Document Title

1st amendment of

Summary of the ecotoxicological studies
Fluopyram + Trifloxystrobin SC 500 (250 + 250 g/L)

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EU) No 284/2013

Document MCP

Section 10: Ecotoxicological studies

According to the Guidance Document SANCO/10181/2013 for applicants on preparing dossiers for the approval of a chemical active substance

Date

2021-07-05

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Version history

Date [yyyy-mm-dd]	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2021-03-25	Original MCP as submitted by applicant	M-766068-01-1
2021-07-05	Addition of reliability assessments for aquatic organisms and update of aquatic macrophyte endpoint and risk assessment. All changes by the applicant have been highlighted in yellow colour	M-766068-02-1

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

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CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

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), abbreviation FLU+TFS SC 500 (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 names such as Luna Sensation, Luna Sensation SC and Moon Sensation. FLU+TFS SC 500 (250+250) was not a representative formulation of Bayer AG for the Annex I inclusion of Fluopyram under Council Directive 91/414/EEC.

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

Use pattern considered in this risk assessment

Table 10.1- 1: Intended application pattern

Crop	Timing of application (range)	Number of applications	Application interval [days]	Maximum label rate (range) [L prod./ha]	Maximum application rate, individual treatment (ranges) [kg a.s./ha] Fluopyram
Grapes	BBCH 53-73	2	7	0.2	0.050
Lettuce (soil-less cultivation, high-tech greenhouse)	BBCH 12-49	2	7	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 relevant environmental compartments. The residue definition for risk 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, Trifluoroacetic acid (TFA)
Surface water	Fluopyram, Fluopyram-7-hydroxy, Trifluoroacetic 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 ecotoxicological risk assessment.

As part of this guidance, a template was provided for a "questionnaire" for the use of residue data extracted from Vol. 3 B.7 to support the ecotoxicological assessment of pesticides.

According to EFSA (2019), the respective RMS may consider this questionnaire as useful in their assessments.

Therefore, the questionnaire with the information from the relevant studies with fluopyram is provided on the following pages.

Data Point:	KCP Section 10/01
Report Author:	[redacted]
Report Year:	2021
Report Title:	Fluopyram: Residue information supporting the ecotoxicological assessment
Report No:	EnSa-21-0155
Document No:	M-763894-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
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 the 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 application, dose rate, BBCH growth stage, PHI). They are presented in Vol. 3 B.7 of the DAR. No data gap 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).

The following metabolism studies are available for Fluopyram:

Report reference	Author, Year	Crop Category	Crop	Application	Fluopyram label
M-282177-01-1	[redacted] 2006	Fruit (F)	Grapes	Foliar	[UL-^{14}C-phenyl]
M-282460-01-1	[redacted] 2006				[2,6-^{14}C-pyridyl]
M-286400-01-1	[redacted] 2007	Root crops (R)	Potato	Foliar	[UL-^{14}C-phenyl]
M-286531-01-1	[redacted] 2007				[2,6-^{14}C-pyridyl]
M-283161-02-1	[redacted] 2011	Pulses and oilseeds (P/O)	Bean	Foliar	[UL-^{14}C-phenyl]
M-299067-01-1	[redacted] 2008				[2,6-^{14}C-pyridyl]
M-298790-01-1	[redacted] 2008	Fruit (F)	Bell pepper	Drip irrigation	[UL-^{14}C-phenyl]
M-298741-01-1	[redacted] 2008				[2,6-^{14}C-pyridyl]
M-345948-01-1	[redacted] 2009	Cereal/Grass crops (C/G)	Wheat	Seed treatment	[UL-^{14}C-phenyl] & [2,6-^{14}C-pyridyl]
M-615284-01-1	[redacted] 2018	Miscellaneous	Rice	Foliar	[UL-^{14}C-phenyl]
M-615282-01-1*	[redacted] 2018				[2,6-^{14}C-pyridyl]

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

Metabolism studies have been conducted in three crop groups with foliar applications, namely fruit (F), root (R) and Pulses and oilseed (P/O). Since the metabolism is similar in all three crop groups thus all other 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:

EFSA Journal 2013;11(4):3052: "After foliar applications, fluopyram constitutes the major component of the radioactive residues, accounting for more than 85% TRR in grape, potato 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 the residues were mostly composed of the metabolites resulting from the cleavage of the parent molecule; fluopyram-benzamide (M25), fluopyram-PAA (M40) and fluopyram-PCA (M43).

A similar metabolic profile was observed in pepper following drip irrigation with fluopyram, fluopyram-PCA and fluopyram-PAA-glycosides accounting for 16% to 44% TRR in fruits. […]

Globally, the metabolism of fluopyram can be regarded as similar in all plant groups.

RMS comment:

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 is the major component of the residue.

The metabolism of fluopyram consists as a first step, of the hydroxylation of the parent compound to the metabolites fluopyram- 7-OH (M08) and fluopyram-8-OH (M18), which undergo further hexose conjugations. Cleavage of the hydroxylated metabolites and subsequent oxidation give two distinct groups of metabolites; those containing the trifluoromethyl-phenyl moiety [fluopyram-benzamide (M25), fluopyram-benzoic acid (M33)] and those containing the pyridyl moiety [fluopyram-PAA (M40), fluopyram-PCA (M43)].

Metabolite	Overall Maximum Concentration (foliar and drip)		
	%TRR	mg parent eq./kg	Comment
Fluopyram-7-hydroxy AE C656948-7-hydroxy / M08 BCS-AA10065	1.0	0.43	Grape leaves
	0.3	0.20	Grape (Summer Cut: leaves BBCH71)
	1.6	0.60	Bean foliage
	1.1	0.30	Bean straw
	1.5	0.63	Pepper (intermediate plant) drip irrigation
	6.8	0.24	Pepper (rest of plant) drip irrigation
	0.8	0.36	Potato leaves
Fluopyram-7-hydroxy-glc AE C656948-7-hydroxy-glc / M11 **(conjugate of M08)**	0.7	0.35	Grape leaves
	0.2	0.12	Grape (Summer Cut: leaves BBCH71)
	0.9	0.31	Pepper (rest of plant) drip irrigation
	0.6	0.25	Bean foliage
	0.3	0.14	Bean straw
Fluopyram-7-hydroxy-glc-MA AE C656948-7-hydroxy-glc-MA / M12 **(conjugate of M08)**	3.2	1.22	Bean foliage
	4.7	0.90	Bean straw
Fluopyram-8-hydroxy AE C656948-8-hydroxy / M18	0.8	0.34	Grape leaves
	0.2	0.13	Grape (Summer Cut: leaves BBCH71)
	0.5	0.21	Bean foliage

[2] These trigger values of 0.05 mg/kg or 10%TRR of total radioactive residues are only meant as guidance. In some circumstances, generally governed by toxicological concerns, it may be necessary to identify terminal metabolites, which are present at 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).

	0.9	0.17	Bean straw
Fluopyram-hydroxy-glyc-gluc AE C656948-hydroxy-glyc-gluc M22	10.4	0.013	Dry beans
Fluopyram-benzamide AE C656948-benzamide AE F148815 BCS-AA10014 M25	51.6	0.04	Succulent bean
	64.0	0.08	Dry bean
	0.5	0.17	Bean foliage
	0.6	0.10	Bean straw
	10.1	0.36	Pepper (rest of plant) drip irrigation
	16.1	0.006	Pepper (fruit) drip irrigation
	0.5	0.23	Potato leaves
Fluopyram-hydroxyethyl-glc AE C656948-hydroxyethyl-glc M35	0.2	0.06	Bean foliage
Fluopyram-hydroxyethyl-di-glc AE C656948-hydroxyethyl-di-glc M36	7.0	0.16	Pepper (rest of plant) drip irrigation
Fluopyram-pyridyl-acetic acid AE C656948-pyridyl-acetic acid PAA / BCS-AA10139 / M40	29.5	0.05	Succulent bean
	22.6	0.07	Dry bean
Fluopyram-PAA-glycoside AE C656948-PAA-glycoside / M42	38.0	0.023	Pepper (fruit) drip irrigation
Fluopyram-pyridyl-carboxylic acid AE C656948-pyridyl-carboxylic acid / PCA / AE C657188 / M43	31.0	0.05	Succulent bean
	32.5	0.10	Dry bean
	0.6	0.11	Bean straw
	0.5	0.19	Bean foliage
	43.5	0.026	Pepper (fruit) drip irrigation
	0.8	0.33	Grape leaves
	0.3	0.21	Grape (Summer Cut: leaves BBCH71)
	49.8	0.006	Potato tuber

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

	Residue definition		Reference
Food of plant origin	Monitoring	fluopyram (parent only)	EFSA Scientific Report EFSA Journal 2013;11(4):3052
	Risk assessment	fluopyram and fluopyram-benzamide (M25) expressed as fluopyram	

RMS comment:

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, grains, 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 older leaves. On the other hand, phloem mobility moves a chemical to sites of utilization of products from photosynthesis, particularly to roots, growing points, developing seeds and fruits.

Following application of radiolabelled Fluopyram to grapevine, potatoes, beans, red bell pepper and wheat employing both ^{14}C-labels, the highest radioactive residue levels (TRR 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 leaves and foliage; low levels were observed in fruits, tubers and seeds. This residue pattern shows that Fluopyram is xylem mobile, at least to a certain extent.

However, it should be admitted that most of the residue in/on the leaves after foliar application is assumed to consist of immobilized residue on the plant surface. This behaviour can be derived from the relative high residue levels in/on foliage of grapevine, potatoes and beans (foliar application) compared to the far lower residue levels in foliage of red bell pepper and wheat (drip application and seed treatment). On the other hand, the Fluopyram 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).

RMS comment:

Metabolism in rotational crops
Reference material: Test No. 502: Metabolism in Rotational Crops (OECD 2007b), Test No. 504: Residues in Rotational Crops (OECD, 2007d)

Question 4: Do results of the rotational crops show any translocation of residues (uptake from soil) from roots to the aerial parts of the plant[5]? 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 (leafy, roots, cereals)/crop parts is the accumulation observed?

Applicant response:

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

[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 ≥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 PBI, but decreased at the 280-day PBI, to ~2x the initial value. The TRR values for all RACs are given in the following table (Table B.7.9-1 from DAR).

Table B.7.9-1 : Total Radioactive Residues (TRRs) in the different RACs of the three rotations (expressed as parent compound equivalents, mg/kg)

TRR	Wheat				Swiss	turnip	
[mg/kg]	forage	hay	straw	Grain	chard	leaves	roots
1st rotation (30 days)	0.100	1.783	6.156	0.167	0.540	0.884	0.065
2nd rotation (139 days)	0.783	1.120	3.450	0.054	0.377	0.113	0.013
3rd rotation (280 days)	0.197	1.527	1.032	0.023	0.164	0.103	0.009

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 plant-back intervals. AE C656948-7-hydroxy and its various conjugates with glucose, malonic acid (2 isomers) and sulphuric 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 in which AE C656948-7-hydroxy accounted for 12.3-12.6% TRR. The sulphuric acid conjugate of AE C656948-7-hydroxy, AE C656948-7-OH-SA, was also a prominent metabolite in Swiss chard increasing from 7% of TRR in the 1st rotation to 16% 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 139-day PBIs), but not in the other rotated crop RACs.

AE C656948-8-hydroxy and its conjugate were only of minor importance. Both or at least one of them were detected in all RACs but at very low levels of <2.7% of the TRR in sum. AE C656948-phenol-glc was detected in turnip leaves 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 C656948-benzamide 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 10.3-11.7% TRR in Swiss chard and turnip leaves from the 280-day PBI.

The metabolism of [phenyl-UL-^{14}C]AE C656948 in confined rotational crops corresponds very well with the metabolism in confined rotational crops after application of [pyridyl-2,6- ^{14}C] 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:

Metabolite	Overall Maximum Concentration (CRC)		
	%TRR	mg parent eq./kg	Comment
Fluopyram-phenol-glc AE C656948-phenol-glc / M06	10.4	0.092	Turnip leaf
Fluopyram-7-hydroxy AE C656948-7-hydroxy / M08 / BCS-AA10065	12.6	0.193	Wheat Hay
	7.4	0.494	Wheat Straw
	28.0	0.160	Swiss chard
Fluopyram-7-OH-SA AE C656948-7-OH-SA / M10	16.8	0.058	Swiss chard
Fluopyram-7-hydroxy-glc AE C656948-7-hydroxy-glc / M11 **(conjugate of M08)**	3.1	0.203	Wheat Straw
	3.4	0.052	Wheat Hay
Fluopyram-7-hydroxy-glc-MA AE C656948-7-hydroxy-glc-MA / M12 **(conjugate of M08)**	14.1	0.170	Wheat Hay
	6.7	0.448	Wheat Straw
Fluopyram-8-hydroxy AE C656948-8-hydroxy / M18	1.4	0.087	Wheat Straw
Fluopyram-benzamide AE C656948-benzamide AE F148815 / BCS-AA10014 / M25	6.2	0.095	Wheat Hay
	2.8	0.169	Wheat straw
	10.1	0.06	Swiss chard
	9.7	0.086	Turnip leaf
Fluopyram-benzoic acid AE C656948-benzoic acid / M33	13.6	0.007	Wheat grain
Fluopyram-pyridyl-carboxylic acid AE C656948-pyridyl-carboxylic acid / PCA / AE C657188 / M43	4.9	0.088	Wheat Hay
	16.5	0.026	Wheat forage
	0.9	0.060	Wheat straw
	55.9	0.230	Wheat grain
Fluopyram-methyl-sulfoxide AE C656948-methyl-sulfoxide AE 1344122 / M45	49.0	0.035	Wheat grain

RMS comment:

Question 5: If the GAP is for a seed treatment or other pre-emergency[6] 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 [phenyl-UL-^{14}C]AE C656948 and [pyridyl-2,6-^{14}C]AE C656948 formulated as SC 500. Due to the low intended dressing rate of 1 g a.s./dt (decitonne = 100 kg) in agricultural 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 straw and grain were harvested at maturity.

Parent compound was the predominant residue in all plant matrices. Hydroxylation of the test item was detected as the main metabolic path, resulting in AE C656948-7-hydroxy and AE C656948-8-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-hydroxy-glc-MA were detected. Hydrolytic cleavage of the hydroxylated metabolites was observed, as well. AE C656948-benzamide was identified as direct cleavage product of AE C656948-8-hydroxy. Subsequent hydrolysis of the metabolite resulted in AE C656948-benzoic acid. AE C656948-pyridyl-carboxylic acid was detected as corresponding counterpart to AE C656948-benzamide. AE C656948-pyridyl-carboxylic acid was further transformed by substitution of the chlorine to form AE C656948-methyl-sulfoxide.

Because this is a seed treatment, the parent compound fluopyram (AE C656948) is also subjected to metabolic conversion in the soil. Metabolites formed by molecule cleavage may also be related to the degradation of the test item in the soil. Uptake of these metabolites via the roots could be - at least in part - the reason for their occurrence in the plant matrices.

Metabolite	Overall Maximum Concentration (seed treatment)		
	%TRR	mg parent eq. /kg	Comment
Fluopyram-7-hydroxy AE C656948-7-hydroxy / M08 BCS-AA10066	11.1	0.053	Wheat straw (seed treatment 10X overdose)
Fluopyram-7-hydroxy-glc-MA AE C656948-7-hydroxy-glc-MA M12 **(conjugate of M08)**	12.6	0.017	Wheat forage (seed treatment 10X overdose)
	15.2	0.073	Wheat straw (seed treatment 10X overdose)
	11.9	0.034	Wheat hay (seed treatment 10X overdose)
Fluopyram-benzamide AE C656948-benzamide AE F148815: BCS-AA10014 / M25	10.4	0.01	Wheat grain (seed treatment 10X overdose)

RMS comment:

[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 residue 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 residue 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 73 (in 8 trials), 79, 85 and 89. The last sampling was performed between 57 and 104 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. A gradual decline in the residue level of fluopyram was observed in all trials with a slight upturn observed in some cases at the last sampling. Residue levels of the metabolite AE C656948-benzamide were <LOQ (<0.01 mg/kg) with only few exceptions.

Residue levels of fluopyram were also found to decline gradually in all 13 trials performed 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, 14 and 21 (in 4 trials) days after that. While the residue level of the parent compound remained above the LOQ, AE C656948-benzamide was often below or slightly above the LOQ (<0.01 mg/kg), with no clear decline. Exceptions to this pattern were observed in the 6 trials performed in 2014, in which decline was observed after the last application, with some upturns 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 harvest interval (PHI). By the time of the last sampling, lettuce plants BBCH 49 (typical size, form and firmness of heads reached) and were appropriate for consumption.

The residues field trials 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 extraction), were covered by separate storage stability studies.

RMS comment:

[7] Please report if the residue trials were fully validated in terms of storage stability, GAP compliance, etc.
[8] It is mentioned 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 Livestock (OECD, 2007a); Test No. 505: Residues in Livestock (OECD, 2007e); Test No. 305: Bioaccumulation in Fish (OECD, 2012)

Question 8: Is a metabolism study in fish/bioaccumulation study part of the residue section? If the fish metabolism study is available, does it indicate an accumulation of residues in fish tissues? [12]

Applicant response:

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

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

The average percent lipids 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 µg [pyridyl-2,6-^{14}C]-fluopyram/L) and 35.9 (edible tissue) and 65.7 (whole fish) for the high treatment (60 µg [pyridyl-2,6-^{14}C]-fluopyram/L).

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 fluopyram normalized to 6% lipid content was 16.

[10] The minimum number of supervised residue trials considers for MRL setting might not be applicable for the ecotox. We might build a residue decline curve with less than 4 residue data points. For this consideration, please do not disregard the residue data only based on the minimum number of residue trials. If the residue trials are compliant with the GAP table, ecotox experts might use them for further refinements.

[11] Ecotox colleagues 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, 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 14), 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-OH-GA 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).

Substance uptake and depuration constants and bioconcentration factors

Parameter (based on TRR)	6.0 µg [pyridyl-2,6-^{14}C]-fluopyram/L			60 µg [pyridyl-2,6-^{14}C]-fluopyram/L		
	Edible tissue	**Non-edible tissue**	**Whole fish**	**Edible tissue**	**Non-edible tissue**	**Whole fish**
Kinetic bioconcentration factor (BCF_{TRR})	47.6	156.4	87.9	35.9	121.6	65.7
Time to reach 95 % of steady state [days]	30.6	8.1	14.8	18.1	4.6	7.7
$t_{(1/2)}$ for clearance [days]	7.1	1.9	3.4	4.2	1.1	1.8
Uptake rate constant (k_u) [1/Day]	4.67 (± 0.42)	58.2 (± 2.0)	17.8 (± 1.1)	5.96 (± 0.57)	78.7 (± 3.62)	25.6 (± 1.59)
Depuration rate constant (k_d) [1/Day]	0.098 (± 0.03)	0.37 (± 0.16)	0.20 (± 0.08)	0.17 (± 0.06)	0.65 (± 0.26)	0.39 (± 0.175)

The OriginTM calculated kinetic BCFTRR values for edible parts and whole fish (calculated as the ratio of uptake and depuration rate constant) correspond 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 [pyridyl-2,6-^{14}C]-fluopyram/L and of 41.6 X (edible parts) and 79.2 X (whole fish) for 60 µg [pyridyl-2,6-^{14}C]-fluopyram/L, respectively.

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 µg [pyridyl-2,6-^{14}C]-fluopyram/L and of 2.49 mg/kg edible parts and 4.75 mg/kg whole fish for 60 µg [pyridyl-2,6-^{14}C]-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 viscera 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 16.

RMS comment:

Question 9: Can the metabolism in animals (mammals/fish/hens) bring any information on accumulation/exposure[13] to different metabolites in addition to those present in the plants? Is it 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 animals 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 livestock metabolism studies were performed at 2 mg/kg bw/d, corresponding to 21N for ruminant and 83N for poultry with the representative uses.

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

There is no potential for accumulation (goat, hen). This was also the conclusion based on rat (ADME) 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 [pyridyl-2,6-^{14}C] or [phenyl UL-^{14}C] labelled fluopyram at nominal rates of 2 mg/kg bw/day. One metabolism study was conducted with [pyridyl-2,6-^{14}C] fluopyram in fish [see question 8]. All studies were well performed and fulfilled the acceptability criteria of EC and OECD 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 ethylene bridge of the molecule resulting in fluopyram-7- hydroxy, fluopyram-8-hydroxy, and a dihydroxylated compound,
- hydroxylation of the phenyl ring leading to fluopyram-phenol
- conjugation of the hydroxylated metabolites with glucuronic acid
- elimination of water from compounds hydroxylated in the ethylene bridge leading to fluopyram-Z-olefine and E-olefine (E- and Z-olefine can isomerise into each other),
- molecular cleavage of fluopyram-8-hydroxy to fluopyram-pyridyl-hydroxyethyl (pyridyl label specific) followed by either conjugation with glucuronic acid or oxidation to fluopyram-pyridyl-acetic acid (PAA)
- molecular cleavage of fluopyram-8-hydroxy to fluopyram-benzamide (phenyl label specific) and formation of fluopyram-benzamide sulfate or fluopyram-benzoic acid.

Parent fluopyram is intensively metabolised in the animal. Main metabolites in the goat and hen were fluopyram-benzamide (M25) and fluopyram-E- and Z-olefins (M02 and M03). In the goat, fluopyram-7-OH-GA (M09; sum of isomers) and fluopyram-8-OH-GA (M20b; isomer 2) exceed 10% of TRR."

RMS comment:

[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, 2013).

Question 1: Are data on the magnitude of residues on pollen and bee products part of the residue section? If so, please indicate which data are available and sampling times? [14]

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 sites 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 C656948) and its metabolites fluopyram-benzamide (AE F148815, FLU-benzamide), fluopyram-pyridyl-carboxylic-acid (AE C657188, FLU-PCA), fluopyram-pyridyl-acetic-acid (BCS-AA 10139, FLU-PAA) and fluopyram-7-hydroxy.

No residues of fluopyram and its five metabolites (FLU-benzamide, FLU-PCA, FLU-PAA, FLU-7OH and FLU-methylsulfoxide) were found above the LOQ (LOQ = 0.01 mg/kg) in any honey samples originating from treated or untreated tunnels. Detailed data on test methodology and findings from these trials are presented in section CA 6.10.1.

Residues of fluopyram and its metabolites fluopyram-pyridyl-acetic acid (BCS-AA10189) and fluopyram-benzamide (AE F148815) were also analysed in flowers, bee-collected nectar and bee-collected 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 *Phacelia tanacetifolia* at rates of 560 mL product/ha (corresponding to 140 g fluopyram/ha 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-treated tunnels. Pollen samples were collected from foraging honey bees on the day of the 2nd test item application and the following day. Residues of fluopyram in pollen ranged from 3 to 30 mg/kg. Residues of FLU-PAA did not exceed 0.01 mg/kg pollen, while those of FLU-benzamide 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).

RMS comment:

[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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Appendix 1

Metabolites seen in the confined rotational crop study (M-240707-03-1)

Phenyl label - metabolites														
Plot	Crop Part	PBI (days)	DALA (days)	M-04			M-01			M-06			Fluopyram	
				%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg
29	Lettuce	29	83	-	-	-	81.2	0.822	0.407	-	-	-	11.1	0.112
	Radish Tops	29	74	-	-	-	65.3	4.381	2.170	-	-	-	24.5	1.644
	Radish Roots	29	71	-	-	-	43.2	0.062	0.031	-	-	-	47.9	0.069
	Wheat Forage	29	68	33	1.619	0.870	6	0.312	0.155	1.0	0.049	<0.051	36.6	1.812
	Wheat Grain	29	93	-	-	-	3.6	0.006	0.003	13.1	0.021	0.022	27.3	0.043
	Wheat Straw	29	93	13.6	1.844	0.990	3.4	0.461	0.228	-	-	-	23.1	3.132
133	Lettuce	133	216	-	-	-	60.9	0.070	0.035	-	-	-	26.6	0.031
	Radish Tops	133	196	-	-	-	77.3	0.186	0.092	-	-	-	15.1	0.036
	Radish Roots	133	196	-	-	-	54.9	0.013	0.006	-	-	-	28.2	0.006
	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	19.0	0.003	0.003	-	-	-	7.0	0.001
	Wheat Straw	133	335	14.6	0.123	0.067	25.5	0.215	0.107	-	-	-	15.5	0.131
365	Lettuce	365	421	-	-	-	87.0	0.539	0.267	-	-	-	2.1	0.013
	Radish Tops	365	421	-	-	-	87.5	1.755	0.869	-	-	-	3.8	0.076
	Radish Roots	365	421	-	-	-	60.9	0.022	0.011	-	-	-	24.2	0.009
	Wheat Forage	365	410	59.3	0.513	0.276	14.8	0.128	0.063	-	-	-	4.8	0.042
	Wheat Grain	365	449	24.5	0.013	0.007	17.9	0.010	0.005	-	-	-	7.3	0.004
	Wheat Straw	365	449	28.0	0.063	0.356	5.1	0.121	0.060	-	-	-	7.2	0.172

Pyridyl label- metabolites

Plot	Crop Part	PBI (days)	DALA (days)	M-08			M-05			M-02			M-09			M-06			Fluopyram	
				%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg
29	Lettuce	29	83	-	-	-	13.0	0.039	0.026	17.4	0.053	0.031	5.3	0.016	0.008	-	-	-	35.8	0.108
	Radish Tops	29	71	-	-	-	3.3	0.069	0.046	10.4	0.217	0.128	4.8	0.100	0.052	-	-	-	51.1	1.072
	Radish Roots	29	71	-	-	-	9.6	0.011	0.007	35.6	0.039	0.023	-	-	-	-	-	-	41.1	0.048
	Wheat Forage	29	68	-	-	-	3.8	0.163	0.108	43.0	1.844	1.087	-	-	-	1.4	0.060	0.063	33.7	1.445
	Wheat Grain	29	93	-	-	-	13.1	0.341	0 225	69.6	0.809	1.064	-			-	-	-	1.8	0.046
	Wheat Straw	29	93	-	-		7.7	0.544	0 359	7.0	0.494	0.291			-			-	34.9	2.462
133	Lettuce	133	217	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	79.9	0.027
	Radish Tops	133	197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	72.2	0.171
	Radish Roots	133	197	-	-	-	2.9	0.001	0.001	9.6	0.002	0.002	19.4	0.005	0.003	-	-	-	54.9	0.014
	Wheat Forage	133	282	-	-	-	41.0	0.064	0.042	5.4	0.008	0.005	10.5	0.016	0.008	-	-	-	26.2	0.041
	Wheat Grain	133	336	-	-	-	66.6	0.064	0.042	10.9	0.010	0.006				-	-	-	3.2	0.003
	Wheat Straw	133	336	9.4	0.033	0.019	12	0.004	0.003	2 1	0.007	0.004	21.5	0.075	0.039	-	-	-	25.7	0.089
365	Lettuce	365	421	9.0	0.005	0.003	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	-	-	-	5.1	0.022	0.015	27.1	0.114	0.067	6.0	0.025	0.013	-	-	-	25.2	0.106
	Radish Roots	365	421	9.5	0.003	0.002	5.3	0.002	0.001	10.0	0.003	0.002	-	-	-	-	-	-	55.8	0.018
	Wheat Forage	365	410	6.3	0.015	0.009	18.3	0.045	0.030	8 2	0.020	0.012	9.9	0.024	0.012	-	-	-	27.8	0.068
	Wheat Grain	365	449	-	-	-	64.9	0.116	0.077	14.2	0.025	0.015	-	-	-	-	-	-	2.9	0.005
	Wheat Straw	365	449	4.8	0.048	0.028	14.2	0.143	0.094	4 1	0.042	0.025	-	-	-	-	-	-	27.5	0.277

Appendix 2

Summary of the residue decline trials for fluopyram treated grapes

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948	AE C656948-benzamide
Report No RA- 17-2112 (M-653354-02-1) Trial No 17-2112-01	2 x 0.038 kg a.s./ha	Bunch of grapes	-0	0.078	<0.01
		Bunch of grapes	0	0.22	0.015
		Bunch of grapes	17	0.028	<0.01
		Bunch of grapes	80	<0.01	<0.01
		Bunch of grapes	104	<0.01	<0.01
Report No RA- 17-2112 (M-653354-02-1) Trial No 17-2112-02	2 x 0.038 kg a.s./ha	Bunch of grapes	-0	0.075	<0.01
		Bunch of grapes	0	0.19	<0.01
		Bunch of grapes	15	0.11	<0.01
		Bunch of grapes	44	0.043	<0.01
		Bunch of grapes	72	0.03	<0.01
Report No RA- 17-2112 (M-653354-02-1) Trial No 17-2112-03	2 x 0.038 kg a.s./ha	Bunch of grapes	-0	0.052	<0.01
		Bunch of grapes	0	0.11	<0.01
		Bunch of grapes	45	0.024	<0.01
		Bunch of grapes	67	0.016	<0.01
		Bunch of grapes	93	0.014	<0.01
Report No RA- 17-2112 (M-653354-02-1) Trial No 17-2112-04	2 x 0.038 kg a.s./ha	Bunch of grapes	-0	0.02	<0.01
		Bunch of grapes	0	0.078	<0.01
		Bunch of grapes	12	0.018	<0.01
		Bunch of grapes	62	<0.01	<0.01
		Bunch of grapes	76	<0.01	<0.01
Report No RA- 16-2157 (M-614532-02-1) Trial No 16-2157-01	2 x 0.1 kg a.s./ha (last treatment at BBCH 69)	Bunch of grapes	29	0.039	<0.01
		Bunch of grapes	51	0.024	<0.01
		Bunch of grapes	65	0.014	<0.01
	2 x 0.1 kg a.s./ha (last treatment at BBCH 79)	Bunch of grapes	-0	0.12	<0.01
		Bunch of grapes	0	0.30	<0.01
		Bunch of grapes	22	0.13	<0.01
		Bunch of grapes	36	0.085	<0.01
Report No RA- 16-2157 (M-614532-02-1) Trial No 16-2157-02	2 x 0.1 kg a.s./ha (last treatment at BBCH 69)	Bunch of grapes	35	0.028	<0.01
		Bunch of grapes	51	0.021	<0.01
		Bunch of grapes	78	<0.01	<0.01
	2 x 0.1 kg a.s./ha (last treatment at BBCH 79)	Bunch of grapes	-0	0.33	0.042
		Bunch of grapes	0	0.63	0.044
		Bunch of grapes	16	0.28	0.046
		Bunch of grapes	43	0.12	0.026
Report No RA- 16-2157 (M-614538-02-1) Trial No 16-2157-03	2 x 0.1 kg a.s./ha (last treatment at BBCH 69)	Bunch of grapes	18	0.15	<0.01
		Bunch of grapes	44	0.052	<0.01
		Bunch of grapes	74	0.087	<0.01
	2 x 0.1 kg a.s./ha (last treatment at BBCH 79)	Bunch of grapes	-0	0.16	<0.01
		Bunch of grapes	0	0.38	<0.01
		Bunch of grapes	22	0.070	<0.01
		Bunch of grapes	36	0.062	<0.01
Report No RA- 16-2157 (M-614532-02-1) Trial No 16-2157-04	2 x 0.1 kg a.s./ha (last treatment at BBCH 69)	Bunch of grapes	32	0.22	<0.01
		Bunch of grapes	85	0.07	<0.01
		Bunch of grapes	94	0.052	<0.01
	2 x 0.1 kg a.s./ha (last treatment at BBCH 79)	Bunch of grapes	-0	0.20	<0.01
		Bunch of grapes	0	0.46	<0.01
		Bunch of grapes	53	0.13	<0.01
		Bunch of grapes	62	0.11	<0.01
Report No RA- 16-2157 (M-614532-02-1) Trial No 16-2157-05	2 x 0.1 kg a.s./ha (last treatment at BBCH 69)	Bunch of grapes	31	0.10	<0.01
		Bunch of grapes	84	0.054	<0.01
		Bunch of grapes	93	0.041	<0.01
	2 x 0.1 kg a.s./ha (last treatment at BBCH 79)	Bunch of grapes	-0	0.16	<0.01
		Bunch of grapes	0	0.36	<0.01
		Bunch of grapes	53	0.14	<0.01
		Bunch of grapes	62	0.14	<0.01

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948	AE C656948-benzamide
Report No RA- 16-2157 (M-614532-02-1) Trial No 16-2157-06	2 x 0.1 kg a.s./ha (last treatment at BBCH 69)	Bunch of grapes	36	0.073	<0.01
		Bunch of grapes	57	0.035	<0.01
		Bunch of grapes	79	0.049	<0.01
	2 x 0.1 kg a.s./ha (last treatment at BBCH 79)	Bunch of grapes	-0	0.15	<0.01
		Bunch of grapes	0	0.30	<0.01
		Bunch of grapes	21	0.18	<0.01
		Bunch of grapes	43	0.12	<0.01
Report No RA- 16-2157 (M-614532-02-1) Trial No 16-2157-07	2 x 0.1 kg a.s./ha (last treatment at BBCH 69)	Bunch of grapes	29	0.11	<0.01
		Bunch of grapes	68	0.085	<0.01
		Bunch of grapes	82	0.056	<0.01
	2 x 0.1 kg a.s./ha (last treatment at BBCH 79)	Bunch of grapes	-0	0.089	<0.01
		Bunch of grapes	0	0.21	<0.01
		Bunch of grapes	39	0.13	0.011
		Bunch of grapes	53	0.098	0.011
Report No RA- 16-2157 (M-614532-02-1) Trial No 16-2157-08	2 x 0.1 kg a.s./ha (last treatment at BBCH 69)	Bunch of grapes	30	0.11	<0.01
		Bunch of grapes	61	0.065	<0.01
		Bunch of grapes	88	0.03	<0.01
	2 x 0.1 kg a.s./ha (last treatment at BBCH 79)	Bunch of grapes	-0	0.091	<0.01
		Bunch of grapes	0	0.19	<0.01
		Bunch of grapes	31	0.11	<0.01
		Bunch of grapes	58	0.096	<0.01
Report No RA- 17-2128 (M-688233-01-1) Trial No 17-2128-01	2 x 0.05 kg a.s./ha (21 days interval, last treatment at BBCH 73)	Bunch of grapes	0	0.068	<0.01
		Bunch of grapes	26	0.035	<0.01
		Bunch of grapes	57	0.017	<0.01
		Bunch of grapes	68	0.021	<0.01
	2 x 0.05 kg a.s./ha (12 days interval, last treatment at BBCH 73)	Bunch of grapes	0	0.094	<0.01
		Bunch of grapes	26	0.023	<0.01
		Bunch of grapes	57	<0.01	<0.01
		Bunch of grapes	68	0.01	<0.01
Report No RA- 17-2128 (M-688233-01-1) Trial No 17-2128-02	2 x 0.05 kg a.s./ha (21 days interval, last treatment at BBCH 73)	Bunch of grapes	0	0.031	<0.01
		Bunch of grapes	21	0.018	<0.01
		Bunch of grapes	43	0.011	<0.01
		Bunch of grapes	57	<0.01	<0.01
	2 x 0.05 kg a.s./ha (12 days interval, last treatment at BBCH 73)	Bunch of grapes	0	0.036	<0.01
		Bunch of grapes	21	0.016	<0.01
		Bunch of grapes	43	0.011	<0.01
		Bunch of grapes	57	0.01	<0.01
Report No RA- 17-2128 (M-688233-01-1) Trial No 17-2128-03	2 x 0.05 kg a.s./ha (21 days interval, last treatment at BBCH 73)	Bunch of grapes	0	0.11	<0.01
		Bunch of grapes	14	0.063	<0.01
		Bunch of grapes	56	0.038	<0.01
		Bunch of grapes	98	0.028	<0.01
	2 x 0.05 kg a.s./ha (12 days interval, last treatment at BBCH 73)	Bunch of grapes	0	0.21	<0.01
		Bunch of grapes	14	0.11	<0.01
		Bunch of grapes	56	0.074	<0.01
		Bunch of grapes	98	0.038	<0.01
Report No RA- 17-2128 (M-688233-01-1) Trial No 17-2128-04	2 x 0.05 kg a.s./ha (21 days interval, last treatment at BBCH 73)	Bunch of grapes	0	0.32	<0.01
		Bunch of grapes	32	0.057	<0.01
		Bunch of grapes	53	0.024	<0.01
		Bunch of grapes	80	0.027	<0.01
	2 x 0.05 kg a.s./ha (12 days interval, last treatment at BBCH 73)	Bunch of grapes	0	0.67	<0.01
		Bunch of grapes	32	0.074	<0.01
		Bunch of grapes	53	0.03	<0.01
		Bunch of grapes	80	0.033	<0.01

Summary of the residue decline trials for fluopyram treated lettuce

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948 (Fluopyram)	AE C656948-benzamide
Report No RA-2620/07 (M-308622-01-1) Trial No R 2007 0266/3	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	1.5	< 0.01
		head	0	5.8	0.01
		head	3	4.7	0.01
		head	7	2.1	0.01
		head	14	0.73	< 0.01
			21	0.51	0.01
Report No RA-2620/07 (M-308622-01-1) Trial No R 2007 0642/1	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	0.81	0.02
		head	0	5.5	0.02
		head	3	1.2	0.02
		head	7	0.92	0.02
		head	14	0.44	0.01
			21	0.24	0.01
Report No RA-2620/07 (M-308622-01-1) Trial No R 2007 0644/8	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	0.39	< 0.01
		head	0	3.4	0.01
		head	3	1.5	0.01
		head	7	1.4	0.02
		head	14	0.37	0.01
			21	0.07	< 0.01
Report No RA-2620/07 (M-308622-01-1) Trial No R 2007 0645/6	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	0.69	< 0.01
		head	0	3.5	< 0.01
		head	3	0.63	< 0.01
		head	7	0.23	< 0.01
		head	13	0.19	< 0.01
			21	0.07	< 0.01
Report No RA- 14-2028 (M-534623-01-1) Trial No 14-2028-01	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	1.6	< 0.01
		head	0	5.8	0.014
		head	3	2.5	< 0.01
		head	7	1.6	0.011
		head	14	0.84	< 0.01
Report No RA- 14-2028 (M-534623-01-1) Trial No 14-2028-02	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	2.0	0.015
		head	0	12	0.039
		head	3	4.8	0.027
		head	7	3.6	0.028
		head	14	1.1	0.011
Report No RA- 14-2028 (M-534623-01-1) Trial No 14-2028-03	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	0.42	< 0.01
		head	0	5.3	0.014
		head	3	1.5	< 0.01
		head	7	0.83	< 0.01
		head	14	0.47	< 0.01
Report No RA- 14-2028 (M-534623-01-1) Trial No 14-2028-04	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	1.2	< 0.01
		head	0	7.6	0.030
		head	3	5.5	0.016
		head	7	0.94	0.010
		head	14	0.17	< 0.01
Report No RA- 14-2028 (M-534623-01-1) Trial No 14-2028-05	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	6.0	< 0.01
		head	0	13	0.025
		head	3	12	0.014
		head	7	10	0.013
		head	14	7.1	< 0.01
Report No RA- 14-2028 (M-534623-01-1) Trial No 14-2028-06	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	1.1	< 0.01
		head	0	5.7	0.017
		head	3	5.4	0.011
		head	7	3.9	0.010
		head	14	0.85	< 0.01
Report No RA-18-2048 (M-675904-01-1) Trial No 18-2048-01	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	2.9	< 0.01
		head	0	9.1	< 0.01
		head	3	6.7	< 0.01
		head	7	5.5	< 0.01
		head	14	1.3	< 0.01

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948 (Fluopyram)	AE C656948-benzamide
Report No RA- 18-2048 (M-675904-01-1) Trial No 18-2048-02	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	2.2	< 0.01
		head	0	6.6	< 0.01
		head	3	7.6	< 0.01
		head	7	5.0	< 0.01
		head	14	4.6	< 0.01
Report No RA- 18-2048 (M-675904-01-1) Trial No 18-2048-03	2 x 0.2 kg a.s./ha 14 and 7 days before harvest	head	-0	0.97	< 0.01
		head	0	5.4	0.01
		head	3	2.3	0.010
		head	7	1.3	0.010
		head	14	0.46	0.014

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 DT_{50} values for foliage dwelling arthropods (3 DT_{50}s in vines, 3 DT_{50}s in OSR and 6 DT_{50}s in apple orchards), 9 DT_{50} values for flying insects (3 DT_{50}s in vines, 1 DT_{50} in OSR and 5 DT_{50}s in apple) and ground dwelling arthropods (1 DT_{50} extended lab, 6 DT_{50}s in apple).
- **Residue decline in foliage: 143 trials** and 6 kinetic evaluation reports providing DT_{50} values for various types of vegetables (surrogates for non-grass weeds: 118 DT_{50}s) and young cereals (surrogates for grass and cereals: 25 DT_{50}s). Due to the size of the kinetic evaluation reports, these DT_{50}s are reported in 4 reports for the vegetables and 2 reports for the cereals.

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

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 formulated product).

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

It should be noted that the data set of surrogates for non-grass weeds also includes onions and leek, which are monocots. However, onion and leek are not grasses (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 surrogates for non-grass weeds.

In the summaries for these studies, an attempt is made to visualize and assess the influence of rainfall on the residue time course according to the recommendations of EFSA 2019. For that purpose, the DT_{50} values from the trials have been assigned to 3 categories:

Category 1: no discernible influence of precipitation

Category 2: influence possible / slight

Category 3: marked influence

Influence of rainfall on arthropod residue decline

The evaluation of the arthropod residue decline trials demonstrated that rainfall occurred in the majority of trials. Thus, rainfall (and/or irrigation) is a typical element for exposure assessment in realistic bird and wild mammal scenarios under EFSA GD 2009. However, there was hardly any discernible impact of rainfall on the insect residue decline, so that nearly all trials can be assigned to rainfall category 1. The difference between the geomean DT_{50} for category 1 trials and for both category 1 & 2 trials is negligible (< 5%). Therefore, it is proposed to pool all trials per foliage dwelling arthropods (n= 12), flying insects (n= 9) or ground dwelling arthropods (n= 7), respectively.

DT_{50} of fluopyram 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).

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

Group Plot Edition no.	Crop	Zone	Kinetic model	DT$_{50}$	Cat	Rainfall	Source DT$_{50}$
Ground dweller Extended lab M-545010-02-1	Bare soil	na	HS slow phase	5.58	1	none	M-545010-02-1
Foliage dweller plot 1 M-453376-01-2	Vines	N	FOMC DT$_{90}$/3.32	5.94 a)	1	No discernible influence of rainfall on residue time course	EnSa-15-0934
Foliage dweller plot 2 M-453376-01-2	Vines	N	SFO	5.57	1	No discernible influence of rainfall on residue time course	EnSa-15-0934
Foliage dweller plot 3 M-453376-01-2	Vines	N	FOMC DT$_{90}$/3.32	2.37	2	Frequent early rainfall without consistent correlation with the residue time course	EnSa-15-0934
Flying insects plot 1 M-453376-01-2	Vines	N	SFO	5.55	1	No discernible influence of rainfall on residue time course	EnSa-15-0934
Flying insects plot 2 M-453376-01-2	Vines	N	HS DT$_{90}$/3.32	2.85 b)	1	No discernible influence of rainfall on residue time course	EnSa-15-0934
Flying insects plot 3 M-453376-01-2	Vines	N	DFOP DT$_{90}$/3.32	3.38 a)	2	Frequent early rainfall without consistent correlation with the residue time course	EnSa-15-0934
Foliage dweller plot 1 M-544190-01-1	Oilseed rape	N	SFO	0.685	1	No discernible influence of rainfall on residue time course	EnSa-16-0035
Foliage dweller plot 2 M-544190-01-1	Oilseed rape	N	HS DT$_{90}$/3.32	1.078	1	No discernible influence of rainfall on residue time course	EnSa-16-0035
Foliage dweller plot 3 M-544190-01-1	Oilseed rape	N	HS DT$_{90}$/3.32	1.594	1	No discernible influence of rainfall on residue time course	EnSa-16-0035
Flying insects plots 1+2+3 M-544190-01-1	Oilseed rape	N	SFO	2.13	1	Very little rain	EnSa-16-0035
Foliage dweller plot 1 M-644049-01-1	Apple orchard	N	SFO	4.1	1	No discernible influence of rainfall on residue time course	M-644049-01-1
Foliage dweller plot 2 M-644049-01-1	Apple orchard	N	FOMC DT$_{90}$/3.32	2.7	1	No discernible influence of rainfall on residue time course	M-644049-01-1
Foliage dweller plot 3 M-644049-01-1	Apple orchard	N	FOMC DT$_{90}$/3.32	5.3	2	Slight influence of rainfall from day 8	M-644049-01-1
Flying insects plot 1 M-644049-01-1	Apple orchard	N	FOMC DT$_{90}$/3.32	2.4	1	No discernible influence of rainfall on residue time course	M-644049-01-1
Flying insects plot 2 M-644049-01-1	Apple orchard	N	SFO	2.2	1	No discernible influence of rainfall on residue time course	M-644049-01-1
Flying insects plot 3 M-644049-01-1	Apple orchard	N	SFO	1.9	1	No discernible influence of rainfall on residue time course	M-644049-01-1
Ground dweller plot 1	Apple orchard	N	Pseudo SFO DT$_{50}$	8.3	1	No discernible influence of rainfall on residue time course	EnSa-20-0891

Group Plot Edition no.	Crop	Zone	Kinetic model	DT_{50}	Cat	Rainfall	Source DT_{50}
M-644049-01-1							
Ground dweller plot 2 M-644049-01-1	Apple orchard	N	Pseudo SFO DT_{50}	4.4	1	No discernible influence of rainfall on residue time course	EnSa-20-0891
Ground dweller plot 3 M-644049-01-1	Apple orchard	N	Pseudo SFO DT_{50}	9.4	1	No discernible influence of rainfall on residue time course	EnSa-20-0891
Foliage dweller plot 1 M-644048-01-1	Apple orchard	S	SFO	6.1	1	No discernible influence of rainfall on residue time course	M-644048-01-1
Foliage dweller plot 2 M-644048-01-1	Apple orchard	S	FOMC $DT_{90}/3.32$	5.5	1	No discernible influence of rainfall on residue time course	M-644048-01-1
Foliage dweller plot 3 M-644048-01-1	Apple orchard	S	SFO	4.4	1	No discernible influence of rainfall on residue time course	M-644048-01-1
Flying insects plot 1 M-644048-01-1	Apple orchard	S	SFO	4.9	1	No discernible influence of rainfall on residue time course	M-644048-01-1
Flying insects plot 3 M-644048-01-1	Apple orchard	S	SFO	3.8	1	No discernible influence of rainfall on residue time course	M-644048-01-1
Ground dweller plot 1 M-644048-01-1	Apple orchard	S	Pseudo SFO DT_{50}	7.1	1	No discernible influence of rainfall on residue time course	EnSa-20-0890
Ground dweller plot 2 M-644048-01-1	Apple orchard	S	Pseudo SFO DT_{50} [d]	5.5	2	Moderate rainfalls on days 4 and 5 coincide with a visible drop in residues, influence likely	EnSa-20-0890
Ground dweller plot 3 M-644048-01-1	Apple orchard	S	Pseudo SFO DT_{50}	5.8	1	No discernible influence of rainfall on residue time course	EnSa-20-0890

[a] it is proposed to use FOMC as the best fit (instead of DFOP as selected in EnSa-15-0934) because the visual fit rating is identical but the χ^2 -error is lower

[b]: it is proposed to use HS as the best 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 χ^2-error is lower

[c]: it is proposed to use DFOP as 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 χ^2 -error is lower

[d] it is proposed to use the pseudo-SFO DT_{50} of 5.5 days instead of the FOMC $DT_{90}/3.32$ of 7.9 days as suggested in the original report. Justification: both the pseudo-SFO of 5.5 days and the FOMC $DT_{90}/3.32$ 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 FOMC $DT_{90}/3.32$ of 7.9 days is an overestimation as it results in a 21-d f_{TWA} much larger than the 21d- f_{TWA} calculated with the FOMC parameter alpha and beta. The 21-d f_{TWA} calculation with the pseudo SFO-DT_{50} of 5.5 days still 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:

Approach	Calculated with	Parameter values	Resulting 21-d f_{TWA}
FOMC-DT_{90}/3.32	Surrogate SFO DT_{50}	7.9 days	0.46
Pseudo SFO-DT_{50}	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-$DT_{90}/3.32$ = 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 irrigation) is a typical element for exposure assessment in realistic bird and wild mammal scenarios under EFSA GD 2009. The comparison of DT_{50}s for the 3 rainfall categories indicate slower residue dissipation in category 1 than in categories 2 or 3, which is not surprising since rainfall may influence residue decline by various 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 DT_{50}s in plant foliage per rainfall category and feed category

Category 1		Category 2		Category 3		
young cereals	**non-grass herbs**	**young cereals**	**non-grass herbs**	**young cereals**	**non-grass herbs**	
4.60 d	3.39 d	3.58 d	3.22 d	2.50 d	2.76 d	**geomean DT_{50}**
11	34	6	36	8	48	**number of trials**
44%	29%	24%	31%	32%	48%	**% of trials**

Table 10.1- 5: Overview on foliage residue decline DT_{50} sorted per rainfall influence categories

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
R 2006 0655/9 M-290825-01-1	Beans	N	SFO	2.729	1	late rain, no influence	Ensa-20-8029
R 2006 0722/8 M-291180-01-1	Beans	N	SFO	13.88	1	very little rain, no influence	Ensa-20-8029
R 2006 0723/7 M-291180-01-1	Beans	N	SFO	3.636	1	late rain, no influence	Ensa-20-8029
08-2096-01 T1 M-365542-01-1	Beans	S	SFO	2.969	1	irrigation d5 and d11, no discernible influence	Ensa-20-8029
R 2006 0378/9 M-290827-01-1	Beans	S	SFO	8.872	1	no rain, no influence	Ensa-20-8029
R 2006 0657/5 M-290827-01-1	Beans	S	HS	0.883	1	no rain, no influence	Ensa-20-8029
R 2006 0658/3 M-290827-01-1	Beans	S	SFO	3.548	1	no rain, late irrigation, no influence	Ensa-20-8029
R 2007 0550/6 M-297564-01-1	Beans	S	SFO	0.7695	1	no rainfall, no influence	Ensa-20-8030
R 2007 0551/4 M-297564-01-1	Beans	S	SFO	8.169	1	no rainfall, no influence	Ensa-20-8030
R 2007 0552/2 M-297564-01-1	Beans	S	DFOP	11.166	1	nearly no rainfall (1.2mm day 7), no influence	Ensa-20-8030
R 2007 0599/9 M-302401-01-1	Cabbage	N	SFO	1.979	1	marked decline, unlikely to be influenced by very little early rainfall	EnSa-20-0832
R 2006 0644/7 M-293182-01-1	Cabbage	S	FOMC	3.148	1	Little rainfall until day 14, no influence discernible	EnSa-20-0832
R 2006 0605/2 M-292048-01-1	Lettuce	N	HS	3.587	1	very little rain, no influence	Ensa-20-8029

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
R 2007 0244/2 M-304280-01-1	Lettuce	N	SFO	3.09	1	late rain, no marked influence	Ensa-20-8030
R 2007 0540/9 M-304280-01-1	Lettuce	N	SFO	1.368	1	late rain, no discernible influence	Ensa-20-8030
14-2029-02 M-534202-01-1	Lettuce	N	SFO	2.892	1	little rainfall, irrigation without discernible impact. Influence unlikely.	Ensa-20-8031
14-2029-04 M-534202-01-1	Lettuce	N	SFO	2.034	1	no rainfall until day 6, without discernible impact. Influence unlikely	Ensa-20-8031
18-2086-01-T1 M-675005-01-1	Lettuce	S	SFO	8.248	1	nearly no rain, no influence	Ensa-20-8029
18-2086-01-T2 M-675005-01-1	Lettuce	S	SFO	7.754	1	nearly no rain, no influence	Ensa-20-8029
18-2086-02-T1 M-675005-01-1	Lettuce	S	SFO	3.419	1	nearly no rain, no influence	Ensa-20-8029
18-2086-02-T2 M-675005-01-1	Lettuce	S	SFO	4.04	1	nearly no rain, no influence	Ensa-20-8029
18-2086-03-T1 M-675005-01-1	Lettuce	S	SFO	4.378	1	no rain, no influence	Ensa-20-8029
18-2086-03-T2 M-675005-01-1	Lettuce	S	SFO	4.339	1	no rain, no influence	Ensa-20-8029
18-2086-04-T1 M-675005-01-1	Lettuce	S	SFO	1.186	1	nearly no rain until d7, no influence	Ensa-20-8029
18-2086-04-T2 M-675005-01-1	Lettuce	S	SFO	1.174	1	nearly no rain until d7, no influence	Ensa-20-8029
R 2006 0376/2 M-292050-01-1	Lettuce	S	SFO	1.52	1	little rain, late irrigation, no influence	Ensa-20-8029
R 2006 0608/7 M-292050-01-1	Lettuce	S	SFO	2.57	1	very little rain, no influence	Ensa-20-8029
R 2006 0610/9 M-292050-01-1	Lettuce	S	SFO	3.529	1	late rain, no influence	Ensa-20-8029
R 2006 0611/7 M-292050-01-1	Lettuce	S	SFO	3.02	1	late rain, no influence	Ensa-20-8029
14-2030-01 M-534595-01-1	Lettuce	S	SFO	4.804	1	virtually no rain, no influence	Ensa-20-8031
14-2030-02 M-534595-01-1	Lettuce	S	SFO	5.522	1	no rain, no influence	Ensa-20-8031
14-2185-02 M-536963-01-1	Lettuce	S	SFO	4.578	1	virtually no rain, no influence	Ensa-20-8031
14-2185-03 M-536963-01-1	Lettuce	S	SFO	4.78	1	no rain until day 9, no influence	Ensa-20-8031
R 2007 0568/9 M-302325-01-1	Onion	S	SFO	4.203	1	No rainfall and no influence from irrigation day 10	EnSa-20-0832
18-2951-02 M-678413-01-1	Young cereals	N	SFO	3.214	1	very little rain, no influence	EnSa-20-0834
18-2951-03 M-678413-01-1	Young cereals	N	SFO	3.523	1	no rain, no influence	EnSa-20-0834
E19RP102-01 M-758824-01-1	Young cereals	N	SFO	6.419	1	no rain, no influence	EnSa-20-0834
E19RP102-02 M-758824-01-1	Young cereals	N	SFO	8.185	1	no rain, no influence	EnSa-20-0834
15-2952-01 M-566830-01-1	Young cereals	N	SFO	3.37	1	rain only late, no influence	EnSa-17-0484

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
15-2952-02 M-566830-01-1	Young cereals	N	SFO	7.59	1	no rain, no influence	EnSa-17-0484
18-2954-03 M-675129-02-1	Young cereals	S	SFO	3.607	1	no rain, no influence	EnSa-20-0834
E19RP087-01 M-758649-01-1	Young cereals	S	SFO	10.18	1	no rain, no influence	EnSa-20-0834
E19RP087-02 M-758649-01-1	Young cereals	S	SFO	1.782	1	rain d4 and d5 but no discernible influence	EnSa-20-0834
15-2952-04 M-566830-01-1	Young cereals	S	SFO	4.19	1	very little rain, no influence	EnSa-17-0484
15-2953-03 M-566828-01-1	Young cereals	S	SFO	4.64	1	no rain, no influence	EnSa-17-0484
R 2006 0377/0 M-290825-01-1	Beans	N	SFO	4.674	2	late rain, influence possible	Ensa-20-8029
R 2006 0656/7 M-290825-01-1	Beans	N	SFO	2.84	2	frequent rainfall, slight influence possible	Ensa-20-8029
R 2007 0546/8 M-297562-01-1	Beans	N	SFO	2.969	2	frequent but little rain, influence possible	Ensa-20-8030
R 2007 0547/6 M-297562-01-1	Beans	N	SFO	2.744	2	late irrigation and rainfall, possibly slight influence	Ensa-20-8030
R 2007 0548/4 M-297562-01-1	Beans	N	HS	3.639	2	little early and more rain on day 6, no marked influence	Ensa-20-8030
R 2006 0347/9 M-292103-01-1	Cabbage	N	HS	4.729	2	frequent rainfall but in small amounts which are unlikely to have markedly influenced residue levels.	EnSa-20-0832
R 2006 0543/9 M-292103-01-1	Cabbage	N	SFO	5.78	2	Little rainfall until day 8, no influence discernible	EnSa-20-0832
R 2006 0348/7 M-293182-01-1	Cabbage	S	FOMC	3.693	2	Little rainfall until day 8, no influence discernible	EnSa-20-0832
R 2007 0079/2 M-302044-01-1	Cabbage	S	FOMC	4.084	2	marked decline until 2nd sampling but little early rain until day 7 (influence questionable)	EnSa-20-0832
R 2007 0600/6 M-302044-01-1	Cabbage	S	SFO	3.981	2	Moderate early rainfall but no marked decline (influence unlikely)	EnSa-20-0832
10-2099-01 M-423901-01-1	Endive	N	SFO	2.252	2	frequent heavy rainfall, influence not discernible but likely	Ensa-20-8029
R 2006 0343/6 M-292101-02-1	Leek	N	SFO	8.282	2	frequent rainfall after day 5 did not seem to have any discernible influence on residue dissipation	EnSa-20-0832
R 2006 0466/1 M-292101-02-1	Leek	N	SFO	5.836	2	frequent rainfall after day 7 did not seem to have any discernible influence	EnSa-20-0832
R 2006 0468/8 M-292101-02-1	Leek	N	SFO	8.99	2	Frequent late rainfall and heavy irrigation coincide with a moderate drop of residue levels on day 15	EnSa-20-0832
R 2006 0344/4 M-292082-01-1	Leek	S	SFO	6.01	2	rainfall on day 6 and 7 may have slightly influenced residue dissipation	EnSa-20-0832
R 2006 0469/6 M-292082-01-1	Leek	S	SFO	7.054	2	frequent irrigation and occasional rainfall may have	EnSa-20-0832

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
						markedly influenced residue dissipation, although this is not discernible in the decline pattern	
R 2006 0604/4 M-292048-01-1	Lettuce	N	SFO	1.409	2	rain after 75% already declined, at most slight influence	Ensa-20-8029
R 2006 0606/0 M-292048-01-1	Lettuce	N	SFO	2.452	2	some rain after 2nd sampling but no visible influence	Ensa-20-8029
R 2007 0011/3 M-304280-01-1	Lettuce	N	SFO	1.048	2	little rain during first days, influence possible	Ensa-20-8030
R 2007 0537/9 M-304280-01-1	Lettuce	N	FOMC / DFOP	1.949	2	Little but early rain, influence possible	Ensa-20-8030
R 2007 0539/5 M-304280-01-1	Lettuce	N	SFO	2.129	2	Very early rainfall, no influence discernible	Ensa-20-8030
14-2029-01 M-534202-01-1	Lettuce	N	SFO	2.921	2	frequent irrigation and rainfall. Slight influence possible	Ensa-20-8031
14-2029-03 M-534202-01-1	Lettuce	N	SFO	1.682	2	little rainfall until day 6 (8 mm). Slight influence possible.	Ensa-20-8031
14-2029-05 M-534202-01-1	Lettuce	N	SFO	1.8	2	several rainfalls without discernible impact, slight influence cannot be excluded	Ensa-20-8031
14-2184-02 M-536965-01-1	Lettuce	N	SFO	4.71	2	rainfall coincides with slight drop of residue levels. Influence possible.	Ensa-20-8031
14-2184-03 M-536965-01-1	Lettuce	N	SFO	2.406	2	irrigation coincides with slight drop of residue levels. Influence possible	Ensa-20-8031
R 2006 0609/5 M-292050-01-1	Lettuce	S	SFO	0.8444	2	little rain during first days, but influence possible	Ensa-20-8029
R 2007 0012/1 M-304278-01-1	Lettuce	S	SFO	1.813	2	no rain but daily irrigation. Influence likely.	Ensa-20-8030
R 2007 0245/0 M-304278-01-1	Lettuce	S	SFO	1.204	2	little but very early rain after application, influence possible	Ensa-20-8030
R 2007 0541/7 M-304278-01-1	Lettuce	S	SFO	5.797	2	frequent but little rain, influence possible	Ensa-20-8030
14-2030-03 M-534595-01-1	Lettuce	S	SFO	3.233	2	Frequent rainfall and regular sprinkler irrigation. Marked influence not discernible but slight impact likely	Ensa-20-8031
14-2185-04 M-536963-01-1	Lettuce	S	SFO	2.403	2	Several rainfalls around 2nd sampling, no influence discernible	Ensa-20-8031
R 2006 0339/8 M-292098-01-1	Onion	S	SFO	4.448	2	Irrigation coincides with a moderate drop of residue levels, slight influence likely	EnSa-20-0832
R 2007 0555/7 M-298639-01-1	Peas	N	SFO	5.468	2	Only little rainfall but coinciding with residue decline. Influence possible.	Ensa-20-8030
R 2007 0556/5 M-298639-01-1	Peas	N	SFO	7.032	2	many days with little rainfall. Influence possible	Ensa-20-8030
R 2007 0557/3 M-297487-01-1	Peas	S	SFO	3.275	2	early irrigation and rainfall, influence possible	Ensa-20-8030
E19RP102-03 M-758824-01-1	Young cereals	N	SFO	7.01	2	moderate rain d4, slight influence	EnSa-20-0834
E19RP102-04 M-758824-01-1	Young cereals	N	FOMC	4.319	2	moderate rain d2, slight influence	EnSa-20-0834

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
13-2950-01 M-471216-01-1	Young cereals	N	SFO	1.95	2	little early rainfall, very little impact on DT50	EnSa-17-0484
15-2953-02 M-566828-01-1	Young cereals	N	SFO	4.23	2	heavy rain day 5, visible but slight influence	EnSa-17-0484
E19RP087-04 M-758649-01-1	Young cereals	S	SFO	2.956	2	"moderate rain d3, d4; slight influence"	EnSa-20-0834
15-2952-03 M-566830-01-1	Young cereals	S	SFO	2.86	2	no rain before day 5, only slight influence	EnSa-17-0484
08-2034-01 T1 M-365530-01-1	Beans	N	SFO	4.053	3	moderate rain d3, d5, marked influence	Ensa-20-8029
08-2034-02 T2 M-365530-01-1	Beans	N	SFO	3.992	3	moderate rain d3, d5, marked influence	Ensa-20-8029
R 2006 0380/0 M-291180-01-1	Beans	N	SFO	17.32	3	late rain but influence likely	Ensa-20-8029
R 2006 0654/0 M-290825-01-1	Beans	N	SFO	0.7187	3	marked influence by early heavy rain	Ensa-20-8029
R 2007 0014/8 M-297562-01-1	Beans	N	SFO	2.733	3	Heavy rain on days around 2nd sampling, influence likely	Ensa-20-8030
R 2007 0549/2 M-297562-01-1	Beans	N	SFO	3.172	3	marked influence of rainfall days 3 and 4 likely	Ensa-20-8030
08-2096-02 T2 M-365542-01-1	Beans	S	SFO	3.648	3	irrigation d5 and d11, marked influence	Ensa-20-8029
R 2006 0620/6 M-290827-01-1	Beans	S	SFO	0.7254	3	marked influence of early rainfall likely	Ensa-20-8029
R 2007 0035/0 M-297564-01-1	Beans	S	SFO	3.176	3	large rainfall days 4 and 5, influence likely	Ensa-20-8030
R 2007 0078/4 M-302101-01-1	Cabbage	N	SFO	2.062	3	early rainfall coincides with marked drop (influence possible)	EnSa-20-0832
10-2099-02 M-423901-01-1	Endive	N	FOMC	2.659	3	early rainfall, marked decline, influence likely	Ensa-20-8029
10-2099-03 M-423901-01-1	Endive	N	SFO	1.228	3	early rainfall, marked decline, influence likely	Ensa-20-8029
10-2099-04 M-423901-01-1	Endive	N	SFO	1.481	3	early rainfall, marked decline, influence likely	Ensa-20-8029
11-2029-01 M-442996-01-1	Leek	N	SFO	2.279	3	Heavy rainfall coincides with a marked drop of residue levels, influence likely	EnSa-20-0832
11-2029-02 M-442996-01-1	Leek	N	SFO	2.657	3	Heavy rainfall coincides with a marked drop of residue levels, influence likely	EnSa-20-0832
11-2029-03 M-442996-01-1	Leek	N	SFO	2.620	3	Early rainfall coincides with a marked drop of residue levels, influence likely	EnSa-20-0832
11-2029-04 M-442996-01-1	Leek	N	SFO	2.543	3	Early rainfall coincides with a marked drop of residue levels, influence likely	EnSa-20-0832
R 2006 0465/3 M-292101-02-1	Leek	N	SFO	2.346	3	Frequent early rainfall coincides with a marked drop of residue levels, influence likely.	EnSa-20-0832
R 2007 0056/3 M-304288-01-1	Leek	N	DFOP	4.184	3	Early rainfall coincided with a moderate drop (influence likely).	EnSa-20-0832
R 2007 0249/3 M-304276-01-1	Leek	N	HS	3.392	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832

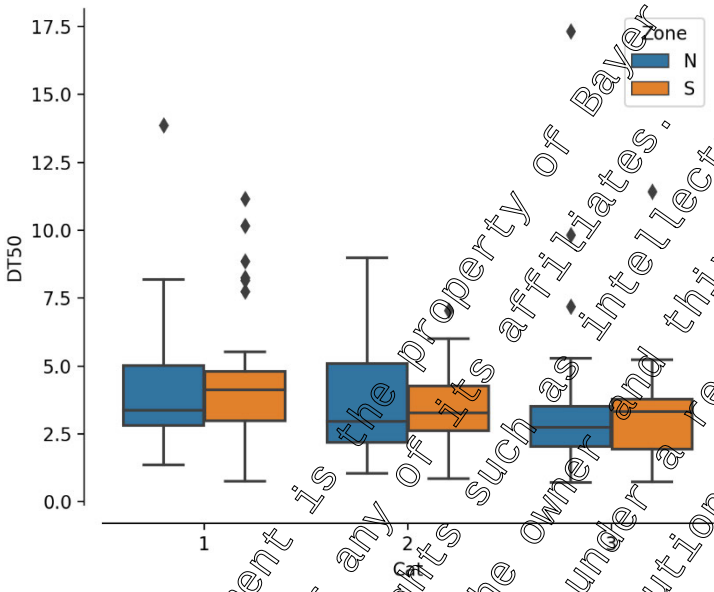
Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
R 2007 0569/7 M-304288-01-1	Leek	N	FOMC	3.551	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832
R 2007 0570/0 M-304288-01-1	Leek	N	FOMC	3.675	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832
R 2007 0571/9 M-304288-01-1	Leek	N	SFO	2.557	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832
R 2007 0573/5 M-304276-01-1	Leek	N	SFO	3.321	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832
R 2007 0574/3 M-304276-01-1	Leek	N	SFO	2.916	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832
R 2007 0057/1 M-302775-01-1	Leek	S	FOMC	5.241	3	early irrigation coincides with marked drop (influence likely	EnSa-20-0832
R 2007 0250/7 M-302780-01-1	Leek	S	HS	11.434	3	early irrigation coincides with moderate drop (influence likely)	EnSa-20-0832
R 2007 0572/7 M-302775-01-1	Leek	S	SFO	1.952	3	early irrigation coincides with marked drop (influence likely)	EnSa-20-0832
R 2006 0375/4 M-292048-01-1	Lettuce	N	HS	2.198	3	early rainfall and sprinkler irrigation, marked influence	Ensa-20-8029
R 2006 0607/9 M-292048-01-1	Lettuce	N	SFO	1.129	3	marked influence by sprinkler irrigation	Ensa-20-8029
R 2007 0538/7 M-304280-01-1	Lettuce	N	SFO	0.7255	3	marked influence of early rainfall	Ensa-20-8030
14-2184-01 M-536965-01-1	Lettuce	N	SFO	1.095	3	Early rainfall coincides with marked drop of residue levels. Influence likely	Ensa-20-8031
14-2184-04 M-536965-01-1	Lettuce	N	SFO	1.592	3	frequent early rainfall may have markedly influenced residue levels	Ensa-20-8031
R 2007 0246/9 M-304278-01-1	Lettuce	S	SFO	0.8952	3	early rain and irrigation, influence likely	Ensa-20-8030
14-2030-04 M-534595-01-1	Lettuce	S	FOMC	1.918	3	Early heavy rainfall, marked influence likely	Ensa-20-8031
14-2030-05 M-534595-01-1	Lettuce	S	SFO	3.779	3	Heavy rainfall before 3rd sampling, marked influence likely	Ensa-20-8031
14-2185-01 M-536963-01-1	Lettuce	S	SFO	1.057	3	early rainfall and irrigation, marked influence likely.	Ensa-20-8031
R 2006 0337/1 M-292996-01-1	Onion	N	FOMC	7.184	3	Irrigation and rainfall coincide with a moderate drop of residue levels, influence likely	EnSa-20-0832
R 2006 0504/8 M-292996-01-1	Onion	N	SFO	4.91	3	Irrigation coincides with moderate drops of residue levels, influence likely.	EnSa-20-0832
R 2007 0567/0 M-302330-01-1	Onion	N	SFO	2.992	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832
R 2006 0505/6 M-292098-01-1	Onion	S	SFO	3.282	3	Irrigation coincides with moderate drops of residue levels, influence likely.	EnSa-20-0832
R 2007 0043/1 M-302325-01-1	Onion	S	FOMC	4.584	3	Likely marked influence from irrigation at day 3	EnSa-20-0832
R 2007 0036/9 M-298639-01-1	Peas	N	SFO	5.287	3	large rainfall days 4 and 5, influence likely	Ensa-20-8030
R 2007 0553/0 M-298639-01-1	Peas	N	HS	3.401	3	Rain on day 2, influence likely	Ensa-20-8030

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
R 2007 0554/9 M-298639-01-1	Peas	N	FOMC	9.837	3	Large rainfall on days 2 and 3, influence likely	Ensa-20-8030
15-2030-01 M-566823-03-1	Peas	N	SFO	3.346	3	Heavy rainfall coincides with a marked drop in residue levels, impact likely	Ensa-20-8031
R 2007 0037/7 M-297487-01-1	Peas	S	SFO	3.329	3	Large rainfall on day 3, influence likely.	Ensa-20-8030
15-2030-04 M-566823-03-1	Peas	S	SFO	2.928	3	Rainfall on days 3 and 4 coincides with a drop in residue levels, influence likely.	Ensa-20-8031
18-2951-01 M-678413-01-1	Young cereals	N	SFO	2.747	3	early rain, marked decline	EnSa-20-0834
13-2950-02 M-471216-01-1	Young cereals	N	HS	2.03	3	rainfall day0, marked decline	EnSa-17-0484
13-2950-03 M-471216-01-1	Young cereals	N	HS	1.2	3	early rainfall, marked decline	EnSa-17-0484
13-2950-04 M-471216-01-1	Young cereals	N	SFO	1.25	3	early rainfall, marked decline	EnSa-17-0484
15-2953-01 M-566828-01-1	Young cereals	N	HS	3.48	3	early rain, marked decline	EnSa-17-0484
18-2954-01 M-675129-02-1	Young cereals	S	SFO	4.201	3	heavy rain d4, marked decline	EnSa-20-0834
18-2954-02 M-675129-02-1	Young cereals	S	SFO	3.599	3	heavy rain d4, marked decline	EnSa-20-0834
E19RP087-03 M-758649-01-1	Young cereals	S	SFO	3.415	3	heavy rain d3, marked decline	EnSa-20-0834

Influence of the residue zone on foliage DT_{50}

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 DT_{50}s from trials conducted in the Northern EU residue zone with the DT_{50} values from trials conducted in the Southern EU residue zone.



Influence of metabolite fluopyram-benzamide on the DT_{50} in foliage

In a part of the residue trials evaluated here for the purpose 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 toxicological assessment for plant material.

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

	Residue definition		Reference
Food of plant origin	Monitoring	fluopyram (parent only)	EFSA Scientific Report EFSA Journal 2013;11(4):3052
	Risk assessment	fluopyram and fluopyram-benzamide (M25) expressed as fluopyram	

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.

Kinetic evaluation report	Matrix	# of trials with analysis for BNZ	Geomean DT_{50} FLU	Geomean DT_{50} FLU+BNZ	Difference in DT_{50}
EnSa-20-0829	Vegetables	37	2.765	2.857	~ 3%
EnSa-20-0830	Vegetables	26	2.845	2.926	~ 3%
EnSa-20-0831	Vegetables	20	2.673	2.697	~ 1%
EnSa-20-0832	Vegetables	35	3.921	4.144	~ 5%
EnSa-20-0834	Young cereals	8	4.820	4.821	~ 1%

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 outcomes at screening or tier 1 level.

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 drift is 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 tier 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.

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

The non-target-terrestrial-plant off-field risk assessments resulted in acceptable outcomes 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+TFS 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 fluopyram and the representative lead formulation, the applicant does not foresee 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; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA" (EFSA Journal 2009; 7(12):1438. doi:10.2903/j.efsa.2009.1438), referred to in the following as "EFSA GD 2009".

CP 10.1.1 Effects on birds

Table 10.1.1- 1: Studies for fluopyram and endpoints used in the risk assessment for birds

Test substance	Test design	Test species	Endpoint		Reference
Fluopyram tech.	Acute oral toxicity	Bobwhite quail (*Colinus virginianus*)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2011) M-263049-04-1 KCA 8.1.1.1/01
	Acute oral toxicity	Zebra finch (*Taeniopygia guttata*)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2008) M-305871-02-1 KCA 8.1.1.1/02
			LD_{50}	= 3036 mg a.s./kg bw [A]	Extrapolated acc. to chapter 2.1.2 of EFSA Journal 2009; 7(12):1438
	Acute oral toxicity	Chicken (*Gallus domesticus*)	LD_{50}	> 5000 mg a.s./kg bw	[redacted] (2011) M-446344-01-1 KCA 8.1.1.1/03
	Dietary toxicity (short-term)	Bobwhite quail (*Colinus virginianus*)	LC_{50} LD_{50}	> 5000 mg a.s./kg feed > 1415.4 mg a.s./kg bw/d	[redacted] (2007) M-264902-02-1 KCA 8.1.1.2/01
	Dietary toxicity (short-term)	Mallard duck (*Anas platyrhynchos*)	LC_{50} LD_{50}	> 5000 mg a.s./kg feed > 1643 mg a.s./kg bw/d	[redacted] (2005) M-262710-01-1 KCA 8.1.1.2/02
	20-weeks feeding chronic, reproduction	Bobwhite quail (*Colinus virginianus*)	NOEC NOED	< 250 mg a.s./kg feed < 23 mg a.s./kg bw/d	[redacted] (2008) M-299245-02-1 KCA 8.1.1.3/01
	22-weeks feeding chronic, reproduction	Bobwhite quail (*Colinus virginianus*)	LOAEC NOAED NOEC NOED	80 mg a.s./kg feed 7.2 mg a.s./kg bw/d 50 mg a.s./kg feed 4.5 mg a.s./kg bw/d	[redacted] (2008) M-298723-01-1 KCA 8.1.1.3/02
	19-weeks feeding chronic, reproduction	Mallard duck (*Anas platyrhynchos*)	NOEC NOED NOEC NOED	500 mg a.s./kg feed 40 mg a.s./kg bw/d 200 mg a.s./kg kg feed 18 mg a.s./kg bw/d	[redacted] (2008) M-299277-01-1 KCA 8.1.1.3/03 DAR
	Chronic, reproduction: EC_{10} calculation	Bobwhite quail (*Colinus virginianus*) – both chronic studies combined	Lowest EC_{10}	7.8 mg a.s./kg bw/d (14-day survivors per eggs set)	[redacted] (2019) M-667209-01-1 KCA 8.1.1.3/04

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)	[redacted] (2019) M-667211-01-1 KCA 8.1.1.3/05
FLU+TFS SC 500	Acute oral toxicity	Japanese quail (*Coturnix japonica*)	LD_{50}	> 2000 mg prod./kg bw	[redacted] (2012) M-442023-01-1 KCA 8.1.1.1/08
			LD_{50}	= 3228 mg prod./kg bw [B]	Extrapolated acc. to chapter 2.1.2 of EFSA Journal 2009; 7(12):1438
Fluo-pyram + Trifloxy-strobin	Acute	Bobwhite quail	LD_{50} (mix)	> 2000 mg total a.s./kg bw	See Table 10.1.1-6

Note:
Studies referring to KCA are filed in the dossier for the active substance
Studies written in grey type are referring to studies in the corresponding Baseline-dossier, whereas studies in black type are studies of the Supplemental dossier
a.s. = active substance, prod. = product
[A] Factor 1.518 for 10 birds/dose level with a single mortality (study result: 12 individuals and 1 mortality)
[B] Factor 1.614 for 5 birds/dose level for no mortality

Table 10.1.1- 2: Relevant indicator species for screening risk assessment

Crop	Indicator species	Shortcut value (SV)	
		Acute RA based on RUD_{90}	Long-term RA based on RUD_m
Vineyards (grapes)	Small omnivorous bird	95.3	38.9

Table 10.1.1- 3: Relevant generic focal species for first-tier risk assessment

Crop	Generic focal species	Shortcut value (SV)	
		Acute RA based on RUD_{90}	Long-term RA based on RUD_m
Vineyards (grapes) BBCH 53-73	Small insectivorous species "redstart" BBCH ≥ 20	Not needed	9.9
	Small granivorous bird "finch" BBCH ≥ 40	Not needed	3.4
	Small omnivorous bird "lark" BBCH ≥ 40	Not needed	3.3

<u>Greenhouse use in lettuce</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, 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)

Crop	Indicator species	DDD			DDD	LD_{50} [mg a.s./ kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
Vineyards (grapes)	Small omnivorous bird	0.050	95.3	1.4	6.67	> 2000	> 300	10

Table 10.1.1- 5: Screening acute risk assessment for birds (FLU+TFS SC 500)

Crop	Indicator species	DDD			DDD	$LD_{50\,mix}$ [mg total a.s./ kg bw]	TER_A	Trigger
		Appl. rate [kg total a.s./ha]	SV_{90}	MAF_{90}				
Vineyards (grapes)	Small omnivorous bird	0.100	95.3	1.4	13.3	> 2000	> 150	10

For fluopyram and the calculated $LD_{50\,mix}$ of FLU+TFS SC 500 the TER_A values are above the trigger of 10. Therefore, a Tier 1 risk assessment is not required.

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 acute effects (mortality), a surrogate LD_{50} (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 LD_{50} (mix) for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

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

$LD_{50}\,(a.s._i)$ = Acute toxicity for the active substance (i)

The 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 LD_{50} (mix) of fluopyram and trifloxystrobin when mixed 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.

Table 10.1.1- 6: Avian LD_{50} (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
Fraction in the a.s. mixture	0.50	0.50
LD_{50} of a.s. [mg a.s./kg bw]	> 2000	> 2000 [A]
Fraction / LD_{50}	< 0.00025	< 0.00025
Sum	< 0.00050	
1/sum = predicted LD_{50} (mix) [mg total a.s./kg bw]	> 2000	

[A] LD_{50} for Bobwhite quail 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 10.1.1- 7: Avian "tox per fraction" for FLU+TFS SC 500 (step 1 in Appendix B of EFSA GD 2009)

	Fluopyram	Trifloxystrobin	"Mix"
Content of a.s. in product [g a.s./L prod.]	250	250	500
Fraction in the a.s. mixture	0.5	0.5	1
LD_{50} of a.s. [mg a.s./kg bw]	> 2000	> 2000 [A]	> 2000
Tox per fraction	> 4000	> 4000	> 8000
Contribution to predicted toxicity	50 %	50 %	100 %

[A] LD_{50} for Bobwhite quail given in EFSA Journal 2017;15(10):4989

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

According to EFSA GD 2009 the following equation should be used for the comparison:

$$\sum_{i} \frac{X(a.s._i)}{LD_{50}(a.s._i)} = \frac{1}{LD_{50}(mix)}$$

With:

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

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

$LD_{50}(mix)$ = 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 LD_{50} 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 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 unambiguously ascribed to a factor that would not be relevant under environmental exposure conditions.

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

Left side of the equation: $\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} = \frac{0.5}{> 2000 \frac{mg\ a.s.}{kg\ bw}} + \frac{0.5}{> 2000 \frac{mg\ a.s.}{kg\ bw}} = < 0.0005$

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

< 0.0005 appears lower than < 0.001

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 fluopyram and trifloxystrobin. There are only unbound LD50 values for the active substances (> 2000 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 screening level risk assessment (please refer to Table 10.1.1- 5).

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

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

With a K(f)oc of 232.1 L/kg, fluopyram belongs to the group of less sorptive substances.

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

Crop	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 concern
Vineyards (grapes) 2 × 0.100 kg total a.s./ha	FLU+TFS SC 500	232.1 [C]	200 [D]	> 1000	0.200	≤ 50	No concern

[A] K_{OC} value given in MCP 9.2.4.1 (Table 9.2.4- 1)
[B] MAF_m = 2.0, calculated for 2 applications with a 7-day interval considering the DT_{50} in soil of 298.1 days given in MCP 9.2.4.1 (Table 9.2.4- 1)
[C] K_{oc} of fluopyram
[D] MAF_m calculated considering the DT_{50} in soil of 298.1 days for fluopyram

According to the EFSA 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 + trifloxystrobin. Therefore, the acute risk for birds from drinking water that may contain residues from fluopyram and trifloxystrobin is acceptable.

LONG-TERM REPRODUCTIVE RISK 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.

Screening step

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

Crop	Indicator species	DDD				DDD	NOEL [mg a.s./kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
Vineyards (grapes)	Small omnivorous bird	0.050	38.9	1.6	0.53	1.65	4.5	**2.73**	5

The screening level TER_{LT} value is below the trigger of 5. Therefore, a Tier 1 risk assessment is required.

Tier 1

Table 10.1.1- 10: First-tier long-term reproductive risk assessment for birds (fluopyram)

Crop	Generic focal species	DDD				DDD	NOEL [mg a.s./ kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
Vineyards (grapes) BBCH 53-73	Small insectivorous species "redstart" BBCH ≥ 20	0.05	9.9	1.6	0.53	0.420	4.5	10.7	5
	Small granivorous bird "finch" BBCH ≥ 40	0.05	3.4	1.6	0.53	0.144	4.5	31.3	5
	Small omnivorous bird "lark" BBCH ≥ 40	0.05	2.3	1.6	0.53	0.140	4.5	32.2	5

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

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

Table 10.1.1- 11: Evaluation of potential concern for exposure of birds from drinking water (long-term, escape clause)

Crop	Compound	Koc [L/kg]	AR_{eff} (Appl. rate × MAF_m) [g a.s./ha]	NO(A)EL [mg a.s./ kg bw/d]	Ratio (AR_{eff}/ NOED)	"Escape clause" No concern if ratio	Conclusion
Vineyards (grapes)	Fluopyram	232 [A]	100 [B]	4.5	22.2	≤ 50	No concern

[A] K_{OC} value given in MCP 9.2.4.1 (Table 9.2.4- 1)

[B] MAF_m = 2.0, calculated for 2 applications with a 7-day interval considering the DT_{50} 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 necessary when the ratio of effective application rate (in 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 (Koc > 500 L/kg).*" This is the case for fluopyram. Therefore, the long-term risk for birds from drinking water that may contain residues from fluopyram is acceptable.

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 fluopyram

Substance	Log Pow	Compartment	Reference
Fluopyram	3.3 (20 °C)	Soil, surface water	[redacted] (2006) M-280089-01-1 MCA, 2.7

The log P_{OW} value of fluopyram is 3.3 and thus, effects on secondary poisoning have been assessed.

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

Generic avian indicator species	Body weight (g)	Example	FIR/bw
Earthworm eater	100	Thrush	1.05
Fish eater	1000	Heron	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 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 10.1.1- 14: Tier 1 long-term DDD and TER calculation for earthworm-eating birds in vineyards

	Fluopyram	
	Tier 1	**Refinement**
K_{OW}	2060	2060
K_{OC} [mL/g]	232.1 [A]	232.1 [A]
f_{OC}	0.02	0.02
BCF_{worm}	5.51	0.85 [C]
$PEC_{soil, accu}$ (mg/kg)	0.238 [B]	0.238 [B]
PEC_{worm} (mg/kg)	1.31	0.202
FIR/bw	1.05	1.05
DDD (mg/kg bw/d)	1.38	0.212
NO(A)EL (mg/kg bw/d)	4.5	4.5
TER_{LT}	**3.3**	21.2
Trigger	5	5

[A] K_{OC} value given in MCP 9.2.4.1 (Table 9.2.4- 1)

[B] $PEC_{soil, accu}$ value given in MCP 9.1.3, Table 9.1.3- 3 (vines, 2 × 30 g a.s./ha): 21-day-TWA of 0.053 mg a.s./kg + plateau concentration (5 cm) of 0.185 mg a.s./kg

[C] Measured BCF resulting from a bioaccumulation study in earthworms, please refer to MCA 8.1.3

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 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.

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

	Fluopyram
BCF_{fish}	16 [A]
FOCUS Step 2 PEC_{SW} (twa, 21 d) (mg/L)	0.00576 [B]
PEC_{fish} (mg/kg)	0.092
FIR/bw	0.159
DDD (mg/kg bw/d)	0.0147
NO(A)EL (mg/kg bw/d)	4.5
TER_{LT}	307
Trigger	5

[A] Measured BCF resulting from a bioconcentration study in fish, please refer to MCA 8.2.2.3

[B] 21 d twa PEC_{sw} value given in MCP 9.2.5, Table 9.2.5- 6 (vines, 2 × 50 g a.s./ha), FOCUS Step 2, Southern Europe, spring application scenario as worst case

Table 10.1.1- 16: Tier 1 long-term DDD and TER calculation for fish-eating birds in lettuce (greenhouse use)

	Fluopyram
BCF_{fish}	16 [A]
GEM PEC_{SW} (mg/L)	0.04359 [B]
PEC_{fish} (mg/kg)	0.697
FIR/bw	0.159
DDD (mg/kg bw/d)	0.111
NO(A)EL (mg/kg bw/d)	4.5
TER_{LT}	40.6
Trigger	5

[A] Measured BCF resulting from a bioconcentration study in fish, please refer to MCA 8.2.2.3

[B] PEC_{sw} value given in MCP 9.2.5, Table 9.2.5- 25 (lettuce, 2 × 200 g a.s./ha) calculated with exposure assessment model GEM, soilless cultivation with reuse of filter cleaning water (0% mitigation), application dates 22.04. + 29.04. as worst case

The TER values for fluopyram are above the trigger of concern of 5, indicating no risk from secondary poisoning for earthworm- and fish-eating birds.

CP 10.1.1.1 Acute oral toxicity

Data Point:	KCP 10.1.1.1/01
Report Author:	[redacted]
Report Year:	2012
Report Title:	Acute oral toxicity of fluopyram & trifloxystrobin SC 500 (250 + 250 g/L) to Japanese quail (Coturnix japonica) (according to OECD 223)
Report No:	EnSa-12-0495
Document No:	M-442023-01-1
Guideline(s) followed in study:	OECD-Guideline for the Testing of Chemicals No. 223; US EPA OCSPP not applicable; Regulation (EC) No 1107/2009 of the European Parliament and of the Council
Deviations from current test guideline:	Current Guideline: OECD 223 (2016) Deviations: The photoperiod was 12 hours light, above the 8 hours light as 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 recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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

Executive summary

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 held 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 necropsies were performed on all survivors at test termination.

The study fulfilled all validity criteria of OECD 223 guideline.

There were no mortalities in the control group and in 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 comparable to those of the control animals. Two female and two male birds 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. At sacrifice, one female bird of the dosed group had yellowish discoloured lower part of the esophagus and stomach contents, probably triggered by reflux of bile fluid. 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.

I. MATERIAL AND METHODS

Test item: FLU + TFS SC 500 (250+250 g/L), specification No.: 102000012886 – 03; origin Batch ID.: 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 dosed 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 cage had floor space that measured approximately 38 × 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 recorded at day -1 prior to dosing, on study days 3, 7, 14 and at test termination (day 21). Food consumption was determined by pen for each dosage group and control group daily until day 3, then 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_{50} calculation was not necessary.

Dates of experimental work: September 11th to October 16th, 2012.

II. RESULTS AND DISCUSSION

Validity criteria:

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

Validity criteria	Required	Obtained
Control mortality	≤ 10 %	0 %

Mortality and clinical observations:

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.

Table 10.1.1.1- 2: Summary of mortalities and clinical symptoms

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

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 fourth day onwards, the body weights were comparable to those of the control animals.

Table 10.1.1.1- 3: Mean body weight of surviving birds

Treatment level [mg product/kg bw]	Mean body weight [g]				
	Day -1	Day 3	Day 7	Day 14	Day 21
Control	209	216	219	220	222
2000	218	212	218	222	221

Day-1: 1 day prior to dosing.
Day 0: Day of oral administration.

Two female and two male birds 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 10.1.1.1- 4: Mean feed consumption

Treatment level [mg product/ kg bw]	Mean food consumption [g/bird/day]					
	Day 0 - 1	Day 1 - 2	Day 2 - 3	Day 3 - 7	Day 7 - 14	Day 14 - 21
Control	26.3	26.0	21.7	24.7	23.2	23.7
2000	13.4	21.8	23.3	26.0	24.2	22.9

Gross pathology:

At sacrifice, only one female 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 500 (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]	≥ 2000

III. CONCLUSION

Based on this study the LD_{50} for Japanese Quail exposed to FLU + TFS SC 500 (250+250 g/L) was determined to be > 2000 mg product/kg bw. The NOED was ≥ 2000 mg product/kg bw.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is:

LD_{50} > 2000 mg product/kg bw. Based on zero mortalities among 5 dosed birds, the LD_{50} can be extrapolated to 3228 mg a.s./kg bw.

CP 10.1.1.2 Higher tier data on birds

Insect and foliage residue decline studies and kinetic evaluations to generate a DT_{50} for higher tier risk assessment on birds and mammals are in the MCA point 8.9.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

Table 10.1.2- 1: Endpoints used in the risk assessment for mammals

Test substance	Test design	Test species	Endpoint		Reference
Fluopyram	Acute oral	Rat	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2005) M-259398-01-1 KCA 5.2.1/01
	Two-generation study	Rat	NOAEL	= 14.5 mg a.s./kg bw/d	[redacted] (2008) M-299334-01-1 KCA 5.6.1/02
FLU+TFS SC 500	Acute oral	Rat	LD_{50}	> 2000 mg prod./kg bw > 1000 mg total a.s./kg bw [A]	[redacted] (2007) M-287410-01-1 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

[A] Considering 25% fluopyram and 25% trifloxystrobin within the product

Table 10.1.2- 2: Relevant indicator species for screening risk assessment

Crop	Indicator species	Shortcut value (SV)	
		Acute RA based on RUD_{90}	Long-term RA based on RUD_m
Vineyards (grapes)	Small herbivorous mammal	136.4	72.3

Table 10.1.2- 3: Relevant generic focal species for first-tier risk assessment

Crop	Generic focal species	Shortcut value (SV)	
		Acute RA based on RUD_{90}	Long-term RA based on RUD_m
Vineyards (grapes) BBCH 53-73	Large herbivorous mammal "lagomorph" BBCH ≥ 40	Not needed	3.3
	Small insectivorous mammal "shrew" BBCH ≥ 20	Not needed	4.9
	Small herbivorous mammal "vole" BBCH ≥ 40	Not needed	21.7
	Small omnivorous mammal "mouse" BBCH ≥ 40	Not needed	2.3

Greenhouse use in lettuce

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 drinking water risk assessment for the soil-less cultivation use in lettuce is not considered below.

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.2- 4: Screening acute risk assessment for mammals (fluopyram)

Crop	Indicator species	DDD			DDD	LD_{50} [mg a.s./ kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
Vineyards (grapes)	Small herbivorous mammal	0.050	136.4	1.4	9.55	> 2000	> 209	10

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

Crop	Indicator species	DDD			DDD	LD_{50} (product) [mg total a.s./ kg bw]	TER_A	Trigger
		Appl. rate [kg total a.s./ha]	SV_{90}	MAF_{90}				
Vineyards (grapes)	Small herbivorous mammal	0.100	136.4	1.4	19.1	> 2857	> 150	10

The TER_A values for fluopyram and the product FLU+TFS SC 500 are above the trigger of 10. Therefore, a Tier 1 risk assessment is not required.

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 acute effects (mortality), a surrogate LD_{50} (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 LD_{50} (mix) for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

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

LD_{50} ($a.s._i$) = Acute toxicity for the active substance (i)

The 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 LD_{50} (mix) of fluopyram and trifloxystrobin when mixed in these proportions (step 1 in Appendix B of EFSA GD 2009).

Table 10.1.2- 6: Mammalian LD_{50} (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
Fraction in the a.s. mixture	0.50	0.50
LD_{50} of a.s. [mg a.s./kg bw]	> 2000	> 5000 [A]
Fraction / LD_{50}	< 0.00025	< 0.0001
Sum	< 0.00035	
1/sum = predicted LD_{50} (mix) [mg total a.s./kg bw]	> 2857	

[A] LD_{50} 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 500 (step 1 in Appendix B of EFSA GD 2009)

	Fluopyram	Trifloxystrobin	"Mix"
Content of a.s. in product [g a.s./L prod.]	250	250	500
Fraction in the a.s. mixture	0.5	0.5	1
LD_{50} of a.s. [mg a.s./kg bw]	> 2000	> 5000	> 2857
Tox per fraction	> 4000	> 10000	> 14000
Contribution to predicted toxicity	71.4 %	28.6 %	100 %

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

According to EFSA GD 2009 the following equation should be used for the comparison:

$$\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._i)} = \frac{1}{LD_{50}(mix)}$$

With:

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

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

LD_{50} (mix) = 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 LD_{50} 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 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 unambiguously ascribed to a factor that would not be relevant under environmental exposure conditions.

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

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

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

< 0.00035 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 fluopyram and trifloxystrobin. There are only unbound 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 screening level risk assessment (please refer to Table 10.1.1- 5).

Acute risk assessment for mammals drinking contaminated water from puddles

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

With a K(f)oc of 232.1 L/kg, fluopyram belongs to the group of less sorptive substances.

Table 10.1.2- 8: Evaluation of potential concern for exposure of mammals from drinking water (acute, escape clause)

Crop	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 concern
Vineyards (grapes) 2 × 0.100 kg total a.s./ha	Fluopyram + Trifloxystrobin	232.1 [C]	200 [D]	> 1000	< 0.200	≤ 50	No concern

[A] K_{OC} value given in MCP 9.2.4.1 (Table 9.2.4- 1)
[B] MAF_m = 2.0, calculated for 2 applications with a 7-day interval considering the DT_{50} in soil of 298.1 days given in MCP 9.2.4.1 (Table 9.2.4- 1)
[C] K_{oc} of fluopyram
[D] MAF_m calculated considering the DT_{50} in soil of 298.1 days for fluopyram

According to the EFSA 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 reproductive risk assessment for mammals

Crop	Indicator species	DDD				DDD	NOAEL [mg a.s./kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
Vineyards (grapes)	Small herbivorous mammal	0.050	72.3	1.6	0.53	3.07	14.5	**4.73**	5

The screening level TER_{LT} value is below the trigger of 5. Therefore, a Tier 1 risk assessment is required.

Tier 1

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

Crop	Generic focal species	DDD				DDD	NOEL [mg a.s./ kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
Vineyards (grapes) BBCH 53-73	Large herbivorous mammal "lagomorph" BBCH ≥ 40	0.050	3.3	1.6	0.53	0.140	14.5	104	5
	Small insectivorous mammal "shrew" BBCH ≥ 20	0.050	1.9	1.6	0.53	0.081	14.5	180	5
	Small herbivorous mammal "vole" BBCH ≥ 40	0.050	21.7	1.6	0.53	0.920	14.5	15.8	5
	Small omnivorous mammal "mouse" BBCH ≥ 40	0.050	2.3	1.6	0.53	0.098	14.5	149	5

The Tier 1 TER_{LT} values 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 (long-term, escape clause)

Crop	Compound	Koc [L/kg]	AReff (Appl. rate × MAFm) [g a.s./ha]	NOAEL [mg a.s./ kg bw/d]	Ratio (AReff/ NOAEL)	"Escape clause" No concern if ratio	Conclusion
Vineyards (grapes)	Fluopyram	232.1 [A]	100 [B]	14.5	6.90	≤ 50	No concern

[A] K_{OC} value given in MCP 9.2.4.1 (Table 9.2.4- 1)
[B] MAF_m = 2.0, calculated for 2 applications with a 7-day interval considering the DT_{50} 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 necessary when the ratio of effective application rate (in 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 (Koc > 500 L/kg)*." This is the case for fluopyram. Therefore, the long-term risk for mammals from drinking water that may contain residues from fluopyram is acceptable.

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 10.1.2- 12: Log Pow value of fluopyram

Substance	Log Pow	Compartment	Reference
Fluopyram	3.3 (20 °C)	Soil, surface water	[redacted] (2006) M-280089-01-1 MCA, 2.7

The log P_{OW} value of fluopyram is 3.3 and thus, effects on secondary poisoning have been assessed.

Table 10.1.2- 13: Mammalian generic focal species for the Tier 1 risk assessment of secondary poisoning

Generic mammalian indicator species	Body weight (g)	Example	FIR/bw
Earthworm eater	100	Common shrew	1.28
Fish eater	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 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 10.1.2- 14: Tier 1 long-term DDD and TER calculation for earthworm-eating mammals in vineyards

	Fluopyram	
	Tier 1	**Refinement**
K_{OW}	2060	2060
K_{OC} [mL/g]	232.1	232.1 [A]
f_{OC}	0.02	0.02
BCF_{worm}	0.51	0.85 [C]
$PEC_{soil, accu}$ (mg/kg)	0.238 [B]	0.238 [B]
PEC_{worm} (mg/kg)	1.31	0.202
FIR/bw	1.28	1.28
DDD (mg/kg bw/d)	1.68	0.259
NO(A)EL (mg/kg bw/d)	14.5	14.5
TER_{LT}	8.6	56.0
Trigger	5	5

[A] K_{OC} value given in MCP 9.2.4.1 (Table 9.2.4- 1)

[B] $PEC_{soil, accu}$ value given in MCP 9.1.3 (Table 9.1.3- 3 (vines, 2 x 50 g a.s./ha): 21-day-TWA of 0.053 mg a.s./kg + plateau concentration (5 cm) of 0.185 mg a.s./kg

[C] Measured BCF resulting from a bioaccumulation study in earthworms, please refer to MCA 8.1.3

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 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.

Table 10.1.2- 15: Tier 1 long-term DDD and TER calculation for fish-eating mammals in vineyards

	Fluopyram
BCF_{fish}	16 [A]
FOCUS Step 2 PEC_{SW} (twa, 21 d) (mg/L)	0.00576 [B]
PEC_{fish} (mg/kg)	0.092
FIR/bw	0.142
DDD (mg/kg bw/d)	0.0131
NO(A)EL (mg/kg bw/d)	14.5
TER_{LT}	1108
Trigger	5

[A] Measured BCF resulting from a bioconcentration study in fish, please refer to MCA 8.2.2.3

[B] 21 d twa PEC_{sw} value given in MCP 9.2.5, Table 9.2.5- 6 (vines, 2 × 50 g a.s./ha), FOCUS Step 2, Southern Europe, spring application scenario as worst case

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

	Fluopyram
BCF_{fish}	16 [A]
GEM PEC_{SW} (mg/L)	0.04359 [B]
PEC_{fish} (mg/kg)	0.697
FIR/bw	0.142
DDD (mg/kg bw/d)	0.099
NO(A)EL (mg/kg bw/d)	14.5
TER_{LT}	146
Trigger	5

[A] Measured BCF resulting from a bioconcentration study in fish, please refer to MCA 8.2.2.3

[B] PEC_{sw} value given in MCP 9.2.5, Table 9.2.5- 25 (lettuce, 2 × 200 g a.s./ha) calculated with exposure assessment model GEM, soil-less cultivation, with reuse of filter cleaning water (0% mitigation), application dates 22.04. + 29.04. as worst case

The TER values for fluopyram are above the trigger of concern of 5, indicating no risk from secondary poisoning for earthworm- and fish-eating mammals.

CP 10.1.2.1 Acute oral toxicity to mammals

Table 10.1.2- 17: Mammalian toxicity data of the formulated product FLU+TFS SC 500

Test substance	Test design	Species	Endpoint	Reference
FLU+TFS SC 500	Acute	Rat	LD_{50} > 2000 mg prod./kg bw	[redacted] (2007) M-287410-01-1 KCA 7.1.1.01

CP 10.1.2.2 Higher tier data on mammals

Insect and foliage residue decline studies and kinetic evaluations to generate a DT_{50} for higher tier risk assessment on birds and mammals are in the MCA point 8.9.

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

Information on effects of fluopyram on reptiles or amphibians is not available. No guidelines for studies with terrestrial amphibian life stages and reptiles are available and no risk assessments schemes are established so far. Therefore no further studies can be suggested for these groups of organisms.

CP 10.2 Effects on aquatic organisms

The risk assessment is based on the current guidance: EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013;11(7):3290.

Table 10.2- 1: Endpoints used in risk assessment

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
FLU+TFS SC 500	Fish, acute *Oncorhynchus mykiss*	96 h / static	LC_{50} = 0.091 mg prod./L (nom)	[redacted] (2007) M-294350-01-1 KCP 10.2.1/01
	Fish, acute *Oncorhynchus mykiss*	96 h / semi-static	**LC_{50} = 0.0884 mg prod./L (nom)**	[redacted] (2018) M-636236-01-1 KCP 10.2.1/02
	Invertebrate, acute *Daphnia magna*	48 h / static	EC_{50} = 0.086 mg prod./L (nom)	[redacted] (2007) M-292365-01-1 KCP 10.2.1/03
	Invertebrate, acute *Daphnia magna*	48 h / semi-static	**EC_{50} = 0.051 mg prod./L (nom)**	[redacted] (2018) M-636231-01-1 KCP 10.2.1/04
	Green algae *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	**E_rC_{50} = 0.292 mg prod./L (nom)** E_bC_{50} = 0.0497 mg prod./L (nom) E_yC_{50} = 0.0425 mg prod./L (nom)	[redacted] (2021) M-292579-02-1 KCP 10.2.1/05 Recalculation by [redacted] (2020) M-757720-01-1 KCP 10.2.1/06
	Green algae *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	E_rC_{50} = 4.25 mg prod./L (nom) E_rC_{10} = 0.005 mg prod./L (nom) E_bC_{50} = 0.069 mg prod./L (nom) E_yC_{50} = 0.040 mg prod./L (nom)	[redacted] (2018) M-615579-01-1 KCP 10.2.1/07
	Green algae *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	E_rC_{50} = 0.419 mg prod./L (nom) E_rC_{10} = 0.0230 mg prod./L (nom) E_bC_{50} = 0.0405 mg prod./L (nom) E_yC_{50} =0.0432 mg prod./L (nom)	[redacted] (2018) M-636234-01-1 KCP 10.2.1/08

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
Fluopyram tech.	Fish, acute *Oncorhynchus mykiss*	96 h / static	LC_{50} > 1.89 mg a.s./L (nom)	[redacted] (2008) M-277770-02-1 KCA 8.2.1/01
	Fish, acute *Lepomis macrochirus*	96 h / static	LC_{50} > 5.68 mg a.s./L (nom)	[redacted] (2008) M-278441-02-1 KCA 8.2.1/02
	Fish, acute *Pimephales promelas*	96 h / static	LC_{50} > 4.95 mg a.s./L (nom) [A]	[redacted] (2008) M-298918-01-1 KCA 8.2.1/03
	Fish, acute *Cyprinus carpio*	96 h / static	LC_{50} = 3[redacted] mg a.s./L (nom) [B]	[redacted] (2008) M-280108-01-1 KCA 8.2.1/04
	Fish, acute *Cyprinodon variegatus*	96 h / static	LC_{50} > 0.[redacted] mg a.s./L (nom)	[redacted] (2006) M-279167-01-1 KCA 8.2.1/05
	Fish, acute Geometric mean	96 h / static	Geometric mean **LC_{50} = 4.37 mg a.s./L**	
	Fish, chronic (ELS) *Pimephales promelas*	33 d / flow-through	**NOEC = 0.135 mg a.s./L (mm)** EC_{10} = 0.162 mg a.s./L (mm)	[redacted] (2006) M-279440-01-1 KCA 8.2.2.1/01 Recalculation by [redacted] (2020) M-758375-01-1 KCA 8.2.2.1/02
	Fish, BCF flow-through *Lepomis macrochirus*	28 d exposure / 14 d depuration / flow-through	BCF (whole fish, wet weight) = 18 BCF (whole fish, normalized to 6 % lipid content) = 16	[redacted] (2008) M-298506-01-1 KCA 8.2.2.3/01
	Invertebrate, acute *Daphnia magna*	48 h / static	EC_{50} > 20 mg a.s./L (nom)	[redacted] (2006) M-278709-01-1 KCA 8.2.4.1/01
	Sediment dweller, sub-chronic *Leptocheirus plumulosus* (spiked sediment)	10 d / static	LC_{50} > 100 mg a.s./kg (mm) NOEC = 100 mg a.s./kg (mm)	[redacted] (2008) M-297751-01-1 KCA 8.2.4.2/01
	Invertebrate, acute *Crassostrea virginica*	96 h / flow-through	EC_{50} > 0.44 mg a.s./L (mm) (shell deposition and mortality)	[redacted] (2006) M-282691-01-1 KCA 8.2.4.2/02
	Invertebrate, acute *Americamysis bahia*	96 h / flow-through	LC_{50} > 0.50 mg a.s./L (mm)	[redacted] (2007) M-282839-01-2 KCA 8.2.4.2/03
	Invertebrate, acute Geometric mean	-	Geometric mean **EC_{50} = 1.638 mg a.s./L**	

Test substance	Test species	Time 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]	[redacted] (2007) M-282102-02-1 KCA 8.2.5.1/01 Recalculation by [redacted] (2020) M-758376-01-1 KCA 8.2.5.1/02
	Sediment dweller, chronic (54 d, Life cycle) *Chironomus tentans* (spiked sediment)	54 d / static-renewal	**NOEC = 2.6 mg a.s./kg (nom)** EC_{10}: not determined [c]	[redacted] (2008) M-298809-01-1 KCA 8.2.5.3/01 Recalculation by [redacted] (2020) M-758569-01-1 KCA 8.2.5.3/02
	Sediment dweller, chronic (28 d, Life Cycle) *Chironomus riparius* (spiked water)	28 d / static	NOEC = 1.39 mg a.s./L (nom) **EC_{10} = 1.54 mg a.s./L (nom)** EC_{15} = 1.37 mg a.s./L (nom) EC_{50} > 32 mg a.s./L (nom)	[redacted] (2008) M-298266-01-1 KCA 8.2.5.4/01
	Sediment dweller, chronic *Leptocheirus plumulosus* (spiked sediment)	28 d / static-renewal	NOEC = 38 mg a.s./kg (nom) LC_{50} > 94 mg a.s./kg (nom)	[redacted] (2008) M-298810-02-1 KCA 8.2.5.4/02
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 - 72 h / static	E_rC_{50} = 8.9 mg a.s./L (mm) E_rC_{10} = 7.1 mg a.s./L (mm) E_bC_{50} = 3.97 mg a.s./L (mm) **E_yC_{50} = 4.26 mg a.s./L (nom)**	[redacted] (2007) M-286541-01-1 KCA 8.2.6.1/01 Recalculation by [redacted] [redacted] (2020) M-757659-01-1 KCA 8.2.6.1/03
	Freshwater diatom *Navicula pelliculosa*	0 - 72 h / static	E_rC_{50} = 9.08 mg a.s./L (mm) E_rC_{10} = 5.23 mg a.s./L (mm) E_bC_{50} = 5.62 mg a.s./L (mm) **E_yC_{50} = 5.64 mg a.s./L (mm)**	[redacted] (2007) M-289899-01-1 KCA 8.2.6.2/01 Recalculation by [redacted] [redacted] (2020) M-757699-01-1 KCA 8.2.6.2/04
	Marine diatom *Skeletonema costatum*	0 - 72 h / static	**E_rC_{50} > 1.13 mg a.s./L (mm)** E_rC_{10} > 1.13 mg a.s./L (mm) E_bC_{50} > 1.13 mg a.s./L (mm) **E_yC_{50} > 1.13 mg a.s./L (mm)**	[redacted] (2007) M-287289-01-1 KCA 8.2.6.2/03 Recalculation by [redacted] [redacted] (2020) M-757680-01-1 KCA 8.2.6.1/06
	Aquatic macrophyte, *Lemna gibba*	7 d / static	**E_rC_{50} = 2.51 mg a.s./L (nom)** E_rC_{10} = 1.58 mg a.s./L (nom) E_yC_{50} = 2.12 mg a.s./L (nom)	[redacted] (2021) M-283647-02-1 KCA 8.2.7/01

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
Fluopyram-7-hydroxy	Invertebrate, acute *Daphnia magna*	0 - 48 h / static	**EC_{50} > 88.7 mg p m./L (nom)**	[redacted] (2020) M-759029-01-1 KCA 8.2.4.1/02
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	**E_rC_{50} = 20.9 mg p m./L (nom)** E_rC_{10} = 20.2 mg p m./L (nom) E_yC_{50} = 13.0 mg p m./L (nom)	[redacted] (2020) M-758708-01-1 KCA 8.2.6.1/05
		0 -96 h / static	E_rC_{50} = 21.1 mg p m./L (nom) E_rC_{10} = 20.4 mg p m./L (nom) E_yC_{50} = 13.7 mg p m./L (nom) E_bC_{50} = 12.6 mg p m./L (nom)	
	Aquatic macrophyte *Lemna gibba*	7 d static	**E_rC_{50} = 9.2 mg p m./L (mm)** E_rC_{10} = 5.2 mg p m./L (mm) E_yC_{50} = 7.1 mg p m./L (mm)	[redacted] (2020) M-759030-01-1 KCA 8.2.7/02
Trifluoro-acetic acid (TFA)	Fish, acute *Brachydanio rerio*	96 h / static	**LC_{50}** > 1200 mg p m./L (nom Na-TFA) **> 1008 mg p m./L (nom TFA)** [E]	[redacted] (1992) M-247889-01-1 KCA 8.2.1/06
	Invertebrate, acute *Daphnia magna*	0 - 48 h / static	**EC_{50}** > 1200 mg p m./L (nom Na-TFA) **> 1008 mg p m./L (nom TFA)** [E]	[redacted] (1992) M-247890-01-1 KCA 8.2.4.1/03
	Invertebrate, chronic *Daphnia magna*	21 d Semi-static	**NOEC** ≥ 30 mg p m./L (nom Na-TFA) **≥25.2 mg p m./L (nom TFA)** [E] EC_{10}: not determined [C]	[redacted] (2010) M-615126-01-1 KCA 8.2.5.1/03
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	**E_rC_{50}** > 1.2 mg p m./L (nom Na-TFA) **> 1.01 mg p m./L (nom TFA)** [E] E_rC_{10} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E] E_bC_{50} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E] E_yC_{50} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E]	[redacted] (1993) M-247818-02-1 KCA 8.2.6.1/06 Re-evaluation by [redacted] (2021) M-762268-02-1 KCA 8.2.6.1/07

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	E_rC_{50} = 160 mg p m./L (nom Na-TFA) = 134.4 mg p.m./L (nom TFA) [E] E_rC_{10} = 2.239 mg p m./L (nom Na-TFA) = 1.881 mg p.m./L (nom TFA) [E] E_bC_{50} > 4.8 mg p m./L (nom Na-TFA) > 4.03 mg p.m./L (nom TFA) [E] E_yC_{50} = 4.190 mg p m./L (nom Na-TFA) = 3.52 mg p.m./L (nom TFA) [E]	[redacted] (1992) M-247820-01-1 KCA 8.2.6.1/08 Re-evaluation by [redacted] (2021) M-762208-02-1 KCA 8.2.6.1/09
	Green algae *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*)	0 -72 h / Static	E_rC_{50} = 237.07 mg p.m./L (nom) E_rC_{50} = 241.95 mg p.m./L (mm) E_bC_{10} = 5.59 mg p.m./L (nom) E_rC_{10} = 5.80 mg p.m./L (mm) E_bC_{50} = 26.866 mg p.m./L (mm) E_yC_{50} = 18.956 mg p m./L (mm)	[redacted] (2017) M-615180-01-1 KCA 8.2.6.1/12 Re-evaluation by [redacted] (2021) M-762267-01-1 KCA 8.2.6.1/13
	Aquatic macrophyte *Lemna gibba*	7 d / static	E_yC_{50} = 1100 mg p.m./L (nom Na-TFA) = 924 mg p.m./L (nom TFA) [E] **E_rC_{50} = >2016 mg p.m./L (nom TFA)** [E] NOE_rC = 252 mg p m./L (nom TFA) [E] E_rC_{10} = 308 mg p m./L (nom TFA) [E]	[redacted] (1993) M-247900-01-1 KCA 8.2.7/03 Endpoint recalculation by [redacted] (2021) M-768038-01-1 KCA 8.2.7/06

Note:
Studies referring to KCA are filed in the dossier for the active substance
Studies written in grey text 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 metabolite, prod. = product
mm = mean measured; nom = nominal
Bold values used in risk assessment

A Practical limit of water solubility
B In all test levels precipitations were observable so the LC_{50} is clearly above the water solubility of the test item.
C Not determined due to mathematical reasons
D Formerly known as *Selenastrum capricornutum*
E As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to Trifluoroacetic acid with factor 0.84.

Metabolites

Metabolites Fluopyram-7-hydroxy and Trifluoroacetic acid (TFA) are relevant for the aquatic risk 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.

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

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

Substance name	Fluopyram	Fluopyram-7-hydroxy	TFA
Endpoint (mg/L)	8.9 [A]	20.9	>1.01
Molecular Weight (g/mol)	396.7	412.7	114.0
Parent endpoint recalculated on a molar basis (mg/L) $10^{\frac{M_{met}}{M_{ai}}} \cdot LC_{50ai}$	N.A.	92.59	25.58

[A] The bound value for green algae 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 discreet value is used.

The green algae endpoints for TFA and Fluopyram 7-hydroxy is much greater than 10 times the parent endpoint recalculated on a molar basis => Step 5

For TFA and Fluopyram 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.
Is RACsw; ac > PECsw and RACsw; ch > PECsw?

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

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

Species	Endpoints [mg/L]	
	Fluopyram-7-hydroxy	Trifluoroacetic acid (TFA)
Acute fish	LC_{50} = 0.437 *	LC_{50} > 1008
Acute invertebrates	EC_{50} > 88.7	EC_{50} > 1008
Chronic fish	NOEC = 0.0135 *	NOEC = 0.0135 *
Chronic invertebrates	NOEC = 0.125 *	NOEC ≥ 25.2
Algae	E_rC_{50} = 20.9	E_rC_{50} > 1001
Macrophyte	E_rC_{50} = 9.2	~~E_yC_{50} = 924~~ E_rC_{50} > 2016

* 1st tier parent endpoint divided by 10

Selection of endpoints – Tier 1

Acute toxicity to fish

The acute toxicity of fluopyram to fish has been investigated in total with five different fish species. The 96 h LC_{50} 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 (LC_{50} 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 sensitivity, the trout, resulted in a 96 h LC_{50} of >1.98 mg a.s./L.

As acute toxicity data are available for five different fish species a geometric mean endpoint was derived according to the Tier 2A approach of the Aquatic Guidance Document (EFSA PPR Panel Guidance, 2013; 11(7):3290). Therefore, a Tier 2A Geomean-LC_{50} of 4.37 mg a.s./L was calculated and used for the fish acute risk assessment in connection with an assessment factor of 100.

Table 10.2- 3: Summary of acute endpoints for fish

Test species	Test system	Endpoint
Fish, acute *Oncorhynchus mykiss*	96 h LC_{50}	> 1.89 mg a.s./L (nom)
Fish, acute *Pimephales promelas*	96 h LC_{50}	> 4.95 mg a.s./L (mm) [A]
Fish, acute *Lepomis macrochirus*	96 h LC_{50}	> 5.68 mg a.s./L (nom)
Fish, acute *Cyprinus carpio*	96 h LC_{50}	30.5 mg a.s./L (mm) [B]
Marine fish, acute *Cyprinodon variegatus*	96 h LC_{50}	> 0.98 mg a.s./L (mm)
Geometric mean	96 h LC_{50}	**4.37 mg a.s./L**

A Practical limit of water solubility

B In all test levels precipitations were observable so the LC_{50} 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 clearly lower fish toxicity of metabolites compared to the active substance fluopyram.

Chronic toxicity to fish

According to the AGD, EC_{10} 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 EC_{10} is 0.162 mg a.s./L based on fry survival. It is 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 investigated on Daphnids as well as on the estuarine species Mysid Shrimp and Eastern Oyster. In addition, subchronic tests with spiked sediment have been conducted on two sediment dwelling organisms: *Chironomus tentans* and *Leptocheirus plumulosus*. Chronic testing was done with Daphnids and *Chironomus riparius*.

The EC_{50} for the standard species *Daphnia magna* was > 20 mg a.s./L. The LC_{50} for the mysid shrimp *Americamysis bahia* was > 0.50 mg a.s./L. The test on the Eastern Oyster (*C. virginica*) resulted in an LC_{50} > 0.44 mg a.s./L for mortality (i.e. no effects on mortality up to the highest test concentration) and shell deposition.

As acute toxicity data are available for three different aquatic invertebrate species a geometric mean endpoint was derived according to the Tier 2A approach of the Aquatic Guidance Document (EFSA PPR Panel Guidance, 2013; 11(7):3290). Therefore, a Tier 2A Geomean-EC_{50} 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 subchronic tests with sediment dwellers *C. tentans* and *L. plumulosus* 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-hydroxy and trifluoroacetic acid (TFA) were of low acute toxicity to invertebrates with EC_{50} values of >88.7 mg p.m./L and to >1008 mg p.m./L, respectively.

Chronic toxicity to invertebrates

Chronic testing on *Daphnia magna* resulted in a NOEC of 1.25 mg a.s./L. The life cycle test with *Chironomus tentans* revealed a NOEC of 1.39 mg a.s./L and an EC_{10} of 0.54 mg a.s./L that is considered for use in the risk assessment.

Primary producers

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 461, 1-44, 2007), the EFSA supporting publication 2015 (EN-924 published 22 December 2015) and also the EFSA Aquatic Guidance Document (AGD, 2013, noted by SCFCAH on July 10-11th, 2014), list growth rate 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-hydroxy showed lower toxicity than the parent with 72-hour E_rC_{50} value 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 (fluopyram-7-hydroxy and trifluoroacetic acid (TFA)). Consistent with metabolite testing in algae, the metabolites (fluopyram-7-hydroxy and trifluoroacetic acid (TFA)) were far less toxic than the parent (by a factor of ca. 3.3 to 475 or 3.7 to 803).
The 7-day E_yC_{50} values of the metabolites were 7.1 mg p.m./L (fluopyram-7-hydroxy) and >1008 mg p.m./L (trifluoroacetic acid (TFA)) and the 7-day E_rC_{50} values of the metabolites were 9.2 mg p.m./L (fluopyram-7-hydroxy) and >2016 mg p.m./L (trifluoroacetic acid (TFA)).

Uncertainty factors for isomer composition of metabolites
The metabolite Fluopyram-7-hydroxy has a chiral center. Ecotoxicological 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 its metabolites in surface water were calculated according to FOCUS Steps 1-2 for the use in grapes.

For the application in lettuce predicted environmental concentrations in surface water (PECsw) were estimated after use in high-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_{sw} 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 latest by end of March 2022.

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

Table 10.2- 4: Initial max PEC_{sw} values – FOCUS Steps 1 and 2 – Multiple application in grapes (spring and summer, 2 x 50 g a.s./ha)

Compound	FOCUS Scenario	PECsw, max [µg/L]	
		Grapes - Spring (Mar. - May) 2 × 50 g a.s./ha, BBCH 53-73	Grapes – Summer (Jun. - Sep.) 2 × 50 g a.s./ha, BBCH 71-89
Fluopyram	STEP 1	28.1	28.1
	STEP 2 – North	3.96	3.96
	STEP 2 - South	**5.96**	**4.96**
Fluopyram-7-hydroxy	STEP 1	1.77	1.77
	STEP 2 – North	0.141	0.141
	STEP 2 - South	**0.282**	**0.212**
Trifluoroacetic acid (TFA)	STEP 1	1.42	142
	STEP 2 – North	0.113	0.113
	STEP 2 - South	**0.226**	**0.169**

Bold values used in risk assessment

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

Compound	FOCUS Scenario	PECsed, max [µg/L]	
		Grapes - Spring (Mar. - May) 2 × 50 g a.s./ha, BBCH 53-73	Grapes – Summer (Jun. - Sep.) 2 × 50 g a.s./ha, BBCH 71-89
Fluopyram	STEP 1	63.8	63.8
	STEP 2 – North	8.82	8.82
	STEP 2 - South	**13.5**	11.1
Fluopyram-7-hydroxy	STEP 1	1.78	1.78
	STEP 2 – North	0.142	0.142
	STEP 2 - South	0.283	0.212
Trifluoroacetic acid (TFA)	STEP 1	<0.001	<0.001
	STEP 2 – North	<0.001	<0.001
	STEP 2 - South	<0.001	<0.001

Bold values used in risk assessment

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

Table 10.2- 6: Maximum PEC_{sw} values (50th perc. of 7 annual peak concentrations) – Application in lettuce (soil-less cultivation, 2 x 200 g a.s./ha)

Compound	Mitigation (end-of-pipe reduction)	PECsw, max [µg/L]	
		Lettuce - no reuse of filter cleaning water 2 × 200 g a.s./ha	Lettuce – With reuse of filter cleaning water 2 × 200 g a.s./ha
Fluopyram	0 %	**20.75**	**43.59**
	95 %	**0.996**	**2.178**
Trifluoroacetic acid (TFA)	0 %	**0.557**	**0.895**
	95 %	**0.028**	**0.045**

Bold values used in risk assessment

Predicted environmental concentrations for the formulation FLU+TFS SC 500

Application in grapes, 2 x 0.2 L prod./ha (2 x 50 g a.s./ha)

Table 10.2- 7: Initial PEC_{sw} values for the formulation FLU+TFS SC 500 following the single application in grapes – 2 × 0.2 L prod./ha

Compound	Maximum use rate	No spray buffer (Drift)	Drift reducing nozzles	PEC_{sw} [A] [µg product/ha]
FLU+TFS SC 500	2 × 0.2 L prod./ha	0 m	0 %	**11.317**
		5 m	90 %	**0.504**
		10 m	50 %	0.837
		15 m	0 %	0.877

Bold values used in risk assessment

[A] Calculation based on Rautmann drift values for grape vines, late, assuming a specific density of 1.174 g/mL (please refer to study M-294350-01-1).

Application in lettuce 2 x 0.8 L prod./ha (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.

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 assessment:

$RAC_{sw, ac} = LC_{50}$ or EC_{50} / 100

The risk is considered acceptable, if the $RAC_{sw, ac} \geq PEC_{sw, max}$.

Chronic risk assessment:

$RAC_{sw, ch} = NOEC$ or EC_{10} / 10

$RAC_{sw, ch} = E_rC_{50}$ / 10

The risk is considered acceptable, if the $RAC_{sw, ch} \geq PEC_{sw, max}$

To summarise, these abbreviations are used in subscript following the term PEC or RAC:

ac: acute, ch: chronic, sw: surface water, max: maximum.

ACUTE RISK ASSESSMENT FOR AQUATIC ORGANISMS

Important remark by the applicant: The PEC_{sw} 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.

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

Table 10.2-8: Acute risk assessment based on FOCUS Step 2 for the application in grapes (2 × 50 g a.s./ha)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
Spring application					
FLU+TFS SC 500	Fish, acute *Oncorhynchus mykiss*	LC_{50} 88.4	0.884	11.317 [A]	**No**
	Invertebrate, acute *Daphnia magna*	EC_{50} 51	0.51		**No**
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	5.96	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		Yes
Fluopyram-7-hydroxy	Fish, acute	LC_{50} 437 [B]	2.19 [C]	0.282	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >88700	>443.5 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.226	Yes

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes
Summer application					
FLU+TFS SC 500	Fish, acute *Oncorhynchus mykiss*	LC_{50} 88.4	0.884	11.317 [A]	**No**
	Invertebrate, acute *Daphnia magna*	EC_{50} 51	0.51		**No**
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	4.96	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		Yes
Fluopyram-7-hydroxy	Fish, acute	LC_{50} 437 [B]	2.19 [C]	0.212	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >88700	>443.5 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.169	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes

A Formulation-specific PEC_{sw}
B 1st tier parent endpoint divided by 10
C For the metabolite fluopyram-7-hydroxy the RAC has been corrected in addition according to an uncertainty factor of 2 in consideration of two enantiomers

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 fluopyram and its metabolites.

For the formulation the acute trigger was not met for fish and aquatic invertebrates for the application in grapes (spring and summer application) at 2 × 50 g a.s./ha. Therefore, a refinement is presented below considering drift mitigation measures.

Refined risk assessment for the formulation FLU+TFS SC 500 considering drift mitigation - grapes (2 × 0.2 L prod./ha)

Table 10.2- 9: Refined risk assessment for the formulation based on mitigation measures- grapes (2 × 0.2 L prod./ha)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	Mitigation	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
FLU+TFS SC 500	Fish, acute *Oncorhynchus mykiss*	LC_{50} 88.4	0.884	5 m drift buffer + 90 % drift reducing nozzles	0.504 [A]	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 51	0.51	5 m drift buffer + 90 % drift reducing nozzles		Yes

A Formulation-specific PEC_{sw}

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 identified in water-sediment 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 fluopyram-7-hydroxy.

Table 10.2- 10: Acute risk assessment based on FOCUS Step 2 for the application in lettuce — no mitigation (2 × 200 g a.s./ha, soil-less cultivation)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
No reuse of filter cleaning water					
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	20.75	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		**No**
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.557	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes
With reuse of filter cleaning water					
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	43.59	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		**No**
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.895	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes

For the application in lettuce (with and without reuse of filter cleaning water) at 2 × 200 g a.s./ha the acute trigger was not met for aquatic invertebrates for fluopyram. A risk assessment for fluopyram using mitigation removal fraction of 0.99 is presented below.

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

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

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	Mitigation	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
No reuse of filter cleaning water						
Fluopyram tech.	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38	95 % (end-of-pipe reduction)	0.996	Yes
With reuse of filter cleaning water						
Fluopyram tech.	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38	95 % (end-of-pipe reduction)	2.178	Yes

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

CHRONIC RISK ASSESSMENT FOR AQUATIC ORGANISMS

Important remark by the applicant: The PEC_{sw} 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.

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

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

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
Spring application					
FLU+TFS SC 500	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} 292	29.2	11.317 [A]	Yes
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC 135	13.5	5.96	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC 1250	125		Yes
	Invertebrate, chronic *Chironomus riparius*	EC_{10} 540	54		Yes
	Algae *Skeletonema costatum*	E_rC_{50} >1130	>113		Yes

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} 2510	251		Yes
Fluopyram-7-hydroxy	Fish, chronic *Pimephales promelas*	NOEC 13.5 [B]	0.675 [C]	0.282	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC 125 [B]	6.25 [C]		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} 20900	1045 [C]		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} 9200	460.5 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC 13.5 [B]	1.35	0.226	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC ≥25200	≥2520		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} >1010	>101		Yes
	Aquatic macrophyte *Lemna gibba*	~~E_rC_{50} 924000~~ E_rC_{50} >2016000	~~9240~~ >201600		Yes
Summer application					
FLU+TFS SC 500	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} 292	29.2	11.317 [A]	Yes
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC 135	13.5	4.96	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC 1250	125		Yes
	Invertebrate, chronic *Chironomus riparius*	EC_{10} 540	54		Yes
	Algae *Skeletonema costatum*	E_rC_{50} >1130	>113		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} 2510	251		Yes
Fluopyram-7-hydroxy	Fish, chronic *Pimephales promelas*	NOEC 13.5 [B]	0.675 [C]	0.212	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC 125 [B]	6.25 [C]		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} 20900	1045 [C]		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} 9200	460.5 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC 13.5 [B]	1.35	0.169	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC ≥25200	≥2520		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} >1010	>101		Yes

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
	Aquatic macrophyte *Lemna gibba*	~~E_yC_{50} 924000~~ E_rC_{50} >2016000	~~92400~~ >201600		Yes

A Formulation-specific PEC_{sw}

B 1[st] tier parent endpoint divided by 10

C For the metabolite fluopyram-7-hydroxy the RAC has been corrected according to an uncertainty factor of 2 in consideration of two enantiomers.

Table 10.2- 13: Chronic risk assessment for sediment organisms based on FOCUS Step 2 for the application in in grapes (2 × 50 g a.s./ha)

Compound	Species	Endpoint [µg/kg]	RAC [µg/kg]	$PEC_{sed.max}$ [µg/kg]	RAC ≥ PEC_{sed}
Spring cereals					
Fluopyram tech.	Sediment dweller, *Chironomus tentans*	NOEC 26000	2600	13.5	Yes
Winter cereals					
Fluopyram tech.	Sediment dweller, *Chironomus tentans*	NOEC 26000	2600	13.5	Yes

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 fluopyram and its metabolites.

<u>Application in lettuce, 2 x 200 g a.s./ha</u>

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 identified in water-sediment 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 fluopyram-7-hydroxy.

Table 10.2- 14: Chronic risk assessment based on FOCUS Step 2 for the application in lettuce - no mitigation (2 × 200 g a.s./ha, soil-less cultivation)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
No reuse of filter cleaning water					
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC 135	13.5	20.75	**No**
	Invertebrate, chronic *Daphnia magna*	NOEC 1250	125		Yes
	Invertebrate, chronic *Chironomus riparius*	EC_{10} 540	54		Yes
	Algae *Skeletonema costatum*	E_rC_{50} >1130	>113		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} 2510	251		Yes

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC 13.5 [A]	1.35	0.557	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC ≥25200	≥2520		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} >1010	>101		Yes
	Aquatic macrophyte *Lemna gibba*	~~E_yC_{50} 924000~~ E_rC_{50} >2016000	~~92400~~ >201600		Yes
With reuse of filter cleaning water					
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC 135	13.5	43.59	**No**
	Invertebrate, chronic *Daphnia magna*	NOEC 1250	125		Yes
	Invertebrate, chronic *Chironomus riparius*	EC_{10} 540	54		Yes
	Algae *Skeletonema costatum*	E_rC_{50} >1130	>113		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} 2510	251		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC 13.5 [A]	1.35	0.895	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC ≥25200	≥2520		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} >1010	>101		Yes
	Aquatic macrophyte *Lemna gibba*	~~E_yC_{50} 924000~~ E_rC_{50} >2016000	~~92400~~ >201600		Yes

[A] 1st tier parent endpoint divided by 10

For the application in lettuce (with and without reuse of filter cleaning water) at 2 × 200 g a.s./ha the chronic trigger was not met for fish for fluopyram. 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 (soil-less cultivation, 2 x 200 g a.s./ha)

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

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	Mitigation	$PEC_{sw,max}$ [µg/L]	RAC > PEC_{sw}
No reuse of filter cleaning water						
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC 135	13.5	95 % (end-of-pipe reduction)	0.996	Yes
With reuse of filter cleaning water						
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC 135	13.5	95 % (end-of-pipe reduction)	2.178	Yes

For the application in lettuce (with and without reuse of filter cleaning water) at 2 x 200 g a.s./ha the chronic trigger was met for fish for fluopyram considering a mitigation removal fraction of 0.95.

Combined toxicity risk assessment

According to the EFSA "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013;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 ecotoxicology" (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 formulated product (PPP) FLU+TFS SC 500 and the active substances fluopyram and trifloxystrobin.

Is the formulation three times more toxic than fluopyram?

Table 10.2- 16: Comparison of endpoints available for the formulated product (PPP) FLU+TFS SC 500 and the active substance fluopyram

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 endpoint / Recalculated formulation endpoint
O. mykiss	LC_{50}, acute, 96 h	0.0884	> 1.89	not calculated	-
D. magna	EC_{50}, acute, 48 h	0.051	> 20	not calculated	-
Pseudokirchneriella subcapitata	E_rC_{50}, short-term, 72 h	0.292	8.9	0.06	142

[A] Amount of fluopyram in the test item used in formulation studies: 21.4 % (please refer to study M-292579-02-1)

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

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

For algae the formulation (endpoint expressed in terms of fluopyram) is more than 3 times more toxic than fluopyram. Therefore, a risk assessment for the formulation is 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- 17: Overview of endpoints available for the formulated product (PPP) FLU+TFS SC 500 and the active substances fluopyram and trifloxystrobin

Test species	Endpoint and test system	Measured toxicity of PPP [mg prod./L]	FLU [mg a.s./L]	TFS [A] [mg a.s./L]
O. mykiss (PPP / TFS) *C. variegatus* (FLU)	LC_{50}, acute, 96 h	0.0884	> 0.98	0.015
D. magna (PPP / TFS) *C. virginica* (FLU)	EC_{50}, acute, 48 h	0.051	> 0.44	0.016

[A] Please refer for endpoints to EFSA Journal 2017;15(10):4989: Peer review of the pesticide risk assessment of the active substance trifloxystrobin

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

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) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$ [mg total a.s./L]	Model deviation ratio (MDR = $EC_{50\ mix\text{-}CA}$ / $EC_{50\ PPP}$)
Fish	LC_{50}, acute, 96 h	0.038	0.029	0.77
Aquatic invertebrates	EC_{50}, acute, 48 h	0.022	0.031	1.40

[A] The mixture toxicity of the formulation was re-calculated based on the measured contents of fluopyram (251.3 g/L), and trifloxystrobin (253.5 g/L) within the formulation and the product density (1.074 g/mL) (please refer to study M-294350-01-1).

The calculated MDR values are between 0.2 and 5 for fish and aquatic 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 due to the co-formulants.

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 been performed 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

Data Point:	KCP 10.2.1/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Acute toxicity of fluopyram & trifloxystrobin SC 500 (250+250) G to fish (Oncorhynchus mykiss) under static conditions
Report No:	EBGMP030
Document No:	M-294350-01-1
Guideline(s) followed in study:	EPA-FIFRA § 72-1/SEP-EPA-540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Directive 92/69/EEC, C.1 (1992) OECD No. 203 (rev.1992); Equivalent to US EPA OPPTS Guideline No. 850.1075 SUPP
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The fish length at test start was not reported. The missing information was 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

Executive Summary

An acute toxicity test was performed with the Rainbow trout (*Oncorhynchus mykiss*) in a static system. Juvenile fish were exposed to FLU + TFS SC 500 (250+250 g/L) in groups of 10 (one replicate of 10 fish per test level) to an aqueous solution of the product at nominal concentrations of 0.0313, 0.0625, 0.125, 0.250 and 0.500 mg prod./L for a 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.

Concentrations of fluopyram were verified by HPLC – MS/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 lowest standard concentration during the study. The mean measured concentrations were: 7.26, 13.6, 26.1, 53.1, 103 µg fluopyram a.s./L

The study fulfils all validity criteria of OECD 203 guideline.

There were behavioural abnormalities of the fish in the three highest test concentrations (0.125, 0.250 and 0.500 mg prod./L). In the controls no mortalities or sub-lethal finding were observed.

The endpoints based on nominal product concentrations of FLU + TFS SC 500 (250+250 g/L) were: LC_{50} – 96 hours (95 % C.I.): 0.091 mg prod./L (n.d.) and NOEC – 96 hours: 0.0625 mg prod./L.

I. MATERIALS AND METHODS

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 (*Oncorhynchus mykiss*)
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 age/size	Mean length: 55 ± 6 mm at test start Mean body weight: 1.6 ± 0.6 g at test start
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 nominal a.s. concentrations Control: water Evidence of undissolved material: During the whole exposure period no test material was observed, and the test medium appeared clear in all test concentrations.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 96 hours
Test Vessel Loading	0.40 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 11.7 - 12.2 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: not reported pH: 6.8 - 7.2 Water hardness: 40 - 60 mg $CaCO_3$/L Dissolved oxygen: 88 - 100 % of saturation (aeration was added on study to reach oxygen saturation) Conductivity: < 0.2 µS/cm Alkalinity: not reported
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4 hours and then once daily (day 1 to 4). Discrete measurements of dissolved oxygen, water temperature and pH were obtained at test initiation and after 24, 48, 72, and 96 hours. Temperature was additionally measured hourly by a calibrated data logger in one control replicate.
Sampling for Chemical analysis	Samples of test solutions were taken at test initiation (0 hour), after 48 hours and at test termination (96 hours) for analysis of test substance. 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 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 96 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 AND DISCUSSION

Table 10.2.1- 1: Validity criteria

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 60 %	88 - 100 %

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, day 1, day 2 and day 4 were between 95 and 113 % (see table below). Biological results are based on nominal product concentrations of FLU + TFS SC 500 (250+250 g/L).

No residues of fluopyram were found in the control samples above 0.521 µg a.s./L, which was used as the lowest standard concentration during the study.

Table 10.2.1- 2: Analytical results for fluopyram

Nominal concentration		Mean measured concentration [µg a.s./L]				% of nominal				Mean measured conc. [µg a.s./L] [A]	Mean % of nominal
[mg prod./L]	[µg a.s./L]	Day 0	Day 1	Day 2	Day 4	Day 0	Day 1	Day 2	Day 4		
0.0313	6.70	6.38	- [B]	7.56	6.85	110	-	113	102	7.26	108
0.0625	13.4	14.2	- [B]	13.4	13.3	106	-	100	99	13.6	102
0.125	26.8	25.5	- [B]	26.6	- [C]	95	-	99	-	26.1	97
0.250	53.5	54.0	52.1	- [C]	-	101	97	-	-	53.1	99
0.500	107	103	- [C]	- [C]	- [C]	96	-	-	-	103	96

[A] Not given in report. Calculations based on measured concentrations on each sampling day.
[B] No measurements performed.
[C] No measurements performed as all fish were dead.

Biological results:

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.

Table 10.2.1- 3: Mortality

Nominal concentration [mg prod./L]	Dead fish No. (%)				
	Exposure time				
	4 h	24 h	48 h	72 h	96 h
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0313	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0625	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.125	0 (0)	4 (40)	10 (100)	10 (100)	10 (100)
0.250	3 (30)	10 (100)	10 (100)	10 (100)	10 (100)
0.500	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)

III. CONCLUSION

The study meets the validity criteria according to OECD 203 (2019) and the endpoints based on nominal product concentrations were:

LC_{50} - 96 hours (95 % C.L.)	**0.091 mg prod./L** (n.d. [A])
NOEC - 96 hours: highest concentration without an effect (based on mortality and sublethal effects)	0.0625 mg prod./L

[A] Not determined due to mathematical reasons.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (96 hours) = 0.091 mg prod./L

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Data Point:	KCP 10.2.1/02
Report Author:	[redacted]
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:	M-636236-01-1
Guideline(s) followed in study:	- Commission Regulation (EC) No 440/2008, Annex, Part C, C.1: "Acute Toxicity for Fish", Official Journal of the European Union, May 30, 2008 - EPA Guideline 712-C-16-007: OCSPP 850.1075, " Freshwater and Saltwater Fish Acute Toxicity Test" October 2016 - Japanese MAFF, Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7-1-1, Fish acute toxicity studies, 2005 - OECD Guideline for Testing of Chemicals, Section 2, No. 203: "Daphnia sp. “Fish, Acute Toxicity Test" adopted July 17, 1992 - SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistration data requirements for Annex II (part A, Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The temperature ranged between 13.9 and 14.8 °C and thus above the maximum 14 °C recommended in OECD 203. This deviation was 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

Executive Summary

An acute toxicity test was performed with the Rainbow Trout (*Oncorhynchus mykiss*) in a semi-static system. Juvenile fish were exposed to FLU + TFS SC 500 (250 + 250) in groups of 10 (one replicate of 10 fish per test level) to an aqueous solution of the product at nominal 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 trifloxystrobin were verified in the freshly prepared and aged test media by using HPLC – MS/MS 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 (fluopyram and trifloxystrobin: 0.4 µg a.s./L).

The study fulfils all validity criteria of the OECD 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 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.

The endpoints based on nominal concentrations were: LC_{50} – 96 hours (95 % C.I.): 0.0884 mg prod./L (0.0625 - 0.125 mg prod./L), 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) adaptation	None specified
Test species	Rainbow Trout (*Oncorhynchus mykiss*)
Acclimation	At least 12 days to test conditions Health during acclimation: no mortality in the 48-hour acclimation period before testing.
Organism age/size	Mean length: 46.8 ± 2.5 mm at test start Mean body weight: 1.11 ± 0.18 g at test start
Test solutions	Nominal concentrations: 0.0156 – 0.0313 – 0.0625 – 0.125 – 0.250 mg prod./L Mean measured recoveries based on a.s. content ranged from 81 to 116 % of nominal a.s. concentrations for trifloxystrobin and between 97 and 109 % for fluopyram Control: water 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 (replicates): 1 No. of vessels per control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Semi-static (daily renewal) Total exposure duration: 96 hours
Test Vessel Loading	0.79 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 13.9 - 14.8 °C Photoperiod: 16 hours light, 8 hours dark; with 30 minute transition periods Light intensity: 560 - 680 lux pH: 7.4 - 8.0 Water hardness: 250.0 mg $CaCO_3$/L Dissolved oxygen: 93 - 101 % of saturation (the test media were slightly aerated during the test) Conductivity: ≤ 10 µS/cm Alkalinity: 0.8 mmol/L
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects at test start and after 2, 24, 48, 72 and 96 hours. The temperature, dissolved oxygen and pH were determined daily in the freshly prepared and aged test media of each treatment group.
Sampling for Chemical analysis	Duplicate samples of test solutions were taken from the freshly prepared test media of all test concentrations and the control at test start and each test medium renewal (day 1, 2, and 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.
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 96 h. 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 AND DISCUSSION

Table 10.2.1- 4: Validity criteria

Validity criteria	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen saturation	≥ 60 %	93 - 101 %

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 in freshly prepared and aged test media on each sampling (day 0, 1, 2, 3 and 4) were between 97 and 110 % of nominal a.s. concentrations for trifloxystrobin and between 77 and 116 % for fluopyram (see table below). Biological results are based on nominal product concentrations of FLU + TFS SC 500 (250 + 250).

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

Table 10.2.1- 5: Analytical results for fluopyram

Nominal concentration		Mean measured concentration [µg a.s./L] [A]							
[mg prod./L]	[µg a.s./L]	Day 0	Day 1		Day 2		Day 3		Day 4
		New	Aged	New	Aged	New	Aged	New	Aged
0.0156	3.316	3.471	3.427	3.645	3.393	3.597	3.484	3.392	3.314
0.0313	6.653	7.074	6.913	6.891	6.613	7.039	7.126	6.891	6.461
0.0625	13.285	14.002	14.089	13.567	13.393	14.132	14.306	14.610	13.614
0.125	26.571	28.083	27.649	28.171	26.693	- [B]	- [B]	- [B]	-
0.250	53.141	54.422	54.769	- [B]	- [B]	- [B]	- [B]	- [B]	- [B]
		% of nominal [B]							
0.0156	3.316	105	104	110	102	108	105	103	100
0.0313	6.653	107	104	104	100	106	107	104	97
0.0625	13.285	106	106	102	101	107	108	110	103
0.125	26.571	106	104	106	101	-	-	-	-
0.250	53.141	103	103	-	-	-	-	-	-

[A] Not given in report. Calculation based on measured concentrations of 2 replicate samples.
[B] No measurements performed as all fish died.

Table 10.2.1- 6: Analytical results for trifloxystrobin

Nominal concentration		Mean measured concentration [µg a.s./L] [A]							
[mg prod./L]	[µg a.s./L]	Day 0	Day 1		Day 2		Day 3		Day 4
		New	Aged	New	Aged	New	Aged	New	Aged
0.0156	3.343	3.859	2.622	3.742	2.733	3.641	2.897	3.523	2.711
0.0313	6.707	6.846	5.285	6.913	5.168	6.964	5.638	7.065	5.302
0.0625	13.392	14.365	10.455	13.201	10.421	13.526	11.780	15.372	11.277
0.125	26.784	28.043	22.303	28.026	24.770	- [B]	- [B]	- [B]	- [B]
0.250	53.568	53.586	49.558	- [B]	- [B]	- [B]	- [B]	- [B]	- [B]
		% of nominal [B]							
0.0156	3.343	116	79	112	82	109	87	106	81
0.0313	6.707	102	79	103	77	104	84	105	79
0.0625	13.392	108	78	100	78	101	88	115	85
0.125	26.784	105	84	105	93	-	-	-	-
0.250	53.568	100	93	-	-	-	-	-	-

[A] Not given in report. Calculation based on measured concentrations of 2 replicate samples.
[B] No measurements performed as all fish died.

Biological results:

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.

Table 10.2.1- 7: Mortality

Nominal concentration [mg prod./L]	Dead fish No. (%)					
	Exposure time					
	0 h	2 h	24 h	48 h	72 h	96 h
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0156	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0313	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.0625	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.125	0 (0)	0 (0)	8 (80)	10 (100)	10 (100)	10 (100)
0.250	0 (0)	8 (80)	10 (100)	10 (100)	10 (100)	10 (100)

III. Conclusion

The study meets the validity criteria according to OECD 203 (2019) and the endpoints based on nominal product concentrations were:

LC_{50} – 96 hours (95 % C.I.):	**0.0884 mg prod./L** (0.0625 – 0.125 mg prod./L)
LOEC – 96 hours: lowest concentration with an effect	0.125 mg prod./L
NOEC – 96 hours: highest concentration without an effect	0.0625 mg prod./L

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LC_{50} (96 hours) = 0.0884 mg prod./L

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Data Point:	KCP 10.2.1/03
Report Author:	[redacted]
Report Year:	2007
Report Title:	Acute toxicity of AE C656948 + trifloxystrobin SC 250 + 250 G to the waterflea Daphnia magna in a static laboratory test system
Report No:	EBGMP031
Document No:	M-292365-01-1
Guideline(s) followed in study:	OECD guideline 202,(2004); EEC Directive 92/69/EWG, part C.2 (1992); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72 2 (1982), OPPTS Guideline 850.1010 public draft 1996 (modified); JMAFF 12 Nousan No. 8147 (2000); Equivalent to US EPA OPPTS Guideline No. 850.1010 SUPP
Deviations from current test guideline:	Current Guideline: 202 (2004) Deviations: None. 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

Executive Summary

An acute toxicity test was performed with daphnids (*Daphnia magna*) under static conditions to determine the 48-hour EC_{50}. First-instar neonate daphnids (< 24 hours old) were exposed to FLU + TFS SC 500 (250+250 g/L) in groups 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, a control was included. Immobilisation and sub-lethal behavioural effects were determined after 24 and 48 hours.

Concentrations of fluopyram were verified by HPLC – 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 µg a.s./L, which was used as the lowest standard concentration during this study.

The study fulfils all validity criteria of OECD 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 µg prod./L) and after 48 hours in the two highest test concentrations (80 and 160 µg prod./L).

The endpoint based on nominal concentrations was: EC_{50} – 48 hours (95 % C.I.): 0.086 mg prod./L (0.077 – 0.095 mg prod./L).

MATERIAL AND METHODS

Test material	FLU+TFS SC 500 (250+250 g/L) Specification No.: 102000012886 Batch No.: 2007-00044 Content of a.s.: 21.4 % w/w fluopyram 21.6 % w/w trifloxystrobin
Guideline(s) 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 No. of vessels per control (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 – 21.2 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: max. 1200 lux pH: 8.1 (at test start) Water hardness: 231 mg/L as $CaCO_3$ (at test start) Dissolved oxygen: 8.3 – 8.8 mg/L (92 – 99 % of saturation) Conductivity: 579 µS/cm (at test start) Alkalinity: 53 mg/L as $CaCO_3$/L (at test start)
Parameters Measured / Observations	Observations for immobility and sub-lethal behavioural effects were made after 24 and 48 hours of exposure. Prior to test initiation, conductivity, total hardness, pH and alkalinity of the dilution media (Elendt M7) were determined. Additionally, the dissolved oxygen and pH values were measured in the freshly prepared test solutions of each treatment level and control and repeatedly in the pooled replicates of the aged media at test termination (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 study. 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 test levels and the control immediately after preparation and again from pooled replicates of the corresponding aged media at the end of the 48 hours exposure interval. The chemical analyses were performed by high-performance liquid chromatograph (HPLC – MS/MS).
Data analysis	For EC_x 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_x and 95 % confidence limits for immobility rates if possible (mathematical limits based on quality of the dose-response pattern). Calculations (mean and standard deviation) were performed using Microsoft Excel The statistical analysis was carried out using the ToxRat Professional® Software (Vers.2.10, ToxRat Solutions GmbH, Germany).

II. RESULTS AND DISCUSSION

Table 10.2.1- 8: Validity criteria

Validity criteria acc. to OECD 202	Required	Obtained
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	8.3 – 8.5 mg/L

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 on day 0 and 2 ranged from 96 to 104 % of nominal values. The biological results are based on nominal product concentrations of FLU+TFS SC 500 (250+250 g/L).

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+TFS 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 active ingredient.

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

Table 10.2.1- 9: Analytical results for fluopyram

Nominal concentration		Measured concentration [µg a.s./L]		% of nominal	
[mg prod./L]	[mg a.s./L]	Day 0 (New)	Day 2 (Aged)	Day 0 (New)	Day 2 (Aged)
0.010	2.14	2.051	2.151	96	100
0.020	4.28	4.224	4.276	99	100
0.040	8.56	8.611	8.383	101	98
0.080	17.1	17.07	17.84	100	104
0.160	34.2	33.82	34.84	99	102

Biological results:

Observations

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

Table 10.2.1- 10: Immobilisation of daphnids

Nominal concentration [mg prod./L]	No. of immobilized (cumulative %)	
	Exposure time	
	24 h	48 h
Control	0 (0)	0 (0)
0.010	0 (0)	0 (0)
0.020	0 (0)	0 (0)
0.040	0 (0)	0 (0)
0.080	0 (0)	11 (36.7)
0.160	14 (46.7)	30 (100)

III. CONCLUSION

The study meets the validity criteria according to OECD 202 and the endpoints based on nominal product concentrations were:

EC_{50} – 48 hours (95 % C.I.):	**0.086 mg prod./L** (0.077 – 0.095 mg prod./L)
EC_{50} – 24 hours (95 % C.I.):	Not determined [A]

[A] Not determined due to 46.7 % effects at the highest test concentration.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: EC_{50} (48 hours) = 0.086 mg prod./L

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Data Point:	KCP 10.2.1/04
Report Author:	[redacted]
Report Year:	2018
Report Title:	Fluopyram + trifloxystrobin SC 500 (250 + 250 g/L): Acute toxicity to Daphnia 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 Immobilsisation Test", Official Journal of the European Union (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, 2-7-2-1, Daphnia acute immobilization studies, 2005 - OECD Guideline for Testing of Chemicals No. 202: "Daphnia sp., Acute Immobilisation Test" adopted April 13, 2004 - SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistration for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations from current test guideline:	Current Guideline: Current guideline: 202 (2004) Deviations: None. 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

Executive Summary

An acute toxicity test was performed with daphnids (*Daphnia magna*) under semi-static conditions to determine the 48-hour EC_{50}. First-instar neonate daphnids (< 24 hours old) 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, 0.04, 0.08 and 0.16 mg prod./L. Additionally, a control was included. Immobilisation and sub-lethal behavioural effects were determined after 24 and 48 hours.

Concentrations of fluopyram 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 (fluopyram: 0.27 µg a.s./L, trifloxystrobin: 0.86 µg a.s./L).

The study fulfils all validity criteria of OECD 202 guideline.

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 prod./L). 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./L (0.041 - 0.063 mg prod./L), NOEC – 48 hours: 0.01 mg prod./L, LOEC– 48 hours: 0.02 mg prod./L.

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 hours 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 concentration (replicates): 4 No. of vessels per control (replicates): 4
Organisms per replicate	No. of organisms per vessel: 5
Exposure	Semi-static (renewal on day 1) Total exposure duration: 48 hours
Feeding during test	None
Test conditions	Temperature: 20.8 - 21.0 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: 780 - 840 lux pH: 7.8 - 78.0 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 µS/cm Alkalinity: 0.9 mmol/L as $CaCO_3$/L (reconstituted water)
Parameters Measured / Observations	Observations for immobility and sub-lethal 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	Duplicate samples were taken from freshly prepared batch solutions at test start and on day 1. Duplicate samples from aged test 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. The chemical analyses were performed by LC-MS/MS.
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
Mortality in control during test	≤ 10 %	0 %
Dissolved oxygen concentration at the end of the test	≥ 3 mg/L	8.7 mg/L

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 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 trifloxystrobin (see table below). Biological results are based on nominal product concentrations of FLU + TFS SC 500 (250 + 250 g/L).

No residues of fluopyram and trifloxystrobin were found in the control samples above the limit of quantification (LOQ for fluopyram: 0.27 µg a.s./L, LOQ for trifloxystrobin: 0.86 µg a.s./L).

Table 10.2.1- 12: Analytical results for fluopyram

Nominal concentration		Measured concentration [A] [µg a.s./L]				% of nominal [A]			
[mg prod./L]	[µg a.s./L]	Day 0	Day 1		Day 2	Day 0	Day 1		Day 2
		New	Aged	New	Aged	New	Aged	New	Aged
0.01	2.126	2.190	2.040	2.024	2.007	103	96	95	95
0.02	4.251	4.347	4.064	4.123	3.948	103	96	97	93
0.04	8.503	8.712	8.578	8.587	8.420	103	101	101	99
0.08	17.005	17.716	17.132	16.883	16.366	105	101	100	97
0.16	34.010	34.790	35.040	33.458	33.125	102	103	99	98

[A] Not given in report. Calculated based on measured concentrations of 2 replicate samples.

Table 10.2.1- 13: Analytical results for trifloxystrobin

Nominal concentration		Measured concentration [A] [µg a.s./L]				% of nominal [A]			
[mg prod./L]	[µg a.s./L[A]]	Day 0	Day 1		Day 2	Day 0	Day 1		Day 2
		New	Aged	New	Aged	New	Aged	New	Aged
0.01	2.143	2.062	1.778	1.848	1.755	97	83	87	82
0.02	4.285	3.957	3.398	3.610	3.351	92	80	85	78
0.04	8.571	8.360	7.720	7.450	6.844	98	90	87	80
0.08	17.142	16.410	15.029	14.962	13.614	96	88	88	79
0.16	34.284	33.555	29.310	31.096	27.424	98	85	91	80

[A] Not given in report. Calculated based on measured concentrations of 2 replicate samples.

Biological results:

Observations

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 prod./L). 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.

Table 10.2.1- 14: Immobilisation of daphnids

Nominal concentration [mg prod./L]	No. of immobilized (cumulative %)	
	Exposure time	
	24 h	48 h
Control	0 (0)	0 (0)
0.01	0 (0)	0 (0)
0.02	1 (5)	1 (5)
0.04	3 (15)	8 (40)
0.08	4 (20)	14 (70)
0.16	10 (50)	20 (100)

III. CONCLUSION

The study meets the validity criteria and the endpoints based on nominal product concentrations were:

Endpoints 0 to 24 hours	
EC_{50} – 24 hours (95 % C.I.):	> 0.16 mg prod./L (not determined)
EC_{20} – 24 hours (95 % C.I.):	0.062 mg prod./L (0.041 - 0.093 mg prod./L)
EC_{10} – 24 hours (95 % C.I.):	0.036 mg prod./L (0.022 - 0.061 mg prod./L)
NOEC – 24 hours:	0.01 mg prod./L
LOEC – 24 hours:	0.02 mg prod./L
Endpoints 0 to 48 hours	
EC_{50} – 48 hours (95 % C.I.):	**0.051 mg prod./L** (0.041 - 0.063 mg prod./L)
EC_{20} – 48 hours (95 % C.I.):	0.031 mg prod./L (0.024 - 0.041 mg prod./L)
EC_{10} – 48 hours (95 % C.I.):	0.024 mg prod./L (0.018 - 0.033 mg prod./L)
NOEC – 48 hours:	0.01 mg prod./L
LOEC – 48 hours:	0.02 mg prod./L

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: EC_{50} (48 hours) = 0.051 mg prod./L

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Data Point:	KCP 10.2.1/05
Report Author:	[redacted]
Report Year:	2021
Report Title:	Amendment no. 01 to final report: Pseudokirchneriella subcapitata growth inhibition test with fluopyram & trifloxystrobin SC 500 (250 + 250)G
Report No:	E 323 3111-4
Document No:	M-292579-02-1
Guideline(s) followed in study:	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006); Equivalent to US EPA OPPTS Guideline No. 850.5400 SUPP
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: None. 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

Executive Summary

The green alga *Pseudokirchneriella subcapitata* were exposed to FLU+TFS SC 500 (250+250 g/L) under static conditions for 72 hours. Algal cultures with an initial nominal 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 cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram were verified by HPLC– MS/MS on day 0 and 3 for each concentration and control. Measured concentrations were in the 90 - 98 % range of nominal 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 did not influence the outcome of the study negatively. No residues were found in the control samples above 0.5214 µg a.s./L, which was used as the lowest standard concentration during the study. The biological results are based on nominal concentrations of FLU+TFS SC 500 (250+250 g/L).

The study fulfils all validity criteria of OECD 201 guideline.

No physical abnormalities were observed 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. Material and Methods

Test material	FLU + TFS SC 500 (250 + 250 g/L) Specification No. 102000012886 Batch ID: 2007-000447 Content of a.s.: 21.4 % w/w fluopyram (251.5 g/L) 21.6 % w/w trifloxystrobin (253.5 g/L) Density: 1.174 g/mL
Guidelines adaptation	Not specified.
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
Test conditions	Temperature: 22.0 - 22.7 °C Photoperiod: 24 hours light Light intensity: 5620 - 7090 lux Type of light: bank light containing cool white fluorescent lamps pH of control: 8.0 - 9.4 Conductivity: not reported Growth medium same as culture medium: 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 test 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 counting under a microscope.
Sampling for chemical analysis	Samples of test solutions were taken at test initiation (0 hour) in all treatment levels and the control. At test termination (72 hours) samples were collected from composite samples of all replicates for each test concentration. Samples were analysed by using a high performance liquid chromatograph (HPLC) – MS/MS.
Data analysis	Probit analysis using linear 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 Microsoft Excel sheets and the further statistical evaluations with the commercial program ToxRat Professional (version 2.09).

II. RESULTS AND DISCUSSION

Table 10.2.1- 15: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 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 cultures must not exceed 35 %.	< 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 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+TFS 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 active ingredient.

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.

Table 10.2.1- 16: Analytical results for fluopyram

Nominal concentration		Measured a.s. concentration [µg a.s./L]		% of nominal	
[mg prod./L]	[µg a.s./L]	Day 0	Day 3	Day 0	Day 3
0.0286	6.12	6.00	9.31 [A]	98	152 [B]
0.0916	19.6	18.3	17.9	93	91
0.293	62.7	59.3	59.1	95	94
0.938	201	189	184	94	92
3.00	642	603	578	94	90

A Calculations based on 3 measured samples. Sample A was measured again, and sample B was also measured to confirm the first measurement.

B Most likely an outlier based on a handling error while sampling.

Biological results:

Observations:

No physical abnormalities were observed in any test concentration and the control.

Table 10.2.1- 17: Cell density

Nominal concentration [mg prod./L]	Mean cell density [x 10^4 cells/mL]		
	24 h	48 h	72 h
Control	3.7	14.0	85.9
0.0286	3.2	11.7	53.7
0.0916	1.8	5.7	24.0
0.293	1.7	3.3	10.7
0.938	1.5	2.2	3.0
3.00	0.7	2.0	1.7

Table 10.2.1- 18: Algae growth rate

Nominal concentration [mg prod./L]	Mean growth rate [1/d]	% Inhibition
	0 - 72 h	0 - 72 h
Control	1.482	-
0.0286	1.324 *	10.7
0.0916	1.059 *	28.5
0.293	0.782 *	47.2
0.938	0.363 *	75.5
3.00	0.167 *	88.7

* Significantly (α=0.05, one-sided smaller) reduced, based on Dunnett's Multiple t-test Procedure

III. CONCLUSION

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

E_rC_{50} -72 hours (95 % C.I.):	**0.292 mg prod./L** (0.236 – 0.361 mg prod./L)
E_rC_{20} -72 hours (95 % C.I.):	Not determined [A]
E_rC_{10} -72 hours (95 % C.I.):	Not determined [A]
E_bC_{50} -72 hours (95 % C.I.):	**0.0497 mg prod./L** (0.034 – 0.067 mg prod./L)
E_bC_{20} -72 hours (95 % C.I.):	0.0151 mg prod./L (0.006 – 0.024 mg prod./L)
E_bC_{10} -72 hours (95 % C.I.):	Not determined [A]
E_yC_{50} - 96 hours (95 % CI): [B]	0.0425 mg prod./L (0.0351 – 0.0500 mg prod./L)
E_yC_{20} - 96 hours (95 % CI): [B]	Not determined [A]
E_yC_{10} - 96 hours (95 % CI): [B]	Not determined [A]
LOE$_r$C - 72 hours: lowest concentration with an effect	≤ 0.0286 mg prod./L
NOE$_r$C - 72 hours: highest concentration without an effect (based on growth rate and yield [B])	< 0.0286 mg prod./L

[A] Not determined due to mathematical reasons.

[B] Please refer to recalculation document M-757720-01-1

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 0.292 mg prod./L

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Data Point:	KCP 10.2.1/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	evaluation (non-GLP) of the study M-292579-01-1 ([redacted] 2007, EBGMP032) on the chronic toxicity of fluopyram & trifloxystrobin SC 500 (250 + 250) G to Pseudokirchneriella subcapitata (currently known as: Raphidocelis subcapitata) under static conditions
Report No:	M-757720-01-1
Document No:	M-757720-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

In the existing report M-292579-01-1 (amended report M-292579-02-1) endpoints for yield were statistically determined at 72 h.

A statistical evaluation addressing the calculation of valid 72-h EC_{10}, EC_{20}, and EC_{50} values as well as NOEC values for yield was conducted to fulfill the data requirements according to regulation EU 283/2013. Furthermore, the validity criteria for the study were re-evaluated according to the current guideline OECD 201 (2011).

The recalculations were performed with the software ToxRat Professional (Version 3.3.0) based on nominal concentrations (EFSA 2015).

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 on yield of the test subjects (EC_{10}, EC_{20}, and EC_{50}), a probit analysis using linear maximum-likelihood regression was performed.

NOEC was determined by Williams 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.

Table 10.2.1- 19: Re-calculated EC_{10}, EC_{20}, EC_{50} and NOEC values based on nominal concentrations

Endpoint	FLU+TFS SC 500 [mg product/L]	Fluopyram [mg a.s./L]	Trifloxystrobin [mg a.s./L]
	Yield	Yield	Yield
72 hours - EC_{10} (95 % C.I.)	not determined	not determined	not determined
72 hours - EC_{20} (95 % C.I.)	not determined	not determined	not determined
72 hours - EC_{50} (95 % C.I.)	0.0425 (0.0351 – 0.0500)	9.10 (7.51 – 10.7)	9.18 (7.58 – 10.8)
72 hours - NOEC	< 0.0286	< 6.12	< 6.18

C.I.: Confidence interval

Assessment and conclusion by applicant:

The data are considered as acceptable and reliable without use in risk assessment.

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Data Point:	KCP 10.2.1/07
Report Author:	[redacted]
Report Year:	2018
Report Title:	Pseudokirchneriella subcapitata growth inhibition test with fluopyram + trifloxystrobin SC 500 G - final report
Report No:	EBGM0016
Document No:	M-615579-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation1107/2009 (Europe) OECD Test Guideline 201 US EPA OCSPP 850.4500
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: The pH increase in the control was 1.8 units and thus higher than the maximum 1.5 units as recommended in OECD 201. This deviation was 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

Executive Summary

The green alga *Pseudokirchneriella subcapitata* were exposed to FLU+TFS SC 500 (250+250 g/L) under static conditions for 96 hours. Algal cultures with an initial nominal cell count of approximately 1.0 × 10^4 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.

Concentrations of fluopyram and trifloxystrobin were verified by HPLC – MS/MS on day 0, 3 and 4 for each concentration and control. For fluopyram 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 (0.0625 µg 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 fulfils 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 Batch ID: PAIS005241 Content of a.s.: 21.1 % w/w fluopyram 21.6 % w/w trifloxystrobin Density: 1.167 g/mL
Guidelines adaptation	Not specified.
Test species	Freshwater Green alga: *Pseudokirchneriella subcapitata* Strain SAG 61.81
Culturing conditions	In-house 3-day old pre-culture held under test conditions.
Test solutions	Nominal product concentrations: of 0.00182 – 0.00582 – 0.0186 – 0.0596 – 0.191 – 0.610 – 1.95 – 6.25 – 20.0 mg prod./L Corresponding mean measured concentrations: not relevant Control: untreated medium Evidence of undissolved material: The highest test concentration (20.0 mg prod./L) was turbid over the whole testing period. All other test concentrations and the control showed clear media.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Exposure	Static Total exposure duration: 96 hours
Initial cell density	1×10^4 cells/mL in each test group
Test conditions	Temperature: 23.1 - 23.7°C Photoperiod: 24 hours light Light intensity: 4510 - 4820 lux Type of light: band light containing cool white fluorescent lamps pH of control: 8.1 - 9.9 Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	pH- values were measured at test start and additionally after 72 and after 96 hours. Temperature was determined by a continuous measurement in one additional incubated glass vessel. 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 determining the extinctions at a wave length of 578 nm. Cell numbers were computed from extinction values using the conversion formula: $\log_{10}$ (cell no.) = 6.3593 + 0.85307 × $\log_{10}$ (extinction).
Sampling for chemical analysis	Duplicate samples were taken at test initiation (0 hour) from bulk solution. After 72 hours and at test termination (96 hours) duplicate samples were collected from the pooled replicates from each test concentration and the control. Samples were analysed by using a high-performance liquid chromatograph (HPLC) – MS/MS.

Data analysis	Probit analysis using linear 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 Microsoft Excel sheets and the further statistical evaluations with the commercial program ToxRat Professional (version 3.2.1)

II. RESULTS AND DISCUSSION

Table 10.2.1- 20: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	103.7
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 %.	35 %	31.6 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	7 %	0.3 %

Analytical results:

Full details and acceptable validation data to support the analytical methods 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, 3 and 4 were between 91 and 113 % of nominal concentrations (see table below). For trifloxystrobin the recoveries ranged between 97 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 FLU + TFS SC 500.

No residues of fluopyram and trifloxystrobin were detected in the control samples above the limit of quantification (LOQ: 0.0625 µg a.s./L).

Table 10.2.1- 21: Analytical results for fluopyram

Nominal concentration		Measured a.s. concentration [µg a.s./L]			% of nominal		
[mg prod./L]	[µg a.s./L]	Day 0	Day 3	Day 4	Day 0	Day 3	Day 4
0.00182	0.384	0.413	0.387	0.420	108	101	109
0.00582	1.23	1.38	1.37	1.14	112	111	93
0.0186	3.93	4.39	3.80	4.41	112	97	112
0.0596	12.6	14.3	14.2	14.2	113	113	113
0.191	40.3	45.7	45.4	43.5	113	113	108
0.610	129	145	140	142	112	109	110
1.95	405	437	417	422	108	103	104
6.25	1319	1420	1400	1440	108	106	109
20.0	4220	4240	3830	4560	100	91	108

Table 10.2.1- 22: Analytical results for trifloxystrobin

Nominal concentration		Measured a.s. concentration [µg a.s./L]			% of nominal		
[mg prod./L]	[µg a.s./L]	Day 0	Day 3	Day 4	Day 0	Day 3	Day 4
0.00182	0.393	0.454	0.481	0.177	116	122	45
0.00582	1.26	1.41	0.915	0.193	112	73	15
0.0186	4.02	4.38	2.50	1.47	109	62	37
0.0596	12.9	15.1	9.09	7.02	117	70	54
0.191	41.3	45.9	28.7	23.3	109	69	56
0.610	132	136	86.4	79.9	103	65	61
1.95	421	408	263	230	97	62	55
6.25	1350	1620	959	938	120	71	69
20.0	4320	5100	3270	3450	118	76	80

Biological results:

Observations:

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

Table 10.2.1- 23: Cell density

Nominal concentration [mg prod./L]	Mean cell density [x 10^4 cells/mL]			
	24 h	48 h	72 h	96 h
Control	8.2	31.3	103.7	226.3
0.00182	8.0	29.8	97.6	213.6
0.00582	7.8	28.3	90.3	209.9
0.0186	6.9	24.3	68.9	158.7
0.0596	6.4	16.1	40.3	93.7
0.191	5.4	10.6	20.8	40.8
0.610	4.3	6.4	12.3	19.1
1.95	4.4	6.1	8.7	13.4
6.25	5.9	6.8	9.0	10.9
20.0	13.0	10.6	12.5	6.0

Table 10.2.1- 24: Algae growth rate

Nominal concentration [mg prod./L]	Mean growth rate [1/d]		% Inhibition	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	1.547	1.355	-	-
0.00182	1.527	1.341	1.3 *	1.1 *
0.00582	1.501	1.337	3.0*	1.4 *
0.0186	1.410	1.267	8.8 *	6.6 *
0.0596	1.232	1.135	20.4 *	16.3 *
0.191	1.012	0.927	34.6 *	31.5 *
0.610	0.836	0.737	45.9 *	45.6 *
1.95	0.722	0.649	53.3 *	52.1 *
6.25	0.731	0.597	52.7 *	55.9 *
20.0	0.818	0.407	47.1 *	70.0 *

* Significantly (α=0.05, one-sided smaller) reduced, based on multiple sequentially-rejective Welch- t-test after Bonferroni-Holm

Table 10.2.1- 25: Biomass

Mean measured concentration [µg a.s./L]	Biomass [A]		% Inhibition of biomass	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	2131.2	6067.0	-	-
0.00182	2019.4	5730.0	5.2 *	5.6 *
0.00582	1890.6	5468.8	11.3 *	9.9 *
0.0186	1515.5	4223.7	28.9 *	30.4 *
0.0596	961.6	2545.5	54.9 *	58.0 *
0.191	574.3	1289.6	73.1 *	78.7 *
0.610	345.7	698.6	83.8 *	88.5 *
1.95	296.4	538.2	86.1 *	91.1 *
6.25	353.5	567.3	83.5 *	90.6 *
20.0	800.6	1008.5	62.4 *	83.4 *

[A] Biomass is equal to the area under the growth curve.

* Significantly (α=0.05, one-sided smaller) reduced, based on multiple sequentially-rejective Welch- t-test after Bonferroni-Holm for the 0-72 hour period and based on Williams multiple sequential t-test procedure for the 0-96 hour period

Table 10.2.1- 26: Yield

Nominal concentration [mg prod./L]	Mean yield [x 10^4 cells/ml]		% Inhibition	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	102.7	225.3	0.0	0.0
0.00182	96.6	212.6	5.9 *	5.6 *
0.00582	89.3	208.9	13.0 *	7.3 *
0.0186	67.9	157.7	33.9 *	30.0 *
0.0596	39.2	92.7	61.8 *	58.8 *
0.191	19.8	39.8	80.7 *	82.3 *
0.610	11.3	18.1	89.0 *	92.0 *
1.95	7.7	12.4	92.5 *	94.5 *
6.25	8.0	9.9	92.2 *	95.6 *
20.0	11.5	5.9	88.8 *	97.4 *

* Significantly (α=0.05, one-sided smaller) reduced, based on multiple sequentially-rejective Welch- t-test after Bonferroni-Holm for the 0-72 hour period and based on Williams multiple sequential t-test procedure for the 0-96 hour period

III. CONCLUSION

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 hours	
E_rC_{50} - 72 hours (95 % CI):	**4.25 mg prod./L** (3.72 - 4.90 mg prod./L)
E_rC_{20} -72 hours (95 % C.I.):	0.048 mg prod./L (0.040 - 0.056 mg prod./L)
E_rC_{10} -72 hours (95 % C.I.):	0.005 mg prod./L (0.003 - 0.006 mg prod./L)
E_yC_{50} - 72 hours (95 % CI):	**0.040 mg prod./L** (0.038 - 0.041 mg prod./L)
E_yC_{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.):	0.003 mg prod./L (0.003 - 0.004 mg prod./L)
E_bC_{50} - 72 hours (95 % CI):	**0.069 mg prod./L** (0.066 - 0.072 mg prod./L)
E_bC_{20} -72 hours (95 % C.I.):	0.006 mg prod./L (0.006 - 0.006 mg prod./L)
E_bC_{10} -72 hours (95 % C.I.):	0.002 mg prod./L (0.002 - 0.002 mg prod./L)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate, biomass integral and yield)	≤ 0.00182 mg prod./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate, biomass integral and yield)	< 0.00182 mg prod./L

Results – 0 to 96 hours	
E_rC_{50} - 96 hours (95 % CI):	**1.99 mg prod./L** (1.67 - 2.41 mg prod./L)
E_rC_{20} - 96 hours (95 % C.I.):	0.077 mg prod./L (0.058 - 0.099 mg prod./L)
E_rC_{10} -96 hours (95 % C.I.):	0.014 mg prod./L (0.009 - 0.020 mg prod./L)
E_yC_{50} - 96 hours (95 % CI):	**0.044 mg prod./L** (0.043 - 0.045 mg prod./L)
E_yC_{20} - 96 hours (95 % C.I.):	0.011 mg prod./L (0.011 - 0.012 mg prod./L)
E_rC_{10} -96 hours (95 % C.I.):	0.005 mg prod./L (0.005 - 0.006 mg prod./L)
E_bC_{50} - 96 hours (95 % CI):	**0.048 mg prod./L** (0.046 - 0.049 mg prod./L)
E_bC_{20} - 96 hours (95 % C.I.):	0.009 mg prod./L (0.009 - 0.010 mg prod./L)
E_bC_{10} -96 hours (95 % C.I.):	0.004 mg prod./L (0.004 - 0.004 mg prod./L)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate, biomass integral and yield)	≤ 0.00182 mg prod./L
NOEC - 96 hours: highest concentration without an effect (based on growth rate, biomass integral and yield)	< 0.00182 mg prod./L

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Pseudokirchneriella subcapitata*.

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate	0.005	0.003 – 0.006	0.6 (fair)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)
Yield	0.003	0.003 – 0.004	0.333 (good)	EC_{10} < EC_{20}, low (high)
Biomass	0.002	0.002 – 0.002	0.0 (excellent)	EC_{10} < EC_{20}, low (high)

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 4.25 mg prod./L

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Data Point:	KCP 10.2.1/08
Report Author:	[redacted]
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:	M-636234-01-1
Guideline(s) followed in study:	-OECD Guidelines for the Testing of Chemicals, Section 2, No. 201, "Feshwater Alga and Cyanobacteria, Growth Inhibition Test", adopted March 23, 2006, corrected July 28, 2011 - Commission Regulation (EC) No 761/2009, Annex, Part C, C.3: "Feshwater Alga and Cyanobacteria, Growth Inhibition Test", Official Journal of the European Union (EN), dated August 24, 2009 - EPA Guideline 712-C-006: OCSPP 850.4500, "Algal Toxicity", January 2012 - Japanese MAFF, Gueidelines for preparation of Study Results, Algae growth Inhibition studies. Notification No. 12-Nousan-8147, JMAFF Test Guideline, 2-7-7, Algae growth Inhibition, 2005 - SANCO/3029/99 rev. 4 11/07/00: Residues: Guidance for generating and reporting methods of Analysis in Support of preregistration data requiremetns for Annex II (part A; Section 4) and Annex III (part A; Section 5) of directive 91/414
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: None. 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

Executive Summary

The green alga *Pseudokirchneriella subcapitata* were 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 × 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 study design included 4 replicates for each test concentration and 6 replicates for the control. At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram and trifloxystrobin were verified by LC – MS/MS on day 0, 1, 2, 3 and 4 for each concentration and control. For fluopyram measured concentrations were in the 85.0 - 100 % range of nominal concentrations. For trifloxystrobin the recoveries showed a decrease over time with recoveries ranging between 90.5 and 111 % 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 trifloxystrobin were found in the control samples above their LOQ (LOQ for fluopyram: 0.2 µg a.s./L, LOQ for trifloxystrobin: 0.75 µg 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 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 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) Specification No.: 102000012886 Batch ID: PA1S0Q5 1 73 Content of a.s.: 21.3 % w/w fluopyram 21.4 % w/w trifloxystrobin Density: 1.170 g/mL
Guidelines adaptation	Not specified.
Test species	Freshwater Green alga *Pseudokirchneriella subcapitata*
Culturing conditions	In-house 3 day old pre-culture held under test conditions.
Test solutions	Nominal product concentrations: 0.0089 – 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: There were no remarkable observations of the test medium during the exposure. The test medium appeared clear.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 6
Exposure	Static Total exposure duration: 96 hours
Initial cell density	1×10^4 cells/mL in each test group
Test conditions	Temperature: 22.5 - 23.8 °C Photoperiod: 24 hours light Light intensity: 4550 - 5990 lux Type of light: fluorescent lamps pH of control: 8.2 - 9.7 Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	The pH was measured in all test item concentrations and the control at the start, after 72 h and the end of the test. Temperature was determined by a continuous measurement in one additional incubated glass vessel. Cell density and morphological 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 chemical analysis	Duplicate samples of test solutions were taken at test initiation (0 hour) in all treatment levels and the control and then daily thereafter till test termination (96 hours). Samples were analysed by using liquid chromatograph (LC) – MS/MS.
Data analysis	Probit analysis using linear max. likelihood regression was used for EC_x-value estimation and where possible their 95 %-confidence limits. LOEC/ NOEC determinations were done using Welch t-test after Bonferroni-Holm. Calculations were done with Microsoft Excel sheets and the further statistical evaluations with the commercial program ToxRat Professional (version 3.2.1).

II. RESULTS AND DISCUSSION

Table 10.2.1- 27: Validity criteria

Validity criteria acc. to OECD 201 (adopted 2006)	Required	Obtained
The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.	≥ 16	183
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 %.	35 %	22 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	7 %	1.6 %

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 nominal concentrations (see table below). For trifloxystrobin the measured concentrations showed a decrease over time: recoveries ranged between 90.5 and 111 % 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 111 % 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 FLU + TFS SC 500 (250+250 g/L).

No residues of fluopyram and trifloxystrobin were detected in the control samples above their limit of quantification (LOQ for fluopyram: 0.2 µg a.s./L, LOQ for trifloxystrobin: 0.75 µg a.s./L).

Table 10.2.1- 28: Analytical results for fluopyram

Nominal concentration		Measured a.s. concentration [A] [µg a.s./L]					% of nominal [A]				
[mg prod./L]	[µg a.s./L]	Day 0	Day 1	Day 2	Day 3	Day 4	Day 0	Day 1	Day 2	Day 3	Day 4
0.0089	1.892	1.71	1.60	1.69	1.77	1.70	90.5	85.0	89.5	93.5	90.0
0.0286	6.079	5.51	5.30	5.50	5.49	5.39	90.5	87.5	90.5	90.5	88.5
0.0916	19.471	18.6	18.33	18.17	19.2	19.0	95.5	94.0	93.5	98.5	97.5
0.293	62.281	59.5	60.3	60.0	60.9	62.3	96.0	96.5	96.5	97.5	100
0.938	199.385	188	184	192	190	191	94.5	92.5	96.0	95.5	96.0
3.00	637.692	620	622	632	624	634	97.0	97.5	99.0	98.0	99.5

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

Table 10.2.1- 29: Analytical results for trifloxystrobin

Nominal concentration		Measured a.s. concentration [A] [µg a.s./L]					% of nominal [A]				
[mg prod./L]	[µg a.s./L]	Day 0	Day 1	Day 2	Day 3	Day 4	Day 0	Day 1	Day 2	Day 3	Day 4
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	45.5	1
0.0916	19.627	19.1	17.21	14.98	12.8	6.71	97	87.5	76.5	65.5	34.5
0.293	62.782	60.1	55.7	48.4	42.8	38.9	96	89.0	77.0	68	62
0.938	200.989	188	165	151	133	130	93.5	82.0	75.5	66.5	65
3.00	642.821	581	507	452	452	443	90.5	83.5	79.0	70.5	69

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

Biological results:

Observations:

After 72 hours there were no deviations of the shape of the algal cells in the 4 lowest test concentrations (0.0089, 0.0286, 0.0916 and 0.293 mg prod./L) compared to the cells in the control. However, 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 concentrations (0.0089, 0.0286 and 0.0916 mg prod./L). However, the three highest test concentrations (0.293, 0.938 and 3.00 mg prod./L) showed smaller cells compared to the cells in the control and the cells were elongated instead of sickle-shaped.

Table 10.2.1- 30: Cell density

Nominal concentration [mg prod./L]	Mean cell density [x 10^4 cells/mL]			
	24 h	48 h	72 h	96 h
Control	7.28	49.5	183	335.0
0.0089	6.43	37.2	170.8	340.2
0.0286	5.20	26.1	111.2	275.6
0.0916	4.03	13.6	52.6	155.3
0.293	3.50	6.01	14.4	39.8
0.938	3.12	3.79	6.40	12.2
3.00	2.96	3.18	3.37	4.80

Table 10.2.1- 31: Algae growth rate

Nominal concentration [mg prod./L]	Mean growth rate (based on cell density) [1/d]		% Inhibition [A]	
	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	-0.3
0.0286	1.57	1.405	9.5 *	3.3 *
0.0916	1.32	1.26	23.9 *	13.3 *
0.293	0.889	0.921	48.8 *	36.6 *
0.938	0.618	0.625	64.4 *	57.0 *
3.00	0.404	0.391	76.7 *	73.1 *

A -% inhibition means increase in growth relative to the control
* Mean value significantly different from the control (α= 0.05, one-sided, Welch t-test after Bonferroni-Holm)

Table 10.2.1- 32: Biomass

Nominal concentration [mg prod./L]	Cumulative biomass [A]		% Inhibition of cumulative biomass [B]	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	145.785	403.791	-	-
0.0089	126.562	381.054	13.2 *	5.6
0.0286	84.423	276.849	42.1 *	31.4 *
0.0916	41.685	144.445	71.5 *	64.2 *
0.293	14.190	40.295	90.3 *	90.0 *
0.938	7.082	15.407	95.1 *	96.2 *
3.00	6.231	9.314	95.7 *	97.7 *

A Cumulative biomass is equal to the area under the growth curve.
B -% inhibition means increase in growth relative to the control
* Mean value significantly different from the control (Welch t-test after Bonferroni-Holm, α = 0.05, one-sided)

Table 10.2.1- 33: Algae yield

Nominal concentration [mg prod./L]	Mean yield [x 10^4 cells/mL]		% Inhibition [A]	
	0 - 72 h	0 - 96 h	0 - 72 h	0 - 96 h
Control	181.995	334.017	-	-
0.0089	169.804	339.179	6.7	-1.5
0.0286	110.240	274.611	39.4 *	17.8 *
0.0916	51.828	154.291	71.6 *	53.8 *
0.293	13.388	38.822	92.6 *	88.4 *
0.938	5.406	11.244	97.0 *	96.6 *
3.00	2.368	3.798	98.7 *	98.8 *

A -% inhibition means increase in growth relative to the control
* Mean value significantly different from the control (α= 0.05, one-sided, Welch t-test after Bonferroni-Holm)

III. Conclusion

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

Results – 0 to 72 hours	
E_rC_{50} - 72 hours (95 % CI):	**0.419 mg prod./L** (0.405 – 0.433 mg prod./L)
E_rC_{20} -72 hours (95 % C.I.):	0.0624 mg prod./L (0.0592 – 0.0657 mg prod./L)
E_rC_{10} -72 hours (95 % C.I.):	0.0230 mg prod./L (0.0214 – 0.0248 mg prod./L)
E_yC_{50} - 72 hours (95 % CI):	**0.0432 mg prod./L** (0.0412 – 0.0453 mg prod./L)
E_yC_{20} -72 hours (95 % C.I.):	0.0147 mg prod./L (0.0136 – 0.0159 mg prod./L)
E_yC_{10} -72 hours (95 % C.I.):	0.0084 mg prod./L (0.0076 – 0.0093 mg prod./L)
E_bC_{50} - 72 hours (95 % CI):	**0.0405 mg prod./L** (0.0391 – 0.0420 mg prod./L)
E_bC_{20} -72 hours (95 % C.I.):	0.0117 mg prod./L (0.0110 – 0.0124mg prod./L)
E_bC_{10} -72 hours (95 % C.I.):	0.0061 mg prod./L (0.0056 – 0.0066 mg prod./L)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate and yield)	0.0286 mg prod./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate and yield)	0.0089 mg prod./L
LOE_bC - 72 hours: lowest concentration with an effect (based on biomass)	≤ 0.0286 mg prod./L
NOE_bC - 72 hours: highest concentration without an effect (based on biomass)	< 0.0089 mg prod./L

Results – 0 to 96 hours	
E_rC_{50} - 96 hours (95 % CI):	**0.709 mg prod./L** (0.686 – 0.734 mg prod./L)
E_rC_{20} - 96 hours (95 % C.I.):	0.128 mg prod./L (0.122 – 0.135 mg prod./L)
E_rC_{10} -96 hours (95 % C.I.):	0.0525 mg prod./L (0.0488 – 0.0564 mg prod./L)
E_yC_{50} - 96 hours (95 % CI):	**0.0815 mg prod./L** (0.0782 – 0.0849 mg prod./L)
E_yC_{20} - 96 hours (95 % C.I.):	0.0320 mg prod./L (0.0300 – 0.0342 mg prod./L)
E_yC_{10} -96 hours (95 % C.I.):	0.0197 mg prod./L (0.0181 – 0.0214 mg prod./L)
E_bC_{50} - 96 hours (95 % CI):	**0.0563 mg prod./L** (0.0545 – 0.0583 mg prod./L)
E_bC_{20} - 96 hours (95 % C.I.):	0.0188 mg prod./L (0.0178 – 0.0198 mg prod./L)
E_bC_{10} -96 hours (95 % C.I.):	0.0106 mg prod./L (0.0099 – 0.0114 mg prod./L)
LOEC - 96 hours: lowest concentration with an effect (based on growth rate, biomass and yield)	0.0286 mg prod./L
NOEC - 96 hours: highest concentration without an effect (based on growth rate, biomass and yield)	0.0089 mg prod./L

Reliability assessment (EFSA 2015):

The following table provides reliability indicators for EC_{10} values for *Pseudokirchneriella subcapitata*.

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate	0.023	0.0214 – 0.0248	0.148 (excellent)	$EC_{10} < EC_{20}$, low (high)
Yield	0.0084	0.0076 – 0.0093	0.202 (good)	$EC_{10} < EC_{20}$, low (high)
Biomass	0.0061	0.0056 – 0.0066	0.163 (excellent)	$EC_{10} < EC_{20}$, low (high)

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: E_rC_{50} (72 hours) = 0.419 mg prod./L

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic

invertebrates and sediment dwelling organisms

No new studies were necessary based on the current data requirements. Please refer to Document MCA, Section 8.2.

CP 10.2.3 Further testing on aquatic organisms

No studies were necessary based on the current data requirements. Please refer to Document MCA, Section 8.2.

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

The risk assessment has been performed according to the existing guidance in force at the time of the preparation and submission of this dossier namely the EU Guidance Document on Terrestrial 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 (EU) 283/2013 and 284/2013 require testing of both acute (oral and contact) and chronic toxicity, including sub-lethal effects. Although there are no current testing requirements for any bee other than for the honey bee within 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 adult honey bees (OECD 213 and 214) the following studies are also provided:

- Acute oral and contact toxicity of fluopyram tech. to adult bumble bees under laboratory conditions (OECD 246/OECD 247; M-542447-01-1, M-510849-01-1 and M-763123-01-1)
- Acute and contact toxicity of FLU + TFS SC 500 to adult bumble bees under laboratory conditions (OECD 246/OECD 247; M-738910-01-1)
- Chronic 10-day toxicity test with FLU + TFS SC 500 on adult honey bees under laboratory conditions (OECD 245; M-753793-02-1)
- Toxicity to honey bee larvae under laboratory conditions following repeated exposure to the formulated product FLU + TFS SC 500 (OECD guidance 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 Phacelia covering effects on brood development, adult and pupal mortality, foraging activity, behaviour and colony development and strength. This semi-field study is presented in MCP Section 10, Point 10.3.1.5/01, M-435338-01-1.

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

Test substance	Test species/ study type	Endpoint	References
Fluopyram tech.	*Apis mellifera*, acute test	LD_{50} oral (48 h) **> 102.3 µg a.s./bee** LD_{50} contact (48 h) **> 100 µg a.s./bee**	[redacted] (2005) M-261594-01-1 KCA 8.3.1.1.1/01 KCA 8.3.1.1.2/01
	Bombus terrestris, acute test	LD_{50} oral (48 h) > 92.5 µg a.s./bumble bee	[redacted] (2015) M-542447-01-1 KCA 8.3.1.1.1/06
	Bombus terrestris, acute test	LD_{50} contact (48 h) > 100 µg a.s./bumble bee	[redacted] (2015) M-510849-01-1 KCA 8.3.1.1.2/04
	Bombus terrestris, acute test	LD_{50} oral (48 h) > 90.5 µg a.s./bumble bee LD_{50} contact (48 h) > 100 µg a.s./bumble bee	[redacted] (2021) M-763123-01-1 KCA 8.3.1.1.1/07 KCA 8.3.1.1.2/05
FLU+ TFS SC 500	*Apis mellifera*, acute test	LD_{50} oral (48 h) **> 208.8 µg prod./bee** LD_{50} contact (48 h) **> 200 µg prod./bee**	[redacted] (2007) M-288193-01-1 KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01
	Bombus terrestris, acute test	LD_{50} oral (48 h) > 1216.23 µg prod./bee LD_{50} contact (48 h) > 1185.12 µg prod./bee	[redacted] (2020) M-738910-04-1 KCP 10.3.1.1.1/02 KCP 10.3.1.1.2/02
	Apis mellifera, 10-day oral feeding test	LDD_{50} 16.95 µg prod./bee/day NOEDD < 16.73 µg prod./bee/day LC_{50} 902.13 mg product/kg diet NOEC < 829.91 mg product/kg diet	[redacted] (2020) M-753795-02-1 KCP 10.3.1.2/01
	Apis mellifera, larva 22-day repeated feeding test	NOEC 240.48 mg prod./kg diet NOED 37.03 µg prod./larva EC_{50} > 601.21 mg prod./kg diet ED_{50} > 92.59 µg prod./larva	[redacted] (2020) M-738909-02-1 KCP 10.3.1.3/01
Higher Tier			
FLU + TFS SC 500	Honeybee colony development – Semi-Field (EPPO 170)	No adverse effects on mortality, foraging activity, behaviour, nectar- and pollen storage, brood-abundance and development, colony strength as well as on queen survival after two applications at 0.56 L prod./ha each (corresponding to 140 g fluopyram/ha and 140 g trifloxystrobin/ha). The first application to the bee-attractive crop *Phacelia tanacetifolia* was carried out at BBCH 59-61 without bees present, while the second application occurred during full flowering (BBCH 64-65) with bees actively foraging on the crop during application.	[redacted] (2012) M-435338-01-1 KCP 10.3.1.5/01

Bold values used in risk assessment
a.s.: active substance
prod.: product

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 (LD_{50} 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 laboratory contact and oral LD_{50} (expressed in µg 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.

Hazard Quotient, oral:

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

Hazard Quotient, contact:

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

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

Compound	Oral LD_{50} [µg./bee]	Max. application rate [g/ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Fluopyram tech.	> 102.3	50	< 0.49	50	yes
FLU + TFS SC 500	> 208.8	234.8 [A]	< 1.12	50	yes

[A] Based on an application rate of 200 mL 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 10.3.1- 3: Hazard quotients for bees for application in grapes, 2 x 50 g a.s./ha – contact exposure

Compound	Contact LD_{50} [µg/bee]	Max. application rate [g/ha]	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
Fluopyram tech.	> 100	50	< 0.50	50	yes
FLU + TFS SC 500	> 200	234.8 [A]	< 1.17	50	yes

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

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

Further considerations regarding the risk to bees

The active substance fluopyram and the formulated product FLU + TFS SC 500 are both of low toxicity to bees. The technical material exhibits acute LD_{50} values for adult bees of > 100 µg a.s./bee (contact) and > 102.3 µg a.s./bee (oral). The formulated product FLU + TFS SC 500 is of low toxicity with acute oral and contact LD_{50} 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 levels 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 (FLU + TFS SC 500). Hence, the findings indicate that bumble bees do not exhibit greater sensitivity to FLU + TFS 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 non-*Apis* bees such as *Bombus terrestris.*

In addition, a chronic oral toxicity test (10-day feeding) as per 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 or larvae is considered to be low, the applicant recognizes the new requirements for chronic 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 life stages, respectively, in accordance with the data requirements as set out in Commission Regulation (EU) No. 284/2013.

Chronic adult toxicity

A 10-day laboratory feeding study investigating the effects of FLU + TFS SC 500 was conducted to assess chronic toxicity to honey bees in accordance with OECD Guideline No. 245.

The study concluded that continuous *ad libitum* feeding at 829.91 mg product/kg diet over a period of 10 days led to 44.68 % mortality. The LDD_{50} was determined as 16.95 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-finding test used to determine the range of doses that should be tested in the actual study to derive $LC_{10/20/50}$ values. As a result, all but the lowest test item dose yielded 10-day cumulative mortalities ≥ 90%. The regression analysis thus relied on only two data points to estimate the LC_{50} and only a subset of treatments was selected for the Weibull regression as inclusion of all treatments did not show any dose-response relationship. Details of the study are presented in KCP 10.3.1.2/01, M-753795-02-1.

Chronic larval toxicity/effects on brood

A honey bee larval toxicity 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 laboratory dose-response 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/kg diet. The corresponding cumulative doses were 2.37, 5.93, 14.81, 37.03 and 92.59 µg product/larva. 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 tests did not indicate a risk to bees due to the use of FLU+ TFS SC 500, further assessment of the chronic 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) (2010)) 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 trifloxystrobin/ha) (KCP 10.3.1.5; M-435338-01-1). The first application to the bee-attractive crop *Phacelia tanacetifolia* was carried out at BBCH 59-61 without bees present, while the second application occurred during full 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 colonies.

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 *Phacelia tanacetifolia*.

Furthermore, the results of the chemical residue analysis of bee-relevant matrices (flowers, nectar and pollen) indicated maximum residues of fluopyram ranging from 1.1 mg a.s./kg in nectar to 30 mg a.s./kg in pollen. These concentrations are well below the larval NOEC of ≥ 520 mg a.s./kg (KCA 8.3.1.3/01; M-617279-01-1) or the chronic adult NOEC of 3333 mg a.s./kg (KCA 8.3.1.2/01; M-540072-01-1), 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 crop treated with the formulated product FLU + TFS SC 500 at rates well above the proposed foliar spray rate for grapes.

Therefore, it can be concluded that the representative formulation FLU + TFS SC 500 does not adversely affect honey bees and honey bee colonies when applied at a rate of 0.56 L/ha during honey bees actively foraging on a highly bee-attractive, flowering crop, indicating 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

Data Point:	KCP 10.3.1.1.1/01
Report Author:	[redacted]
Report Year:	2007
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 study:	OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test, (adopted 21st September 1998) ; OECD 214: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Contact Toxicity Test, (adopted 21st September 1998); Equivalent to US EPA OPPTS Guideline No. 850.3020 SUPP
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 213: The guideline-specified relative humidity range of 50-70% was not met. This deviation is not expected to have impacted the study results. All validity criteria were met. Deviations from OECD Guideline 214: The guideline-specified relative humidity range of 50-70% was not met. An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL 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
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute contact and oral toxicity of FLU + TFS SC 500 (250+250 g/L) to the honey bee (*Apis mellifera* 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 on intake 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_{50} value (48 h) was determined to be > 200.0 µg product/bee. The oral LD_{50} value was determined to be > 208.8 µg product/bee.

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

I. MATERIAL AND METHODS

Test item: 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;

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

Test design: 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 on intake of the test item was 208.8 µg product/bee).

Tap water with 0.5 % Adhäsit (improves spreading of the test droplet on the water-repellent 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 sugar solution. The toxic reference dimethoate (Perfekthion EC 400, 400.0 g/L nominal, 414.4 g/L analytical) was applied at nominal dose levels of 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee in the contact test and 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee in the oral test.

In the contact and oral toxicity tests each treatment group (test item, controls and reference item) comprised 5 replicates including 10 bees each.

Application in the contact test: A single 5 µL droplet of the control, test item and toxic standard (vehicle: 0.5 % v/v Adhäsit) was placed on the dorsal bee thorax using a Burkard – Applicator following anaesthetization of bees with CO_2.

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

Application in the oral test: The test item and reference item were applied in 50 % ready-to-use syrup (30 % Saccharose, 31 % Glucose, 39 % Fructose). The untreated sugar solution was offered to bees in the control group. The treated diet was offered in syringes, which were weighed before and after introduction into the cages (duration of feeding 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:	200.0 µg product/bee (contact limit test)
	200 µg product/bee (oral limit test)
Actual dose of the test item (oral test):	208.8 µg product/bee
Nominal doses of the reference item:	0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee (contact test)
	0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee (oral test);

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

Test conditions: Temperature: 25 °C; relative humidity: 26 – 46 %; photoperiod: 24 h darkness (except during observations).

Statistics: 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 LD_{50} values of the reference item were estimated according to moving average computations (Thompson and Weil, 1952). The LD_{50} 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), there was no mortality at 200.0 µg product/bee. No mortality occurred in the control (water + 0.5 % Adhäsit).

No behavioural abnormalities of the bees were observed during the entire trial at 200 µg product/bee.

Table 10.3.1.1.1- 1: Mortality and behavioural abnormalities of the bees in the contact toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg product/bee]						
200.0	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
0.10	0.0	0.0	4.0	0.0	6.0	0.0
0.15	4.0	2.0	10.0	12.0	20.0	4.0
0.20	2.0	2.0	22.0	8.0	34.0	0.0
0.30	14.0	10.0	68.0	6.0	72.0	8.0

Results are mean values based on 5 replicates (control, test item and reference item) containing 10 bees each
Behav. abnorm. = behavioural 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 nominal dose of 200 µg FLU + 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 abnormalities occurred.

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

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Control	0.0	0.0	0.0	0.0	2.0	0.0
Test item [µg product/bee]						
208.8	4.0	0.0	4.0	0.0	6.0	0.0
Reference item [µg a.s./bee]						
0.06	0.0	0.0	0.0	0.0	0.0	0.0
0.08	4.0	2.0	14.0	2.0	18.0	0.0
0.17	36.0	20.0	90.0	2.0	94.0	0.0
0.33	82.0	18.0	98.0	2.0	98.0	2.0

Results are mean values of 5 replicates (control, test item and reference item) containing 10 bees each
Behav. abnorm. = behavioural abnormalities;
Test item= FLU + TFS SC 500 (250+250 g/L), reference item= dimethoate; control = 50 % w/v sugar solution

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

Table 10.3.1.1.1- 3: Contact and oral toxicity of FLU + TFS SC 500 (250+250 g/L) to honey bees

Test item	FLU + TFS SC 500 (250+250 g/L)	
Test species	Honey bee *Apis mellifera* L.	
Exposure	**Contact**	**Oral**
Test duration	48 h	48 h
Dose rate [µg product/bee]	200.0	Nominal dose: 200.0 Actual dose: 208.8
LD_{50} [µg product/bee]	> 200.0	> 208.8

<u>Reference item</u>

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.30 and 0.14 µg a.s./bee, respectively.

Validity criteria:

The contact and oral tests were considered valid as the control mortality in each case was ≤ 10 % and the LD_{50} values obtained with the reference item (dimethoate) were within the required ranges.

Table 10.3.1.1.1- 4: Validity criteria

Validity Criteria	Recommended		Obtained
Control mortality	Contact Test		
	Water control	≤ 10 %	0 %
	Oral Test		
	Control	≤ 10 %	2 %
LD_{50} of reference item (24 h)	Contact Test		
	Dimethoate	0.10 - 0.30 µg a.s./bee	0.30 µg a.s./bee
	Oral Test		
	Dimethoate	0.10 - 0.35 µg a.s./bee	0.14 µg a.s./bee

III. CONCLUSION

The toxicity of FLU + TFS SC 500 (250+250 g/L) was tested in both, an acute contact and an acute oral toxicity test on honey bees.

The contact LD_{50} value (48 h) was determined to be > 200.0 µg product/bee. The oral LD_{50} value was determined to be > 208.8 µg product/bee.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment. The endpoints are:

LD_{50} contact (48 hours) > 200.0 µg product/bee

LD_{50} oral (48 hours) > 208.8 µg product/bee

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Bumble bees

Data Point:	KCP 10.3.1.1.1/02
Report Author:	[redacted]
Report Year:	2020
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 laboratory
Report No:	144951105
Document No:	M-738910-04-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 246 and 247 (2017)
Deviations from current test guideline:	Current Guidelines: OECD 246 and 247 (2017) Deviations from OECD Guideline 246: No information on bumble bee colonies concerning size, brood stages and number of adults are reported. The limit dose 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 recommendation of 2 µL. These deviations are not expected to have impacted the study results. All validity criteria of the current guideline were met. Deviations from OECD Guideline 247: No information on bumble bee colonies concerning size, brood stages and number of adults are reported. The limit dose was 250 µg a.s./bee instead of the recommended dose of 100 µg a.s./bee. These deviations are not expected to have impacted the study results. All validity criteria of the current guideline were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the acute contact and oral toxicity of FLU + TFS SC 500 (250 + 250) to the bumble bee (*Bombus terrestris* 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 bumble 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 bee by feeding (oral limit test; actual dose based on the intake of the test item was 1216.23 µg product/bumble bee).

The contact test comprised 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) was included.
The purpose of the analytical part of this study was to verify the concentrations of the active ingredients (fluopyram and trifloxystrobin) in the single contact application solution (limit test) and in the single oral feeding solution (limit test).

In the contact toxicity test the LD_{50} value (48 h) of FLU + TFS SC 500 (250+250 g/L) was estimated to be 1185.12 µg product/bumble bee. The contact NOED value (48 h) was calculated to be ≥ 1185.12 µg product/bumble bee.

The oral LD_{50} value (48 h) of FLU + TFS SC 500 (250+250 g/L) was > 1216.23 µg product/bumble bee. The oral NOED value (48 h) was calculated to be ≥ 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

Test item: 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).

Test species: 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 company. 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 CO_2 to prove a consistent distribution among the treatment groups. Bumblebees were acclimatised to test conditions (contact test: 24 hours; oral test: 45 hours) with *ad libitum* access to untreated 50% w/v sucrose solution.

Test design: Acute contact toxicity of FLU + TFS SC 500 (250+250 g/L) to adult bumble bees was assessed by exposing 50 worker bumble bees to 1185.12 µg product/bumble bee dissolved in tap water containing 0.1 % v/v Triton X-100 (contact limit test). Additional groups of 50 and 30 adult bumble bees each were assigned to either a 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 + TFS SC 500 (250+250 g/L) to adult bumble bees was assessed by exposing 50 worker bumble bees to 1185.12 µg product/bumble bee in 50 % w/v sucrose solution (oral limit test). This nominal treatment dose corresponded to an actual mean oral dose of 1216.23 µg product/bumble bee based on the actual mean intakes of the test item. In addition, 50 and 30 adult bumble bees each were assigned to either a water control (50 % w/v sucrose solution) and reference item (mean oral dose of 4.3 µg dimethoate/bumble bee) treatment 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).

Application in the contact test: In the contact toxicity test the test item was dissolved in tap water with 0.1 % v/v Triton X-100 and applied as one 4 µL droplet onto the dorsal thorax of bumble bees using a calibrated pipette. The reference item was applied as one 4 µL droplet of dimethoate, dissolved in tap water with 0.1 % v/v Triton X-100. For the control, one 4 µL droplet of tap water containing 0.1 % v/v Triton X-100 was used. A 4 µL droplet was chosen instead of 2 µL due to a technical error during the preparation of the test item application solution.
Application in the oral test: The test item and reference item were applied in 50 % w/v sucrose solution, which was used as carrier (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, all syringes containing remaining food were removed, weighed and afterwards replaced by fresh, untreated food. The calculation of the target dose was based on 40 mg food uptake. The ingested 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:	1185.12 µg product/bumble bee (contact limit test)
	1185.12 µg product/bumble bee (oral limit test)
Actual dose of the test item (oral test):	1216.23 µg product/bumble bee (based on the actual food intake)
Nominal doses of the reference item:	10 µg dimethoate/bumble bee (contact limit test)
	4.0 µg dimethoate/bumble bee (oral limit test)
Actual doses of the reference item (oral test):	4.3 µg dimethoate/bumble bee

Test conditions: Temperature: 22.7 – 25.4 °C; relative humidity: 44.9 – 65.3 %; photoperiod: 24 h darkness (except during observation).
Statistics: Results obtained from the bumble bees treated with the test item and the reference item were compared to those obtained from the control in both the contact and oral test. For the evaluation of the results of the oral test, bumble bees which did not consume at least 80 % of the mean food uptake per treatment group were excluded from the evaluation of mortality and behavioural abnormalities, as well as from the calculation of the final actual doses in the test item treatment group. Acute contact and oral toxicity endpoints (*e.g.* LD_{50}, LD_{20}, LD_{10}) could 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 Fisher Test 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 ToxRat Professional, Version 3.2.1 (® ToxRat Solutions GmbH).

Analytics: Freshly prepared application solution and feeding solution (20 mL per specimen) of the control and the test item treatment group were sampled in duplicates on the day of application. The chemical analysis was performed by using LC-MS/MS-method.

Dates of work: August 20th to August 23rd, 2019 (biological phase)
April 21st to May 01st, 2020 (analytical 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 trifloxystrobin in the test 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, due to a technical error during the preparation of the test item application solution. With regard to the increased application volume (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 a.s./L; trifloxystrobin-LOD: 0.9 µg a.s./L).

Table 10.3.1.1.1- 5: Analytical results

Test system	Nominal test concentration		Recovery
	[µg product/bee]	[µg a.s./L application solution]	
Contact Test	1185.12	125 g fluopyram/L application solution	50 % [A]
		126 g trifloxystrobin/L application solution	46 % [A]
Oral Test	1185.12	6.25 g fluopyram/kg feeding solution	101 %
		6.3 g trifloxystrobin/kg feeding solution	104 %

[A] A technical error occurred during the preparation of the test item application solution of the contact test. A 4 µL droplet was applied instead of 2 µL 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 findings:

Contact Test:

At the end of the contact toxicity test (48 hours after application) there was 2.0 % mortality at 1185.12 µg product/bumble bee. No mortality occurred in the water control group (water with 0.1 % Triton X 100).

No test item related behavioural abnormalities occurred at any time during the test.

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

Dose	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]	Mean [%]
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg product/bumble bee]						
1185.12	0.0	0.0	0.0	0.0	2.0	0.0
Reference item [µg a.s./bumble bee]						
10	0.0	56.7	76.7	100.0	93.3	100.0

The contact test was conducted at 250 µg fluopyram per bumble bee. The concentration was based on the active substance fluopyram, therefore, 1185.12 µg product/bumble bee was equivalent to 250 µg fluopyram/bumble bee.
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/L); reference item = dimethoate; water control = tap water containing 0.1 % Triton X-100

<u>Oral Test</u>

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

Table 10.3.1.1.1-7: Mortality and behavioural abnormalities of the bees in the oral toxicity test

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Control	0.0	0.0	0.0	0.0	2.2	0.0
Test item [µg product/bee]						
1216.23 [A]	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bee]						
4.3	0.0	100.0	100.0	-	100.0	-

[A] The oral test was conducted at 250 µg fluopyram per bumble bee. The concentration was based on the active substance fluopyram, therefore, 1216.23 µg 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= mean of dead individuals per treatment group, considering only those bumble bees, which achieved at least 80 % of the mean food uptake per treatment group (test item: n= 20, water control: n= 45, reference item: n=19)

Behav. Abnorm =Behavioural abnormalities

Test item = FLU + TFS SC 500 (250+250 g/L); Reference item = dimethoate; control = 50 % sucrose solution

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 sucrose solution) (based on recorded consumption considering bumble bees with food uptake of at least 80 % of the mean uptake per treatment group [1])	
Target (nominal) dose rates [µg product/bumble bee*]	1185.12		1185.12	
Actual dose rates [µg product/bumble bee*]	1185.12		1216.23	
Test duration	24 h	48 h	24 h	48 h
$LD_{50, 20, 10}$ [µg product/bumble bee] [2, 3]	> 1185.12	> 1185.12	> 1216.23	> 1216.23
NOED [µg product/bumble bee] [2, 4]	≥ 1185.12	≥ 1185.12	≥ 1216.23	1216.23
LOED [µg product/bumble bee] [2, 4]	> 1185.12	> 1185.12	> 1216.23	> 1216.23

* The contact and oral tests were conducted at 250 µg fluopyram per bumble bee. The concentrations were based on the active substance fluopyram; therefore, 1185.12 µg product/bumble bee was equivalent to 250 µg fluopyram/bumble bee).

[1] For the 1216.23 µg product/bumble bee test item treatment group 20 bumble bees were considered for the evaluation.

[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 oral test did not show mortality above 50.0, 20.0 and 10.0 %, no statistical evaluation on the LD_{50}, LD_{20} and LD_{10} was carried out.

[4] The NOED/LOED was estimated using Fisher's Exact Test after Bonferroni-Holm (pairwise comparison, one-sided greater, $\alpha = 0.05$).

Reference item

The bumble bees of the reference item group were treated with 10 µg dimethoate/bumble bee in the contact test and 4.3 µg 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.

Validity criteria:

The contact and oral toxicity test were considered valid as the control mortality in each case was ≤ 10 % and ≥ 50 % in the reference item.

Table 10.3.1.1.1- 9: Validity criteria

Validity criteria	Recommended		Obtained
Control mortality	Contact Test		
	Control	≤ 10 %	0.0 %
	Oral Test		
	Control	≤ 10 %	2.2 %
LD_{50} of reference item (24 h)	Contact Test		
	Dimethoate	≥ 50 %	93.3 %
	Oral Test		
	Dimethoate	≥ 50 %	100.0 %

III. Conclusion

The toxicity of FLU + TFS SC 500 (250+250 g/L) was tested in an acute contact and oral toxicity test on bumble bees.

The contact NOED value was calculated to be ≥ 1185.12 µg product/bumble bee. The contact LD_{50} value was > 1185.12 µg product/bumble bee.

The oral NOED value was calculated to be ≥ 1216.23 µg product/bumble bee. The oral LD_{50} value was estimated to be > 1216.23 µg product/bumble bee.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment. The endpoints are:

LD_{50} contact (48 hours) > 1185.12 µg product/bumble bee

LD_{50} oral (48 hours) > 1216.23 µg product/bumble bee

CP 10.3.1.1.2 Acute contact toxicity to bees

Honeybees

Data Point:	KCP 10.3.1.1.2/01
Report Author:	[redacted]
Report Year:	2007
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 study:	OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test, (adopted 21st September 1998) ; OECD 214: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Contact Toxicity Test, (adopted 21st September 1998); Equivalent to US EPA OPPTS Guideline No. 850.3020 SUPP
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 213: The guideline-specified relative humidity range of 50-70% was not met. This deviation is not expected to have impacted the study results. All validity criteria were met. Deviations from OECD Guideline 214: The guideline-specified relative humidity range of 50-70% was not met. An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL 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
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For study summary on acute contact and oral toxicity of FLU +TFS SC 500 to honey bees, please refer to CP 10.3.1.1.1/01.

Bumble bees

Data Point:	KCP 10.3.1.1.2/02
Report Author:	[redacted]
Report Year:	2020
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 laboratory
Report No:	144951105
Document No:	M-738910-04-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 246 and 247 (2017)
Deviations from current test guideline:	Current Guidelines: OECD 246 and 247 (2017) Deviations from OECD Guideline 246: No information on bumble bee colonies concerning size, brood stages and number of adults are reported. The limit dose 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 recommendation of 2 µL. These deviations are not expected to have impacted the study results. All validity criteria of the current guideline were met. Deviations from OECD Guideline 247: No information on bumble bee colonies concerning size, brood stages and number of adults are reported. The limit dose was 250 µg a.s./bee instead of the recommended dose of 100 µg a.s./bee. These deviations are not expected to have impacted the study results. All validity criteria of the current guideline were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

For study summary on acute contact and oral toxicity of FLU+TFS SC 500 to bumble bees, please refer to CP 10.3.1.1.1/02.

CP 10.3.1.2 Chronic toxicity to bees

Data Point:	KCP 10.3.1.2/01
Report Author:	[redacted]
Report Year:	2020
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 mellifera L. under laboratory conditions
Report No:	S19-20249
Document No:	M-753795-02-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 (Oct 2009) OECD Guideline for the Testing of Chemicals No. 245 (2017). SANCO/3029/99, rev. 4 (2000)
Deviations from current test guideline:	Current Guideline: OECD 245 (2017) Deviations: None. 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

Executive Summary

The objective of this study was to determine the chronic oral toxicity (LDD$_{50}$/LC$_{50}$ and NOEDD/NOEC) of (FLU+TFS SC 500 (250+250 g/L)) applied on 10 consecutive days to young adults of the honey bee (*Apis mellifera* L.).

Worker honey bees (*Apis mellifera* L.) aged two days or less were orally exposed to a daily application of FLU + TFS SC 500 (250+250 g/L) diluted in the bee food (50 % w/v aqueous 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, 146.90, 82.36, 51.77, 27.17 and 16.73 µg 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 with 0.1% Xanthan), as well a reference item (dimethoate) were included.

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

The LDD$_{50}$ and LC$_{50}$ was determined to be 16.95 µg product/bee/day and 902.13 mg product/kg feeding solution, respectively. The LDD$_{10/20}$ and LC$_{10/20}$ were not determined.

The NOEDD and NOEC were determined to be < 16.73 µg product/bee/day and < 829.91 mg product/kg feeding solution, respectively.

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

I. Material and Methods

Test item: 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, 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

Test species: 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/kg 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 µg dimethoate/bee/day

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

Each group (test item, controls and reference item) comprised 5 replicates containing 10 bees each.

Test design: 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 FLU + TFS SC 500 (250+250 g/L) diluted in the bee food (50 % w/v aqueous sucrose solution with 0.1 % Xanthan).

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 amount 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 aliquots 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 moribund and affected bees of that group will die by the end of the test.

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

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

Statistics: Results obtained from bees treated with the test item were compared to those obtained from the thickener control group. A statistical significance of $\alpha = 0.05$ was considered for all tests, except where stated. To estimate the LC_{50} and the LDD_{50}, 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.
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.

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 3 until day 6 resulted for fluopyram 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 fluopyram (LOQ: 0.01 mg a.s./kg) and above the Limit of Detection for trifloxystrobin (LOD: 0.003 mg a.s./kg).

Table 10.3.1.2- 1: Analytical results for fluopyram

Treatment group [mg product/kg diet]	Nominal concentration [mg a.s./kg diet]	Mean measured concentration [mg a.s./kg diet]	Daily recovery range [A] [%]	Mean recovery from target [%]
Control	0	< LOQ	-	-
2655.26	5602.24	5879.90	68 - 144 (A) 113 - 154 (R)	105 (A) 137 (R) Combined: 112
13278.63	2801.12	2691.83	81 - 126 (A) 98 (R) [B]	96 (A)
6639.32	1400.56	1256.31	72 – 117 (A) 104 (R) [C]	90 (A)
3319.66	700.28	657.66	80 – 117 (A)	94 (A)
1659.83	350.14	274.52	54 - 90 (A) 81 - 108 (R)	78 (A) 95 (R) Combined: 87
829.91	175.07	125.35	54 - 87 (A) 80 - 101 (R)	72 (A) 88 (R) Combined: 80

LOD: Limit of Detection: fluopyram = 0.003 mg a.s./kg
LOQ: Limit of Quantification: fluopyram = 0.01 mg a.s./kg diet.
(A): Main samples;
(R): Retain samples
A Retain samples (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.

Table 10.3.1.2- 2: Analytical results for trifloxystrobin

Treatment group [mg product/kg diet]	Nominal concentration [mg a.s./kg diet]	Mean measured concentration [mg a.s./kg diet]	Daily recovery range [A] [%]	Mean recovery from target [%]
Control	0	< LOD	-	-
26557.26	5647.68	5264.57	71 - 118 (A) 108 - 119 (R)	93 (A) 113 (R) Combined: 98
13278.63	2823.84	2573.01	82 – 99 (A) 102 (R) [B]	91 (A)
6639.32	1411.92	1261.58	82 – 97 (A) 97 (R) [C]	89 (A)
3319.66	705.96	656.18	80 – 103 (A)	93(A)
1659.83	352.98	308.22	65 - 96 (A) 85 – 109 (R)	87 (A) 93 (R) Combined: 90
829.91	176.49	147.79	63 – 101 (A) 88 – 136 (R)	84 (A) 102 (R) Combined: 93

LOD: Limit of Detection: 0.003 mg a.s./kg
LOQ: Limit of Quantification: trifloxystrobin = 0.01 mg a.s./kg
(A): Main samples
(R): Retain samples
A Retain samples (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 one single analysed sample (D8-T5).
C Result based on one single analysed sample (D1-T4).

Biological results:

Summary of mean mortality and toxicity of FLU + TFS SC 500 (250+250 g/L) to adult honey bees after 10 days of chronic exposure:

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

Treatment group	Daily dose Consumed [A] [µg prod./bee/day]	Concentration [mg prod./kg diet]	At day 10 Mean mortality [%]	At day 10 Corrected mortality [%]
Control	-	-	6.0	-
Thickener control	-	-	6.0	-
Test item (FLU+TFS SC 500 (250+250 g/L))	343.64	26557.26	100	100.00
	146.90	13278.63	98	97.87
	82.36	6639.32	100	100.00
	51.77	3319.66	100	100.00
	27.17	1659.83	90	89.36
	16.73	829.91	48	44.68
Reference Item	[µg a.s./bee/day]	[mg a.s./kg diet]		
	0.0130	0.90	100	100

Endpoints		10 d
Test item doses	LDD_{50} [µg prod./bee/day] (95 % C.I.) [B]	16.95 (13.66 – 19.47)
	LDD_{20} [µg prod./bee/day]	n.d.
	LDD_{10} [µg prod./bee/day]	n.d.
	LOEDD [µg prod./bee/day]	16.73
	NOEDD [µg prod./bee/day]	< 16.73
Test item concentrations	LC_{50} [mg prod./kg feeding solution] (95 % C.I.) [B]	902.13 (684.03 – 1054.71)
	LC_{20} [mg prod./kg feeding solution]	n.d.
	LC_{10} [mg prod./kg feeding solution]	n.d.
	LOEC [mg prod./kg feeding solution]	829.91
	NOEC [mg prod./kg feeding solution]	< 829.91

Results are mean values of 5 replicates, containing 10 bees each. Calculations are corrected for evaporation
Corrected mortality (according to ABBOTT (1925), modified by SCHNEIDER-ORELLI 1947)
C.I.: Confidence interval
n.d.: not determined
A Actual dose per bee per day; dose measured based on consumed feeding solution
B Estimated using Weibull analysis considering a subset of four treatments (T1-T4)

Bees were recorded as affected and moribund in all the test item treated groups, on different assessment days, starting on 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, 51.77, 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 LC/LDD$_{10/20}$ values could not be reliably estimated.

Reference item

The reference item (dimethoate) was administered in one dosage of 0.0130 µg a.s./bee/day (actual average intake based on food consumption) which caused a continuously increasing mortality leading to 100 % mortality at day 10.

Validity criteria:

All validity criteria of the current OECD Guideline 245 (2017) were met in this study.

Table 10.3.1.2- 4: Validity criteria

Validity criteria according to OECD GD 245 (2017)	Recommended		Obtained
Mortality after 10 days of exposure	Control	≤ 15 %	6.0 %
	Thickener control	≤ 15 %	6.0 %
Mortality after 10 days of exposure	Dimethoate	≥ 50 %	100 %

III. CONCLUSION

The chronic oral toxicity of FLU + TFS SC 500 (250+250 g/L) was tested on young adult honey bees (*Apis mellifera* L.) in a 10-day feeding study under laboratory conditions.

The LDD$_{50}$ and LC$_{50}$ were determined to be 16.95 µg product/bee/day and 902.13 mg product/kg feeding solution, respectively. The LDD$_{10/20}$ and LC$_{10/20}$ could not be determined due to mathematical reasons.

The NOEDD and NOEC were determined to be < 16.73 µg product/bee/day and < 829.91 mg product/kg feeding solution, respectively.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment. The endpoints are:

LDD$_{50}$ oral (10 days) = 16.95 µg product/bee/day

LC$_{50}$ oral (10 days) = 902.13 mg product/kg diet

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

Data Point:	KCP 10.3.1.3/01
Report Author:	[redacted]
Report Year:	2020
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 exposure under laboratory conditions
Report No:	S19-21288
Document No:	M-738909-02-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 (Oct 2009) OECD Guidance Document 239 (2016) SANCO/3029/99, rev.4 (2000)
Deviations from current test guideline:	Current Guidance Document: OECD 239 (2016) Deviations: None. 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

Executive Summary

The purpose of this study was to determine the chronic toxicity ($ED_{50/20/10}$, $EC_{50/20/10}$, NOED/NOEC for adult emergence at day 22) of FLU + TFS SC 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-well cell culture plates 2 days before the start of the exposure period (D1, grafting). Larvae were exposed to 5 concentrations of FLU + TFS SC 500 (250+250 g/L) (nominal 601.21, 240.48, 96.19, 38.48 and 15.39 mg product/kg diet corresponding to nominal cumulative doses of 92.59, 37.03, 14.81, 5.93 and 2.37 µg product/larva) via the larval diet on 4 consecutive days (day 3 to day 6). No additional feeding of the larvae took place after day 6.

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

The larval mortality was recorded daily from day 4 to day 8. Additionally, other observations such as small body size or unconsumed diet on D8 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 larval 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 µg and 92.59 µg product/larva (based on adult emergence), respectively. The NOEC and LOEC were 240.48 mg and 601.21 mg product/kg diet, respectively. The LC_{50} and ED_{50} values (based on adult 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 $EC_{10/20}$ and $ED_{10/20}$ for the emergence at 22 days could be determined.

The study fulfils all validity criteria of the current OECD Guidance Document 239 (2016).

I. Material and Methods

Test item: 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, 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

Test species: Honey bee (*Apis mellifera* L.), synchronized first instar (L1, ≤ 30 hours after grafting) larvae originating from three adequately fed, healthy (free of clinical symptoms) queen-right bee colonies. The larvae were taken from hives that had not received treatments with chemical substances 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, 14.81, 5.93 and 2.37 µg product/larva

One reference item group exposed to a cumulative dose of 7.39 µg dimethoate/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 3 replicates, with 16 larvae each (each colony represented a replicate).

Test design: 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.

Table 10.3.1.3- 1: Feeding scheme

Test day	1[1]	2	3[2]	4[2]	5[2]	6[2]
Artificial diet	A	-	B	C	C	C
Volume of diet per larva	20 µL	-	20 µL	30 µL	40 µL	50 µL
Composition of diets:						
Royal jelly	50 % w/w	-	50 % w/w	50 % w/w		
Sugar solution	50 % w/w		50 % w/w	50 % w/w		
Composition of sugar solution:						
Glucose	12 % w/v	-	15 % w/v	18 % w/v		
Fructose	12 % w/v		15 % w/v	18 % w/v		
Yeast extract	2 % w/v		3 % w/v	4 % w/v		

[1] Day of grafting
[2] Days of exposure

The daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period. The cumulative feeding volume from day 3 until day 6 of 140 µL 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 15: 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 specified in the guidleine lasted ≤ 30 mins per 24 hour period for temperature and < 2 hours for relative humidity. 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 stepdown procedure based on the Cochran-Armitage test (one-sided greater). The NOED and the LOED were 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 ToxRat Professional version 3.2.1.

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 (biological phase)
February 20th to May 13th, 2020 (analytical 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.
The analytical dose verification of the larval diet from day 3 until day 6 resulted in mean measured recoveries of 95 - 99 % for fluopyram and 104 - 115 % for trifloxystrobin. In the control samples, the concentration was below the Limit of Detection for both active substances (LOD fluopyram: 0.003 mg a.s./kg, LOD trifloxystrobin: 0.003 mg a.s./kg).

Table 10.3.1.3- 2: Analytical results for fluopyram

Treatment group	Nominal conc. of fluopyram [mg a.s./kg diet]	Sampling Time	Measured conc. of fluopyram [mg a.s./kg diet]	Recovery from target [%]	Mean recovery from target [%]
Control	0.00	D 3	< LOD	-	-
		D 4	< LOD	-	
		D 5	< LOD	-	
		D 6	< LOD	-	
Test item	3.25	D 3	2.94	90	97
		D 4	2.73	84	
		D 5	3.71	114	
		D 6	3.22	99	
	8.12	D 3	7.94	98	96
		D 4	6.77	83	
		D 5	8.20	101	
		D 6	8.11	100	
	20.29	D 3	20.67	102	96
		D 4	17.12	84	
		D 5	20.03	99	
		D 6	19.91	98	
	50.73	D 3	46.94	93	95
		D 4	54.47	107	
		D 5	40.86	81	
		D 6	50.01	99	
	126.82	D 3	129.92	102	99
		D 4	146.12	115	
		D 5	113.30	89	
		D 6	112.84	89	

LOD: Limit of Detection: 0.003 mg fluopyram/kg

Table 10.3.1.3- 3: **Analytical** results for trifloxystrobin

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 [%]
Control	0.00	D 3	< LOD	-	-
		D 4	< LOD		
		D 5	< LOD	-	
		D 6	< LOD	-	
Test item	3.27	D 3	3.29	101	107
		D 4	3.52	108	
		D 5	3.54	108	
		D 6	3.55	109	
	8.18	D 3	9.34	114	111
		D 4	9.62	118	
		D 5	8.87	108	
		D 6	8.50	104	
	20.46	D 3	21.05	103	108
		D 4	23.89	117	
		D 5	21.87	107	
		D 6	21.30	104	
	51.14	D 3	56.66	111	104
		D 4	52.92	103	
		D 5	49.24	96	
		D 6	53.53	105	
	127.85	D 3	147.51	115	115
		D 4	150.07	117	
		D 5	148.89	116	
		D 6	144.11	113	

LOD: Limit of Detection: 0.003 mg trifloxystrobin/kg

Biological results:

On day 8, no individuals were observed with unconsumed food or 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).

On day 8, larval mortality was 0.0 % in the control group and 97.92 % in the reference item group. In the test item group larval mortalities on day 8 were 50.0 %, 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, an adult emergence rate of 95.83% was determined for the honey bees in the control group. In the test item treated group, the adult honey bees emerged at rates of 50.0 %, 95.83 %, 89.58 %, 93.75 % and 87.50 % following exposure to test item concentrations of 601.21, 240.48, 96.19, 38.48 and 15.39 mg product/kg diet during the larval stages, corresponding to cumulative doses of 92.59, 37.03, 14.81, 5.93 and 2.37 µg product/larva, respectively.

Table 10.3.1.3- 4: Mortality of larvae and adult emergence in the repeated exposure toxicity test

Treatment group	Cumulative dose (nominal) [A]	Concentration (nominal)	Day 8	Day 22		
			Larval mortality Day 3 - 8	Total mortality Day 3 - 22		Adult Emergence rate
			abs.	abs.	corr. [B]	actual
	[µg product/larva]	[mg product/kg diet]	[%]	[%]		[%]
Control	-	-	0.00	4.17		95.83
Test item (FLU+TFS SC 500 (250+250 g/L))	2.37	15.39	4.17	12.50	8.70	87.50
	5.93	38.48	2.08	6.25	2.17	93.75
	14.81	96.19	4.17	10.42	6.52	89.58
	37.03	240.48	2.08	4.17	0.00	95.83
	92.59	601.21	50.00	50.00	47.83	50.00 *
Reference Item (Dimethoate)	7.39 [C]	52.8 [D]	97.92	100.00	100.00	0.00

A Based on the cumulative feeding volume from day 3 to day 6 of 140 µL diet/larva
B Corrected for control mortality according Abbott modified by Schneider-Orelli
C µg dimethoate/larva
D mg Dimethoate/kg diet
* Statistically significant difference compared to control (Step-down Cochran-Armitage Test; $p \leq 0.05$; one sided greater)

Table 10.3.1.3- 5: Calculated endpoints of the repeated exposure larvae toxicity test

Treatment	Endpoint: Adult emergence at day 22	
Test item cumulative doses [µg product/larva]	ED_{50} [1]	> 92.59
	ED_{20} [2]	n.d.
	ED_{10} [2]	n.d.
	LOED [3]	92.59
	NOED [3]	37.03
Test item concentrations [mg product/kg diet]	EC_{50} [1]	> 601.21 mg prod./kg diet
	EC_{20} [2]	n.d.
	EC_{10} [2]	n.d.
	LOEC	601.21 mg prod./kg diet
	NOEC [3]	240.48 mg prod./kg diet

n.d.: Not determined
1 As no corrected mortality above 50% was observed, the EC/ED50 values were empirically estimated to be greater than the highest concentration tested.
2 No clear dose response was observed during the definitive test, therefore, the EC/ED10 and EC/ED20 values could not be reliably calculated.
3 Based on the cumulative feeding volume from day 3 until day 6 of 140 µL diet/larva.

Validity criteria:

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)	Recommended		Obtained
Larval mortality between day 3 and day 8 in the control group (across all replicates)	Control	≤ 15 %	0.0 %
Adult emergence rate until day 22 in the control group (across all replicates)	Control	≥ 70 %	95.83 %
Larval mortality between day 3 and day 8 in the reference group (across all replicates)	Dimethoate	≥ 50 %	97.92 %

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 37.03 µg and 92.59 µg product/larva (based on adult emergence), respectively. The NOEC and LOEC were 240.48 mg and 601.21 mg product/kg diet, respectively.

The EC_{50} and ED_{50} values (based on adult 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 $EC_{10/20}$ and $ED_{10/20}$ for the emergence at 22 days could be determined.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment. The endpoints are:

NOED - emergence (22 days) = 37.03 µg product/larva

NOEC - emergence (22 days) = 240.48 mg product/kg diet

CP 10.3.1.4 Sub-lethal effects

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

CP 10.3.1.5 Cage and tunnel tests

Data Point:	KCP 10.3.1.5/01
Report Author:	[redacted]
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 - tunnel test -
Report No:	64841037
Document No:	M-435338-01-1
Guideline(s) followed in study:	OEPP/EPPO, No.170(4) (OEPP/EPPO, 2010)
Deviations from current test guideline:	Current Guideline: EPPO 170 (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

Executive summary

This semi-field tunnel study was designed to evaluate the acute, short-term and long-term effects on honey bees (*Apis mellifera* L.) following repeated foliar applications of FLU + TFS SC 500 (250+250 g/L) onto bee-attractive *Phacelia tanacetifolia*.

The test was conducted under confined exposure conditions (tunnel) with each tunnel comprising a 40 m² plot of flowering *Phacelia 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 test 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 2nd application. 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.

No adverse effects on mortality, foraging activity, behaviour, nectar- and pollen storage, brood-abundance 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 foraging on a bee-attractive, flowering crop.

I. Material and methods

Test item: FLU + TFS SC 500 (250+250 g/L), Specification No.: 102000012866 - 03, Batch-code: 2011-002701; 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: 1.169 g/mL (20°C).

Test species: 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 honeycombs.

Location of the field site: Germany

Test concentrations:

Test item treatment: 560 mL product in 400 L water/ha (1.64 g/L; corresponding to nominally 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 Perfekthion 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/mL).

A water volume of 400 L/ha was considered for all treatment groups.

Test design: The test was conducted under confined exposure conditions (tunnel) in order to assess acute, short-term and long-term effects of repeated foliar applications of FLU + TFS SC 500 (250+250 g/L) on honey bee colonies under semi-field conditions. A plot of *Phacelia tanacetifolia* with an effective crop size of 40 m² was prepared for each tunnel. 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 flowering 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 2nd test item application treated with tap water (controls) and three tunnels were treated with a reference item (Perfekthion EC (BAS 152 11 I), 400 g/L dimethoate), respectively, during honey bees actively foraging on the crop.

The honey 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 honey bee colony was used per tunnel.

Three of the six tunnels 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 2nd test item application (Day 0) as well as on the day following the 2nd test item application (Day 1).

The confined 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 reference item group, respectively. The conditions of the colonies were examined until day 42 following the 2nd test item application.

Endpoints: 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, dis-coordinated movement, exaggerated motility, aggressiveness, lethargy, apathy, obvious symptoms of intoxication, etc.) were also monitored. 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.

Test conditions: 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), Student`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 analytical method are presented 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 trifloxystrobin and its metabolite CGA321113 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-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 Limit of Quantitation (LOQ) was 0.01 mg/kg (i.e.10 µg 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 µg/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- 1: Summary: residues of fluopyram and its metabolites in pollen, nectar and flowers

Sample material	Tunnel	Concentration range (min – max) [mg/kg]		
		Fluopyram	fluopyram-pyridyl-acetic acid (BCS-AA10189)	fluopyram- benzamide (AEF148815)
Flowers	1 - 3	3.0 – 5.0	<LOD	<LOD - <LOQ
Nectar	1 - 3	0.025 – 1.1	<LOD - <LOQ	<LOD - <LOQ
Pollen	1 - 3	3.0 - 30	<LOQ – 0.01 [A]	<LOQ - 0.017

LOQ: Limit of Quantification for for all compounds = 0.01 mg/kg (= 10 µg/kg = 10 ppb)
[A] Value was corrected for mean of LOQ recovery level of 59 %.

Table 10.3.1.5- 2: Summary: residues of trifloxystrobin and its metabolite in pollen, nectar and flowers

Sample material	Tunnel	Concentration range (min – max) [mg/kg]	
		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 – 0.051

LOQ: Limit of Quantification for for all compounds = 0.01 mg/kg (= 10 µg/kg = 10 ppb)
LOD: Limit of Detection for for all compounds ≈ LOQ/3 (i.e. ≈ 3.5 µg/kg)
A Value was corrected for mean of LOQ recovery level of 59 %.

<u>Biological results:</u>

Mortality

Starting conditions of the experiment were ideal, indicating similar natural mortality levels among the different treatment groups before the application during full flowering (no statistically significant difference of the colonies, Student t-test, pairwise comparison to the control, two-sided, α = 0.05).

<u>Exposure phase in the tunnels (day 0 after application to day 7):</u>

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, α = 0.05, one-sided greater).

Over the course of the remaining exposure period, mortality rates in test item treatment and control groups were similar without any statistically significant differences (Student t-test, pairwise comparison, one-sided greater, α = 0.05) at any assessment day.

The overall daily mean number of dead bees (Day 0 to Day 7) also showed no statistically significant differences between treatment (35.4 ± 13.9 bees) and control (33.5 ± 10.8 bees) (Student t-test, pairwise comparison, one-sided greater, α = 0.05).

In contrast to the observations in the test item treatment group and the control group, application of the reference item (dimethoate at a rate of 600 g a.s./ha) resulted in a markedly increased number of dead bees found in the traps and on the gauze strips in the crop between day 0 and day 4, which was statistically significantly different from the control (Student t-test, pairwise comparison, α = 0.05, one-sided greater). Mortality increased up to approximately 24 x the levels of the control values on day 1 following the application.

Table 10.3.1.5- 3: Summarised mortality data for exposure phase in the tunnels (day 0 after application to day 7)

Time [A]	Mean dead bees ± S.D. [B]				
	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 ± 51.7	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	n.s.	51.7 ± 8.5	n.s.
Day 0 b.a. [A]	**31.0 ± 24.3**	**35.3 ± 14.6**	**n.s.**	**45.3 ± 14.0**	**n.s.**
Daily mean day -3 to 0 b.a.	**36.8 ± 18.4**	**49.5 ± 30.2**	**n.s.**	**51.4 ± 5.7**	**n.s.**
Day 0 a.a.	**19.7 ± 5.0**	**27.3 ± 8.1**	**n.s.**	**407.3 ± 78.7**	*****
Day 1	35.5 ± 13.8	25.7 ± 7.5	n.s.	831.7 ± 132.0	*
Day 2	20.3 ± 5.5	23.0 ± 7.2	n.s.	201.0 ± 90.4	*
Day 3	49.7 ± 5.8	49.7 ± 35.0	n.s.	163.3 ± 44.8	*
Day 4	39.7 ± 19.7	54.3 ± 7.2	n.s.	112.0 ± 31.2	*
Day 5	43.3 ± 21.5	49.3 ± 11.0	n.s.	41.7 ± 19.8	n.s.
Day 6	26.7 ± 9.6	18.7 ± 7.6	n.s.	45.7 ± 17.8	n.s.
Day 7	33.0 ± 13.9	35.0 ± 22.6	n.s.	61.3 ± 4.9	*
Daily mean day 0 to day 7 a.a.	**33.5 ± 10.8**	**35.4 ± 13.9**	**n.s.**	**233.0 ± 270.0**	*****

b.a.: Before application;
a..a.: After application;
S.D.: Standard deviation
[A] Days -3 to -1 = days before 2nd application; day 0 = day of 2nd application; day 1 to 7 = days after 2nd application
[B] Mean values (rounded) of three tunnels of each treatment group;
Statistics: Student t-test, pairwise, two sided (before application); pairwise, one-sided greater (after application), α = 0.05
n.s.: Not statistically significantly different from the control;
* Statistically significant difference to the control

Foraging activity

Two days before the 2nd test item 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, α = 0.05, two sided), indicating ideal starting conditions.

<u>Exposure phase in the tunnels (day 0 after application to day 7):</u>

After the 2nd test item application of FLU + TFS SC 500 (250+250 g/L), the foraging activity of the bees was comparable or even higher in the test item treatment group compared to the control group. An overall comparison of the mean flight activity did 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, α = 0.05). In contrast, the application of the reference item (dimethoate) resulted in a clear decrease of flight intensity until the end of the confined exposure period (day 7), which was statistically significantly lower compared to the control (Student t-test, pairwise comparison, one-sided smaller, α = 0.05).

Table 10.3.1.5- 4: Summary of foraging activity findings

Time [A]	Mean flight density [Bees/m² [B]]				
	Water Control	FLU + TFS SC 500 (250+250 g/L)		Reference item	
Day -3	25.0 ± 2.6	27.4 ± 0.7	-	26.4 ± 2.1	-
Day -2	0.0 ± 0.0	0.2 ± 0.4	-	0.1 ± 0.2	-
Day -1	15.0 ± 4.2	12.7 ± 1.9	-	18.9 ± 1.8	-
Day 0 b.a.	**12.2 ± 3.2**	**13.0 ± 0.9**	**-**	**9.3 ± 1.7**	**-**
Daily mean day -3 to 0 b.a.	**13.1 ± 10.3**	**13.3 ± 11.1**	**n.s.**	**13.7 ± 11.4**	**n.s.**
Day 0 a.a.	**10.3 ± 1.1**	**11.8 ± 1.5**	**-**	**0.8 ± 1.1**	**-**
Day 1	10.6 ± 1.1	17.6 ± 4.0	-	0.0 ± 0.0	-
Day 2	23.0 ± 2.3	21.3 ± 1.3	-	0.0 ± 0.0	-
Day 3	19.6 ± 3.2	21.6 ± 2.2	-	0.1 ± 0.2	-
Day 4	22.1 ± 2.0	23.2 ± 1.4	-	0.0 ± 0.0	-
Day 5	21.4 ± 2.5	22.3 ± 0.7	-	0.2 ± 0.4	-
Day 6	13.4 ± 1.0	15.7 ± 1.5	-	0.1 ± 0.2	-
Day 7	3.7 ± 1.5	3.9 ± 1.3	-	0.3 ± 0.3	-
Daily mean day 0 to day 7 a.a.	**15.5 ± 7.0**	**17.2 ± 6.6**	**n.s.**	**0.2 ± 0.3**	*****

b.a.: Before application;
a.a.: After application;
[A] Days -3 to -1 = days before 2nd application ; day 0 = day of 2nd application; day 1 to 7 = 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), α = 0.05
" - ": No statistics were performed,
n.s.: Not statistically significantly different from the control;
* Statistically significant difference to the control

Behavioural abnormalities

No behavioural abnormalities occurred in the FLU + TFS SC 500 (250+250 g/L) and in the control group at any assessment day, respectively.

The reference item treatment caused behavioural abnormalities (moving coordination problems, abnormal cleaning) at least through the first day following application.

Brood assessment

Over the entire assessment period of 42 days (i.e. 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 brood 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 queens 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 a clear sign of the presence of a healthy queen.

Table 10.3.1.5- 5: Mean number of all brood stages (eggs+larvae+pupae) in the colonies

Time [A]	Water Control		FLU + TFS SC 500 (250+250 g/L)			Reference item		
	Mean [B]	SD [C]	Mean [B]	SD [C]	Statistic	Mean [B]	SD [C]	Statistic
Day -6	8820	1487	7560	3541	n.s.	9540	2509	n.s.
Day +7	5940	2210	6750	3274	n.s	4140	1535	n.s
Day +14	6120	1953	6660	1091	n.s	6570	1582	n.s
Day +21	8280	1535	8280	1217	n.s	6660	1091	n.s
Day +28	4320	1429	4860	810	n.s	4050	1177	n.s
Day +42	2700	270	2880	562	n.s	3870	412	n.s

A Time in relation to the 2nd test item application
B Mean number of cells with all brood stages per colony (mean value of 3 colonies)
C SD: Standard Deviation
n.s.: Not statistically significantly different from the control;
Statistic: Student t-test, pairwise comparison to the control, one-sided smaller, α = 0.05

Strength of colonies

The mean number of honey bees per colony in all test item groups including the colonies to be used for residue analysis was very similar six days before application. Population development was similar in the control and test item treatment group. There was no statistical significant difference to the control group at any assessment date (Student t-test, pairwise comparison, α = 0.05, one-sided 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.

Table 10.3.1.5- 6: Summary of the strength of the colonies

Time [A]	Water Control			FLU + TFS SC 500 (250+250 g/L)			Reference item		
	Mean [B]	SD [C]	[%] [D]	Mean [B]	SD [C]	[%] [D]	Mean [B]	SD [C]	[%] [D]
Day -6	2430	412	100	2625	689	100	2235	264	100
Day +7	2400	425	99	3090	579	118	1365	213	61 *
Day +14	2385	392	98	3090	901	118	2265	506	101
Day +21	2040	594	83	2820	927	107	1710	315	77
Day +28	1530	707	63	1725	767	66	1485	429	66
Day +42	1560	158	64	1935	585	74	1440	119	64

A Time in relation to the 2nd test item application
B Mean value of 3 colonies
C SD: 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, α = 0.05

Validity criteria:

All validity criteria were met in this study.

Table 10.3.1.5- 7: Validity criteria

Validity Criteria	Treatment Group	Recommended	Obtained
Foraging activity shortly before application	All groups	≥ 5 bees/m²	Mean flight densities before application: 12.2 bees per m² in the control tunnels, 13.0 bees per m² in the test item tunnels, 9.3 bees per m² 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.
Control Mortality	Control	Should not have been considerable.	As the mean mortality after 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 under confined semi-field conditions. Mortality at the colony (in the traps) was consistently low, indicating that colonies were healthy and adapted to the tunnel conditions.
Reference Item Mortality	Reference Item (dimethoate)	There should be a high number 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 1 after application: 35.3) by a factor of approximately 24. Statistically significant increased mortality rates compared to the control occurred on all days following the application (except on 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.

III. Conclusion

In order to assess the risk of FLU + TFS SC 500 (250+250 g/L) to honey bees and honey bee colonies, honey bees were exposed under the realistic but severe (forced) exposure conditions of a semi-field test (confinement in gauze tunnels). The test item was applied two times to the highly bee attractive surrogate crop *Phacelia tanacetifolia*, 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 test 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 a.s. fluopyram/ha + 140 g a.s. trifloxystrobin/ha).

No adverse effects on mortality, foraging activity, behaviour, nectar- and pollen storage, brood-abundance 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 product/ha during honey 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.

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Data Point:	KCP 10.3.1.5/02
Report Author:	[redacted]
Report Year:	2012
Report Title:	Determination of residue levels of fluopyram + trifloxystrobin in nectar and pollen, collected by honey bees under confined semi-field conditions
Report No:	E 319 4290-8
Document No:	M-433544-01-1
Guideline(s) followed in study:	US EPA OCSPP Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Summary

This study aimed to examine the honey bee exposure levels of fluopyram- and trifloxystrobin in nectar, pollen and flowers of *Phacelia tanacetifolia* after foliar applications of FLU+TFS SC 500 (250+250 g/L) at a rate of 560 mL product in 400 L water/ha (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 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 trifloxystrobin and its metabolite CGA321113 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-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 2^{nd} test item application.

The Limit of Quantitation (LOQ) was 0.01 mg/kg (i.e.10 µg 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 µg/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.

A summary of the residues of fluopyram and trifloxystrobin (and their metabolites) in flowers, nectar and pollen of *Phacelia tanacetifolia* is presented in the tables below.

Table 10.3.1.5- 8: Summary: residues of fluopyram and its metabolites in pollen, nectar and flowers

Sample material	Tunnel	Concentration range (min – max) [mg/kg]		
		Fluopyram	fluopyram-pyridyl-acetic acid (BCS-AA10189)	fluopyram-benzamide (AEF148815)
Flowers	1 - 3	3.1 – 5.0	<LOD	<LOD - <LOQ
Nectar	1 - 3	0.025 – 1.1	<LOD - <LOQ	<LOD - <LOQ
Pollen	1 - 3	3.0 - 30	<LOQ – 0.01	<LOQ - 0.017

LOQ: Limit of Quantification) for for all compounds = 0.01 mg/kg (= 10 µg/kg = 10 ppb)
A Value was corrected for mean of LOQ recovery level of 59 %.

Table 10.3.1.5- 9: Summary: residues of trifloxystrobin and its metabolite in pollen, nectar and flowers

Sample material	Tunnel	Concentration range (min – max) [mg/kg]	
		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 – 0.051

LOQ: Limit of Quantification for for all compounds = 0.01 mg/kg (= 10 µg/kg = 10 ppb)
LOD: Limit of Detection for for all compounds = LOQ/3 (i.e. ≈ 3.5 µg/kg)
A Value was corrected for mean of LOQ recovery level of 59 %.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The residue levels of fluopyram 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 in nectar and within the range of 3.0 to 30 mg/kg in pollen, respectively.

The residue levels of trifloxystrobin were 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 nectar and within the range of 2.4 to 25 mg/kg in pollen, respectively.

CP 10.3.1.6 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 *Orius laevigatus* and *Coccinella septempunctata* were conducted to determine potential effects on non-target arthropods.

Table 10.3.2- 1: Ecotoxicological endpoints relevant for the risk assessment for non-target arthropods for FLU + TFS SC 500

Test species, Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint		
Aphidius rhopalosiphi [redacted] (2007) M-283599-01-1 KCP 10.3.2.1/01	FLU + TFS SC 500 Laboratory, glass plates 200 mL product/ha 400 mL product/ha 800 mL product/ha 1600 mL product/ha 3200 mL product/ha	LR_{50} > 3200 mL product/ha Corr. Mortality [%] [A] 0.0 0.0 3.3 0.0 3.3	 Effects on reproduction [%] [B] 0.0 5.2 9.0 14.2 22.6	
Typhlodromus pyri [redacted] (2007) M-283552-01-1 KCP 10.3.2.1/02	FLU + TFS SC 500 Laboratory, glass plates 200 mL product/ha 400 mL product/ha 800 mL product/ha 1600 mL product/ha 3200 mL product/ha	LR_{50} > 3200 mL product/ha Corr. Mortality [%] [A] 2.1 1.0 3.1 6.2 13.4	 Effects on reproduction [%] [B] 0.8 + 0.8 + 6.2 0.3 1.0	
Orius laevigatus [redacted] (2007) M-297476-01-1 KCP 10.3.2.2/01	FLU + TFS SC 500 Extended laboratory, exposure on excised grape-vine leaf discs Control 25 mL product/ha 50 mL product/ha 100 mL product/ha 200 mL product/ha 400 mL product/ha	LR_{50} = 139 mL product/ha Corr. Mortality [%] [A] - 0 17.6 35.3 58.8 92.2	 Effect on fecundity [%] [B] - 4.6 10.7 17.6 - -	 Effects on fertility [%] [B] - 3.7 11.0 16.8 - -

Test species, Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Orius laevigatus [redacted] (2008) M-297471-01-1 KCP 10.3.2.2/02	FLU + TFS SC 500 Extended laboratory, exposure to semi-field aged residues on leaf discs prepared from sprayed grape-vine plants 2 × 0.8 L/ha (7 d interval)	Corr. Mortality [%]: 96.1 at 0 DA(L)T; 88.7 at 7 DA(L)T; 51.9 at 14 DA(L)T; 7.0 at 21 DA(L)T; 7.3 at 28 DA(L)T Effect on fecundity [%] [C]: 2.9 at 21 DA(L)T; -5.6 at 28 DA(L)T Effects on fertility [%] [C]: -1.8 at 21 DA(L)T; -2.1 at 28 DA(L)T
Chrysoperla carnea [redacted] (2014) M-482453-01-1 KCP 10.3.2.2/03	FLU + TFS SC 500 Extended laboratory, exposure on detached bean leaves	LR_{50} > 2340 mL product/ha
		Corr. Mortality [%] [A] / Eggs/Female/Day / Hatching [%]
	Control	– / 19.9 / 72.4
	300 mL product/ha	2.6 / 19.5 / 73.5
	500 mL product/ha	5.1 / 20.2 / 72.8
	840 mL product/ha	5.1 / 19.4 / 72.6
	1400 mL product/ha	7.7 / 20.0 / 73.2
	2340 mL product/ha	5.1 / 19.6 / 72.8

DA(L)T: Days after last application
[A] Positive values indicate increased mortality compared to the control; Negative values indicate better survivorship compared to the control
[B] Positive values indicate reduced performance compared to the control; Negative values indicate better performance compared to the control
[C] Due to high mortality at 0, 7 and 14 DA(L)T effects on fecundity and fertility were not assessed.

The exposure scenario is based on the use pattern as given in Table 10-1. The product FLU + TFS SC 500 is intended to be applied at a rate of 0.2 L product/ha (2 applications in grapes).

According the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev. 2 final, 2002) and the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi *et al.* 2000[15]) the exposure is calculated as:

In-field: Application rate × MAF

Off-field: Max. single application rate × MAF × drift factor/VDF × correction factor

Application rate: 0.2 L product/ha in grapes

MAF (multiple application factor) = 1.7 (2 applications)

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

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 (Tier 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 risk at Tier 2 is considered acceptable if the PER is below the application rate causing 50 % effect.

Table 10.3.2- 2: Exposure calculation for in-field assessment (Tier 1 and 2)

Crop	No. of appl.	Appl. rate [L prod./ha]	MAF	$PER_{in\text{-}field}$ [L prod./ha]
Grapes	2	0.2	1.7	0.340

MAF: Multiple application factor; PER: Predicted environmental rate

Table 10.3.2- 3: Exposure calculation for the off-field scenario (Tier 1)

Crop	No. of appl.	Appl. rate [L prod./ha]	MAF	Drift [%]	VDF	Correction factor	$PER_{off\text{-}field}$ [L prod./ha]
Grapes, late	2	0.2	1.7	7.23	5	10	0.049

MAF: Multiple application factor; VDF: Vegetation distribution factor; PER: Predicted environmental rate

Table 10.3.2- 4: Exposure calculation for the off-field scenario (Tier 2)

Crop	No. of appl.	Appl. rate [L prod./ha]	MAF	Drift [%]	Test system	VDF	Correction factor	$PER_{off\text{-}field}$ [L prod./ha]
Grapes, late	2	0.2	1.7	7.23	2D [A]	5	5	0.025

MAF: Multiple application factor; VDF: Vegetation distribution factor; PER: Predicted environmental rate

[A] Relevant for the extended lab studies with *Orius laevigatus* (M-297476-01-1) and *Chrysoperla carnea* (M-482453-01-1)

Risk assessment for non-target arthropods

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.* 2000[16]).

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

Tier 1 in-field risk assessment for non-target arthropods

Table 10.3.2- 5: Tier 1 in-field risk assessment for non-target arthropods

Crop and application rate	Species	$PER_{in\text{-}field}$ [L prod./ha]	LR_{50} [L prod./ha]	HQ	Trigger
Grapes 2 × 0.2 L prod./ha	*Aphidius rhopalosiphi*	0.340	>3.2	< 0.106	2
	Typhlodromus pyri		>3.2	< 0.106	2

PER: Predicted environmental rate;
HQ: Hazard quotient

For the standard species, the in-field HQ values are below the trigger of concern, indicating an acceptable risk for non-target arthropods.

Tier 1 off-field risk assessment for non-target arthropods

Table 10.3.2- 6: Tier 1 off-field risk assessment for non-target arthropods

Crop and application rate	Species	$PER_{off\text{-}field}$ [L prod./ha]	LR_{50} [L prod./ha]	HQ	Trigger
Grapes, late 2 × 0.2 L prod./ha	*Aphidius rhopalosiphi*	0.048	> 3.2	< 0.015	2
	Typhlodromus pyri		> 3.2	< 0.015	2

PER: Predicted environmental rate;
HQ: Hazard quotient

For the standard species, the off-field HQ values are below the trigger of concern, indicating an acceptable risk for non-target arthropods.

Tier 2 in-field risk assessment for non-target arthropods

Table 10.3.2- 7: Tier 2 in-field risk assessment for non-target arthropods

Crop and application rate	Species	$PER_{in\text{-}field}$ [L prod./ha]	LR_{50}/ER_{50} [L prod./ha]	$PER_{in\text{-}field}$ below rate with ≤ 50% effect
Grapes, late 2 × 0.2 L prod./ha	*Orius laevigatus*	0.340	0.139	No
	Chrysoperla carnea		> 2.34	Yes

PER: Predicted environmental rate

The $PER_{in\text{-}field}$ is below the rate with ≤ 50% effect for *Chrysoperla carnea*, but not for *Orius laevigatus* for the application of 2 × 0.2 L prod./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/10329/2002-final) "*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) pattern of the species…The assessment may be based on field studies or other evidence (e.g. results of aged-residues studies, environmental fate information).*"

For this purpose, an extended laboratory test with *Orius laevigatus* and aged residues of FLU + TFS SC 500 on grapevine leaves, sprayed under semi-field conditions, was conducted for an application rate of 2 x 0.8 L product/ha and a 7 days interval (KCP 10.3.2.2/02, M-297476-01-1). The results showed 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 can be expected.

Tier 2 off-field risk assessment for non-target arthropods

Table 10.3.2- 8: Tier 2 off-field risk assessment for non-target arthropods

Crop and application rate	Species	Off-field PER_{max} [L prod./ha]	LR_{50}/ER_{50} [L prod./ha]	$PER_{off\text{-}field}$ below rate with ≤ 50% effect
Grapes, late 2 × 0.2 L prod./ha	*Orius laevigatus*	0.025	0.139	Yes
	Chrysoperla carnea	0.025	2.340	Yes

PER: Predicted environmental rate

The $PER_{off\text{-}field}$ is below the rate with ≤ 50% effect for all species and uses indicating an acceptable risk for non-target arthropods.

Conclusion:

From the data and risk assessments presented above, it is concluded that unacceptable effects of FLU + TFS SC 500 on non-target arthropods in the in-field and off-field environment are not to be expected for the intended uses of FLU + TFS SC 500.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Data Point:	KCP 10.3.2.1/01
Report Author:	[redacted]
Report Year:	2007
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:	06 10 48 189
Document No:	M-283599-01-1
Guideline(s) followed in study:	IOBC (Mead-Briggs et al. 2000); Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: MEAD-BRIGGS ET AL. (2000) Deviations: None. 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

Executive Summary

In a laboratory study the lethal and sublethal toxicity of FLU + TFS SC 500 (250+250 g/L) on the parasitoid wasp, *Aphidius 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 criteria according to Mead-Briggs *et al.* (2000) were met.

After 2, 24 and 48 hours mortality of the wasps was assessed. After 48 hours no statistically significant differences compared to the control occurred and a corrected mortality up to 3.3 % was found in the treatment rates. The LR$_{50}$ was estimated to be > 3200 mL product/ha.

At 48 hours, 15 surviving females per treatment were confined individually for 24 hours over untreated aphid-infested wheat plants with the host cereal aphids *Rhopalosiphum padi*. After removal of the adult wasps, the aphid-infested plants were left for 11 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

Test item: FLU + TFS SC 500 (250+250 g/L); specification number 102000012886; batch ID 2006-004983; TOX07762-00; analysed content of a.s. = 246.1 g/L fluopyram, 245.8 g/L trifloxystrobin; density 1.165 g/mL.

Test design: Adults of the parasitoid wasp, *Aphidius 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 effects were compared to those of a purified water control. All treatments were applied at a nominal volume rate of 200 L spray solution/ha using a calibrated laboratory track sprayer. A toxic reference (Dimethoate 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/v 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.

Climatic conditions: The climatic test conditions of the mortality assessment phase were 19 - 22 ºC temperature and 65 - 73 % relative humidity with a photoperiod of 16 hours light and a light intensity of 2100 lux. In the reproduction assessment phase (Aphid parasitisation) the temperature ranged between 19 - 22 ºC with a photoperiod of 16 hours light and a light intensity of 4400 lux.

Statistics: Fisher's Exact Binominal-test ($p \leq 0.05$) was applied to mortality data, to compare individual test-item treatments with control. For reproduction data, treatments were compared to control by Dunnett's multiple t-test ($p \leq 0.05$).

Dates of work: November 27, 2006 – December 11, 2006

II. RESULTS AND DISCUSSION

In a worst-case laboratory study, the effects of exposing the parasitoid wasp *Aphidius rhopalosiphi* to fresh dry residues of FLU + TFS SC 500 (250+250 g/L) on glass plates were determined.

Corrected mortalities of 0, 0, 3.3, 0 and 3.3 % were observed after 48 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 (LR_{50}) was estimated to be > 3200 mL product/ha.

At 200, 400, 800, 1600 and 3200 mL product/ha, 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 mummies per female) at all tested rates (DUNNETT`s multiple t-test, 1-sided, $p \leq 0.05$) compared to the control 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 rhopalosiphi* (Hymenoptera, Braconidae) in a laboratory study

Test item:	FLU + TFS SC 500 (250+250 g/L)			
Test organism:	*Aphidius rhopalosiphi*			
Exposure on:	Glass plates			
Treatment	**Rate [1] [mL product/ha]**	**Corrected mortality [2] [%]**	**Reproduction [3] [mummies/ female]**	**Effects on reproduction [4] [%]**
Control	0		15.5	-
Test item	200	0	15.5	0
	400	0	14.7	5.2
	800	3.3	14.1	9.0
	1600	0	13.3	14.2
	3200	3.3	12.0	22.6
Toxic ref.	0.3	100	-	-
LR_{50} [5]	**> 3200 mL product/ha**			

1) Application rate for test item in terms of mL product per 200 L water/ha.
2) Mortality corrected for any control treatment deaths using Abbott's formula.
3) The numbers of parasitized aphids per female in each test-item treatment were compared to the numbers in the control by Dunnett's multiple t-test ($p \leq 0.05$).
4) For effects on reproduction, a negative value indicates an increase relative to the control.
5) Median lethal rate estimated empirically.

Validity criteria:

All of the validity criteria were met (according to Mead-Briggs *et al*., 2000).

Table 10.3.2.1- 2: Validity criteria

Validity criteria	Required	Obtained
Mortality within the control treatment at 48 h	≤ 13 % (i.e. 5 wasps from 40)	0 %
Mortality within the toxic reference treatment at 48 h	> 50 %	100 %
In the reproduction assessments the mean number of mummies in the control	> 5.0 per female not be more than two zero values	15.5 mummies per female, no zero values

III. Conclusion

The LR_{50} was estimated to be > 3200 mL product/ha.

The figures obtained fulfil the validity criteria of the laboratory method using glass plates according to Mead-Briggs *et al*. (2000).

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LR_{50} > 3200 mL product/ha

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Data Point:	KCP 10.3.2.1/02
Report Author:	[redacted]
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 study:	IOBC (Bluemel et al. 2000); Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: BLÜMEL ET AL. (2000) Deviations: The cumulative reproduction per females are counted from day 8 to day 14. Any eggs found on day 7 were removed and not counted in the fecundity assessment. 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

Executive Summary

In a laboratory study the lethal and sublethal toxicity of FLU + TFS SC 500 (250+250 g/L) on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), was investigated. Five test item rates from 200 - 3200 mL product/ha were tested. Per 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 criteria according to Blümel *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 LR_{50} was estimated to be > 3200 mL product/ha.

Assessments of mite reproduction were made at 9, 11 and 14 days after treatment. After 14 days no statistical significant effects on reproduction were found.

I. MATERIAL AND METHODS

Test item: FLU + TFS SC 500 (250+250 g/L); specification No.: 102000012886, batch ID 2006-004983; sample description: TOX No. 07762-00; analysed content of a.s.: 246.1 g/L fluopyram, 245.8 g/L trifloxystrobin; density 1.165 g/mL.

Test design: Protonymphs of the predatory mite *Typhlodromus 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, 400, 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 pollen (*Pinus nigra* and *Betula pendula*) at each assessment day.

On day 3, 7, 9, 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 ºC temperature and 72 – 79 % relative humidity with a photoperiod of 16 h and a light intensity of 2200 lux.

Statistics: Fisher's Exact Binominal-test ($p \leq 0.05$) was applied to mortality data, to compare individual treatments with the control. For comparison of reproduction data from individual treatments with the control DUNNETT`s multiple t-test (1-sided, $p \leq 0.05$) was used.

Dates of work: November 27, 2006 – December 12, 2006

II. RESULTS AND DISCUSSION

The effects of exposing the predatory mite *Typhlodromus pyri* to dried spray residues of FLU + TFS 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, $p \leq 0.05$) and the median lethal rate (LR_{50}) was estimated to be > 3200 mL product/ha, the highest rate tested.

At 200, 400, 800, 1600 and 3200 mL product/ha, the reproductive performance of the mites 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 (DUNNETT's multiple t-test, 1-sided, $p \leq 0.05$) compared to the control group.

The results are summarised in the table below.

Table 10.3.2.1- 3: Effects of dried spray residues on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) in a laboratory study

Test item:	FLU + TFS SC 500 (250+250 g/L)			
Test organism:	*Typhlodromus pyri*			
Exposure on:	Glass plates			
Treatment	**Rate [1] [mL product/ha]**	**Corrected mortality [2] [%]**	**Mean number eggs per female [3] (8-14 DAT)**	**Effects on reproduction [4] [%]**
Control	0		7.27	
Test item	200	2.1	7.21	0.8
	400	1.0	7.33	- 0.8
	800	3.1	7.72	- 6.2
	1600	6.2	7.25	0.3
	3200	13.4	7.20	1.0
Toxic ref.	10	79.4	-	-
LR_{50} [5]	**> 3200 mL product/ha**			

1) Application rate for test item in terms of mL product per 200 L water/ha.
2) Mortality corrected for any control treatment deaths using Abbott's formula. Negative values indicate an increase in survival, relative to the control.
3) Results for reproduction in individual test-item treatments compared to the control by Dunnett's multiple t-test, 1-sided ($p \leq 0.05$).
4) For effects on reproduction, a positive value indicates a decrease, relative to the control.
5) Median lethal rate estimated empirically.

Validity 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	≥ 4.0 per female	7.27 per female

III. CONCLUSION

The LR_{50} was estimated to be > 3200 mL product/ha.

The figures obtained fulfil the validity criteria of the laboratory method using glass plates according to Blümel *et al*. (2000).

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LR_{50} > 3200 mL product/ha

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

Data Point:	KCP 10.3.2.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Dose-response toxicity of AE C656948 & Trifloxystrobin SC 250 + 250 to the predatory bug Orius laevigatus (FIEBER) (Heteroptera: Anthocoridae) under extended laboratory conditions
Report No:	07 10 48 049 A
Document No:	M-297476-01-1
Guideline(s) followed in study:	IOBC (BAKKER et al. 2000) modified for the exposure on natural substrate (excised leaf discs); Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: BAKKER ET AL. (2000) modified Deviations: None. 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

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 laevigatus* were investigated. Five product rates from 25 - 400 mL product/ha were tested. Per product rate 6 replicates of 10 individuals were exposed to FLU + TFS SC 500 (250+250 g/L) on excised grape-vine leaf discs. Mortality was assessed on day 10 after exposure by counting the number of living and dead bugs. The reproduction performance of the surviving bugs in the control and all 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-day 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 above 50 mL/ha resulted in a statistically significant mortality compared to the control. The LR_{50} was estimated to be 139 mL 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.

I. Material and Methods

Test item: 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 trifloxystrobin; density 1.165 g/mL.

Test design: Nymphs of the predatory bugs, *Orius laevigatus* (less than 4 days old), were exposed to excised grape-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 test 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 females 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 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.

Climatic conditions: The climatic test conditions during the study were 24.7 - 27.0 °C temperature and 53 - 65 % relative humidity. The light-dark cycle was 16:8 hours with a light intensity range of 800 Lux.

Statistics: The LR_{50} with respect to the mortality was calculated by Probit analysis according to the maximum likelihood method (FINNEY 1971). The goodness-of-fit of the model was evaluated by Pearson's Chi^2 test. The mortality and fecundity results were analysed using the Chi^2 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 LR_{50} was performed using the computer program ToxRat Professional 2.09 (2006).

Dates of work: October 18, 2007 – November 10, 2007

II. Results and Discussion

In this extended laboratory test the effects of FLU + TFS SC 500 (250+250 g/L) residues on the survival and reproduction of the predatory bug *Orius laevigatus* were determined at the rates of 25, 50, 100, 200 and 400 mL product/ha applied to excised grape-vine leaf discs.

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, 11.7, 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 all rates of FLU + 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 group. 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 hatching success (% 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 when compared to control group. The mean hatching rate was 89.2, 88.4, 88.6 and 90.1 % for the control and the application rates 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 5.4, 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

Test item:	FLU + TFS SC 500 (250+250 g/L)							
Test organism:	*Orius laevigatus*							
Exposure on:	grape-vine leaf discs							
Treatment	**Rate [mL product/ha]**	**Mortality after 10 days [%]**		**Reproduction**				
				Fecundity			**Fertility**	
		Un-corrected	Corrected [1)]	Mean number of eggs per female per day	Mean viable eggs per reproductive female per day	Reduction relative to control [%]	Mean hatching rate [%]	Reduction relative to control [%]
Control		15.0	-	6.1	89.2	-	5.4	-
Test item	25	11.7	0	6.4	88.4	4.6	5.6	- 3.7
	50	30.0*	17.6	5.4	88.6	10.7	4.8	11.0
	100	45.0*	35.3	5.0	90.1	17.6	4.5	16.8
	200	65.0*	58.8	-	-	-	-	-
	400	93.3*	92.2	-	-	-	-	-
Toxic ref.	20	98.3*	98.0	-	-	-	-	-
LR_{50} (95 % CI): 139.0 (113.2 – 170.7) mL product/ha								

* Statistically significant
[1)] Corrected mortality according to ABBOTT

Validity criteria:

All of the validity criteria were met (according to Bakker *et al.*, 2000).

Table 10.3.2.2- 2: Validity criteria

Validity criteria	Required	Obtained
Mortality in control treatment	≤ 25 %	15 %
Mortality in reference item treatment	≥ 40 %	98.3 %
Mean number of eggs per female and day in control treatment	≥ 2	6.1
Number of females in the control treatment producing zero values for reproduction	≤ 5	1
Mean hatching rate in control treatment	≥ 70 %	89.2 %

III. CONCLUSION

The LR_{50} was estimated to be 139.0 (113.2 - 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) were met.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: LR_{50} = 139.0 mL product/ha

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Data Point:	KCP 10.3.2.2/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	Toxicity of AE C656948 & Trifloxystrobin SC 250 + 250 to the predatory bug Orius laevigatus (FIEBER) (Heteroptera: Anthocoridae) under extended laboratory conditions using semi-field-aged residues on grape-vine
Report No:	07 10 48 005 A
Document No:	M-297471-01-1
Guideline(s) followed in study:	IOBC (BAKKER et al. 2000) modified for the exposure on natural substrate. Equivalent to US EPA OPPTS Guideline No. 850.STPP
Deviations from current test guideline:	Current Guideline: BAKKER ET AL. (2000) modified Deviation: None. 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

Executive Summary

In an extended laboratory test the effects on the survival and reproduction of the predatory bug *Orius laevigatus* by semi-field aged FLU + TFS SC 500 (250+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), 7 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 1.6 L/ha per season, were tested. Per test item rate 6 replicates of 10 individuals were exposed to FLU + TFS SC 500 (250+250 g/L) on 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.

All validity criteria according to Bakker *et al.* (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 LR_{50} was determined to be 0.174 L product/ha.

In the 1st, 2nd and 3rd bioassay 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

Test item: 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 trifloxystrobin; density 1.165 g/mL.

Test design: 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 0, 7, 14, 21 and 28 after last application. The test system using nymphs on treated plants (leaf discs) enclosed in an untreated arena (the leaf discs having been prepared from grape-vine and were sprayed and maintained outdoors under semi-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), Mating phase (4 days), Reproduction phase (9 days).

In the exposure phase, pre-imaginal mortality of *O. laevigatus* was determined following exposure of 2nd instar nymphs (4 days after hatching) to dried fresh or aged residues on grape-vine leaf discs. In the exposure phase, 6 replicates each containing 10 second-instar nymphs were established for the control (deionised water), one product treatment and a toxic reference. The toxic reference item Perfekthion 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 leaves 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 exposure 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 the last (5th) nymphal stage and at least 80 % of the insects were adults (usually between 9 - 11 days after start of the exposure).

In the reproduction phase, the sublethal effects of the product on 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 test cages for 4 additional days. After females were 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 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.

Climatic conditions: 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 Lux. The temperature, relative humidity and sun radiation of the semi-field were recorded continuously by “Thies Climastation“ 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 $p \leq 0.05$. The calculation of statistical significance was performed using the computer program ToxRat Professional 2.09 (2006). The LR_{50} 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 *Orius 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 7.0 % mortality of *Orius laevigatus* nymphs until adulthood (corrected according to ABBOTT). The fecundity and fertility 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-vine leaves resulted in 7.3 % mortality of *Orius laevigatus* nymphs until adulthood (corrected according to ABBOTT). The fecundity and fertility 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 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.

In the 1st , 2nd and 3rd bioassay 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.

A summary of the effects observed in this study is given below.

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

Test item:	FLU + TFS SC 500 (250+250 g/L)
Test organism:	*Orius laevigatus*
Exposure on:	Grape-vine leaf discs

Treatment group	Surviving nymphs/ adults (no.)	Mortality (%)		Reproduction				
		Absolute	Corrected [1)]	Mean Eggs/ female/ day	Mean Viable eggs/ female/ day	Mean Hatched nymphs/ egg (%)	Reduction in (%)	
							fecundity	fertility
1st bioassay (DA(L)T 0)								
Control	51	15.0	-	-	-	-	-	-
Toxic ref.	0	100*	100	-	-	-	-	-
Test item	2	96.7*	96.1	[2)]	-	-	-	-
2nd bioassay (DA(L)T 7)								
Control	53	11.7	-	-	-	-	-	-
Toxic ref.	0	100*	100	-	-	-	-	-
Test item	6	90.0*	88.7	[2)]	-	-	-	-
3rd bioassay (DA(L)T 14)								
Control	52	13.3	-	-	-	-	-	-
Toxic ref.	0	100 *	100	-	-	-	-	-
Test item	25	58.3 *	51.9	[2)]	-	-	-	-
4th bioassay (DA(L)T 21)								
Control	57	5.0	-	6.8	5.7	89.7	-	-
Toxic ref.	0	100 *	100	-	-	-	-	-
Test item	53	11.7	7.0	6.6	5.8	93.7	2.9	-1.8
5th bioassay (DA(L)T 28)								
Control	55	8.3	-	5.4	4.8	91.4	-	-
Toxic ref.	1	98.3 *	98.2	-	-	-	-	-
Test item	51	15.0	7.3	5.7	4.9	91.3	- 5.6	-2.1

DA(L)T: Days after last application

Validity criteria:

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
Mortality in control treatment	≤ 25 %	≤ 15 %
Mortality in reference item treatment	≥ 40 %	≥ 98.3 %
Mean number of eggs per female and day in control treatment	≥ 2	6.8 and 5.4
Number of females in the control treatment producing zero values for reproduction	≤ 5	1
Mean hatching rate in control treatment	≥ 70 %	89.7 and 91.4 %

III. Conclusion

In conclusion, potential for recovery can be considered after 14 days following the last application but was evident 21 days following the last application, with exposure to aged residues resulting effects on survival and reproduction below 50 %.
The validity criteria according to Bakker *et al.* (2000) modified for the exposure on natural substrate, under semi-field conditions applied and aged residues on natural substrate were used instead of glass plates were met.

Assessment and conclusion by applicant:

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 14 days following the last application but was evident 21 days following the last application, with exposure to aged residues resulting effects on survival and reproduction below 50 %.

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Data Point:	KCP 10.3.2.2/03
Report Author:	[redacted]
Report Year:	2014
Report Title:	Effects of fluopyram + trifloxystrobin SC 500 (250+250 g/L) on the green lacewing Chrysoperla carnea (STEPH.) under extended laboratory conditions
Report No:	14 10 48 022 A
Document No:	M-482453-01-1
Guideline(s) followed in study:	US EPA OCSPP Guideline No. 850.SUPP IOBC (VOGT et al. 2000), modified for the exposure on natural substrate (extended laboratory test)
Deviations from current test guideline:	Current Guideline: US EPA OCSPP Guideline No. 850.SUPP IOBC (VOGT et al. 2000), modified for the exposure on natural substrate (extended laboratory test Deviation: None.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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 test item rates from 300 - 2340 mL product/ha were tested. Per test item rate 40 replicates of one individual larva were exposed to FLU + TFS SC 500 (250+250 g/L) on kidney bean leaves. The number of dead larvae and pupae and hatched adults as well as the number of eggs laid and larvae hatched (F_1) 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 LR_{50} was estimated to be > 2340 mL product/ha.

No effects on reproduction of *Chrysoperla carnea* 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 product treatments the number of eggs per female per day was > 15 and the hatching rate was > 70 %.

I. MATERIAL AND METHODS

Test item: FLU + TFS SC 500 (250+250 g/L), Spec. no: 102000012886 – 03, Batch ID: NP65CX4182, Sample description: TOX10120-00, Material No.: 06033007, analysed content of active substance: 21.1 % w/w (246.2 g/L) fluopyram, 21.2 % w/w (246.8 g/L) trifloxystrobin, density: 1.167 g/mL.

Test design: 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 300, 500, 840, 1400 and 2340 mL product/ha in 200 L deionised water/ha applied on kidney bean leaves (*Phaseolus 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.

Climatic conditions: The climatic test conditions during the study were 23 - 27 °C temperature and 66 - 74 % relative humidity. The light / dark cycle was 16:8 h with a light intensity of 1270 Lux.

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 normality or homoscedasticity prior to analysis. The accepted significance level was $\alpha = 0.05$. Due to the low effects on mortality in the test item treatment groups, a calculation of the LR_{50} (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 + TFS SC 500 (250+250 g/L) 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 bean leaves (*Phaseolus vulgaris*).

In the water treated control a mortality of 2.5 % was observed. In the test 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 NOER (no observed effect rate) for pre-imaginal mortality was ≥ 2340 mL /ha. The LR_{50} was estimated to be > 2340 mL product/ha.

No effects on reproduction of *Chrysoperla carnea* 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 number of eggs per female 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

Test item		FLU + TFS SC 500 (250+250 g/L)			
Test organism		*Chrysoperla carnea*			
Exposure on		detached bean leaves			
		Preimaginal mortality [%]		**Reproduction**	
Treatment	**Rate [mL product/ha]**	**Uncorrected [2)]**	**Corrected [3)]**	**Eggs per female and day**	**Fertility [hatching rate in %]**
Control	0.0	2.5		19.9	72.4
Test item [1)]	300	5.0	2.6	19.5	73.5
	500	7.5	5.1	20.2	72.8
	840	7.5	5.1	19.4	72.6
	1400	10.0	7.7	20.0	73.2
	2340	7.5	5.1	19.6	72.8
Toxic ref.	40	70 *	69.2	n.d.	n.d.
LR_{50}	**> 2340 mL product/ha**				

n.d. not determined
* statistically significantly different compared to the control
[1)] Application rate in 200 L water/ha
[2)] Mortality after exposure to residues on treated bean leaves. The results for mortality in individual treatments were compared to that in the control using FISHER´S Exact Binomial test (α = 0.05).
[3)] Corrected mortality according to ABBOTT (1925)

<u>Validity criteria:</u>

All of the validity criteria were met (according to Vogt *et al*., 2000).

Table 10.3.2.2- 6: Validity criteria

Validity criteria	Required	Obtained
Mortality in water control	≤ 20%	0 %
Corrected mortality reference item	≥ 50%	69.2 %
Mean number of eggs per female and day in water control	≥ 15	19.9
Mean hatching rate of the eggs (fertility) in water control	≥ 70%	72.4 %

III. CONCLUSION

The LR_{50} was estimated to > 2340 mL product/ha.

The NOER (no observed effect rate) for pre-imaginal mortality was the highest tested application rate, 2340 mL/ha.

All validity criteria according to according to Vogt *et al*. (2000) were met.

<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: LR_{50} > 2340 mL product/ha

CP 10.3.2.3 Semi-field studies with non-target arthropods

In view of the results presented above, no semi-field studies were deemed necessary.

CP 10.3.2.4 Field studies with non-target arthropods

In view of the results presented above, no field studies were deemed necessary.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

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

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (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 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 latest by end of March 2022.

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.

Table 10.4- 1: Maximum PECsoil values for fluopyram, its metabolites and FLU + TFS SC 500 in grapes (for details see MCP Section 9, Point 9.1.3)

Compound	Grapes		
	$PEC_{soil, initial}$ [mg/kg]	$PEC_{soil, plateau, 5 cm}$ [mg/kg]	$PEC_{soil, accu}$*
2 × 50 g a.s./ha			
Fluopyram	0.053	0.185	0.238
Fluopyram-7-hydroxy	0.003	0.001	0.003
Trifluoroacetic acid (TFA)	0.002	0.001	0.002
2 × 0.2 L prod./ha			
FLU + TFS SC 500	0.249		

* $PEC_{soil, accu}$ means the sum of $PEC_{soil, initial}$ and $PEC_{soil, plateau}$

1) The PEC_{soil} value for the product FLU + TFS SC 500 is calculated based on the initial rate of the product (0.2 L/ha) in a multiple application, the portion reaching soil (BBCH 53-73, worst case interception of 60% for vines), the standard soil density (1.5 g/cm³), the standard soil depth (5 cm) and the density of the formulation (1.165 g/mL).

Uncertainty factors for isomer composition of metabolites

The metabolite Fluopyram-7-hydroxy has a chiral center. Ecotoxicological testing was performed with the racemic mixture. Therefore, for this metabolite an additional uncertainty factor of 2 will be applied to the TER available for earthworm, springtails, soil mites and nitrogen transformations in consideration of enantiomers.

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 values.

Table 10.4.1- 1: Ecotoxicological endpoints – earthworm reproduction studies with FLU+TFS SC 500, Fluopyram and its metabolites

Test item	Test species, test design	Ecotoxicological endpoint	Reference
FLU+TFS SC 500	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 23.3 mg prod./kg dws $NOEC_{corr}$ = 11.65 mg prod./kg dws [A] EC_{10} = 24.97 mg prod./kg dws EC_{10corr} = 12.49 mg prod./kg dws [A]	[redacted] (2007) M-283637-01-1 KCP 10.4.1.1/01 Recalculation by [redacted] (2020) M-688422-02-1 KCP 10.4.1.1/02
FLU SC 500	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 134 mg a.s./kg dws [B] $NOEC_{corr}$ = 67 mg a.s./kg dws [A, B] EC_{10} = 117.5 mg a.s./kg dws [A] $EC_{10,corr}$ = 59 mg a.s./kg dws [A, B]	[redacted] (2020) M-680776-01-1 KCA 8.4.1/02 KCP 10.4.1.1/02 [C]
Fluopyram-7-hydroxy	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 18 mg p m./kg dws $NOEC_{corr}$ = 9 mg p m./kg dws [A] EC_{10} = calculation not possible	[redacted] (2021) M-762139-01-1 KCA 8.4.1/03
Trifluoroacetic acid (TFA)	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 320 mg p m./kg dws [D]	[redacted] (2005) M-251328-01-1 KCA 8.4.1/04

dws = dry weight soil, prod. = product
[A] Endpoint corrected by a factor of 2 due to lipophilic substance (log Pow > 2)
[B] Endpoint calculated on the basis of analysed fluopyram content in the formulation (42.4 % w/w; as given in study report)
[C] Full details on this study are described in the corresponding MCP for the formulation FLU SC 500.
[D] NOEC of 320 mg p m./kg dws is based on effects on the body weight in the concentration 1000 mg p m./kg dws.

Risk assessment for earthworms

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 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 10.4.1- 2: TER calculation for earthworms for FLU + TFS SC 500

Compound	Species, study type	Endpoint [mg prod./kg]	PEC_{soil} [mg prod./kg]	TER_{LT}	Trigger
Grapes, 2 × 0.2 L prod./ha					
FLU + TFS SC 500	Earthworm, reproduction	NOEC = 11.65	0.249	47	5

Table 10.4.1- 3: TER calculation for earthworms for fluopyram and its metabolites

Compound	Species, study type	Endpoint [mg/kg]	PEC_{soil} [mg/kg]	TER_{LT}	Trigger
Grapes, 2 × 50 g a.s./ha					
Fluopyram [A]	Earthworm, reproduction	NOEC = 67	0.238	282	5
Fluopyram-7-hydroxy	Earthworm, reproduction	NOEC = 9	0.003	1500 [B]	5
Trifluoroacetic acid (TFA)	Earthworm, reproduction	NOEC = 320	0.002	160000	5

[A] endpoint derived from study performed with FLU SC 500

[B] For the metabolite fluopyram-7-hydroxy the TER has been corrected according to an uncertainty factor of 2 in consideration of two enantiomers.

All TER values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on earthworm are to be expected from the intended use of FLU + TFS SC 500 in grapes.

CP 10.4.1.1 Earthworms sub-lethal effects

Data Point:	KCP 10.4.1.1/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 & trifloxystrobin SC 250 & 250 G: effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial 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 11268-2: 1998(E): „Soil quality - Effects of pollutants on earthworms (Eisenia fetida) 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 fetida / Eisenia andrei)"; Equivalent to US EPA OPPTS 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
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effects of FLU + TFS SC 500 (250+250 g/L) on survival and reproduction of adult earthworms *Eisenia 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 cocoons 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 + TFS 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 earthworms the reproduction was determined by counting the number of off-spring hatched from the cocoons per test vessel.

The study fulfilled all validity criteria of OECD 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 EC_{10} and EC_{20}-values related to reproduction were calculated being 24.97 mg prod./kg and 46.24 mg prod./kg, respectively.

I. MATERIALS AND METHODS

Test item: FLU + TFS SC 500 (250+250 g/L), specification no.: 102000012886; Batch No.: 2006-004983; 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 period of 4 weeks (first part). After this period, the adult earthworms were removed from the test vessels and the cocoons and juvenile earthworms remained in the test vessels for additional 4 weeks (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/vessel (animal 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 % Sphagnum 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 mortality and biomass 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 hatched from the cocoons after this additional test period per test vessel.

Climatic conditions: The climatic conditions were in the temperature range 18 - 22 °C with a photoperiod of 16 hours light and a light intensity of 400 to 800 lux.

Statistics: Changes in body weight were statistically evaluated by means of a Dunnett´s multiple t-test. The reproduction of the surviving test organisms was statistically evaluated by means of a Williams multiple sequential t-test.

Dates of work: October 16, 2006 – December 18, 2006

II. RESULTS AND DISCUSSION

Table 10.4.1.1- 1: Effects of FLU + TFS SC 500 (250+250 g/L) on *Eisenia fetida*

Parameter	Treatment [mg product/kg dry weight artificial soil]*					
	Control	14	23.3	37.3	55.9	83.9
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0
Significance (Mortality)	-	-	-	-	-	-
Mean change of body fresh weight of the adults from day 0 to day 28 [%]	+ 50.4	+ 54.0	+ 52.8	+ 54.8	+ 45.2	+ 52.7
Standard deviation	± 7.6	± 4.0	± 2.2	± 5.0	± 2.8	± 3.6
Significance (body fresh weight)**	-	n.s.	n.s.	n.s.	n.s.	n.s.
Mean number of offspring per test vessel after 56 days	234.8	230.0	217.5	190.0	174.8	159.8
Standard deviation	± 24.5	± 18.6	± 19.3	± 29.1	± 34.6	± 10.5
Statistical comparison to the control ***	-	n.s.	n.s.	s.	s.	s.
Significance (reproduction)***	-	-	-	+	+	+
	Adult Mortality			**Growth**	**Reproduction**	
NOEC [mg test item/kg dry weight soil]	**≥ 83.9**			**≥ 83.9**	**23.3**	
LOEC [mg test item/kg dry weight soil]	**> 83.9**			**≥ 83.9**	**37.3**	
EC_{10} (mg prod.kg dry weight artificial soil) 95% confidence limits****					24.97 (11.09 - 34.41)	
EC_{20} (mg prod./kg dry weight artificial soil) 95% confidence limits****					46.24 (33.10 - 58.04)	

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

* Conversion according to the condition of the test (500 g dry weight artificial soil and 200 cm2 surface area per test vessel; the density of the test item was taken into account)

** statistical significance compared to the control (Dunnett's Multiple t-test, two-sided, α = 0.05)

*** statistical significance compared to the control Williams Multiple Sequential t-test, one-sided smaller, α = 0.05)

**** recalculations were performed with the software ToxRatPro 3.2 (M-688422-02-1)

n.s.: mean value not statistically significantly different compared to the control ($p \geq 0.05$)

s.: mean value statistically significantly different compared to the control ($p < 0.05$)

Mortality:

No mortality of adult earthworms 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 at all application rates. Therefore, the endpoints related to growth were: NOEC: ≥ 83.9 mg prod./kg dry weight artificial soil (corresponding to ≥ 18.0 L prod./ha) and LOEC: ≥ 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 artificial soil (corresponding to 3.0 and 5.0 L prod./ha). Statistically significant different values for the number 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, 12.0 and 18.0 L 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 artificial soil. The EC_{10} (C.I.) and EC_{20} (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 soil dry weight, respectively.

Validity criteria:

All validity criteria of the OECD 222 guideline were met.

Table 10.4.1.1- 2: Validity criteria

Validity criteria acc. to OECD 222 (adopted 2016)	**Required**	**Obtained**
Mortality of the adults in the control	≤ 10 %	0 %
Number of juveniles (earthworms per control vessel)	≥ 30	234.8
Coefficient of variance of reproduction in the control	≤ 30 %	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 earthworms was observed 28 days after application. No statistically significant different values for the biomass relative to the control were observed at any application rate, including the highest rate of 0.50 kg test item/ha (results 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, $\alpha = 0.05$).

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:

$NOEC_{mortality}$: ≥ 83.9 mg test item/kg dry weight artificial soil (corresponding to ≥ 18.0 L prod./ha)

$LOEC_{mortality}$: > 83.9 mg test item/kg dry weight artificial soil (corresponding to ≥ 18.0 L prod./ha)

$NOEC_{growth}$: ≥ 83.9 mg test item/kg dry weight artificial soil (corresponding to ≥ 18.0 L prod./ha)

$LOEC_{growth}$: > 83.9 mg test item/kg dry weight artificial soil (corresponding to ≥ 18.0 L prod./ha)

$NOEC_{reproduction}$: = 23.3 mg prod./kg dry weight artificial soil (corresponding to = 5 L prod./ha)

$LOEC_{reproduction}$: = 37.3 mg prod./kg dry weight artificial soil (corresponding to 8.0 L prod./ha)

$EC_{10reporoduction}$: = 24.97 mg prod./kg dry weight artificial soil

$EC_{20reproduction}$: = 46.24 mg prod./kg dry weight artificial soil

According to EFSA (2015) the level of protection for the EC_{10} is classified as "high". The normalised width of confidence interval (NW) rating for the EC_{10} is "fair".

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: $NOEC_{reproduction}$ = 23.3 mg prod./kg dws

****

Data Point:	KCP 10.4.1.1/02
Report Author:	[redacted]
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, 2007; M-283637-01-1) using the probit analysis
Report No:	M-688422-02-1
Document No:	M-688422-02-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Summary

The *Eisenia fetida* reproduction study with FLU + TFS SC 500 (M-283637-01-1) was statistically re-evaluated based on the number of juveniles of *Eisenia fetida* provided in the study report. Probit analysis was performed in order to derive EC_{10}, EC_{20} and EC_{50} -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: Re-calculated EC_{10} and EC_{20} values (with confidence limits) for % effect on number of juveniles compared to the control

	EC_{10}	EC_{20}	EC_{50}
Value [mg prod./kg]	24.97	46.24	n.d.
Lower 95%-cl	11.09	33.10	n.d.
Upper 95%-cl	34.41	58.04	n.d.

n.d.: not determined due to mathematical reasons (inappropriate data) or value is beyond the tested concentrations

Conclusion:

The EC_{10} and EC_{20}-values were 24.97 mg prod./kg dry 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 (NW) 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} = 24.97 mg prod./kg dws (based on reproduction) and EC_{20} = 46.24 mg prod./kg dws (based on reproduction)

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 metabolites. In case EC_{10} values were lower than the NOEC and the calculation was reliable they were used for the calculations of TER values.

Table 10.4.2- 1: Springtail and soil mite reproduction studies with product FLU+TFS SC 500 and fluopyram metabolites

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
Springtails, reproduction			
FLU+TFS SC 500	*Folsomia candida* reproduction 28 d, mixed	NOEC = 562 mg prod./kg dws $NOEC_{corr}$ = 281 mg prod./kg dws [A] EC_{10} = 605 mg prod./kg dws $EC_{10,corr}$ = 302.5 mg prod./kg dws [A]	[redacted] (2017) M-576685-01-1 KCP 10.4.1.2/01
FLU SC 500	*Folsomia candida* reproduction 28 d, mixed	NOEC = 75.5 mg a.s./kg dws [B] $NOEC_{corr}$ = 37.8 mg a.s./kg dws [A, B] EC_{10} = 102 mg a.s./kg dws [B] $EC_{10,corr}$ = 51 mg a.s./kg dws [A, B]	[redacted] (2019) M-675002-01-1 KCA 8.4.2.1/01 KCP 10.4.2.1/03 [C]
Fluopyram-7-hydroxy	*Folsomia candida* reproduction 28 d, mixed	NOEC = 562 mg p.m./kg dws $NOEC_{corr}$ = 281 mg p.m./kg dws [A] EC_{10} = 611 mg p.m./kg dws	[redacted] (2020) M-755397-01-1 KCA 8.4.2.1/03
Trifluoroacetic acid (TFA)	*Folsomia candida* reproduction 28 d, mixed	NOEC ≥ 100 mg p.m./kg dws (Na-TFA) ≥ 84 mg p.m./kg dws (TFA) [D] EC_{10} calculation not possible	[redacted] (2012) M-436127-01-1 KCA 8.4.2.1/05
Soil mites, reproduction			
FLU+TFS SC 500	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC = 316 mg prod./kg dws $NOEC_{corr}$ = 158 mg prod./kg dws [A] EC_{10} = 457 mg prod./kg dws $EC_{10,corr}$ = 228.5 mg prod./kg dws [A]	[redacted] (2016) M-548820-01-1 KCP 10.4.1.2/02
FLU SC 500	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 424 mg a.s./kg dws [B] $NOEC_{corr}$ ≥ 212 mg a.s./kg dws [A, B] EC_{10} calculation not possible	[redacted] (2020) M-678468-01-1 KCA 8.4.2.1/02 KCP 10.4.2.1/04 [C]
Fluopyram-7-hydroxy	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 1000 mg p.m./kg dws $NOEC_{corr}$ ≥ 500 mg p.m./kg dws [A] EC_{10} calculation not possible	[redacted] (2020) M-754291-01-1 KCA 8.4.2.1/04

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
Trifluoroacetic acid (TFA)	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 100 mg p.m./kg dws (Na-TFA) ≥ 84 mg p m./kg dws (TFA) [D] EC_{10} calculation not possible	[redacted] (2012), M-436326-01-1 KCA 8.4.2.1/05

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 analysed fluopyram content in the formulation (42.4 % w/w; as given in study report)

C Full details on this study are described in the corresponding MCP for the formulation FLU SC 500.

D As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

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

The exposure of soil meso- and macrofauna 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 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: TER calculation for other non-target soil meso- and macrofauna for FLU + TFS SC 500

Compound	Species, study type	Endpoint [mg prod./kg]	PEC_{soil} [mg prod./kg]	TER_{LT}	Trigger
Grapes, 2 × 0.2 L prod./ha					
FLU + TFS SC 500	*Folsomia candida*	NOEC = 281	0.249	1129	5
FLU + TFS SC 500	*Hypoaspis aculeifer*	NOEC = 158	0.249	635	5

Table 10.4.2- 3: TER calculation for other non-target soil meso- and macrofauna for fluopyram and its metabolites

Compound	Species, study type	Endpoint [mg/kg]	PEC_{soil} [mg/kg]	TER_{LT}	Trigger
Grapes, 2 × 50 g a.s./ha					
Fluopyram [A]	*Folsomia candida*	NOEC = 37.8	0.238	159	5
Fluopyram [A]	*Hypoaspis aculeifer*	NOEC ≥ 212	0.238	≥ 891	5
Fluopyram-7-hydroxy	*Folsomia candida*	NOEC = 281	0.003	46833 [B]	5
Fluopyram-7-hydroxy	*Hypoaspis aculeifer*	NOEC ≥ 500	0.003	≥ 83333 [B]	5
Trifluoroacetic acid (TFA)	*Folsomia candida*	NOEC ≥ 84	0.002	≥ 4200	5
Trifluoroacetic acid (TFA)	*Hypoaspis aculeifer*	NOEC ≥ 84	0.002	≥ 4200	5

[A] endpoint derived from study performed with FLU SC 500

[B] For the metabolite fluopyram-7-hydroxy the TER has been corrected according to an uncertainty factor of 2 in consideration of two enantiomers.

All TER values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on soil macro-organisms are to be expected from the intended use of FLU + TFS SC 500 in grapes.

CP 10.4.2.1 Species level testing

Data Point:	KCP 10.4.2.1/01
Report Author:	[redacted]
Report Year:	2017
Report Title:	Fluopyram + trifloxystrobin SC 500 (250+250) G: Effects on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	16 10 48 273 S
Document No:	M-576685-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC; Regulation (EC) No 1107/2009 (2009); US EPA OCSPP Not Applicable; OECD 232 (2009)
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) Deviations: none. 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

Executive Summary

In a laboratory study the effects of FLU + TFS SC 500 (250+250 g/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. Eight test item rates from 18 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 collembolans each were exposed to FLU + TFS SC 500 (250+250 g/L) 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 significantly adverse effects on adult mortality of the collembolan *Folsomia candida* in artificial 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.w.

Therefore, the No-Observed-Effect-Concentration (NOEC) for mortality and reproduction was determined to be 562 mg prod./kg soil d.w. and the Lowest-Observed-Effect-Concentration (LOEC) for reproduction was determined to be 1000 mg prod./kg soil d.w..

I. MATERIALS AND METHODS

Test item: FLU + TFS SC 500 (250+250 g/L); specification no.: 102000012886; Batch No.: PAIS005173; REG40032-00; analytical findings: 246.2 g/L (21.1 % w/w) fluopyram, 250.8 g/L (21.5 % w/w) trifloxystrobin; density: 1.165 g/mL (20 °C).

Test design: Ten collembolans (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, 56, 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 Bonferroni-Holm, one-sided greater, α = 0.05) was applied to mortality data and William's t-test (one-sided smaller, α = 0.05) was applied to reproduction data. The ECx values were calculated with Probit analysis.

Dates of work: October 10, 2016 – November 07, 2016

II. Results and Discussion

Table 10.4.2.1- 1: Effects on mortality and reproduction of Folsomia candida after treatment with FLU + TFS SC 500 (250+250 g/L)

Test concentration [mg prod./dry weight artificial soil]	Adult mortality [%]	Significance (*)	Mean number of juveniles per test vessel ± S.D.	Reproduction [% of control]	Significance (**)
Control	2.5		686 ± 57	-	
18	2.5	-	704 ± 56	103	-
32	2.5	-	685 ± 83	100	-
56	2.5	-	685 ± 144	100	-
100	2.5	-	667 ± 53	97	-
178	2.5	-	681 ± 154	99	-
316	5.0	-	686 ± 33	100	-
562	15.0	-	616 ± 90	90	-
1000	32.5	+	521 ± 63	76	+
Endpoints				**Mortality**	**Reproduction**
NOEC [mg prod./kg dry weight artificial soil]				562	562
LOEC [mg prod./kg dry weight artificial soil]				1000	1000
LC_{50} [mg prod./kg dry weight artificial soil] [1)]				> 1000	
EC_{10} [mg prod./kg dry weight artificial soil] [1)] (95 % C.I.)					605 (511 - 715)
EC_{20} [mg prod./kg dry weight artificial soil] [1)] (95 % C.I.)					887 (808 - 973)

Calculations were done with unrounded values.
n.d.: Not determined
SD: Standard deviation
C.I.: Confidence Interval
1) EC_x = Probit analysis
* Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, one-sided greater, α = 0.05, "-": non-significant; "+": significant
** William's t-test, one-sided smaller, α = 0.05, "-": non-significant; "+": significant

Mortality:

In the control group 2.5 % of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Statistically significant effects on mortality compared to the control were observed at a concentration of 1000 mg test item/kg soil dry weight. No effects on behaviour of the collembolans were observed during the test.

The NOEC 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 juveniles compared to the control group were recorded at a concentration of 1000 mg test item/kg soil d.w.

The NOEC for reproduction was determined to be 562 mg prod./kg soil d.w. and the LOEC 1000 mg prod./kg soil d.w. The EC_{10} and EC_{20} values for reproduction were calculated to be 605 and 887 mg prod./kg soil d.w., respectively.

Validity criteria:

Validity criteria for the untreated control of the study according OECD 232 from July 29, 2016 were used.

Table 10.4.2.1- 2: Validity criteria

Validity criteria acc. to OECD 232 (adopted 2016)	Required	Obtained
Mean adult mortality in control	≤ 20 %	23 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	686
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	8.4 %

All validity criteria of the OECD 232 guideline were fulfilled.

Reference test:

To verify the sensitivity of the test system the reference item boric acid is routinely tested at concentrations of 44, 67, 100, 150 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 16 10 48 003 S, dated 2016-08-08) the EC_{50} was determined to be 104 mg/kg soil dry weight. The LC_{50} 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 EC_{50} value for the reproduction was close to the value of 100 mg/kg soil dry weight as stated in OECD 232 (2009). The EC_{50} therefore showed that the test system was sensitive.

III. CONCLUSION

All validity criteria were met. The endpoints were:

$NOEC_{mortality}$: 562 mg prod./kg dry weight artificial soil

$LOEC_{mortality}$: 1000 mg prod./kg dry weight artificial soil

$NOEC_{reproduction}$: 562 mg prod./kg dry weight artificial soil

$LOEC_{reproduction}$: 1000 mg prod./kg dry weight artificial soil

$EC_{10\ reproduction}$: 605 mg prod./kg dry weight artificial soil

$EC_{20\ reproduction}$: 887 mg prod./kg dry weight artificial soil

According to EFSA (2015) the level of protection for the EC_{10} is classified as "high". The normalised width of confidence interval (NW) rating for the EC_{10} is "good".

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: NOEC = 562 mg prod./kg dws

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Data Point:	KCP 10.4.2.1/02
Report Author:	[redacted]
Report Year:	2016
Report Title:	Fluopyram + trifloxystrobin SC 500 (250+250) G: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 4844-4
Document No:	M-548820-01-1
Guideline(s) followed in study:	OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil; US EPA OCSPP: not applicable
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) Deviations: none. 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

Executive Summary

In a laboratory study the effects of FLU + TFS SC 500 (250+250 g/L) on survival and reproduction of the soil mite species *Hypoaspis aculeifer* was tested during 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 all validity 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 ≥1000 mg prod/kg dws. The Lowest-Observed-Effect-Concentration (LOEC) for mortality is >1000 mg prod./kg dws.

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, α = 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

Test item: FLU + TFS SC 500 (250+250 g/L); specification no.: 102000012886; Batch No.: PAIS005173; REG40032-00; analytical findings: 21.1 % w/w fluopyram equivalent to 246.2 g/L, 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 *Hypoaspis aculeifer* were fed with nematodes bred on watered oat flakes. 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, 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 a fixing solution (20 % ethylene glycol, 80 % deionised water, 2 g detergent/L fixing solution were added). All *Hypoaspis aculeifer* were counted under a binocular.

Climatic conditions: The climatic conditions were in the temperature range 20.0 ± 2 °C with a photoperiod of 16 hours light and a light intensity of 400 - 800 lux.

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 applied to reproduction data as well as Probit analysis for the $EC_{0,20}$ calculation.

Dates of work: November 25, 2015 – December 21, 2015

II. RESULTS AND DISCUSSION

Table 10.4.2.1- 3: Effects on mortality and reproduction of *Hypoaspis aculeifer* after treatment with FLU + TFS SC 500 (250+250 g/L)

Test concentration [mg prod./dry weight artificial soil]	Adult mortality [%]	Significance (*)	Mean number of juveniles per test vessel ± SD		Reproduction [% of control]	Significance (**)
Control	1.3	-	265.6 ±	21.2	-	-
100	2.5	-	320.8 ±	27.5	120.8	-
178	2.5	-	319.5 ±	13.1	120.3	-
316	2.5	-	267.8 ±	14.8	100.8	-
562	7.5	-	214.8 ±	22.0	80.8	+
1000	2.5	-	186.0 ±	30.1	70.0	+

Endpoints	Adult mortality	Reproduction
NOEC [mg prod./kg dry weight artificial soil]	≥1000	316
LOEC [mg prod./kg dry weight artificial soil]	>1000	562
EC_{10} [mg prod./kg dry weight artificial soil] [1)] (95% confidence limits)		457 (25 - 645)
EC_{20} [mg prod./kg dry weight artificial soil] [1)] (95% confidence limits)		702 (275 - 998)

Calculations were done with unrounded values.
SD: Standard deviation
1) EC_x = Probit analysis
* Fisher's exact Binominal Test with Bonferroni Correction, one-sided greater, α = 0.05, "-": non-significant; "+": significant
** Williams t-test, one-sided smaller, α = 0.05, "-": non-significant; "+" significant

Mortality:

In the control group 1.3 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Concerning the mortality of the adult test organisms statistical analysis (Fisher´s Exact Binomial Test with Bonferroni Correction, one-sided greater, α = 0.05) revealed no significant difference between control and any treatment group.

Therefore the NOEC for mortality is ≥1000 mg prod./kg dry weight artificial soil. The LOEC for mortality is >1000 mg prod./kg dry weight artificial soil.

Reproduction:

Concerning the number of juveniles statistical analysis (William's t-test, one-sided smaller, α = 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.

Validity criteria:

Validity criteria for the untreated control of the study according OECD 226 from July 29, 2016 were used.

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)	Required	Obtained
Mean adult mortality	≤ 20 %	1.3 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	265.6
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	8.0 %

Reference test:

The most recent non-GLP-test (LAR/HR-O-21/15) with the reference item dimethoate was performed at test concentrations of 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 1.9 mg a.s./kg 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 artificial 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-sided smaller was used. Dimethoate EC 400 G showed an EC_{50} 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.

This is in the recommended range of the guideline, indicating that an EC_{50} based on the number of juveniles of 3.0 – 7.0 mg a.s./kg dry weight artificial soil shows that the test organisms are sufficiently sensitive.

III. CONCLUSION

All validity criteria were met. The endpoints were:

$NOEC_{adult\ mortality}$: ≥ 1000 mg prod./kg dry weight artificial soil

$LOEC_{adult\ mortality}$: > 1000 mg prod./kg dry weight artificial soil

$NOEC_{reproduction}$: 316 mg prod./kg dry weight artificial soil

$LOEC_{reproduction}$: 562 mg prod./kg dry weight artificial soil

$EC_{10\ reproduction}$: 457 mg prod./kg dry weight artificial soil

$EC_{20\ reproduction}$: 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”.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: $NOEC_{reproduction}$ = 316 mg prod./kg dws

CP 10.4.2.2 Higher tier testing

In view of the results presented in Section CP 10.4.2, no further testing is necessary.

CP 10.5 Effects on soil nitrogen transformation

Table 10.5- 1: Studies on nitrogen transformation with FLU+TFS SC 500, Fluopyram and its metabolites

Test substance	Test species, test design	Ecotoxicological endpoint		Reference
N-transformation				
FLU+TFS SC 500	Study duration 56 d	No unacceptable effects at an appl. rate of:	12.53 mg prod./kg dws [A]	[redacted] (2010) M-295282-01-1 KCP 10.5/01
Fluopyram	Study duration 28 d	No unacceptable effects at an appl. rate of:	**3.33 mg a.s./kg dws**	[redacted] (2006) M-281177-01-1 KCA 8.5/01
Fluopyram-7-hydroxy	Study duration 28 d	No unacceptable effects at an appl. rate of:	**10 mg p.m./kg dws**	[redacted] (2020) M-754927-01-1 KCA 8.5/03
Trifluoroacetic acid (TFA)	Study duration 28 d	No unacceptable effects at an appl. rate of:	1.60 mg p.m./kg dws (Na-TFA) **1.344 mg p.m./kg dws (TFA)** [B]	[redacted] (2013) M-444423-01-1 KCA 8.5/04

Bold values used in risk assessment
dws = dry weight soil, prod. = product
[A] Based on the endpoint 10.67 µL prod./kg dws and a product density of 1.174 g/mL
[B] As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

Risk assessment for Soil Nitrogen Transformation

The exposure of soil microorganisms 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} 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 a revised risk assessment latest by end of March 2022.

Table 10.5- 2: Risk Assessment for FLU + TFS SC 500 for nitrogen transformation

Compound	Species	Endpoint [mg prod./kg]	$PEC_{soil, max}$ [mg prod./kg]	Refinement required
Grapes, 2 × 0.2 L prod./ha				
FLU +TFS SC 500	Soil micro-organisms	12.53	0.249	No

Table 10.5- 3: Risk Assessment for Fluopyram and its metabolites for nitrogen transformation

Compound	Species	Endpoint [mg/kg]	$PEC_{soil, max}$ [mg/kg]	Refinement required
Grapes, 2 × 50 g a.s./ha				
Fluopyram	Soil micro-organisms	3.33	0.238	No
Fluopyram-7-hydroxy	Soil micro-organisms	10	0.002	No [A]
Trifluoroacetic acid (TFA)	Soil micro-organisms	1.344	0.002	No

[A] For the metabolite fluopyram-7-hydroxy the assessment conclusion relies on an uncertainty factor of 2 in consideration of two enantiomers.

According to regulatory requirements, the risk is acceptable if the effect on nitrogen transformation at the maximum PEC_{soil} values is < 25% after 28 days. In no case, deviations from the control exceeded 25% at concentrations which are clearly higher than the PEC_{soil}, indicating low risk to soil micro-organisms.

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Study summaries

Data Point:	KCP 10.5/01
Report Author:	[redacted]
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
Document No:	M-295282-01-1
Guideline(s) followed in study:	OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Microorganisms: Nitrogen Transformation Test, Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) Deviations: At day 42 the soil 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 recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effect of FLU + TFS SC 500 (250+250 g/L) on 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 1.07 and 10.67 µL test item/kg dry weight soil (equivalent to 0.8 and 8 L test item/ha) were tested. Per treatment there were 3 replicates. The soil was enriched by 0.5 % lucerne meal and a water content of 40 - 50 % of the water holding capacity was maintained during the test.

The study fulfilled all validity criteria of OECD 216 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 g/L) (10.67 µL test 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. MATERIALS AND METHODS

Test item: FLU + TFS SC 500 (250+250 g/L), Specification No.: 102000012886; Sample description: TOX07851-00; batch no.: 2007-000441; analytical content: 21.4 % w/w (251.5 g/L) fluopyram, 21.6 % w/w (253.5 g/L) trifloxystrobin; density 1.174 g/mL.

Test design: A loamy sand soil with pH 6.7, 0.94 % C_{org} and with the water holding capacity of 43.27 g/100 g dry soil was exposed for 56 days to 1.07 µL and 10.67 µL 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/cm^3 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 NH_4-N-, NO_3-N- and NO_2-N-contents were determined. NH_4-nitrogen, NO_3- and NO_2-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 soil ranged between 40 - 60 % of WHC. The pH value of the soil ranged between 5.83 and 6.14.

Statistics: Homogeneity of variances was determined by Cochran's Test, $\alpha = 0.05$. Depending on the results the appropriate T-tests were performed. In the T-test, the values of nitrate-N/kg dry weight soil/time interval/day from control soils and treated soils were compared. The statistical calculations were carried out using ToxRatPro 2.09.

Dates of work: August 21, 2007 – October 17, 2007

II. RESULTS AND DISCUSSION

Validity criteria:

According to OECD guideline 216 (2000), the variation of the nitrate-concentrations between control replicates should be less than ± 15 %. In this study, a maximum coefficient of variation of 7 % was obtained. Therefore, the results of the study are considered valid.

Observations:

Table 10.5- 4: Effects on nitrogen transformation/ time interval/ day in soil after treatment with FLU + TFS SC 500 (250+250 g/L)

Time interval [days]	Control			1.07 µL prod./kg soil dry weight equivalent to 0.8 L prod./ha				10.67 µL prod./kg soil dry weight equivalent to 8 L prod./ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0 - 7	- 2.06	±	0.11	- 1.64	±	0.29	20 n s	- 1.15	±	0.22	44*
7 - 14	0.63	±	0.04	0.55	±	0.48	12 n w	0.60	±	0.14	4 n w
14 - 28	1.04	±	0.03	1.14	±	0.14	9 n s	1.32	±	0.13	26*
28 - 42	1.35	±	0.10	1.36	±	0.08	1*	1.70	±	0.07	26 n s
42 - 56	0.67	±	0.14	0.59	±	0.04	12 n s	0.53	±	0.09	21 n s

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. No statistically significant difference to the control (Student-t Test, two-sided, $\alpha = 0.05$)
n.w. No statistically significant difference to the control (Welch-t Test, two-sided, $\alpha = 0.05$)
* Statistically significant difference to the control (Student-t Test, two-sided, $\alpha = 0.05$).

During the 56-day test, 1.07 µL FLU + TFS SC 500 (250+250 g/L) had no relevant influence on nitrogen transformation in a soil amended 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-term (> 28 days) influence 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 soil, which are equivalent to application rates up to 8 L prod./ha.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: 8 L prod./ha, corresponding to 12.53 mg prod./kg dws.

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 vigour 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 > 50% were observed in any species tested. The only exception is buckwheat (see Table 10.6- 19 in the vegetative vigour limit test (M-681185-01-1) at 1.0 L prod./ha. For this species a dry weight reduction of 52% was measured. A follow-up dose-response study was conducted (M-688437-01-1) showing an ER_{50} of > 2 L prod./ha for buckwheat. Thus, the relevant ER_{50} for the risk assessment of all species is set as > 1 L prod./ha.

Table 10.6- 1: Effect values relevant for the risk assessment for non-target terrestrial plants for FLU+TFS SC 500

Test organism	Study type	Endpoint	References
Beta vulgaris [d] *Brassica napus* [d] *Cucumis sativus* [d] *Fagopyrum esculentum* [d] *Glycine max* [d] *Helianthus annuus* [d] *Lycopersicon esculentum* [d] *Allium cepa* [m] *Avena sativa* [m] *Zea mays* [m]	Vegetative vigour; Tier 1, single dose, 21 days	ER_{50} > 0.75 L prod./ha (all species)	[redacted] (2007) M-289527-01-1 KCP 10.6.2/03
Beta vulgaris [d] *Brassica napus* [d] *Cucumis sativus* [d] *Fagopyrum esculentum* [d] *Glycine max* [d] *Helianthus annuus* [d] *Lycopersicon esculentum* [d] *Avena sativa* [m] *Lolium perenne* [m] *Zea mays* [m]	Vegetative vigour; Tier 1, double dose, 21 days	ER_{50} > 2 x 0.8 L prod./ha (all species)	[redacted] (2013) M-464310-01-1 KCP 10.6.2/04
Brassica napus [d] *Glycine max* [d] *Fagopyrum esculentum* [d] *Zea mays* [m] *Lolium perenne* [m] *Avena sativa* [m] *Allium cepa* [m]	Vegetative vigour; Tier 1, single dose, 21 days	ER_{50} > 1.0 L prod./ha (all species except *Fagopyrum esculentum*) ER_{50} < 1.0 L prod./ha (*Fagopyrum esculentum*: 52% reduction of shoot dry weight)	[redacted] (2020) M-681185-01-1 KCP 10.6.2/05
Beta vulgaris [d] *Cucumis sativus* [d] *Solanum lycopersicum* [d]	Vegetative vigour; Tier 2, dose response, 21 days	ER_{50} > 2.0 L prod./ha (all species)	[redacted] (2018) M-612774-01-1 KCP 10.6.2/06
Fagopyrum esculentum [d]	Vegetative vigour; Tier 2, dose response, 21 days	ER_{50} > 2.0 L prod./ha	[redacted] (2020) M-688437-01-1 KCP 10.6.2/07
Beta vulgaris [d] *Brassica napus* [d] *Cucumis sativus* [d] *Fagopyrum esculentum* [d] *Glycine max* [d] *Helianthus annuus* [d] *Lycopersicon esculentum* [d] *Avena sativa* [m] *Lolium perenne* [m] *Zea mays* [m]	Seedling emergence; Tier 1, single dose, 14 days	ER_{50} > 0.75 L prod./ha (all species)	[redacted] (2007) M-289525-01-1 KCP 10.6.2/01
Beta vulgaris [d] *Brassica napus* [d] *Cucumis sativus* [d] *Fagopyrum esculentum* [d] *Glycine max* [d] *Helianthus annuus* [d] *Allium cepa* [m] *Avena sativa* [m] *Lolium perenne* [m] *Zea mays* [m]	Seedling emergence; Tier 1, single dose, 21 days	ER_{50} > 1.0 L prod./ha (all species)	[redacted] (2020) M-681165-01-1 KCP 10.6.2/02

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 non-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./ha does not result in effects > 50 % according to the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev. 2 final, 2002), no risk for non-target terrestrial plants is expected. The limit test rate is higher than the highest field application rate and is thus considered as indicator for an acceptable risk.

A detailed deterministic risk assessment is in addition presented below. Greenhouse uses are not relevant for exposure calculations.

Deterministic risk assessment

Table 10.6- 2: Deterministic assessment of the risk for non-target plants due to the use of FLU+TFS SC 500 in grape

Intended use	Grape, 2 × 0.2 L prod./ha, BBCH 53-73			
product	FLU+TFS SC 500			
Application rate (L prod./ha)	2 × 0.2			
MAF	1.0 Justification: At the recent Pesticide Peer Review Meeting 133** in Sept. 2015 "it was agreed that for the risk assessment of active substances, no MAF values should be used by default, until a guidance document is developed." This approach is in line with the "Guidance Document on Terrestrial Ecotoxicology" currently in use which does not require the use of a MAF value in the context of NTTP risk assessment. Thus, it is not deemed necessary to apply a MAF when calculating the PER.			
Test species	**ER_{50} (L prod./ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L prod./ha)**	**TER criterion: TER ≥ 5***
All species -seedling emergence	>1	7.23	0.014	>69.2
All species -vegetative vigour	>1	7.23	0.014	>69.2

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

* TER ≥ 5 for deterministic risk assessment based on ER_{50}

** EFSA (European Food Safety Authority), 2015. Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. 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 available (see Point 10.6.2 in this MCP Summary).

CP 10.6.2 Testing on non-target plants

Data Point:	KCP 10.6.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Non-target terrestrial plants: an evaluation of the effects of AE C656948 + trifloxystrobin SC 250 + 250 g/L in the seedling emergence and growth test (Tier 1)
Report No:	SE07/03
Document No:	M-289525-01-1
Guideline(s) followed in study:	OECD 208 (July 2006): seedling emergence and growth test (Tier 1); Equivalent to US EPA OPPTS Guideline No. 850.4100
Deviations from current test guideline:	Current Guideline: OECD 208 (2006) Deviations: Temporary deviation from climate condition (temperature). Light intensity and humidity were not reported. Deviation from recommended plant density. All validity criteria were met. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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 dicotyledonous and 3 monocotyledonous species from 8 plant families were tested in this seedling emergence and growth 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), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn). 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 0.75 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 criteria 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 prod./ha. Therefore the ER_{50} (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 beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Lycopersicon esculentum* (tomato), *Avena 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 readily cultivated test organisms 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 item 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 0.75 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 sprayed with 200 L/ha of deionized 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 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 biomass endpoint determinations were performed for plant dry weights. Visual phytotoxicity were 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.

Climatic conditions: Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. Light intensity was not reported. The temperature was regulated to maintain 10 to 31 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was not recorded.

Statistics: Statistical analysis of the dry weight was carried out using the Pairwise Mann-Whitney-U-test (one sided smaller).

Dates of work: March 21, 2007 – April 17, 2007

II. Results and Discussion

Validity criteria:

The validity criteria of OECD 208 were fulfilled.

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 mean 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 15.2 % respectively. Biomass was increased in cucumber, soybean, sugar beet, sunflower, tomato corn and oat 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 differences reached or exceeded 50 % to trigger further testing.).

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- 1: Summary of phytotoxicity and growth stages (BBCH exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day 14

Plant species	Phytotoxicity		BBCH growth stages [Min - Max]	
	Control	0.75 L product/ha	Control	Test item 0.75 L product/ha
Beta vulgaris	0	0	12 - 14	12 - 14
Brassica napus	0	0	12 - 14	12 - 14
Cucumis sativus	0	0	12 - 14	12 - 14
Fagopyrum esculentum	0	0	12 - 14	12 - 14
Glycine max	0	0	12 - 14	12 - 14
Helianthus annuus	0	0	12 - 14	12 - 14
Lycopersicon esculentum	0	0	10 - 12	12
Avena sativa	0	0	12 - 14	12 - 14
Lolium perenne	0	0	12 - 14	12 - 14
Zea mays	0	0	13	13

Phytotoxicity was recorded at each assessment time with the following a rating system:
0: no injury or effect
A: slight symptom (s)
B: moderate symptom (s)
C: severe symptom (s)
D: total-plant symptom (s)
E: moribund
Codes for visual injuries:
a: chlorosis (yellowing of green shoot tissue)
b: necrosis (brown shoot tissue)
c: bleaching (shoot tissue without pigmentation)
d: wilting (loss of turgor of shoot tissue)
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

Plant species	Germination			Survival	
	Control	0.75 L prod./ha		Control	0.75 L prod./ha
	% of sown	% of sown	% inhibition	% of sown	% of sown
Beta vulgaris	90	100	11.1	100	100
Brassica napus	90	80	11.1	100	100
Cucumis sativus	95	95	5.6	100	100
Fagopyrum esculentum	75	90	20	100	100
Glycine max	100	100	0	100	100
Helianthus annuus	100	90	10	100	100
Lycopersicon esculentum	85	90	5.9	100	100
Avena sativa	85	95	11.8	100	100
Lolium perenne	90	100	11.1	90	100
Zea mays	100	90	10	100	100

Table 10.6.2- 3: Summary of shoot dry weight following exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day 14

Plant species	Shoot dry weight		
	Control	0.75 L prod./ha	
	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Beta vulgaris	0.124	0.136	- 9.7
Brassica napus	0.278	0.316	- 14.8
Cucumis sativus	0.278	0.316	- 13.5
Fagopyrum esculentum	0.248	0.217	12.5
Glycine max	0.294	0.343	- 17.0
Helianthus annuus	0.125	0.166	- 32.5
Lycopersicon esculentum	0.099	0.1161	- 16.4
Avena sativa	0.145	0.170	- 17.2
Lolium perenne	0.024	0.020	15.2
Zea mays	0.589	0.648	-10

[A] Negative figures indicate that there was an increase in biomass (dry weight) when compared to the untreated control.

III. CONCLUSION

In this 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, dry weight and phytotoxicity of ten non-target terrestrial 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 ER_{50} was determined to be > 0.75 L prod./ha.

Assessment and conclusion by applicant:

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

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Data Point:	KCP 10.6.2/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram + trifloxystrobin SC 500 (250+250 g/L): Effects on the seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions (tier I)
Report No:	S19-22935
Document No:	M-681165-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) no. 1107/2009 US EPA OCSPP 850.4100 (2012) OECD 208 (2006)
Deviations from current test guideline:	Current Guideline: OECD 208 (2006) Deviations: Temporary deviation from climate condition (light). All validity criteria were met. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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 monocotyledonous species from 8 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn). Planting density included 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/ha 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. 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, visual phytotoxicity, plant growth stage and shoot high and shoot dry weight 21 days post emergence of 50 % of the control seedlings.

The study fulfils all validity criteria of OECD 208 guideline.

Visual phytotoxicity 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

Test item: 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: 1.168 g/mL).

Test design: A total of ten species, 6 dicotyledonous and 4 monocotyledonous species from 8 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Avena 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 readily 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. 1.5 kg soil/pot). The used soil was a loamy sand.

Planting density included 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/ha and a water control. The test 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 variabiltiy in growth conditions.

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. 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, visual 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 colour (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 visual phytotoxicity.

Analysis of the test item solution and the control solution were conducted by LC – UV.

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 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 humidity was regulated to maintain 51 to 88 %.

Statistics: 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 homoscedasticity 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 α = 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.

Dates of work: 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, wilting, leaf and stem deformations) and control plants exhibited normal variation in growth and morphology for that particular species. The mean survival of emerged control seedlings 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 nominal concentration.

Biological findings:

Visual phytotoxicity observed at the final assessment (on day 21 after application) in this seedling emergence and growth study included chlorosis, necrosis, deformation and stunting of the plants. Visual phytotoxicity occurred only in *Fagopyrum esculentum* with a mean effect of 3 % compared to the control group.

No statistically significant effects on the parameter seedling emergence were observed for any of the plant species tested. The highest inhibition occurred for *Lolium perenne* with 5.6 % compared to the control group.

No post-emergence mortality 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 shoot height compared to the control group were observed for *Brassica napus* (11.5 %), *Glycine max* (14.3 %) and *Allium cepa* (19.7 %).

Statistically significant differences in shoot dry weight compared to the control group were observed for *Allium cepa* (45.5 %) 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 50 % emergence of the control seedlings).

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

Plant species	BBCH growth stage [Min - Max]		Phytotoxicity: mean % / Symptoms [A]	
	Control	1.0 L prod./ha	Control	1.0 L prod./ha
Beta vulgaris	13 - 13	13 - 13	0 / -	0 / -
Brassica napus	13 - 13	13 - 13	0 / -	0 / -
Cucumis sativus	12 - 12	12 - 12	0 / -	0 / -
Fagopyrum esculentum	51 - 51	51 - 51	0 / -	3 / CC, DE
Glycine max	21 - 21	21 - 21	0 / -	0 / -
Helianthus annuus	16 - 16	16 - 16	0 / -	0 / -
Allium cepa	12 - 12	12 - 12	0 / -	0 / -
Avena sativa	14 - 14	14 - 14	0 / -	0 / -
Lolium perenne	21 - 21	21 - 21	0 / -	0 / -
Zea mays	15 - 15	15 - 15	0 / -	0 / -

[A] Visual symptoms: None (--), CC = change in colour, DE = deformation

Table 10.6.2- 5: Summary of shoot height and shoot dry weight and corresponding percent inhibitions following exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day 21

Plant species	Shoot height [cm]			Shoot dry weight		
	Control	1.0 L prod./ha		Control	1.0 L prod./ha	
		Mean	% inhibition [A]	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Beta vulgaris	13.4	12.5	6.7	0.338	0.302	10.7
Brassica napus	13.0	11.5	11.5*	0.647	0.595	11.7
Cucumis sativus	14.7	15.1	- 2.7	0.674	0.409	12.4
Fagopyrum esculentum	36.3	35.9	1.1	0.582	0.712	- 22.3
Glycine max	18.9	16.2	14.3*	0.845	0.826	2.2
Helianthus annuus	6.7	6.8	-1.5	0.504	0.541	- 7.3
Allium cepa	17.3	13.9	19.7*	0.033	0.018	45.5*
Avena sativa	41.3	42.9	-3.9	0.289	0.261	9.7
Lolium perenne	32.4	29.7	7.5	0.112	0.070	37.5*
Zea mays	49.2	46.4	5.7	0.650	0.586	9.8

* Statistically significantly different compared to the control (Student's t-test; one-sided smaller, α = 0.05)

[A] 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

Plant species	Seedling emergence			Cumulative mortality	
	Control	1.0 L prod./ha		Control	1.0 L prod./ha
	% emerged	% emerged	% Inhibition[A]	% mortality	% mortality
Beta vulgaris	95	100	- 5.3	0	0
Brassica napus	95	100	- 5.3	0	0
Cucumis sativus	90	100	- 11.1	0	0
Fagopyrum esculentum	100	100	0.0	0	0
Glycine max	80	80	0.0	0	0
Helianthus annuus	85	95	- 11.8	0	0
Allium cepa	90	100	- 11.1	0	0
Avena sativa	85	100	- 17.6	0	0
Lolium perenne	90	85	5.6	0	0
Zea mays	80	100	- 11.1	0	0

[A] Negative values indicate that there was an increase compared to the control

III. CONCLUSION

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 pre-emergence application of the product to the soil surface. No adverse effects on emergence, post-emergence mortality, shoot height and shoot dry weight above the 50 % effect level occurred. Therefore the ER_{50} 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 for use in risk assessment.

The endpoint is: ER_{50} > 1.0 L prod./ha

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Data Point:	KCP 10.6.2/03
Report Author:	[redacted]
Report Year:	2007
Report Title:	Non-target terrestrial plants: an evaluation of the effects of AE C656948 + Trifloxystrobin SC 250 + 250 g/L in the vegetative vigour test (Tier 1)
Report No:	VV07/03
Document No:	M-289527-01-1
Guideline(s) followed in study:	OECD 227 (July 200): vegetative vigour test (Tier 1); Equivalent to US EPA OPPTS Guideline No. 850.4150
Deviations from current test guideline:	Current Guideline: OECD 227 (2006) Deviations: Temporary deviation from climate condition (temperature during night period). Light intensity and humidity was not reported. All validity criteria were 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 above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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 a limit 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: *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). Planting density included 4 or 5 plants per pot with 4 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 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 OECD 227 guideline. 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 ten species tested. No adverse effects (i.e. greater than 50 %) were observed in all species tested in the vegetative vigour test Therefore the ER_{50} was determined to be > 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 (2253.5 g/L); density: 1.174 g/mL

Test design: 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 viability. 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 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 a single application rate of 0.75 L product/ha and a water control. The test solution was 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.

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 plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. The light intensity was not recorded. The temperature was 10 to 31 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was recorded.

<u>Statistics</u>: Statistical analysis was carried out using the Pairwise Mann-Whitney-U-test (one sided smaller).

<u>Dates of work</u>: April 12, 2007 – May 14, 2007

II. Results and Discussion

<u>Validity criteria:</u>

The validity criteria of OECD 227 were fulfilled.

All plant species in this study 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 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.

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.

<u>Biological findings:</u>

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 exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day 21

Plant species	Survival		Shoot dry weight		
	Control	0.75 L prod./ha	Control	0.75 L prod./ha	
	% of survival	% of survival	Mean dry weight [g]	Mean dry weight [g]	% Inhibition[A]
Beta vulgaris	100	100	2.389	1.641	31.3
Brassica napus	100	100	3.870	3.383	12.6
Cucumis sativus	100	100	4.551	3.627	20.3
Fagopyrum esculentum	100	100	2.673	3.498	- 30.9
Glycine max	100	100	2.953	3.089	-4.6
Helianthus annuus	100	100	3.790	3.780	0.3
Lycopersicon esculentum	100	100	1.663	1.289	22.5
Avena sativa	100	100	1.434	1.320	7.9
Allium cepa	100	100	0.045	0.097	- 117.5
Zea mays	100	100	4.945	5.475	- 10.7

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- 8: Summary of phytotoxicity and growth stages (BBCH) following exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day 21

Plant species	Phytotoxicity [A]		BBCH growth stages	
	Control	0.75 L prod./ha	Control	0.75 L prod./ha
Beta vulgaris	0	0	12 - 14	12 - 14
Brassica napus	0	0	12 - 14	12 - 14
Cucumis sativus	0	0	12 - 14	12 - 14
Fagopyrum esculentum	0	0	12 - 14	12 - 14
Glycine max	0	0	12 - 14	12 - 14
Helianthus annuus	0	0	12 - 14	12 - 14
Lycopersicon esculentum	0	0	12 - 14	12 - 14
Allium cepa	0	0	12 - 14	12 - 14
Avena sativa	0	0	12 - 14	12 - 14
Allium cepa	0	0	12 - 14	12 - 14
Zea mays	0	0	12 - 14	12 - 14

[A] Phytotoxicity: 0: no phytotoxicity or effect

Phytotoxicity was recorded at each assessment time with the following a rating system:
0: no injury or effect
A: slight symptom (s)
B: moderate symptom (s)

C: severe symptom (s)
D: total-plant symptom (s)
E: moribund
Codes for visual injuries:
a: chlorosis (yellowing of green shoot tissue)
b: necrosis (brown shoot tissue)
c: bleaching (shoot tissue without pigmentation)
d: wilting (loss of turgor of shoot tissue)
e: leaf deformation (leaf curl, abnormal leaf shape)
f : stunting (plant height reduced with shorter inter-node lengths)

III. Conclusion

As a result of this vegetative vigour and growth study, in which the effects of FLU + TFS SC 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 on the survival and dry weight of the 10 species were determined compared to the control. Therefore the ER_{50} was determined to be > 0.75 L prod./ha.

Assessment and conclusion by applicant:

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

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Data Point:	KCP 10.6.2/04
Report Author:	[redacted]
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:	M-464310-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP 850.4150; OECD 227 guideline for the testing of chemicals, Terrestrial Plant Test: Vegetative vigour (July 2006) and considers the recommendations of US EPA Ecological Effects Test Guideline OCSPP 850.4150
Deviations from current test guideline:	Current Guideline: OECD 227 (2006) 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 lux (referring to day light spectrum 15000 lux result in 345 µmol/s/m2). This had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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 leaf stage. 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). 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 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 OECD 227 guideline. 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

No adverse effects on survival, visual 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 x 0.8 L prod./ha.

I. Material and Methods

Test item: FLU + TFS SC 500 (250 + 250); specification no.: 102000012886 - 03; batch ID.: EM4L011075; Sample description: TOX 10053-00; active substance (analysed content): fluopyram: 20.7 % w/w (241.7 g/L), trifloxystrobin: 21.1 % w/w (246.5 g/L); density: 1.167 g/mL

Test design: 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 × 0.8 L 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 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 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 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 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 (16 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; $p \leq 0.05$), included in ToxRat statistics (ToxRatPro version 2.10).

<u>Dates of work</u>: May 22, 2013 – June 25, 2013

II. Results and Discussion

<u>Validity criteria:</u>

The validity criteria of OECD 227 were fulfilled.

All plant species in this study 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 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.

<u>Analytical results:</u>

The analysis of trifloxystrobin 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 esculentum* that was stunting. For *Cucumis sativus, Fagopyrum esculentum and Glycine max,* there was mostly 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*, *Brassica napus, Cucumis sativus, Glycine max, Lycopersicon esculentum, Avena sativa* and *Zea 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 differences was statistically significantly or biologically meaningful.

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- 9: Summary of survival and shoot dry weight following exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day 21

Plant species	Survival					Shoot dry weight		
	Control		2 × 0.8 L prod./ha			Control	2 × 0.8 L prod./ha	
	No. plants	% Survival	No. plants	% Survival	% inhibition	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Beta vulgaris	20	100	20	100	0.0	2.637	2.747	- 4.2
Brassica napus	20	100	20	100	0.0	5.127	5.138	- 0.2
Cucumis sativus	20	100	20	100	0.0	7.912	8.005	- 1.2
Fagopyrum esculentum	20	100	20	100	0.0	5.341	4.545	14.9
Glycine max	20	100	20	100	0.0	7.173	7.198	- 0.3
Helianthus annuus	20	100	20	100	0.0	3.757	3.607	4.0
Lycopersicon esculentum	20	100	20	100	0.0	3.942	4.328	- 9.8
Avena sativa	20	100	20	100	0.0	3.595	3.786	- 5.3
Lolium perenne	20	100	20	100	0.0	1.714	1.651	3.7
Zea mays	20	100	20	100	0.0	8.303	8.351	-0.6

Statistical significance **-:** Inhibition is statistically not significant (Pairwise Mann-Whitney-U-test, one sided smaller; $p \leq 0.05$).
[A]: A negative inhibition indicates an increase of the shoot 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 + TFS SC 500 (250+250 g/L) at the final assessment on day 21

Plant species	Phytotoxicity		BBCH growth stages	
	Control	2 × 0.8 L prod./ha	Control	2 × 0.8 L prod./ha
Beta vulgaris	0	0	18 - 19	18 - 19
Brassica napus	0	0-A/e	17 - 30	15 - 30
Cucumis sativus	0	A-B/ab	69	69
Fagopyrum esculentum	0	A-B/be	65	65
Glycine max	0	A/abe	51 - 59	51 - 59
Helianthus annuus	0	0	53 - 55	51 - 55
Lycopersicon esculentum	0	0-A/e	61 - 62	61 - 62
Avena sativa	0	0	31 - 32	31 - 32
Lolium perenne	0	0	29	27 - 29
Zea mays	0	0	30 - 31	30 - 31

Phytotoxicity was recorded at each assessment time with the following a rating system:

0: no injury or effect
A: slight symptom (s)
B: moderate symptom (s)
C: severe symptom (s)
D: total-plant symptom (s)
E: moribund

Codes for visual injuries:

a: chlorosis (yellowing of green shoot tissue)
b: necrosis (brown shoot tissue)
c: bleaching (shoot tissue without pigmentation)
d: leaf deformation (leaf curl, abnormal leaf shape)
e : stunting (plant height reduced with shorter inter-node lengths)

III. CONCLUSION

As a result of this vegetative vigour and growth study, in which the effects of FLU + TFS SC 500 (250+250 g/L) on ten non-target 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 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 x 0.8 L prod./ha.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: ER_{50} > 2 x 0.8 L prod./ha

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Data Point:	KCP 10.6.2/05
Report Author:	[redacted]
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:	S19-22936
Document No:	M-681185-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) no. 1107/2009 US EPA OCSPP 850.4150 (2012) OECD 227 (2006)
Deviations from current test guideline:	Current Guideline: OECD 227 (2006) Deviations: Temporary deviation from climate condition (light) All validity criteria were met. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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 seven non-target terrestrial plant species in a limit test, following a post-emergence application of the product onto the foliage of plants at the 2 - 3 leaf stage. 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). 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 injuries were made on day 7, 14 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.

The study fulfils all validity 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 ER_{50} for all species (based on survival, growth stage development and shoot dry weight) was set to be > 1.0 L prod./ha.

I. Material and Methods

Test item: 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

Test design: 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 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. 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 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 necrosis, deformation and change in colour (e.g. chlorosis, bleaching, reddening). The ratings 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 LC – UV.

<u>Climatic conditions</u>: Following application, the pots with plants were maintained under greenhouse conditions and an independent set of LED lamps above each cultivation table ensured an appropriate exposure to light. The light intensity was in the range of 240 - 330 µmol/m²/s. The temperature was 15 to 27 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was 54 to 88 %.

<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 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 in 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 ToxRat Professional Version 3.3.0.

<u>Dates of work</u>: November 19, 2019 – December 16, 2019

II. Results and Discussion

<u>Validity criteria:</u>

The validity criteria of OECD 227 were fulfilled.

All plant species in this study 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 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 ≥ 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 to 102.8 % of the nominal concentration.

Biological findings:

Visual phytotoxicity observed at the final assessment (on day 21 after application) in this vegetative vigour study included chlorosis, necrosis, deformation and stunting of the plants. Visual 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 esculentum* (31.4 %) and shoot dry weight for the plant species *Fagopyrum esculentum* (52.0 %) and *Avena sativa* (23.4 %) at the single application rate of 1.0 L prod./ha.

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).

Table 10.6.2- 11: Summary of shoot height and shoot dry weight following exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day 21

Plant species	Shoot height			Shoot dry weight		
	Control	1.0 L prod./ha		Control	1.0 L prod./ha	
	Mean [cm]	Mean [cm]	% Inhibition [A]	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Brassica napus	15.1	14.4	4.6	0.748	0.771	- 3.1
Fagopyrum esculentum	104.8	112.5	- 7.3	2.934	1.407	52.0 *
Glycine max	50.6	34.7	31.4*	2.905	2.692	7.3
Allium cepa	31.0	36.6	- 18.1	0.508	0.523	- 3.0
Avena sativa	60.7	58.8	3.1	0.997	0.764	23.4 *
Lolium perenne	42.3	43.8	- 3.5	0.863	0.801	7.2
Zea mays	55.8	57.9	- 3.8	1.937	1.916	1.1

[A] Negative figures indicate that there was an increase in biomass (dry weight) when compared to the untreated control.
* Statistically significantly different compared to the control (Student's t-test; one-sided smaller, α = 0.05)

Table 10.6.2- 12: Summary of phytotoxicity and growth stages (BBCH) following exposure to FLU + TFS SC 500 (250+250 g/L) at the final assessment on day 21

Plant species	Phytotoxicity mean % / Symptoms [A]		BBCH growth stages	
	Control	1.0 L prod./ha	Control	1.0 L prod./ha
Brassica napus	0	0 / -	15 / 15	15 / 15
Fagopyrum esculentum	0	10 / NE,CC	67 / 67	65 / 65
Glycine max	0	0 / -	62 / 62	62 / 62
Allium cepa	0	0 / -	15 / 15	15 / 15
Avena sativa	0	0 / -	55 / 55	55 / 55
Lolium 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 SC 500 (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 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 ER_{50} for all species (based on survival, growth stage development and shoot dry weight) was set to be > 1.0 L prod./ha.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: ER_{50} > 1.0 L prod./ha for all species tested, except *Fagopyrum esculentum* with an ER_{50} < 1.0 L prod./ha

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Data Point:	KCP 10.6.2/06
Report Author:	[redacted]
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:	M-612774-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP 850.4150 (2012) OECD 227 (2006)
Deviations from current test guideline:	Current Guideline: OECD 227 (2006) Deviations: Temporary deviation from climate condition (light). All validity criteria were met. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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 three non-target terrestrial plant species, following a post-emergence application of the product onto the foliage of plants at the 2 - 4 leaf stage. 3 dicotyledonous species from 3 plant families were tested in this vegetative vigour test: *Beta vulgaris* (sugar beet), *Cucumis sativus* (cucumber) and *Solanum lycopersicum* (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: 0.06; 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 all validity criteria of OECD 227 guideline.

No adverse effects on survival, visual phytotoxicity, 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.

I. MATERIAL AND METHODS

Test item: FLU + TFS SC 500 (250 + 250); specification no.: 102000012886; supplier batch no.: PAIS005241; Sample description: FAR30164-00; active substance (analysed content): fluopyram: 21.1 % w/w (246.7 g/L), trifloxystrobin: 21.6 % w/w (251.9 g/L); density: 1.167 g/mL.

Test design: 3 dicotyledonous species from 3 plant families were tested in this vegetative vigour test: *Beta vulgaris* (sugar beet), *Cucumis sativus* (cucumber) and *Solanum lycopersicum* (tomato). 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 15 cm plastic pots (filled with approx. 1.2 L soil). The used soil was a 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 tables 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 (e.g. chlorosis, necrosis, bleaching, deformation, reddening, stunting) was recorded from the living plants at each assessment date following a 0 - 90 % (no effect to moribund) rating system in 10 % 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 cycle (16 h) and during the dark cycle (8 h). The relative humidity was 55 to 85 %.

Statistics: Statistical analyses of the data were performed to obtain NOER (No Observed Effect Rate), LOER (Lowest Observed Effect Rate), ER_{25}/ER_{50} (Effect Rate producing 25/50 % effect) for survival, shoot length and shoot dry weight, using the ToxRat statistical software.

Dates of work: October 19, 2017 – November 20, 2017

II. Results and Discussion

Validity criteria:

The validity criteria of OECD 227 were fulfilled.

All plant species in this study 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 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 ≥ 70 %.

Analytical results:

The analysis of trifloxystrobin content in the initial product stock solution revealed measured concentrations of 101.6 % of nominal.

Biological findings:

Typical symptoms observed at the final assessment (on day 21 after application) in vegetative vigor testing include chlorosis, necrosis, deformation and stunting of the plants. Slight visual phytotoxicity was observed sporadically for *Cucumis sativus* (0.06 - 2.0 L product/ha) and *Solanum lycopersicum* (0.144 - 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).

Table 10.6.2- 13: Effects of FLU + TFS SC 500 (250+250 g/L) on survival

Survival				
Plant species	**ER_{25} [L product/ha]** (95 % CI)	**ER_{50} [L product/ha]** (95 % CI)	**LOER [L product/ha]**	**NOER [L product/ha]**
Beta vulgaris	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2	2
Cucumis sativus	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2	2
Solanum lycopersicum	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2	2

n.d.: Confidence limits not determined (outside the range tested)
[A]: No effects were observed up to the highest concentration tested.

Table 10.6.2- 14: Effects of FLU + TFS SC 500 (250+250 g/L) on shoot length

Shoot length				
Plant species	**IR_{25}* [L product/ha]** (95 % CI)	**IR_{50}* [L product/ha]** (95 % CI)	**LOER [L product/ha]**	**NOER [L product/ha]**
Beta vulgaris	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2	2
Cucumis sativus	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2[B]	2[B]
Solanum lycopersicum	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2	2

*: IR corresponds to ER.
n.d.: Confidence limits not determined (outside the range tested)
[A]: Not calculated (outside the range tested).
[B]: Calculated with Dunnett's multiple I-test procedure

Table 10.6.2- 15: Effects of FLU + TFS SC 500 (250+250 g/L) on shoot dry weight

Shoot dry weight				
Plant species	**IR_{25}* [L product/ha]** (95 % CI)	**IR_{50}* [L product/ha]** (95 % CI)	**LOER [L product/ha]**	**NOER [L product/ha]**
Beta vulgaris	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2	2
Cucumis sativus	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2	2
Solanum lycopersicum	> 2[A] (n.d.)	> 2[A] (n.d.)	> 2	2

*: IR corresponds to ER.
n.d.: Confidence limits 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 day 21

Plant species	Growth stage (BBCH) Min - Max [L product/ha]					
	Control	0.06	0.144	0.346	0.832	2
Beta vulgaris	19	19	18 - 19	19	19	19
Cucumis sativus	66 - 69	69	65 - 69	64 - 69	69	68 - 69
Solanum lycopersicum	51 - 52	51 - 52	51 - 52	51 - 52	51 - 52	51 - 52

Table 10.6.2- 17: Phytotoxicity summary at the final assessment on day 21

Plant species	Phytotoxicity summary [mean damage in %][A] [L product/ha]					
	Control	0.06	0.144	0.346	0.832	2
Beta vulgaris	0	0	0	0	0	0
Cucumis sativus	0	1.3 ab	3.1 abe	5.0 abe	9.4 abe	14.4 abe
Solanum lycopersicum	0	0	3.8 de	1.3 de	1.9 e	0

[A] Phytotoxic symptoms:
a: chlorosis (yellowing of green shoot tissue)
b: necrosis (e.g. brown shoot tissue, parts of the plant die)
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)

III. Conclusion

This vegetative vigour and growth study, in which the effects of FLU + TFS SC 500 (250+250 g/L) on three non-target terrestrial plant species were tested under greenhouse 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 ER_{50} (based on survival, shoot height and shoot dry weight) was determined to be > 2 L prod./ha.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: ER_{50} > 2.0 L prod./ha

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Data Point:	KCP 10.6.2/07
Report Author:	[redacted]
Report Year:	2020
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:	M-688437-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) no. 1107/2009 US EPA OCSPP 850.4150 (2012) OECD 227 (2006)
Deviations from current test guideline:	Current Guideline: OECD 227 (2006) Deviations: Temporary deviation from climate condition (humidity). All validity criteria were met. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

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 post-emergence application of the product to the above-ground portion of the plants at the 2 leaf stage. This plant species had shown relevant effects in a preceding limit test and is therefore investigated in this rate-response study. 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 with 5 product rates and a water control. The test concentrations were: 0.060; 0.144; 0.346; 0.832 and 2.0 L prod./ha. The application was done at a volume rate of 200 L/ha. Assessments for mortality and visual injuries were made on day 7, 14 and 21. Additionally, the BBCH growth stage, shoot height and shoot dry weight were determined for all treatment groups on day 21.

The study fulfils all validity criteria of OECD 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 NOER of 0.346 L prod./ha was determined 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 ER_{25} and ER_{50} (based on mortality, shoot height and shoot dry weight) were determined to be > 2.0 L prod./ha, respectively.

I. MATERIAL AND METHODS

Test item: 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).

Test design: *Fagopyrum esculentum* 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. 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 colour (e.g. chlorosis, bleaching, reddening). The ratings 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 HPLC – MS/MS.

Climatic conditions: 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 15 to 35 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was 31 to 72 %.

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 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 Chi² - test was conducted for the parameter shoot dry weight. Statistical analyses of shoot height and shoot dry weight also included the determination of effect rates (ER_{25} and ER_{50}) and their 95 % confidence limits by Probit analysis (based on mean values) using linear max. likelihood regression, where possible. Statistical analysis was performed using the program ToxRat Professional Version 3.3.0.

Dates of work: March 17, 2020 – April 09, 2020

II. Results and Discussion

Validity criteria:

The validity criteria of OECD 227 were fulfilled.

All plant species in this study 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 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 ≥ 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 (21 DAA).

Statistically significant reduction of the shoot height of *Fagopyrum esculentum* at the two highest application rates (0.832 and 2.0 L prod./ha) was observed.

Statistically significant reduction of the shoot dry weight of *Fagopyrum esculentum* at the highest application rate (2.0 L prod./ha) was observed.

The effects on shoot height, dry weight, phytotoxicity as well as plant growth stage are summarized for *Fagopyrum esculentum* in the following tables for the final assessment (on day 21 after application).

Table 10.6.2- 18: Summary for effects of FLU + TFS SC 500 (250+250 g/L) on *Fagopyrum esculentum* at the final assessment on day 21

Endpoints	Mortality	Shoot height[C]	Shoot dry weight[B]
LR_{25} / ER_{25} [L product/ha]	> 2.000	2.000	> 2.000
LR_{50} / ER_{50} [L product/ha]	> 2.000	> 2.000	> 2.000
NOER [L product/ha]	≥ 2.000	0.346	0.832
LOER [L product/ha]	-	0.832	2.000

[A]: determined with: [a] Williams` test; test one-sided smaller, α = 0.05
[B]: determined with: [a] Chi² - test; test one-sided smaller, α = 0.05

Table 10.6.2- 19: Summary of effects on growth stages (BBCH), shoot height and dry weight of *Fagopyrum esculentum* at the final assessment on day 21

Species	*Fagopyrum esculentum*		
Test item [L product/ha]	Growth stages [Min. - Max]	Inhibition [%]	
		Shoot height[A]	Shoot dry weight[B]
Control (0)	63 - 63	-	-
0.060	63 - 63	3.8	- 4.3#
0.144	63 - 63	12.6	6.0
0.346	63 - 63	0.4	0.7
0.832	63 - 63	17.7 *	4.8
2.000	63 - 63	14.8 *	13.1 *

* Statistically significantly different compared to the control
[A]: determined with Williams` test; one-sided smaller, α = 0.05
[B]: determined with Chi² - test; one-sided smaller, α = 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 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 ER_{25} and ER_{50} (based on mortality, shoot height and shoot dry weight) were determined to be > 2.0 L prod./ha, respectively.

Assessment and conclusion by applicant:

The study and its data are considered as acceptable and reliable for use in risk assessment.

The endpoint is: ER_{50} > 2.0 L prod./ha

CP 10.6.3 Extended laboratory studies on non-target plants

In view of the results presented under Point CP 10.6.2 above, no further studies are deemed necessary.

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

In view of the results presented under Point CP 10.6.2 above, no further studies are deemed necessary.

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

In view of the study results presented above no studies on other terrestrial organisms are considered necessary. However, further investigation has been conducted on fungicidal activity with no adverse effects observed; for details see MCA 8.7.

CP 10.8 Monitoring data

No monitoring data has been collected by the applicant nor have they been reported in any of the public literature references as evaluated in Document MCA, Section 9. No monitoring of non-target organism is deemed to be necessary.