*
28 - 4	1.35	جي ا	0.10	1.36	±	0.08	× 1*	1.70	±	0.07	26 ^{ns}
42 56	0.67 🕊)±	6 ,74	0.59		0.04	12 ^{ns}	0.53	±	0.09	21 ^{n s}

The calculations were performed with throunded values

1) Rate: Nitral N in mg/kg soll dry weight/time interval/day, mean of 3 replicates and standard deviation

No statistically significant afference to the control (Student-t Test, two-sided, $\alpha = 0.05$) n.s.

No statistically significant difference to the control (Welch-t Test, two-sided, $\alpha = 0.05$) n.w.

Statistically significant difference to the control (Student-t Test, two-sided, $\alpha = 0.05$).

During the 56 day test, 1.97 µL FLU + TFS SC 500 (250+250 g/L) had no relevant influence on nitrogen tractornation in a soil and ended with lucerne-grass-green meal. The 10-fold dose of the test item caused a temporary stimulation of the daily nitrate rates at the time interval 0 - 7 days, 14 - 28 days and 28 - 42 days after treatment. At the end of the experiment (42 - 56 day interval), differences in the nitrate-N rates between control soil samples and treated soil samples are < 25 % and meet the trigger values of above mentioned guideline for a termination of the study.



Reference test:

Sodium chloride was used as a reference standard in the tests. In tests (non-GLP) with the agricultural soil described above, 16 g NaCl/kg dry weight soil had a distinct and long-terp (> 28 days), afluence on microbial mineralization of nitrogen.

III. CONCLUSION If used as recommended, FLU + TFS SC 500 (250+250 g/L) should not have an impact on nitrogen transformation in soils. The study was performed in a field soil at concentrations up to 10.67 µL prod./kg



CP 10.6 Effects on terrestrial non-target higher plants

For the product FLU + TFS SC 500 three single dose studies (testing 7 and 2×10 species) and two dose response studies (testing 3 and 1 species) on terrestrial plant vegetative vigous and two single dose studies (testing 10 species) on terrestrial plant seedling emergence were conducted to determine possible effects on terrestrial non-target higher plants. In none of the studies listed below adverse effects 20% were observed in any species tested. The only exception is buckwheat usee Table 10.6- 10 in the were observed in any species tested. The only exception is buckwhear tese and ble UP 6- 191n the vegetative vigour limit test (M-681185-01-1) at 1.0 L prodyha. For this species a dryweight addressing and ER₃₀ of > 2 L prodyha for buckwheat. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thus, the relevant ER₃₀ for the risk assessment of all species is set as > 1 L prodyha. Thu vegetative vigour limit test (M-681185-01-1) at 1.0 L prodyha. For this species a dry weight reduction



Table 10.6- 1:	Effect values relevant for the risk assessment for non-target terrestrial plants for
	FLU+TFS SC 500

FLU+TFS SC 500									
Test organism	Study type	Endpoint	References						
Beta vulgaris ^d		*	S P						
Brassica napus ^d									
Cucumis sativus ^d		l i i i i i i i i i i i i i i i i i i i							
Fagopyrum esculentum ^d	TT	1							
<i>Glycine max</i> ^d	Vegetative vigour;	$ER_{50} > 0.75 L \text{ prod}$	(2007)						
Helianthus annuus ^d	lier I, single dose,	(all species)	M-259527-01-1 6						
<i>Lycopersicon esculentum</i> ^d	21 days		K@P 10.6 903 ×						
Allium cepa ^m									
Avena sativa ^m	1								
Zea mays ^m	Ó, y								
Beta vulgaris ^d	. ~	· · · · · · · · ·	A . ~ . ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~ ~						
Brassica napus ^d	4								
Cucumis sativus ^d			P & A co						
Fagopyrum esculentum ^d	A O								
<i>Glycine max</i> ^d	Vegetative sugour,	$ER_{50} \approx x 0.$ T prod. Ra							
Helianthus annuus ^d	21 days	(all species)	2 CD 1 CD						
<i>Lycopersicon esculentum</i> ^d	21 dayso		OKCP 19.0.2/04						
Avena sativa ^m	A O' Y								
Lolium perenne ^m			Õ 'Y						
Zea mays ^m									
Brassica napus ^d		$ER_{30} > 1$ QL prod ha							
<i>Glycine max</i> ^d		Carll species except	Ô						
Fagopyrum esculentum ^d	Vegetative vigou	Fagopyrum esculentum)	(2020)						
Zea mays ^m	Ajer 1, single dese,	ER 1.0 L prod./ha	<u>M-681185-01-1</u>						
Lolium perenne ^m	Ží davs	(Fagopyram esculentum?	KCP 10.6.2/05						
Avena sativa ^m		32% reduction of shoot $%$							
Allium cepa ^m		dry weight) 🔬 🖉							
Beta vulgaris ^d	Wegetative vigour;	$FR_{co} > 2$ or prothing	(2018)						
Cucumis sativus ¹	Tier & dose responses	All sneeps)	<u>M-612774-01-1</u>						
Solanum lycopersicum ^{yd} 🔊	21 days		KCP 10.6.2/06						
	Vegetativewigou		(2020)						
Fagopyann esculentum 🖱	Pier 2, dose response, S	$ER_{s} > 2.0 \mathbb{P}$ prod./ha	<u>M-688437-01-1</u>						
	21 days and the		KCP 10.6.2/07						
Beta vulgaris "									
Brassica napus									
Cucumis sativus	P. C. A. O	1 Alexandree and the second se							
Fagopyrum eseulentum	Seeding emergence?	CD > 0.75 L mod /ha	(2007)						
Glycine max d	Tor 1, single doso,	$PER_{50} > 0.75 L \text{ prod./ha}$	(2007) M 280525 01 1						
Helianinus annuus -	JA days	(all species)	$\frac{M-289525-01-1}{VCP}$						
Lycoperating ^m			KCP 10.6.2/01						
I okum navanna ^m									
Zea mays ^m	67 ~ 0 ⁷								
Bota vulgaris de									
Brassica nantit									
Cucumis satisfies de									
Fagonvran esculantum	S Y								
Glycing max d	Seedling emergence;	$ER_{co} > 1.0 I$ prod /ba (all	(2020)						
Holianthus annus	Tier 1, single dose,	species)	<u>M-681165-01-1</u>						
Altom certom	21 days	species	KCP 10.6.2/02						
Avena salava ^m									
Lolium perenne m									
Zea mays ^m									

m: monocotyledonous; d: dicotyledonous



Risk assessment for Terrestrial Non-Target Higher Plants

The risk assessment is based on the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev. 2 final, 2002). It is restricted to off-field situations, as non-target plants are defined as non-crop plants located outside the treated area. Thus, effects on con-target plants are of concern in the off-field environment, where non-target plants may be exposed to spray drift,

As it is demonstrated by the available set of studies that the single application rate of 0.2 L prod that does not result in effects > 50 % according to the "Guidance Document on Terrestrial EcoOxicology" (SANCO/10329/2002 rev. 2 final, 2002), no risk for pon-target terrestrial plants is expected. The limit test rate is higher than the highest field application rate and is only considered as indicator for acceptable risk.

A detailed deterministic risk assessment is in addition presented below. Greenhouse uses are not relevant for exposure calculations.

Table 10.6- 2:	Deterministic asse	ssmento	f the ris	sk for i	ion-targe	et plants	dae to	theuse	of FLU+TFS
	SC 500 in grape	ÇŐ,	Q	ð	5	L) >	Õ		*

	<u>_0'</u>			
Intended use	Grape, 2×0 2 L pro	od./ha, BBCH 53	\$\$\$. \$	O*
product	FLQ+TFS SC 500			Ş
Application rate (L prod./ha)				/
MAF	1.6 fustification: At the "it was agreet that f should be used by approach is in Cotoxeology" curr in the context of N	recent Pesturide I earthe risk assess defaulty until line with the reactly in use which IPP risk accessed	Peet Review Meetin rent of active subst guidance documen "Guidance Docu broes not require t	ng 133** in Sept. 2015 cances, no MAF values it is developed." This ment on Terrestrial he use of a MAF value
	apply a MAF when	calculating the Pl	ER.	t deemed necessary to
Test species	CR50 (L prôd./ha)	Drift rate	PER _{off-field} (L prod./ha)	TER criterion: TER≥5*
All species -seedling emergence		7.23 ~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.014	>69.2
All pecies		7.23	0.014	>69.2

virgomental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below relevant trigger. 21 resevant trigger. S for Germinicitic risk assessment based on ER₅₀

SA (European Food Safety Quthority), 2015. Technical report on the outcome of the pesticides peer review meeting on

eneral recurring issues in esotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

Conclusion:

From the information presented above it is concluded that the use of FLU + TFS SC 500 will not produce unacceptable effects on terrestrial non-target plants growing near treated fields. No mitigation measures are necessary for the intended use rates.



CP 10.6.1 Summary of screening data

Studies were not necessary since guideline GLP studies for terrestrial non-target plants are shallable (see Point 10.6.2 in this MCP Summary).

CP 10.6.2	Testing on	non-target	plan
	U	0	-

Data Point:	KCP 10.6.2/01
Report Author:	
Report Year:	$2007 \qquad $
Report Title:	Non-target terrestrial plants: an evaluation of the effects of AE 0656948 +
	trifloxystrobin SC 250+250 gL in the seedling emergence and growth test (Yier
Report No:	SE07/03
Document No:	$\underline{M-289525-01-}$
Guideline(s) followed in	OECD 208 (Javy 2006): seedling emergence and growth test Tier 1. Equivalent
study:	to US EPA OPPTS Guideline No: \$50.4100 Q Q
Deviations from current	Current Guidelin@OECD208 (2006) 2 2 2 2
test guideline:	Deviations: Temporary deviation from climate Condition (temperature). Light
	inten @y and wimidit@were pot rep@ded. Deviation from recommended plant
	density. All validity criteria were met. The deviations listed abov Chad no
	influence on the pliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially	No not conducted under QLP/Offizially @cognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes / (x / x / x / x / x / x / x / x / x /
8 <u>4</u>	

Executive Summary

The objective of this specific study was to evaluate the potential effects of FLU + TFS SC 500 (250 + 250) on the seedling emergence and growth of ten species of non-target terrestrial plants in a limit test. A total of ten species, 7 divotyledonous and 3 monocotyledonous species from 8 plant families were tested in this seeding emergence and growth fest: Beta vulgaris (sugar beet), Brassica napus (oilseed rape winter), Cucunts satisfies (occumber), Fagopyrum esculentum (buckwheat), Glycine max (soybean), Helianthus annus (sunflower), Lycopersicon esculentum (tomato), Avena sativa (oat), Lolium perenne (ryegrass) Zea ways (corn). Planting density included 5 seeds per pot, with 4 replicate pots, respectively, for a total of 20 seeds per reatment level. The sown seeds of the plant species were treated with a single application rate of 6.75 L product/ha (equivalent to 500 g a.s./ha) and a water control. The application was done at a volume rate of 200 L/ha. All seeds were planted one day before application and test duration was 14 days after 70 % emergence of the seedling in the controls for each species. Plants were assessed for emergence survival and rated for phytotoxicity on days 7 and 14. Final assessments were made for germination, plant survival, phytotoxicity, plant growth stage and plant biomass.

The study fulfils all validity ofteria of OECD 208 guideline.

There was no adverse effect of the product on the survival and phytotoxicity of the ten species tested.

There were no differences in germination and biomass above the 50 % effect level at the single rate of 0.75 L for od./ha. Therefore the ER₅₀ (based on germination, survival and biomass) was > 0.75 L prod./ha.


I. MATERIAL AND METHODS

Test item: FLU + TFS SC 500 (250 + 250), specification no.: 102000012886; batch ID.: 2007-000441; Sample description: TOX 07851-00; active substance (analysed content): fluopyram: 21.4% w/w (251.5 g/L), trifloxystrobin: 21.6 % w/w; density: 1.174 g/mL.

Test design: A total of ten species, 7 dicotyledonous and 3 monocotyledonous species from 8-plant families were tested in this seedling emergence and growth test: Beta vulgaris (sugar best), Brassica napus (oilseed rape winter), Cucumis sativus (cucumber), Fagopyrum esculentum (buckwheat), Glycine max (soybean), Helianthus annuus (sunflower), Lycopersteon esculentum (tomato), Azena sativa (a), Lolium perenne (ryegrass), Zea mays (corn). The plant species used in this study are representative of a wide range of plant families and were chosen because they are readily cultivated test organisons and widely used in research. Routine germination tests were carried out on the seeds to ensure their viability. The seeds were sown one day prior to application of the test from to the soil surface in 10 cm plastic pots. The used soil was a sandy-silt loam with washed sand.

Planting density included 5 seeds per pot, with 4 replicate pots, respectively, for a total of 20 seeds per treatment level. The sown seeds of the plant species were treated with a single application rate of 0.25 L product/ha (equivalent to 500 g a.s./ha) and a water control. The test solution was applied at a volume rate of 200 L/ha. Control pots were sporyed with 200 L/ha of deioouzed water.

Daily checks were made to identify the date when 70 % of the seedlings emerge in the control for each species. All seeds were planted one day before application and test duration was 44 days after 70 % emergence of the seedling in the controls for each species. Plants were assessed for emergence, survival and rated for phytotoxicity on days 7 and 14. Final assessments were made for biomass endpoint determinations were performed for plant dry weights. Visual phytotoxicity were assessed using a qualitative rating: 0 (no effect), A-E rating (shight effect to moribund). Any plant considered as being dead was not rated for visual prytoto city. \bigcirc

Climatic conditions Following application, the pots with plants were maintained under greenhouse conditions and natural da dight was supplemented by artificial lighting. Light intensity was not reported. The temperature was regulated to manutain 10 to 31%C during the nght cycle (16 h) and during the dark cycle (8 h). The relative humidity was not recorded. Õ Ľ

Statistics: Statistical analysis of the dra weight was carried out using the Pairwise Mann-Whitney-Utest (one sided smaller)

-1, 4907 - April, Dates of work: March 21

DISCUSSION

Validity criteria:

The validity criteria of OECD 208 were for filled

The seedling emergence of control plants was ≥ 70 % (actually between 75 and 100 %). The control seedlings of each species did not exhibit visible visual injuries (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and control plants exhibited normal variation in growth and morphology for that particular species. The non-an survival of emerged control seedlings was ≥ 90 % (actually between 90) and 100 21 days after at least 50 % emergence in the control. The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

Biological findings:

There was no adverse effect of the product on the survival and phytotoxicity of the ten species tested.



Germination was increased in sugar beet, tomato, buckwheat, oat and ryegrass by 11.1 %, 5.9 %, 20 %, 11.8 % and 11.1 % respectively. Germination was inhibited in cucumber, oilseed rape, sunflower and corn by 5.6 %, 11.1 %, 10 % and 10 % respectively.

Biomass was reduced in oilseed rape, buckwheat and ryegrass by 14.8 % 12.5 % and respectively. Biomass was increased in cucumber, soybean, sugar beet, sunflower, tomato corn and on by 13.5 %, 17 %, 9.7 %, 32.5 %, 16.4 %, 10 % and 17.2 % respectively.

None of these differences were significant at the 95% confidence limits. None of these ditte reached or exceeded 50 % to trigger further testing.).

Please note: Phytotoxicity was assessed using a qualitative rating: 0 (60 effect), A-E rating slight of to moribund). Control and treated plants showed the symptoms of phytotoxicity phytotoxicity assessment are presented in the table below.

			Ĉn (r &	402	
Table 10.6.2- 1:	Summary of phytotoxicity	and prowth	strages (R	BCH exh	sure to FI	1 + TES SC 500
14010101012 11	Summary of phytotometer			you cape		
	(250+250 g/L) at the final	assessment of	m dav≥14	A.	N. A.	
	(J 7 0	\heartsuit	

Plant species	Phytoto	stielty 5	ў <u>"</u> ввен а "У <u>ў</u> Імя́	rowth stages
	Control	075 L product/fra	Control O	C Test [≉] item ℃0.75 % /product/ha
Beta vulgaris			~ 12 - 14 ja	12 - 14
Brassica napus				12 - 14
Cucumis sativus 🤞			° ≤42 - 1;4 s	12 - 14
Fagopyrum esculentum		Les Car	12 - 94	12 - 14
Glycine max			\$ \$\$ - 14~\$	12 - 14
Helianthus annuus			12 - 14	12 - 14
Lycopersicon [©] escalèntum			10-12	12
Avena sativa 🔍 🖉			012 - 14	12 - 14
Lolium perenne			12 - 14	12 - 14
Zea mays			v 13	13

as sets ment time with the following a rating system: Phytotoxicity was recorded at each

- 0: no injury or effect
- A: «slight symptom (s)
- B: [≫]moderate symptom_∂(s)
- C: severe symptom (s)
- D: total-plant symptom
- E: moribund 🔬 Codes for isual murie
- chlorosis (yellowing of green shoot tissue) a:
- (pecrosis (brown shoot tissue) b:
- c: ^{Sob} bleaching (shoot tissue without pigmentation)
- withing (loss of turgor of shoot tissue) d:
- e: leaf deformation (leaf curl, abnormal leaf shape)
- f : stunting (plant height reduced with shorter inter-node lengths)



Table 10.6.2- 2:Summary of germination and survival following exposure to FLU + TFS SC 500
(250+250 g/L) at the final assessment on day 14

		Germination	<i></i>	Sui	rvival
Plant species	Control % of sown	0.75 L % of sown	prod./ha % inhibition	Control % of Sown	0.75 L prod./ha % of sown
Beta vulgaris	90	100	11.1	×100	~ 100° ~
Brassica napus	90	80	T 11.1	J 100	
Cucumis sativus	95	95	5.6	100 🖑	Q 100 4
Fagopyrum esculentum	75	90	20	@°100	× 100 ×
Glycine max	100	100	·	× 1890 ~	<u>,</u> ≪100,~Ş
Helianthus annuus	100	090 0	×10 \$	~~100 ~~	100
Lycopersicon esculentum	85	A 90 0	<u>∼</u> 5.9 ^Q	<u>100</u> *	
Avena sativa	85	2.95	149.8 Å	¥00 ~~	لا 100 م
Lolium perenne	90 5	×100 ×	\$11.1 °	2 90 S	2 100
Zea mays	100	20	10	0 100 Č	× 100
	a W	W L	ř di A	<u>, 9, °0</u>	<u>_</u>

 Table 10.6.2-3:
 Summary of short dry veight following exposure to FLU + IFS SC 500 (250+250 g/L) at the final assessment on day 14

	Shoot dry weight	
Plant species	Control 0.45 L	prod./ha 🍼 🎸
Thank species	S Mean dry weight Mean dry Weight	0/ Babibitie A
ć		
Beta vulgaris		- 9.7
Brassica napus	₩ 40.278	
Cucumis _s sativus	× 0.278 0.316 0	13.5
Fagogyrum		12.5
escutentum		× 12.5
Glycine max	\$ 40.294 \$ \$ \$ \$ \$ 0.343 \$	- 17.0
Helianthus 👸		° 27.5
annuus	2 0,025 C X 0,466 X	- 52.5
Lycopersic		16.4
esculentum		- 10.4
Avena sativa		- 17.2
LoliumSperenne	× 0.024 0020	15.2
Zea mays	2,589 m ³ Q 2,648	-10

A Negative figures indicate that there was an increase in bromass (dry weight) when compared to the untreated control.

$\mathbb{Q}^{\mathbb{Q}^{\vee}}$ **III. CONCLUSION**

In this sections emergence and growth study, FLU + TFS SC 500 (250+250 g/L) was tested under greenhouse conditions for effects on the seedling emergence, survival, dry weight and phytotoxicity of ten non-targe perrestrial plant species, following a pre-emergence application of the test item to the soil surface. No adverse effect (i.e. greater than 50 %) for all the tested species in the seedling emergence test were observed. Therefore the ER₅₀ was determined to be > 0.75 L prod./ha.



Assessment and conclu	sion by applicant:
The study and its data an	re considered as acceptable and reliable for use in risk assessment.
The ordnoint is: ED	0.75 L prod /ho
The endpoint is. EK ₅₀ >	

Data Point:	KCP 10.6.2/02
Report Author:	
Report Year:	
Report Title:	Fluopyram + trifloxystroom SC 500 (250+250 g/2): Effects on the seeding
	emergence and seedling growth of ten non-target terrestrial plant species under
	greenhouse conditions (tier 10) & A a a a a a a a a a a a a a a a a a a
Report No:	
Document No:	$\underline{M-681165-01-1}$
Guideline(s) followed in	EU Directive 91/414/EBC
study:	Regulation (BC) no $4/107/2409$ 3 3 3 3 3
	US EPA OCSPP 850.4100(2012)
	OECD 268 (2006)
Deviations from current	Current Guidelfine: OFCD 208 2006 C
test guideline:	Deviations: Temporary deviation from climate condition (light). All validity
	criteria were met. The deviations listed above had no influence on the reliability of
	the stude and endpoints of the stude of the
Previous evaluation:	No, not previously sugmitted a start way and a
X	
GLP/Officially	Yes, conducted under GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Regrability	$ \underline{Y} \underbrace{es}^{*} \underbrace{v}_{\mathcal{V}} \underbrace{v}_{\mathcal{V}}$
Executive Summary	

The objective of this specific study was to evaluate the potential effects of FLU + TFS SC 500 (250 + 250) on the seedling emergence and growth of ten species of non-target terrestrial plants in a limit test. A total of ten species, 6 dicotyledonous and 4 monopolyledonous species from 8 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucutus sativus* (cucumber), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sufflower), *Allum cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn). Planting density factuded 2 or 4 seeds per pot, with 10 or 5 replicate pots, respectively, for a total of 20 seeds per treatment level. The sown seeds of the plant species were treated with a single application rate of 1.0 L product an and a water control. The application was done at a volume rate of 200 L/ha. The control pots of each species were observed daily for the number of seedlings emerged until 50 % of the seedlings had emerged (= day 0). Assessments were made individually for each species on this day (day 0) and 7, 14 and 21 days post emergence of 50 % of the control seedlings. Or day 6, 7 and 14, only seedling emergence, post-emergence mortality and visual phytotoxicity, were recorded. Enal assessments were made for emergence, plant survival, visual phytotoxicity, and tages and shoot high and shoot dry weight 21 days post emergence of 50 % of the control seedlings.

The study falfils all valienty criteria of OECD 208 guideline.

Visual hytotoxicity occurred only in *Fagopyrum esculentum* with a mean effect of 3 % compared to the control group.



There were no adverse effects on emergence, post-emergence mortality, shoot height and shoot dry weight above the 50 % effect level at the single rate of 1.0 L prod/ha. Therefore the ER_{50} was determined to be > 1.0 L prod./ha.

I. MATERIAL AND METHODS

<u>Test item</u>: FLU + TFS SC 500 (250 + 250); specification no.: 102000012886; supplier batch no.: EV57002709; Sample description: TOX 21159-00; active substance (analysed content): flue pyram. 21.1 % w/w (246.6 g/L), trifloxystrobin: 21.3 % w/w (248.6 g/L); density: 1.168 g/m/).

<u>Test design</u>: A total of ten species, 6 dicotyledonous and 4 monocotyledonous pecies from 8 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucamber), *Fagopyrum esculentum* (buckwheat), *Glyetne max* (soybean), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Ivena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn). The plant species used in this study are representative of a wide range of plant families and were chosen because they are cadily cultivated test organisms and widely used in research. The seeds were sown on the day of application of the product to the Soil surface in commercial 15 cm plastic pots (filled with approx. *Co* kg soil/pot). The used soft was a loamy sand.

Planting density included 2 or 4 seeds per pot, with 10 or 5 replicate pots, respectively for agotal of 20 seeds per treatment level. The sown seeds of the plant species were treated with a single application rate of 1.0 L product/ha and a water control. The set solution was applied at a volume rate of 200 L/ha. Control pots were sprayed with 200 L/ha of deionized water.

After application, the pots with seeds were transferred back to the greenhouse. The pots were set up sorted per treatment group after application. All pots were repositioned on the first and second assessment day to compensate for potential variability in growth conditions.

The control pots of each species were observed daily for the number of seedlings emerged until 50 % of the seedlings had onerged (= day 0). Assessments were made individually for each species on this day (day 0) and 7, 14 and 21 days post emergence of 50% of the control seedlings. On day 0, 7 and 14, only seedling emergence, post-emergence, mortality, and visual phytotoxicity were recorded. Final assessments were made for emergence, plant survival, osual phytotoxicity, plant growth stage and shoot high and shoot dry weight 21 days post emergence of 50% of the control seedlings. A gradual rating was assigned to describe the extent of the visual phytotoxicity in comparison to the control, taking into account necrosis, deformation and change in celour (e.g. chlorosis, bleaching, reddening). The ratings referred to the whole plants within a replicate and range from 10 to 90%. Any plant considered as being dead was not rated for Qsual phytotoxicity.

Analysis of the test item solution and the control solution were conducted by LC – UV.

<u>Climatic conditions</u>: Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. The light intensity was in a range of 260-400. The temperature was regulated to maintain 15 to 27 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative homidity was regulated to maintain 51 to 88 %.

<u>Statistics</u>: The data of seedling emergence were tested with Fisher's exact test. As no mortality occurred, no computations were performed. The data of shoot height and shoot dry weight were tested for normality and honoscedisticity using Shapiro-Wilk's test and Levene-test. In case both requirements were fulfilled, Student t-test was conducted. The significance level was set to $\alpha = 0.05$ for all hypothesis tests. In case of an increase in the test item group compared to the control group for seedling emergence, shoot height and shoot dry weight, no statistical evaluation was conducted. Statistical analysis was performed using the program ToxRat Professional Version 3.3.0.

<u>Dates work</u>: November 20, 2019 – December 23, 2019



II. RESULTS AND DISCUSSION

Validity criteria:

The validity criteria of OECD 208 were fulfilled.

The seedling emergence of control plants was ≥ 70 % (actually between 90 and 100 %). The control seedlings of each species did not exhibit visible visual injuries (e.g. chlorosis, necrosis, witting, leaf and stem deformations) and control plants exhibited normal variation in growth and morphology for that particular species. The mean survival of emerged control sedlings was ≥ 90 % (actually 100 %) 21 days after at least 50 % emergence in the control. The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANTE/2020/12830, Rev.1.

The analysed concentration of fluopyram in the product solution corresponded to 99.8 % of the mominal concentration.

Biological findings:

Visual phytotoxicity observed at the final assessment (of day of after application) in this seedling emergence and growth study included chlorosis, necrosis, deformation and studying of the plants. Visual phytotoxicity occurred only in *Fagopyrum esculentum* with a mean effect of the compared to the control group.

No statistically significant effects on the parameter seeding emergence were observed for any of the plant species tested. The Kighest whibition occurred for *Lolfum perenne* with 5.6 % compared to the control group.

No post-emergence mortalit occurred during the course of this study

No differences in the growth stages between the test item and the control group of all ten plant species tested were observed.

Statistically significant differences in short height compared to the control group were observed for Brassica napus (125%), Glycine max (14.3%) and Allium sepa (19.7%).

Statistically significant differences in shoot dry weight compared to the control group were observed for Allium cepa (5.5 %) and Lolium perenne (37.5 %).

The growth stage shoot height and dry weight as well as emergence, survival and symptoms of phytotoxicity are summarized for each of the plant species in the following tables for the final assessment (21 days after 59,% emergence of the control seedlings).

are summarized for each of the



Table 10.6.2- 4: Summary of growth stages (BBCH) and inhibition of shoot height and dry weight following exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day

21				. Å . Å	
	BBCH	growth stage	Dhutatariaituu nõra 9/ / Suma An		
Plant species	[Mi	n - Max]			
	Control	1.0 L prod./ha	Control	1.0 Lorrod./Az	
Beta vulgaris	13 - 13	13 - 13 👸	0 / - 🎝		
Brassica napus	13 - 13	13 - 13 🔊	058	L 07- 5 07	
Cucumis sativus	12 - 12	12 - 12	Q 0 /	20 1 - C C	
Fagopyrum esculentum	51 - 51	54Q 51		9 / CCCDE	
Glycine max	21 - 21	\$21 - 21 [°]	\$ \$ \$/- \$	₩ ¹ ¹ ¹ ¹ ¹ ¹ ¹	
Helianthus annuus	16 - 16	16,46	Q 0 / - O		
Allium cepa	12 - 12	× 42 ² -12~	\mathcal{O}	$\swarrow 0^{7}$	
Avena sativa	14 - 14	ر الأسلام 14 - الأطل الأسلام ال	- <u>-</u> - <u>-</u>		
Lolium perenne	21 - 21	[™] 21 [™] 21		5 0 L	
Zea mays	15 - 15	P5 - 15	5 .49 - of	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
A Visual symptoms: None	e (), 6 C = change	in colour, DE deformati	on 🖉 👸	O A	

Visual symptoms: None (Ŵ Ś Ö

Summary of shoot height and shoot dry weight and corresponding percent inhibitions following exposure to FLIO+ TFSSC 500 (250+250 g/L) at the final assessment on day 210^{-10} 0 Table 10.6.2- 5:

Ø

			7.	V	
	Sboot hei	ght S		boot dry weig	ght
Plant Spains &) (10.1 %)	prod./ha	Control	1.0 L J	orod./ha
Control	Mean	intribition	Mean dry weight	Mean dry weight [g]	% Inhibition ^A
Beta vulgaris 🔿 🍼 13.4	, 12:5	× 6¢7	o.338 €	0.302	10.7
Brassica napus 13,9	_°∿¥1.5 @	v 1 <u>0</u> 5* _∞	0.647	0.595	11.7
Cucumis saltarus 🔿 🖉.7	15.1	~- 2.7 ~	0.674	0.409	12.4
Fagopyrum & culent of 63.3	0 350	0 [°] 1.10 [°]	0.582	0.712	- 22.3
Glycing max 🔍 🔊 🖉 18.9	16.2	× 143*	0.845	0.826	2.2
Helianthus annuus 🔍 😥	A 6.8 Q	Ø.5	0.504	0.541	- 7.3
Allium cepa 🔬 🖉 👌 7.3	Ø 13,	×19.7*	0.033	0.018	45.5*
Ävena sativa 41.3	429	-3.9	0.289	0.261	9.7
Lolium perenne [®] 22,0°	9 .7 ~(7.5	0.112	0.070	37.5*
Zea mays 0° 49.2	¥6.4 O	5.7	0.650	0.586	9.8

* А

 Lea mays
 ""
 49.2
 46.4 $^{\circ}$ 5.7
 0.650
 0.586

 Statisticall/significantly different compared to the control (Student's t-test; one-sided smaller, $\alpha = 0.05$)
 Negative values indicate that there was an increase compared to the control

 Negative values indicate that there was an increase compared to the control



Table 10.6.2- 6:	Summary of emergence and cumulative mortality following exposure to FLU + TFS
	SC 500 (250+250 g/L) at the final assessment on day 21

	. 0	,		-	^ ``````````````````````````````
	Se	odling omorgo	nco	Cum®lati	
Plant species		euning einer ge	nee		A A
	Control	1.0 L p	orod./ha	Control	1.0 C prod ha
	% emerged	% emerged	% Inbibition ^A	% mortality	% mortality
Beta vulgaris	95	100	- 5.3	Q 0	
Brassica napus	95	100	- 5.3		Q 0 O S
Cucumis sativus	90	100 🕰	- 11.1 🔍	°0 K	
Fagopyrum esculentum	100	100	0.0 🥎		
Glycine max	80	80 ×	○ 0.0 ⁰	j 90	
Helianthus annuus	85	25 (- KU.8	, de S	
Allium cepa	90	4 100 🔬	91.1 O		à the the
Avena sativa	85	×7100×	.~-17.6	$\beta = 0$	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Lolium perenne	90	\$ 85	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	× 10 ~	
Zea mays	80	× 00,20 ×	<u>- 151.1 (</u>		
A Negetine realized in diago	ha hla an hla anna 🖓 🤇	La in the second	and the second and the second se	1 &	

Negative values indicate that there was an increase compared to the control

In a seedling emergence and growth study, FLU + TFS SC 500 (250+250 g/L) was tested under greenhouse conditions for effects on the seedling emergence, survival growth and shoot dry weight of ten non-target terrestrial plant species, following a protemergence application of the product to the soil surface. No adverse effects or emergence, post-emergence mortality, shoot height and shoot dry weight above the 50 % effect level occurred. Therefore the ER was determined to be > 1.0 L prod./ha.

Assessment and conclusion by applicant: The study and its data are considered as acceptable and reliable foruse in risk assessment. The encount is: ER 1.0 prod ha



Data Point:	KCP 10.6.2/03
Report Author:	
Report Year:	2007
Report Title:	Non-target terrestrial plants: an evaluation of the effects of AE C656948 + $\sqrt[3]{2}$
	Trifloxystrobin SC 250 + 250 g/L in the vegetative vigour $cost$ (Tier 1)
Report No:	VV07/03
Document No:	<u>M-289527-01-1</u>
Guideline(s) followed in	OECD 227 (July 200): vegetative vigour test (Tier 1); Equivalent to OS EPA
study:	OPPTS Guideline No. 850.4150 🛞
Deviations from current	Current Guideline: OECD 227 (2006) \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O} \mathcal{O}
test guideline:	Deviations: Temporary deviation from climate@ondition (temperature during@ight 0
	period). Light intensity and kernidity was not reported. All validity criteria vere
	met but the germination rate of the seeds used in this study was not reported.
	However, as routine germination tests were carried out on the seeds to ensure their
	viability, the germination rate can be considered to be in the acceptable range. The
	deviations listed abook had the influence on the reliability of the study and
	endpoints.
Previous evaluation:	No, not previously submitted A A A A
GLP/Officially	No, not conducted mder GLP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$Yes \qquad \qquad$

Executive Summary

The objective of this study was to evaluate the potential effects of FLU + TFS SC 500 (250 + 250) on the vegetative vigour of ten non-target terrestrial plant species in ohmit test, following a post-emergence application of the product onto the foliage of plants at the 2 - 4 leaf stage. A total of ten species, 7 dicotyledonous and 3 monocotyledonous species from 9 plant families were tested in this vegetative vigour test: Bela vubcaris (sugar beet), Brassiela napus (oilseed rape winter), Cucumis sativus (cucumber), Cagopytum esculentum (buckwheat), Gycine max (soybean), Helianthus annuus (sunflower), Lycopersicon esculentum (totato), Allian cepa (onion), Avena sativa (oat), Zea mays (corn). Planting density included 4 or Oplants per pot with for 5 toplicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated with a single application rate of 0.75 L product/ha and a water control. The application was done at a volume rate of 200 L/ha. Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and visual phytotoxicity were recorded. Final assessments were made for plant survival, visual phytotoxicity, growth stages and dry weight.

The study fulfils all validity criteria of OECI9227 grideline. However, germination rate of the seeds used in this study was not reported. As routine germination tests were carried out on the seeds to ensure their viability, the germination rate was considered to be in the acceptable range.

There were no adverse effects of the single treatment of 0.75 L prod./hat on the survival and phytotoxicity of the species tested. No adverse effects (i.e. greater than 50 %) were observed in all species tested in the vegetative vigour test. Therefore the ER₅₀ was determined to be > 0.75 L prod./ha.

I. MATERIAL AND METHODS

<u>Test jiem</u>: FU + TFS SC 500 (250 + 250); specification no.: 102000012886; batch ID.: 2007-000441; Sample description: TOX 07851-00; active substance (analysed content): fluopyram: 21.4 % w/w (251.5 g/L), trifloxystrobin: 21.6 % w/w (2253.5 g/L); density: 1.174 g/mL

<u>Test design</u>: A total of ten species, 7 dicotyledonous and 3 monocotyledonous species from 9 plant families were tested in this vegetative vigour test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed



rape winter), *Cucumis sativus* (cucumber), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Lycopersicon esculentum* (tomato), *Allium cepa* (onion), *Avena sativa* (oat), *Zea mays* (corn). The plant species used in this study are representative of a wide range of plant families and were chosen because they are readily cultivated test organisms and widely used in research. Routine germination tests were carried out on the seeds to ensure their viabality. The plants were grown in a greenhouse in commercial 10 and 13 cm plastic pots. The used soil was a sandy-silt loam.

Planting density included 4 or 5 plants per pot with 4 or Speplicate pots respectively, for a total of 20 plants per treatment level. The plant species were treated at the 2 - 4 leaf stage with a single application rate of 0.75 L product/ha and a water control. The test solution was applied onto the folge of plants \bigcirc and above-ground portions at a volume rate of 200 tha. Control pots were sprayed with 200 t/ha at deionized water.

After application, the pots with plants were transferred back to the greenhouse.

Assessments were made 7, 14 and 21 days after application. On day 7 14 and 21 only plant survival and visual phytotoxicity were recorded. Final assessments were made for plant survival, visual phytotoxicity growth stages and dry weight. Visual phytotoxicity was assessed using a qualitative rating 0 (no effect), A-E rating (slight effect to moribund). Any plant considered as being dead was not rated for visual phytotoxicity and removed from the pot.

<u>Climatic conditions</u>: Following application, the pots with points were munitained under greenhouse conditions and natural dayligh was supplemented by artificial to thing. The best intensity was not recorded. The temperature was 10 to 31 °C buring the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was recorded.

Statistics: Statistical analysis was carried out using the Bairwise Mann-Whitney-U-test (one sided smaller).

Dates of work: Apr 12, 2007 - May 14, 2007

II. RESULTS AND DISCUSSION

Validita Criteria

The validity criteria of OECD 22% were tulfilled.

All plant species in this study met the validity criterion of at least 90 % for survival in the control. In accordance with US PA guideline (OCSPP 850 4150) and OECD guideline (OECD 227), there was no visible phytotoxicity in control plants. Normal growth occurred in the controls of the ten species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The pots used for all species of this study were third in equal manner with the same soil.

Routine germination tests were carried out on the seeds to ensure their viability. However, as routine germination tests were carried out on the seeds to ensure their viability, the germination rate was considered to be in the acceptable range \mathcal{Q}

Biological findings:

No visual phytotoxicity was observed at the final assessment (on day 21 after application) in this vegetative vigour study for the control and the treatments.

There was no adverse effect of the treatment at 0.75 L prod./ha on the survival and dry weight of the 10 species tested.



The effects on survival, phytotoxicity, plant growth stage and dry weight are summarized for each of the plant species in the following tables for the final assessment (on day 21 after application).

Table 10.6.2- 7:	Summary of survival and shoot dry weight following expo	osure to FLU	+ TF\$ SC 500
	(250+250 g/L) at the final assessment on day 21	<i>O</i> [*]	

	Surv	vival	Ĉġ	Shoot ary weight	
Plant species	Control	0.75 L prod./ha	Control	0.75 J	prod. that the second sec
	% of	% of	Mean dry weight	Mean dry weight	S% Inhibition
	survival	survival			
Beta vulgaris	100	100	2,389	1.641 ×	√ √31.3 √ ♥
Brassica napus	100	100	S.870 [∞]	3:383	≫ 12.6
Cucumis sativus	100	100	4.55C J	8.627	20 3 °
Fagopyrum esculentum	100	100		3.498	\$0.9 °
Glycine max	100	َّى 000L	2.953	, P 3/089 5	-4.6
Helianthus annuus	100	0100 K	<u> </u>	3.780	Q Q.3
Lycopersicon	100	× 100 °	1.663	0 1.289	22.5
esculentum		Y Ö			× ×
Avena sativa	100		1.434 ⁰	Q 9.320 %	7.9
Allium cepa	100	100	0.045	0.097	O - 117.5
Zea mays	100	100	4.9 45	~ 5. 4 75 Q	- 10.7
				<u> </u>	

Please note: Phytotox with was assessed using a qualitative rating. 0 (no effect), A-E rating (slight effect to moribund). Control and treated plants showed no symptoms of phytotoxicity. Results from the phytotoxicity assessment are presented in the table below.

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Å * 0 Summary of phytotoxicity and growth stages (BBCH) following exposure to FLU + Table 10.6 TFS SC 500 (250+250 g/L) at the final assessment on day 21

Blant anadim ⁹	Phyte	otoxicity A 🖉 🗳	BBCH g	growth stages
Plant species	Controk	0,75 L prod./ha	Control	0.75 L prod./ha
Beta vulgaris		X X0 X	12 - 14	12 - 14
Brassicenapus	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~		12 - 14	12 - 14
Cucumis sativus 🛛 💍			12 - 14	12 - 14
Fagopyrum esculentum	N 0 0		12 - 14	12 - 14
Gycine max 🔊	Q Q		12 - 14	12 - 14
Helianthus annua		<u>مَ</u> حَمَّةً الم	12 - 14	12 - 14
Lycopersicon esculentum		0	12 - 14	12 - 14
Allium cepa		S 0	12 - 14	12 - 14
Avena sotiva 🔬 🔪		∞ 0	12 - 14	12 - 14
Allium cepg y	<u> </u>	0	12 - 14	12 - 14
Zea mays		0	12 - 14	12 - 14

totoxicity: Mno phytoxoxicity or effect

Phytotoxicity was recorded at each assessment time with the following a rating system:

0: fo injury or effect

A: slight symptom (s)

B: moderate symptom (s)



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- C: severe symptom (s)
- D: total-plant symptom (s)
- E: moribund
- Codes for visual injuries:
- chlorosis (yellowing of green shoot tissue) a:
- b: necrosis (brown shoot tissue)
- c: bleaching (shoot tissue without pigmentation)
- d: wilting (loss of turgor of shoot tissue)
- leaf deformation (leaf curl, abnormal leaf shape) e:
- f : stunting (plant height reduced with shorter inter-node lengths

HI. CONCLUSION As a result of this vegetative vigour and growth study, in which the effects of PLU + TFS \$\$ 500 (250+250 g/L) on 10 non-target terrestrial plant species were tested under greenhouse conditions, no adverse effects of the single treatment at 0.75 L prod./ha of the servival and dry weight of the 00 species were determined compared to the control. Therefore the ER_{50} as determined to be > 0.75 \tilde{E} proc ha.

<u>Assessment and conclusion by applicant</u>: The study and its data are considered as acceptable and reliable for use in risk assessment. The endpoint is: $ER_{50} > 0.75 L$ prod./ha



Data Point:	KCP 10.6.2/04
Report Author:	
Report Year:	2013
Report Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L) - Effects on the vegetative
	vigour of ten species of non-target terrestrial plants (Tier 1)
Report No:	VV13/033
Document No:	<u>M-464310-01-1</u>
Guideline(s) followed in	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPAOCS P
study:	850.4150; OECD 227 guideline for the testing of chemicals, Terrestrial Rlant Test?
	Vegetative vigour (July 2006) and considers the ecommendations of USEPA
	Ecological Effects Test Guideline OCSPP 8500 150
Deviations from current	Current Guideline: OECD 22 ⁽²⁾ (2006)
test guideline:	Deviations: None. All validity criteria were met. However the range of light
	intensity was not recorded. Nevertheless, natural daylight was supplemented by
	artificial lighting, when light intensities was \$15000 fpx (referring to day light
	spectrum 15000 lux tesult in 945 µpt ol/s/m2).
	This had no influence on the reliability of the study and endpoints and endpoints
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted under GLP Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes w w w w w w w w w w w w w w w w w w w

Executive Summary

The objective of this study was to evaluate the potential effects of FLU + TFS SC 500 (250 + 250) on the vegetative vigour of ten non-target terrestrial plant species, following a post-emergence application of the product onto the foliage of plants at the 2 4 leapstage. A total of ten species, 7 dicotyledonous and 3 monocotyledonous species from 9 plant families were tested in this vegetative vigour test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Herianthus annuus* (sunflower), *Eagopylum esculentum* (buckwheat), *Lycopersicon esculentum* (tomato), *Avena sativa* (oat), *Lolium perentie* (ryegrass) Zea mays (corn). Planting density included A plants per poi with 5 replicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated twice with the rate of 0.8 L product/ha, at test initiation and one week later, as well as a water control. The application was done at a volume rate of 200 L/ha. Assessments were made 7, 14 and 21 days after second application. Final assessments were made for plant survival, visual phytotoxicity, plant growth stage and shoot dry weight.

The study fulfils all validity criteria of QECD 227 guideline. However, germination rate of the seeds used in this study was not reported. As fourine germination tests were carried out on the seeds to ensure their viability, the germination rate was considered to be in the acceptable range

No adverse effects on survival, vioual phytotoxicity, growth stage development and shoot dry weight above the 50 % effect level were observed. No statistically significant differences between treated plants and plants in the control were found. Therefore the ER_{50} (based on survival, growth stage development and shoot dry weight) was determined to be $>2 \times 0.8 L$ prod./ha.

I. MATERIAL AND METHODS

<u>Test stem:</u> LU + TFS SC 500 (250 + 250); specification no.: 102000012886 - 03; batch ID.: ENAL011075; Sample description: TOX 10053-00; active substance (analysed content): fluopyram: 20.7 % w (241.7 g/L), trifloxystrobin: 21.1 % w/w (246.5 g/L); density: 1.167 g/mL

<u>Test design</u>: A total of ten species, 7 dicotyledonous and 3 monocotyledonous species from 8 plant families were tested in this vegetative vigour test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed



rape winter), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Fagopyrum esculentum* (buckwheat), *Lycopersicon esculentum* (tomato), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn). The germination rates of the seeds used in this study, observed in annual germination tests, were 80 - 100 %. The plants were grown in a greenhouse in 13 cm plastic pots? The used soil was a silt loam.

Planting density included 4 plants per pot with 5 replicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated at the 2 - 4 leaf stage with the rate of 2×0.82 product/ha, at test initiation and one week later, as well as a water control. The test solutions were applied onto the foliage of plants and above-ground portions at a volume rate of 200 b ha. Control pots were sprayed with 200 L/ha of deionized water.

After application, the pots with plants were transferred back to the greenhouse and placed on the tables in a randomized design with all pots of one species arranged together in a species plot. One to four days prior to the final assessment, the pots of the plant species were arranged according to their treatment level to facilitate the final assessment.

Assessments were made 7, 14 and 21 days after second application. Final assessments were made for plant survival, visual phytotoxicity, plant growth stage and shoot dry weight. Visual phytotoxicity were assessed using a qualitative rating: 0 no effect), A-E rating (slight effect to moriburad). Any plant considered as being dead was not rated for yisual phytotoxicity and removed from the pot.

<u>Climatic conditions</u>: Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. The temperature was 18 to 23 °C during the light cycle (46 h) and during the dark cycle (8 h). The relative humidity was 70 %.

<u>Statistics</u>: Statistical analysis of shoot dry weight data was carried out with the Mann-Whitney-U-Test (one sided smaller; $p \le 0.05$), included in Tox Rat statistics (Fox Rat Proversion 2.10).

Y. RESULTS AN

Dates of work: May 2, 2019 June 25, 2013

Validity criferia:

The validity criteria of DECD 227 were fulfilled.

All plant species in this study mer the validity riterior of at least 90 % for survival in the control. In accordance with the SEPA guideline (OCSPP 850.4150) and OECD guideline (OECD 227), there was no visible phytotoxicity in control plants. Normal growth occurred in the controls of the ten species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The pots used for all species of this study were filled in equal manner with the same soil.

The germination rate of the seeds used in this study was not reported. However, as routine germination tests were carried out on the seeds to ensure their viability, the germination rate was considered to be in the acceptable range.

Analytical results

The analysis of trifloxystroom content in the application rate revealed measured concentrations of 94.4 % for the first and the second application of nominal.



Biological findings:

Slight visual phytotoxicity was observed sporadically for *Brassica napus* and *Lycopersicon escule* that was stunting. For Cucumis sativus, Fagopyrum esculentum and Glycine max, there was postly slight to moderate visual phytotoxicity observed i.e. chlorosis and/or necrosis and/or stunting.

No adverse effect on the survival and the growth stage were observed.

Shoot dry weight for Fagopyrum esculentum, Helianthus annuus and Lolium perenne was reduced by 14.9 %, 4.0 % and 3.7 %, respectively. Shoot dry weight for Beta vulgaris, Brassicarnapus, Cucuros sativus, Glycine max, Lycopersicon esculentum, Avena Sativa and Zee mays was increased by 4.2 0.2 %, 1.2 %, 0.3 %, 9.8 %, 5.3 % and 0.6 %, respectively. None of the shoot dry weight difference was statistically significantly or biologically meaningful. Q

The effects on survival, phytotoxicity, plant growth stage and dry weight are summarized for each the plant species in the following tables for the final assessment (on day 21 after application). S.

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Table 10.6.2- 9:	Summary of survival and	l shoot dry	weight follo	wing exposi	re to FL	U +, TFS SC	300
	(250+250 g/L) at the final	l øssessmer	nt on day 21		<u></u>	S O	

		L	Surviva	1 🔊 🤘	j ~	No Shot	ot dry we	ight
	Co	ontrol 🔍	<u>کہ کہ ا</u>	9.8 L prod	l./ha	Control	£0 × گ	L prod./ha
Plant species	No.				~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	Mean dro	Mean d©y	% Inhibition
	plants			Survivan			seight	Α
Beta vulgaris	20	100	20	100	0.Q	2.637	2.747	- 4.2
Brassica napus	20	\$00 ×	20		0.0	\$ 5.127	5.138	- 0.2
Cucumis sativus	QŎ	100		\$100 x	0.0	© 7.912	8.005	- 1.2
Fagopyrum esculentum _(\$ ²⁰			100	\$ 0.0 fg	~\$:341	4.545	14.9
Glycine max	20	0100 C	200	×100 ô		7.173	7.198	- 0.3
Helianthus annuus	20	100	20 e	¥ 1000	9.0 x	3.757	3.607	4.0
Lycopersicon esculentum	200		² 20			3.942	4.328	- 9.8
Avena sativa	<u>\$20</u>	≁ 100\$	>20	100	0.0	3.595	3.786	- 5.3
Lolium perenne @	5 20 A	1,00	20 v	100	0.0	1.714	1.651	3.7
Zea mays	20	200 (20	A00 a	0.0	8.303	8.351	-0.6

Statistical significance -: Thibition is statistically not significant (Pairwise Mann-Whitney-U-test, one sided smaller; p < 0.05).

A: A negative mhibition indicates an increase of the short dry, weight compared to the control.

Please note: Phytotoxicity was assessed using a qualitative rating: 0 (no effect), A-E rating (slight effect to moribund). Control and treated plants showed no symptoms of phytotoxicity. Results from the

phytotoxicity assessment are presented in the table below.



Table 10.6.2- 10:	Summary of phytotoxicity and growth stages (BBCH) following exposure to FLU +

TF	S SC 500 (25	50+250 g/L) at the final as	sessment on	day 21
Diant an asian		Phytotoxicity	B	BCH growth stages 🖉 🖉
Fiant species	Control	2 × 0.8 L prod./ha	Control	2 × 0.8 L prod./ha
Beta vulgaris	0	0	18 - 19	0 18 - 19
Brassica napus	0	0-A/e	17 - 30	15 - 30 4
Cucumis sativus	0	A-B/ab	69	4 69 ° ° °
Fagopyrum esculentum	0	A-B/be	65 💡	
Glycine max	0	A/abe	51 - 59 🖧	5 1 59 ~ 59
Helianthus annuus	0	0 🚿	53 - 55	<u>51 - 55</u> ()
Lycopersicon esculentum	0	0-A/e	61 @2	× 61 - 6 × ×
Avena sativa	0	0,10	36-32	\sim $31 - 32$ \sim 0°
Lolium perenne	0	Č, V	29 🦓	
Zea mays	0		30 - 34	<u>30 - 34</u>
 Phytotoxicity was recorded 0: no injury or effect A: slight symptom (s) B: moderate symptom (C: severe symptom (s) D: total-plant symptom E: moribund Codes for visual injuries: a: chlorosis (yellowing b: necrosis (brown show c: bleaching (showt tiss 	at each ass (s) (s) (s) (s) (s) (s) (s) (s) (s) (essment time with the fo	ollowing a r	rating system:
d: leaf deformation (lea	if curl abno	wonal leaf shape?		
e : stunting (plant hergh	t reduced x	ith shorter inter-node le	ngths) 🖏	Я́

- 0:
- A:
- B:
- C:
- D:
- E:

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- a:
- b:
- c:
- d:
- e : Ś, 0 J

Ļ III. KONCLUSION, Ø

As a result of this vegetative vigout and growth rudy, in which the effects of FLU + TFS SC 500 (250+250 g/L) on ten non-farget terrestrial plant species were tested under greenhouse conditions, no adverse effects on survival, visual phytotoxicity, growth stage development and shoot dry weight above the 50 % effect level wore observed, No statistically significant differences between treated plants and plants in the control were found. Therefore the $ER_0^{(0)}$ (based on survival, growth stage development and shoot dry weight) was determined to be 2 x 08 L prot./ha.

Assessment and conclusion by applicant:

The study and as data are considered as acceptable and reliable for use in risk assessment.



Data Point:	KCP 10.6.2/05
Report Author:	
Report Year:	2020
Report Title:	Fluopyram + trifloxystrobin SC 500 (250+250 g/L): Effects on the vegetative
	vigour of seven non-target terrestrial plant species under greenhouse conditions
	(Tier 1)
Report No:	\$19-22936
Document No:	<u>M-681185-01-1</u>
Guideline(s) followed in	EU Directive 91/414/EEC
study:	Regulation (EC) no. 1107/2009 $\sqrt[5]{2}$
	US EPA OCSPP 850.4150 (2012)
	OECD 227 (2006)
Deviations from current	Current Guideline: OECD 27 (2006)
test guideline:	Deviations: Temporary deviation from climate condition (light)
	All validity criteria were met. The devortions fisted above had no infraence on the
	reliability of the stud@and endpoints?
Previous evaluation:	No, not previously submitted Q Q A A A A A A A A A A A A A A A A A
GLP/Officially	Yes, conducted under GLP/Officially recognized testing facilities 🖉 🌋
recognised testing	
facilities:	
Acceptability/Reliability:	Yes Q' A A A' O O A' A

Executive Summary

The objective of this study was to evaluate the potential effects of FLU + PFS \$6,500 (250 + 250) on the vegetative vigour of seven non-target errestrial plant species in a limit lest, following a postemergence application of the product onto the foldage of plants at the 2 - 3 leaf stage. A total of seven species, 3 dicotylectonous and 4 monocotyledonous species from 5 plant families were tested in this vegetative vigour test; *Brassica napus* (oriseed rape winter), *Fagopy fun esculentum* (buckwheat), *Glycine max* (soybean), *Allium cepo* (onion), *Avena sativa* (oat) *Lolium perenne* (ryegrass), *Zea mays* (corn). Planting density included 2 or 4 plants per pot with 10 or 5 replicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated with a single application rate of 1.0 L product ha and a water control. The application was done at a volume rate of 200 L/ha. Assessments for mortality and visual muries were made on day 7, 14 and 21. Additionally, the BBCH growth stage and shoot height were determined for all treatment proups on day 21. The effects on plant shoot dry weight were determined for all treatment proups on day 21. The effects on plant shoot dry weight

The study fulfits all calidity criteria of OECD 227 guideline.

Visual phytotoxicity and differences in the BBCH growth stage occurred in *Fagopyrum esculentum*. A statistically significant difference in shoot height compared to the control group was observed for *Glycine max*. A statistically significant difference in shoot dry weight compared to the control group was observed for *Avena Sativa*. An adverse effect on shoot dry weight above the 50 % effect level occurred in *Fagopyrum esculentum*, which was also statistically significant. Except for *Fagopyrum esculentum* the ER₅₀ for all species (based of survival, growth stage development and shoot dry weight) was set to be 1.0 product.

I. MATERIAL AND METHODS

<u>Test Item</u>: FLU + TFS SC 500 (250 + 250); specification no.: 102000012886; supplier batch no.: EV57002709; Sample description: TOX 21159-00; active substance (analysed content): fluopyram: 21.1 % w/w (246.6 g/L), trifloxystrobin: 21.3 % w/w (248.6 g/L); density: 1.169 g/mL

<u>Test design</u>: A total of seven species, 3 dicotyledonous and 4 monocotyledonous species from 5 plant families were tested in this vegetative vigour test: *Brassica napus* (oilseed rape winter), *Fagopyrum*



*esculentum (*buckwheat), *Glycine max* (soybean), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn). The germination rate of the seeds used in this study, observed in a seedling emergence test, was 80 - 97 %. The plants were grown in a greenhouse in 15 cm plastic pots (filled with approx. 1.5 kg soil/pot). The used soil was a loamy sand.

Planting density included 2 or 4 plants per pot with 10 or 5 replicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated at the 2 - 3 leaf stage with a single application rate of 1.0 L product/ha and a water control. The test solutions were applied onto the foldage of plants and above-ground portions at a volume rate of 200 L/ha. Control pots were sprayed with 200 L/ha of deionized water.

After application, the pots with plants were transferred back to the greenhouse. The pots were set up sorted per treatment group and within each treatment group following the replicate number after application. All pots were repositioned within each treatment group at the first and second assessment day to compensate for potential variability in growth miditions.

Plants were assessed for mortality and visual injuries on day 7,44 and 21. Additionally, the BBCH growth stage and shoot height were determined for all treatment groups on day 21. The effects on plant shoot dry weight were determined for day 21. A gradual rating was assigned to describe the extent of the visual phytotoxicity in comparison to the control, considering pecrosis, deformation and change in colour (e.g. chlorosis, bleaching, redening). The atings referred to the whole plants within a replicate and range from 10 to 90 %.

Analysis of the product solution and the control solution were conducted by LCSUV.

<u>Climatic conditions</u>: Following application, the pots with plants were maintained under greenhouse conditions and an independent set of LED langes above each cultivation table ensured an appropriate exposure to light. The light intensity was in the range of 240 - 330 μ mol/m²/s. The temperature was 15 to 27 °C during the light cycle (16 k) and during the dark cycle (8 h). The relative humidity was 54 to 88 %.

Statistics: As no mortality occurred, no statistical evaluation was performed for this endpoint.

The data of shoot height and shoot dry weight were tested for normal distribution and homoscedasticity using Shapiro-Wilk's Test and Levene-Test, respectively. For all species tested both requirements were fulfilled, therefore Student t-test was conducted. The significance level was set to $\alpha = 0.05$ for all tests. In case of an increase on the test item group compared to the control group for shoot height and shoot dry weight, no statistical evaluation was conducted.

Statistical analysis was performed using the program ToxRet Professional Version 3.3.0.

Dates of work November 19, 2019 December 18, 2019

RESULTS AND DISCUSSION

Validity criteria:

The validity covering of OEQU 227 were fulfilled.

All plant species in this oudy met the validity criterion of at least 90 % for survival in the control. In accordance with US EPA guideline (OCSPP 850.4150) and OECD guideline (OECD 227), there was no visible phylotoxicity in control plants. Normal growth occurred in the controls of the ten species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The possused for all species of this study were filled in equal manner with the same soil.

The germination rate of the seeds used in this study was \geq 70 % for all species included in this test.



Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANTE/2020/12830, Rev.1.

The analysis of fluopyram concentration in the product solution corresponded (\$102.8 % of the normal concentration.

Biological findings:

Visual phytotoxicity observed at the final assessment (on day 21 ofter application) in this vegetative vigour study included chlorosis, necrosis, deformation and stunting of the planes. Visyal phytotoxicity (mean effect of 10 %) occurred in Fagopyrum esculentum.

All plants survived until test end.

There were statistically significant effects on shoot height for the plant species Fagopyrum & culenum (31.4%) and shoot dry weight for the plant species Fagopyrum esculentum (52.0%) and Avena sativa Ç, (23.4 %) at the single application rate of 1.0 & prod. Aba. Ľ

The effects on shoot height, dry weight, phytotoxicity as well applant prowth stage are summarized for each of the plant species in the following tables for the final assessment (or day 2) after application). Ø 0

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Table 10.6.2- 11:	Summary of shoot heigh and shoot dry weight following exposure to PLU + TFS
	SC 500 (250+250 g/L) at the final assessment on day 21

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	A	Shoot beig	nt 🗳 🛇	Schoot dry weight		
	Control	1.0 L	prod./hà	Control	مَ∾ٍ1.0 L p	rod./ha
Plant species	M	Maan ~		Mean dry	Mean dry	%
J. O			Indibition	weight	weight	Inhibition
	· ⟨				g	
Brassica papus,	. ◎ 15.1 ◎ ″	Q#.4	40	چ ² 0.7 <u>48</u>	0.771	- 3.1
Fagopyrum esculeRtum 🕈	[©] 10 <del>2</del> ∕8	_ 112.5°~√	<b>.</b> 3.3	2:234	1.407	52.0 *
Głącine max 🔬	50.6	s 34.70°	31.4*	<b>209</b> 05	2.692	7.3
Atium cepa 🖉	<b>3</b> 1.0 €	36,6	<b>~</b> - 18 <b>/</b>	<b>0</b> 0.508	0.523	- 3.0
Avena sativa	∽ 60.₹	\$8.8 _^	<b>3</b> .1	🏷 0.997	0.764	23.4 *
Lolium perenne	42,3	× 43.8	<u>3.5</u>	0.863	0.801	7.2
Zea may 🧟 🖂	<b>\$</b> 5.8	S7.9	- 3.8	1.937	1.916	1.1

A Negative figures, indicates that there was a princrease in biomass (droweight) when compared to the untreated control.

* Statistically significantly different compared to the control Student's t-test; one-sided smaller,  $\alpha = 0.05$ )

Table_10.6.2- 12:	Strimmary of phytotoxicity and growth stages (BBCH) following exposure to FLU +
N N	TFS \$ 500 (250+250g/L) at the final assessment on day 21

Plant of sign (	Phytotoxicity 1	nean % / Symptoms ^A	<b>BBCH growth stages</b>		
Flant species	Control	[≫] 1.0 L prod./ha	Control	1.0 L prod./ha	
Brassea napus		0 / -	15 / 15	15 / 15	
Fagopyrum eséptentum		10 / NE,CC	67 / 67	65 / 65	
"Flycine max 🔬		0 / -	62 / 62	62 / 62	
Allium Cepa 🖓 ,	<b>⊘</b> 0	0 / -	15 / 15	15 / 15	
Avega sativa	0	0 / -	55 / 55	55 / 55	
Lofum perenne	0	0 / -	26 / 26	26 / 26	
©Zea mays	0	0 / -	16 / 16	16 / 16	

^A Phytotoxicity: 0: no phytotoxicity or effect; Visual symptoms: None (-), CC = change in colour, NE = necrosis



#### **III. CONCLUSION**

As a result of this vegetative vigour and growth study, in which the effects of FLU + TFS SCOOO (250+250 g/L) on ten non-target terrestrial plant species were tested under greenhouse conditions, no mortality occurred for any of the plant species tested.

Visual phytotoxicity and differences in the BBCH growth stage occurred in Fagopyrum escutentum

A statistically significant difference in shoot height compared to the control group was been defended for *Glycine max.* 

rol group is constructed is



Data Point:	KCP 10.6.2/06
Report Author:	
Report Year:	2018
Report Title:	Effects on the vegetative vigor of three species of non-target terrestrial plants. Tier
	2) fluopyram + trifloxystrobin SC 500 (250 + 250 g/L)
Report No:	VV17/038
Document No:	<u>M-612774-01-1</u>
Guideline(s) followed in	EU Directive 91/414/EEC
study:	Regulation (EC) No. 1107/2009 🖉
	US EPA OCSPP 850.4150 (2012)
	OECD 227 (2006)
Deviations from current	Current Guideline: OECD 22 (2006)
test guideline:	Deviations: Temporary deviation from climate condition (right). All validity
	criteria were met. The declations listed above had no influence on the rehability of
	the study and endpoints, of S & C & C &
Previous evaluation:	No, not previously submitted
GLP/Officially	Yes, conducted ander GDP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	$\underline{\text{Yes}}_{\mathcal{A}} \xrightarrow{\mathcal{O}}_{\mathcal{A}} \xrightarrow{\mathcal{O}}_{\mathcal{O}} \xrightarrow{\mathcal{O}}} \xrightarrow{\mathcal{O}} \mathcal{$
	$a$ , $\pm i$ $a$ , $\pm i$ $a$ , $\pm i$ $a$ , $a$

### **Executive Summary**

The objective of this study was to evaluate the potential effects of FLV + TFS SC 500 (250 + 250) on the vegetative vigour of three non-target errestrial plant species, following a post-emergence application of the product onto the foliage of plants at the 2 - fleaf stage. 3 dicotyledonord species from 3 plant families were tested in this vegetative vigour test: *Beta vulgaris* (sugar beet), *Cucumis sativus* (cucumber) and *Solenum tycopersicum* (tomato). Planting density included 2 plants per pot with 16 replicate pots, respectively, for a total of 32 plants per treatment level. The plant species were treated with 5 product rates and a water control. The test concentrations were: 0.96; 0.144; 0.346; 0.832 and 2.0 L product/ha. The application was done at a volume rate of 200 L/ha. Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and visual phytotoxicity were recorded Final assessments were made for plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight.

The study fulfils an validity criteria of OECD 27 guideline.

No adverse effects on prvivat visual phytonoxicits, growth stage development, shoot length and shoot dry weight above the 25 % effect level were observed. Therefore the  $ER_{50}$  (based on survival, shoot height and shoot dry weight) was determined to be > 2 L prod./ha.

### VI. MATERIAL AND METHODS

<u>Test item</u>: FLU  $\pm$  TFS SC 500 (250 + 250), specification no.: 102000012886; supplier batch no.: PAIS005241; Sample description PAR30(64-00; active substance (analysed content): fluopyram: 21.1 % w/w Q46.7 g/L), two xystrobin: Q1.6 % w/w (251.9 g/L); density: 1.167 g/mL.

<u>Test design</u>. 3 divotyledonous species from 3 plant families were tested in this vegetative vigour test: *Beta vingaris* (sugar beet), *Cucumis sativus* (cucumber) and *Solanum lycopersicum* (tomato). The germitation rates of the seeds used in this study, observed in annual germination tests, were 80 - 100 %. The plants were grown in a greenhouse in 15 cm plastic pots (filled with approx. 1.2 L soil). The used soil was silt loam.

Planting density included 2 plants per pot with 16 replicate pots, respectively, for a total of 32 plants per treatment level. The plant species were treated at the 2 - 4 leaf stage with 5 product rates and a water control. The test concentrations were: 0.06; 0.144; 0.346; 0.832 and 2.0 L product/ha. The test solutions



were applied onto the foliage of plants and above-ground portions at a volume rate of 200 L/ha. Control pots were sprayed with 200 L/ha of deionized water.

After application, the pots with plants were transferred back to the greenhouse and placed on the ables in a randomized design with all pots of one species arranged together in a species plot. During the course of the experimental study part the pots of each plant species were rearranged within each species plot.

Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and visual phytotoxicity were recorded. Final assessments were made for plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight. Visual phytotoxicity fe.g. chlorosis, necrosis, bleaching, deformation, reddening, stunting) was recorded from the riging mants at each assessment date following a 0 - 90 % (no effect to moribund) rating system in 40 % steps. The phytotoxicity is a subjective assessment.

Climatic conditions: Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting the light intensity was in the range from 135.89 to 426.8 µmol/m²/s. The temperature was 14 to 31 °C during the light cole (16 h) and during the dark cycle (8 h). The relative humidity was 55,06 85 %.

Statistics: Statistical analyses of the data were performed to obtain NOER (Not bered Effect Rate), LOER (Lowest Observed Effect Rate), ERE/ER50 (Effect Rate producing 25/50 % effect) for survival, shoot length and shoot dry weight, using the TowRat statistical software.

RESULTS

- November 20, Dates of work: October 19, 2007

Validity criteria:

The validity criteria of QECD 227 were fulfilled.

All plant species in this study met the validity centerion of at least 90% for survival in the control. In accordance with US EPA guideline (OGSPP \$50.4156) and OECD guideline (OECD 227), there was no visible phytotoxicity in control plants. Normal growth occurred in the controls of the ten species tested. The control plants of each species showed or mal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The pots used for all species of this study were filled in equal manner with the same soil.

The germination rate of the seeds usêd in this studo was 2070 %.

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### Analytical results:

The analysis of triffoxystrobin conten@in the initial product stock solution revealed measured concentrations of 101.6 % of nonrinal.

### Biological fordings:

Typical Symptoms observed at the final assessment (on day 21 after application) in vegetative vigor testing include chlorosis, new osis, deformation and stunting of the plants. Slight visual phytotoxicity was Sperver sporadically for Cucumis sativus (0.06 - 2.0 L product/ha) and Solanum lycopersicum (0.444 - 0.832 L product/ha).

All plants survived until test end.

There were no statistically significant effects on shoot length and shoot dry weight for all plant species up to the highest concentration tested. The effects on shoot height, dry weight, phytotoxicity as well as



plant growth stage are summarized for each of the plant species in the following tables for the final assessment (on day 21 after application). 

#### Effects of FLU + TFS SC 500 (250+250 g/L) on survival Table 10.6.2-13:

			108	\$ \$
		Survival	1	
Plant species	ER ₂₅ [L product/ha] (95 % CI)	ER50 [L product/ha] (95 % CI)	EOER [Leroduct/ha]	© [L product/ba]
Beta vulgaris	> 2 ^A (n.d.)	2 ^A (n.d.)		
Cucumis sativus	> 2 ^A (n.d.)	2 ^A (n.d.)		× 2 5
Solanum lycopersicum	$> 2^{A}$ (n.d.)	$\mathcal{O} \geq \mathcal{D}(n.d)$	1 2	L 2A C
n.d.: Confidence limits not dete ^A : No effects were observed up	rmined (outside the range to the highest concentra	tion tested		
Table 10.6.2-14:         Effects	of FLU + PFS SG5	00 (25,0+256Kg/L) on	shoot tength S	Š. 43

#### 300 (250+250Kg/L) Table 10.6.2-14: **Effects of FLU**

A: No effects were observed up to the highest concentration tested	
Table 10.6.2-14: Effects of FLU + FFS SG 500 (250+250Kg/L) on shoot length	Ş. L
Shoot length Shoot length	×
Plant species (1) Product/hal (1) Product/ha	NOER
Beta vulgaris $2^{A}$ (n'd) $2^{A}$ (n'd) $2^{A}$ (n'd) $2^{A}$ (n'd)	2
Cucumis sativus $(n.d)$ $(n.d$	2 ^B
Solanum lycoperstaum $2^{A}$ (n.d.) $2^{A}$ (n.d.) $2^{A}$ (n.d.)	2

*: IR corresponds to ER n.d.: Confidence limits for determined (outside the range tested) A: Not calculated (outside the range tested). B: Calculated with Dunnett' conultiple test procedure

#### $\widetilde{\mathrm{SC}}$ 50 $\widetilde{\mathfrak{g}}$ (250+250 g/b) on shoot dry weight Table 10.6.2- 15: Effects of FLO+ TES

	Sho	oț Øy wei <b>D</b> t		
Plant species	<b>R</b> 25* <b>R</b> [ <b>I</b> product/ha] (95 % CI)	<b>165</b> % <b>[L. product/ha]</b> 95 % CI)	LOER [L product/ha]	NOER [L product/ha]
Beta yulgaris	2 ^A (n.d:)	$S^{*} > 2^{A} (n.d.)$	> 2	2
Cucumis sativus	$2^{A}$ (ard.) $Q$	$> 2^{A}$ (n.d.)	> 2	2
Solanum lycopersicting	$2^{A}$ (n.d.)	$> 2^{A}$ (n.d.)	> 2	2

*: IR corresponds to FR. n.d.: Confidence lines not determined (outside the range tested)

A: Not calculated (outside the range) tested).



Table 10.6.2- 16:	Summary for growth stages (BBCH) of the test plants at the final assessment on	ນໍ 🐎

	day 21					
Plant species						
•	Control	0.06	0.144	0.346	Ø ⁹ 0.832	
Beta vulgaris	19	19	18 - 19	19	» 19 <u>م</u>	
Cucumis sativus	66 - 69	69	65 -	64 - 68	69 🔬	~ <del>68</del> - 695
Solanum lycopersicum	51 - 52	51 - 52	51 - 52	51 52	51, 52	51-52 O
			(0) n			

		- ** -	
Table 10.6.2- 17:	Phytotoxicity summary	at the final asse	essmenut on då¥ 21
1 4010 101012 171	i nytotokiency summary	at the main usse	

Plant species		Phytorox	icitySummary	[mean dau kt/ha]	ige in %] ^A	
Ĩ	Control	Ø.96 ~	0.1.44	0.346	0 0.832	<u>2</u>
Beta vulgaris	0			×00 ×		<u> </u>
Cucumis sativus	0	( ⁰ 1.3 <b>%</b> )	≫ 3.1 abe	5.0 abe	, 9.4 abe	4.4 abe
Solanum lycopersicum	0		3 <b>8</b> de 6	1,3 de 🗞	∮ 1∂e	$\gg 0$

^A Phytotoxic symptoms:

a: chlorosis (yellowing of green shoot tissue)

b: necrosis (e.g. brown shoot tissue, parts of the plant dier

d: deformation (e.g. leaf curl, abnormal leaf shape, abnormal plant habitus)

e: stunting (e.g. plant height reduced with shorter internode length, plant growth reduction)  $\bigcirc$ 

SIII. CONCLASION @ This vegetative vigour and growth study, in which the effects of FU + JFS SC 500 (250+250 g/L) on three non-target terrestrial plant species were tested undergreenhouse conditions, resulted in no adverse effects on survival, Visual phytotoxicity growth stage development shoot length and shoot dry weight above the 25 % effect level. Therefore the ER5 (based on survival shoot height and shoot dry weight) was determined to be 2 L prod./ha O S

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age. The study and its data are considered as acceptable and reliable for use in risk assessment. The endpoint is:  $ER_{50} \ge 2.0 \text{ L}$  prod./ka



Data Dainti	KCD 10 ( 2/07
Data Point:	KCP 10.6.2/0/
Report Author:	
Report Year:	
Report Title:	Fluopyram + trifloxystrobin SC 500 (250+250 g/L): Effects on the vegetative
	vigour of Fagopyrum esculentum under greenhouse conditions (Tier 2)
Report No:	S20-01147
Document No:	<u>M-688437-01-1</u>
Guideline(s) followed in	EU Directive 91/414/EEC
study:	Regulation (EC) no. 1107/2009
	US EPA OCSPP 850.4150 (2012)
	OECD 227 (2006)
Deviations from current	Current Guideline: OECD 22 $\mathcal{P}$ (2006)
test guideline:	Deviations: Temporary deviation from climate condition (fumidity).
	All validity criteria were piet. The deviations listed above had no influence on the
	reliability of the study and endpoints is whether the study and endpoints is the study of the st
Previous evaluation:	No, not previously something in the second s
GLP/Officially	Yes, conducted buder GDP/Officially recognised testing facilities
recognised testing	
facilities:	
Acceptability/Reliability:	Yes & a v v v v v v

#### **Executive Summary**

The objective of this study was to evaluate the potential effects of FLU + TFS SC 500 (250 + 250) on the vegetative vigour of *Fagopyrum esculentum* a non-target terrestrial plant species, following a postemergence application of the product to the above-ground portion of the plants ache 2 leaf stage. This plant species had shown relevant effects in a preceding kinit test and/is therefore investigated in this rate-response study. Planting density included 2 plants per pot with 10 eplicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated with product rates and a water control. The test concentrations were: 0.060, 0.144, 0.346, 0.832 and 20 L prod./ha. The application was done at a volume tate of 200 L/ba. Assessments for portality and visual injuries were made on day 7, 14 and 21. Additionally, the BBCH growth stage, showt height and shoot dry weight were determined for all treatment groups on day 24.

# The study fulfils all validity ofteria of OEGD 227 guideline.

No mortality, no visual phytotoxicity and no differences in the growth stage of the plants compared to the control group occurred after treatment with the product at any rate tested. A LOER of 0.832 L prod./ha and a@IOER@I 0.346 L prod./ha @as defermined based on shoot height. For shoot dry weight the LOER and NOER were determined to be 2.0 L prod./ha and 0.832 L prod./ha, respectively. Due to the lack of inhibition the ER₂₅ and ER₃₆ (based on prostality, shoot height and shoot dry weight) were determined to be > 2.0 L prod./ha, respectively.

# MATERIAL AND METHODS

<u>Test item</u>: FbU + TFS Sc 500 (250 + 250); specification no.: 102000012886; supplier batch no.: EV57002709; Sample description: Test 21159-00; active substance (analysed content): fluopyram: 21.1 % www (246.6 g/L); triflex strobin: 21.3 % w/w (248.6 g/L); density: 1.169 g/mL).

<u>Test design</u>: <u>forgopyrium esculentum</u> was tested in this vegetative vigour tier 2 test as this plant species had shown relevant effects in a preceding limit test and is therefore investigated in this rate-response study. The germination rate of the seeds used in this study, observed in a seedling emergence test, was 98 %. The plants were grown in a greenhouse in 15 cm plastic pots (filled with approx. 1.5 kg soil/pot). The used soil was a loamy sand.

Planting density included 2 plants per pot with 10 replicate pots, respectively, for a total of 20 plants per



treatment level. The plant species were treated at the 2 leaf stage with 5 product rates and a water control. The test concentrations were: 0.060; 0.144; 0.346; 0.832 and 2.0 L product/ha. The test solutions were applied to the above-ground portions at a volume rate of 200 L/ha. Control pots were sprayed with 200 L/ha of deionized water.

After application, the pots with plants were transferred back to the greenhouse. The pots were set up sorted per treatment group and within each treatment group following the replicate number after application. All pots were repositioned within each treatment group at the first and second assessment day to compensate for potential variability in growth conditions.

Plants were assessed for mortality and visual injuries on day 7, 14 and 21. Additionally, the BBCH growth stage, shoot height and shoot dry weight were determined for all treatment groups for day 21. As gradual rating was assigned to describe the extent of the visual phytotoxicity in comparison to the control, taking into account necrosis, deformation and change in colour (e.g. chlorosis bleaching, reddening). The ratings referred to the whole plants within a toplicate and range from 10 to 90 %.

Analysis of the product solution and the control solution were conducted by HPCC - MS/MS

<u>Climatic conditions</u>: Following application, the pots with plants were maintained under greenhouse conditions. An independent set of LED lamps above each cultivation table ensured an appropriate exposure to light. The temperature was 1540 35 % during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was 31 to 72 %.

<u>Statistics</u>: As no mortality occurred, no statistical evaluation was performed for this endpoint. The data of shoot height and shoot dry weight were tested for normality and homoscedasticity using Shapiro-Wilk's Test and Levene-Test. William's test was used for shoot height as both requirements were fulfilled and the trend analysis by contrast, was significant. Since the test on normality and homoscedasticity failed, the  $Ch^2$  - test was conducted for the parameter shoot fry weight. Statistical analyses of shoot height and shoot dry weight also included the determination of effect rates (ER₂₅ and ER₅₀) and their 95% confidence finits by Probit analysis (based on mean values) using linear max. likelihood regression, where possible. Statistical analysis was performed using the program ToxRat Professional Version 33.0.

Dates of work. March 17, 2020 – April 09, 2020

II. RESULTS AND BISCUSSION

Validity criteria:

The validity viteria of OFCD 227 were puffilled

All plant species in this study thet the validity critetion of at least 90 % for survival in the control. In accordance with US EPA guideline (OCSPP 850,4150) and OECD guideline (OECD 227), there was no visible phytotoxicity in control plants. Normal growth occurred in the controls of the species tested. The control plants of the species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The pots used for all species of this study were filled in equal manner with the same soil.

The germination rate of the seeds used in this study was  $\geq 70$  % for *Fagopyrum esculentum*.

Analytical results:

Full details and acceptable validation data to support the analytical method are presented within document. M-CA 4, which comply with the EU regulatory requirements outlined within SANTE 2020/12830, Rev.1.

The analysed concentration of fluopyram in the highest product solution corresponded to 106 % of the nominal concentration.



**Biological findings:** 

No mortality and no visual phytotoxicity was observed in this study. No differences in the BBCH growth stage were observed between all treatment groups on the last assessment day (20DAA).

Statistically significant reduction of the shoot height of Fagopyrum esculentum at the two application rates (0.832 and 2.0 L prod./ha) was observed.

Statistically significant reduction of the shoot dry weight of Fagopyrim esculentian at the application rate (2.0 L prod./ha) was observed. Ø

The effects on shoot height, dry weight, phytotoxicit as well as plant growth stage are summarized Fagopyrum esculentum in the following tables for the final assessment fon day 21 after application

Table 10.6.2- 18:	Summary for effects of FL	Ŭ + TĔS	SC 500	(250+250 §	x) on J	Fagopyrim	esmientum	
	at the final assessment on a	lay 24		s A	, 6	, o 11 e		

Endpoints	Nortauty Shoot reight S	sho@dry weight ^B
LR ₂₅ / ER ₂₅ [L product/ha]		ڳَ <u>&gt; 2</u> 9000
LR ₅₀ / ER ₅₀ [L product/ha]		2.000
NOER [L product/ha]		§ 0.832
LOER [L product/ha]		2.000
A 1 4 1 1 1 1 1 2 W 11 1 1 1 1 1		

^A: determined with: ^a Wilfams` test test on sided gnaller, ^B: determined with: ^a Che² - test test one sided smaller,  $\alpha =$ - test; test one sided smaller,  $\alpha = 0.05$ 

C

n Sommary of effects on growth stages BBCH5 shoot beight and dry weight of Table 10.6.2- 19: Fagopyrum escalentum at the final assessment on day 21 Ô

Species Fa	gopyrum esculentum	
Test item Growth stages	ے Inhi مرکز Inhi	bition %]
[L produ@/ha]	Shoot height ^A	Shoot dry weight ^B
Control (0)	-	-
\$0.060 \$ \$ 63 × 63 × 5	3.8	- 4.3#
0.144 0 4 6 - 63 0	12.6	6.0
0.346 0.346 Q63 - Q63 - Q	0.4	0.7
	17.7 *	4.8
2000 ~ ~ 63	14.8 *	13.1 *

* Statistically significantly different compared to the control

^A: determined with Williams test; on sided smaller,  $\alpha = 0.05$ 

~ ^

^B: determined with Chi² - test; one odd smaller,  $\alpha = 0.05$ # Negative values indicate that there was an increase compared to the control



#### **III. CONCLUSION**

As a result of this vegetative vigour tier 2 study, in which the effects of FLU + TFS SC 500 (250, 250) g/L) on Fagopyrum esculentum were tested under greenhouse conditions, a LOER of 0.832 L prod./ha and a NOER of 0.346 L prod./ha was determined with based on shoot height. For shoot dry wight the LOER and NOER were determined to be 2.0 L prod./ha and 0.832 L prod./ha espectively.

Due to the lack of inhibition the  $ER_{25}$  and  $ER_{50}$  (based on mortality, shoot height and were determined to be > 2.0 L prod./ha, respectively.

#### Assessment and conclusion by applicant:

urse in risk as The study and its data are considered as acceptable and reliable for

The endpoint is:  $ER_{50} > 2.0 L \text{ prod./ha}$ 

#### Extended laberator studies **CP 10.6.3** on non-target plan

In view of the results presented under Point CB 10.6 2 above no further stories are deemed necessary.

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

In view of the results presented under Point CP 10 9.2 above, no further studies are deemed necessary.

#### Effects on other terrestriak organisms (flora and fauna) **CP 10.7**

In view of the study results presented above any studies on other terrestrial organisms are considered necessary. However, further investigation has been conducted on fungicidal activity with no adverse effects berved; for details so MCA

### **CP 10.8**

Monitoring dat

No monitoring data has been collected by the applicant hor have they been reported in any of the public literature preferences as evaluated in Document MCA. Section 9. No monitoring of non-target organism is doomed to be necessary

a pêthe a a în Document.