Document Title

1st amendment of

Summary of the ecotoxicological studies
Fluopyram SC 500 (500 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
2021-03-24	Original MCP as submitted by applicant	M-766069-01-1
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-766069-02-1

[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, Entry into Force on August 22, 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 FLU SC 500 is an SC formulation containing 500 g/kg of Fluopyram. This formulation is registered throughout Europe under trade names such as Luna Privilege. FLU SC 500 was already a representative formulation of Bayer AG for the Annex I inclusion of Fluopyram under Council Directive 91/414/EEC.

FLU SC 500 is an end use product proposed for use in the field on apples 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
Apple	BBCH 71-89	1	-	0.15	0.075

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	10	0.43	Grape leaves
	0.3	0.20	Grape (Summer Cut: leaves BBCH71)
	1.6	0.60	Bean foliage
	1.1	0.20	Bean straw
	3.5	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)
	8.9	0.31	Pepper (rest of plant) drip irrigation
	0.6	0.25	Bean foliage
	0.7	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
	0.9	0.17	Bean straw

[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).

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	0.73	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
	49.5	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:

[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.

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] 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)	0.785	1.120	3.450	0.054	0.377	0.113	0.013
3rd rotation (280 days)	0.197	1.327	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.

[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.

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)**	11.1	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
	11.1	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-AA10065	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)**	12.3	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)

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

RMS comment:

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 apples 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.

Gradual declines in the residue levels of AE C656948 were also detected for apple/pear (fruit) on the 16 trials performed in southern and northern Europe, but in 6 trials an uptum was observed at the last sampling. The application was performed 14 days before harvest and samples were taken at the day of application and 7, 14, 21 and 28 after that. Residue levels of the metabolites AE C656948-benzamide, AE C657188, AE C656948-7-hydroxy and AE C656948-methyl-sulfoxide were always below the LOQ (<0.01 mg/kg), when analysed. Residue levels of the metabolite AE C656948-pyridyl-acetic acid were mostly below the LOQ (<0.01 mg/kg), with exception of two trials, where the levels increased with time.

Samples of apple/pears 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, the apples/pears had reached BBCH 89 (Fruit ripe for consumption, fruit have typical taste and firmness).

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 apples. 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 (250+250) 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 (see M-435338-01-1 submitted in D-020806-01).

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. equiv	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	6.3	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	33.5	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	70	0.491	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	19.1	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	9.1	0.033	0.019	1.2	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	-	-	-	3.1	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	0.0	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 apples/pears

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948	AE C656948-benzamide
Report No RA- E19RP062 (M-757113-01-1) Trial No E19RP062-01	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.0578	<0.01
		Fruit	7	0.0427	<0.01
		Fruit	14	0.0225	<0.01
		Fruit	21	0.0284	<0.01
		Fruit	28	0.0223	<0.01
Report No RA- E19RP062 (M-757113-01-1) Trial No E19RP062-02	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.0435	<0.01
		Fruit	7	0.0341	<0.01
		Fruit	14	0.0279	<0.01
		Fruit	21	0.0215	<0.01
		Fruit	28	0.0141	<0.01
Report No RA- E19RP062 (M-757113-01-1) Trial No E19RP062-03	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.0681	<0.01
		Fruit	7	0.0347	<0.01
		Fruit	14	0.0258	<0.01
		Fruit	21	0.0232	<0.01
		Fruit	28	0.0252	<0.01
Report No RA- E19RP062 (M-757113-01-1) Trial No E19RP062-04	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.0647	<0.01
		Fruit	7	0.0577	<0.01
		Fruit	14	0.0442	<0.01
		Fruit	21	0.0337	<0.01
		Fruit	28	0.0325	<0.01
Report No RA- E19RP083 (M-755638-01-1) Trial No E19RP083-01	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.0943	<0.01
		Fruit	7	0.0937	<0.01
		Fruit	14	0.0462	<0.01
		Fruit	21	0.0402	<0.01
		Fruit	28	0.0298	<0.01
Report No RA- E19RP083 (M-755638-01-1) Trial No E19RP083-02	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.104	<0.01
		Fruit	7	0.100	<0.01
		Fruit	14	0.0738	<0.01
		Fruit	21	0.0619	<0.01
		Fruit	28	0.0311	<0.01
Report No RA- E19RP083 (M-755638-01-1) Trial No E19RP083-03	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.173	<0.01
		Fruit	7	0.0932	<0.01
		Fruit	14	0.0875	<0.01
		Fruit	21	0.0515	<0.01
		Fruit	28	0.0593	<0.01
Report No RA- E19RP083 (M-755638-01-1) Trial No E19RP083-04	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.200	<0.01
		Fruit	7	0.121	<0.01
		Fruit	14	0.0822	<0.01
		Fruit	21	0.0490	<0.01
		Fruit	28	0.0546	<0.01
Report No RA- E19RP105 (M-757080-01-1) Trial No E19RP105-01	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.104	<0.01
		Fruit	7	0.0635	<0.01
		Fruit	14	0.0392	<0.01
		Fruit	21	0.0264	<0.01
		Fruit	28	0.0492	<0.01
Report No RA- E19RP105 (M-757080-01-1) Trial No E19RP105-02	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.0624	<0.01
		Fruit	7	0.0258	<0.01
		Fruit	14	0.0258	<0.01
		Fruit	21	0.0264	<0.01
		Fruit	28	0.0230	<0.01

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948	AE C656948-benzamide
Report No RA- E19RP105 (M-757080-01-1) Trial No E19RP105-03	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.173	<0.01
		Fruit	7	0.116	<0.01
		Fruit	14	0.0988	<0.01
		Fruit	21	0.0804	<0.01
		Fruit	28	0.0870	<0.01
Report No RA- E19RP105 (M-757080-01-1) Trial No E19RP105-04	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.0798	<0.01
		Fruit	7	0.0447	<0.01
		Fruit	14	0.0306	<0.01
		Fruit	21	0.0254	<0.01
		Fruit	28	0.0236	<0.01
Report No RA- E19RP106 (M-757080-01-1) Trial No E19RP106-01	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.158	<0.01
		Fruit	7	0.131	<0.01
		Fruit	14	0.0771	<0.01
		Fruit	21	0.0617	<0.01
		Fruit	28	0.0672	<0.01
Report No RA- E19RP106 (M-757080-01-1) Trial No E19RP106-02	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.347	<0.01
		Fruit	7	0.252	<0.01
		Fruit	14	0.205	<0.01
		Fruit	21	0.175	<0.01
		Fruit	28	0.405	<0.01
Report No RA- E19RP106 (M-757080-01-1) Trial No E19RP106-03	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.150	<0.01
		Fruit	7	0.0984	<0.01
		Fruit	14	0.0752	<0.01
		Fruit	21	0.0519	<0.01
		Fruit	28	0.0412	<0.01
Report No RA- E19RP106 (M-757080-01-1) Trial No E19RP106-04	0.03 kg a.s./ha*m 14 days before harvest	Fruit	0	0.104	<0.01
		Fruit	7	0.120	<0.01
		Fruit	14	0.0751	<0.01
		Fruit	21	0.0133	<0.01
		Fruit	28	<0.01	<0.01

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 c)	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

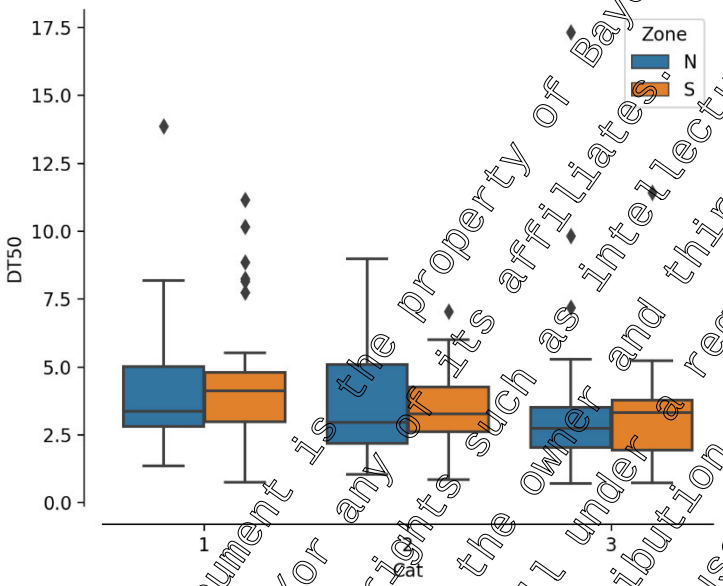
Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
R 2007 0569/7 M-304288-01-1	Leek	N	FOMC	3.551	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832
R 2007 0570/0 M-304288-01-1	Leek	N	FOMC	3.675	3	early rainfall coincides 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 level and refinement data was only needed based on measured data for secondary poisoning.

The risk assessment for aquatic organisms is acceptable when considering FOCUS step 2 PECsw.

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 assessments resulted in acceptable outcomes at tier 1 level, 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 SC 500 (500 g/L) 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-263049-01-1 KCA 8.1.1.1/01
	Acute oral toxicity	Zebra finch (*Taeniopygia guttata*)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2008) M-307871-02-1 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} LDD_{50}	> 5000 mg a.s./kg feed > 1445.4 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} LDD_{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-667212-01-1 KCA 8.1.1.3/05
FLU SC 500	Acute oral toxicity	Bobwhite quail (*Colinus virginianus*)	LD_{50}	> 2000 mg prod./kg bw	[redacted] (2008) M-326987-02-1 KCP 10.1.1.1/01
			LD_{50}	= 3776 mg prod./kg bw [B]	Extrapolated acc. to chapter 2.1.2 of EFSA Journal 2009; 7(12):1438

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 1.888 for 10 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
Orchards (apples)	Small insectivorous bird	46.8	18.2

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.1- 3: Screening acute risk assessment for birds

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}				
Orchards (apples)	Small insectivorous bird	0.075	46.8	1.0	3.51	> 2000	> 570	10

The TER_A value is above the trigger of 10. Therefore, a Tier 1 risk assessment is not required.

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

For the use in the crop under assessment in this evaluation (apples) 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- 4: 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
Orchards (apples)	Fluopyram	232.1 A	75	> 2000	< 0.038	≤ 50	No concern

A K_{OC} value 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) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)."* This is the case for fluopyram. Therefore, the acute risk for birds from drinking water that may contain residues from fluopyram is acceptable.

LONG-TERM REPRODUCTIVE RISK ASSESSMENT

Table 10.1.1- 5: Screening long-term reproductive risk assessment for birds

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}				
Orchards (apples)	Small insectivorous bird	0.075	18.2	1.0	0.53	0.723	4.5	6.22	5

The TER_{LT} value calculated in the chronic risk assessment on screening level exceed the trigger of 5. Therefore, 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- 6: 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}/ NOEL)	"Escape clause" No concern if ratio	Conclusion
Orchards (apples)	Fluopyram	232.1 [A]	75	4.5	16.7	≥ 50	No concern

[A] K_{OC} value 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- 7: 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- 8: 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

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- 9: Tier 1 long-term DDD and TER calculation for earthworm-eating birds in apples

	Fluopyram	
	Tier 1	Refinement
K_{OW}	2060	2060
K_{OC} [mL/g]	2321 [A]	232.1 [A]
f_{OC}	0.02	0.02
BCF_{worm}	5.51	0.85 [C]
$PEC_{soil, accu}$ (mg/kg)	0.157 [B]	0.157 [B]
PEC_{worm} (mg/kg)	0.864	0.133
FIR/bw	1.05	1.05
DDD (mg/kg bw/d)	0.908	0.140
NO(A)EL (mg/kg bw/d)	4.5	4.5
TER_{LT}	**4.96**	32.1
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 (apples, 1 × 75 g a.s./ha), 21-day-TWA of 0.035 mg a.s./kg + plateau concentration (5 cm) of 0.122 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- 10: Tier 1 long-term DDD and TER calculation for fish-eating birds in apples

	Fluopyram
BCF_{fish}	16 [A]
FOCUS Step 2 PEC_{sw} (twa, 21 d) (mg/L)	0.00626 [B]
PEC_{fish} (mg/kg)	0.100
FIR/bw	0.159
DDD (mg/kg bw/d)	0.016
NO(A)EL (mg/kg bw/d)	4.5
TER_{LT}	283
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 (apples, 1 × 75 g a.s./ha), FOCUS Step 2, Northern Europe, autumn application 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:	2011
Report Title:	Acute oral toxicity for bobwhite quail (Colinus virginianus) with the test substance Fluopyram SC 500 G
Report No:	BAR/LD 097
Document No:	M-326987-02-1
Guideline(s) followed in study:	EPA Pesticide Assessment Guidelines § 71-1, Subdivision E (October 1982) with consideration of the recommendation of: EPA Ecological Effects Guidelines OPPTS 850.2100 Avian Acute Oral Toxicity Test (April 1996).
Deviations from current test guideline:	Current Guideline: OECD 223 (2016) Deviations: The photoperiod was 10 hours light, above the 8 hours light as recommended. No information on medication prior to test start was given in the report. The space available for each bird in the pen was about 950 cm2, and thus slightly below the 1000 cm2. Weighing of birds was not performed on day 3 as recommended by the guideline. Observation for regurgitation was performed continuously for the first hour not for the recommended two hours. These deviations 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 SC 500 was administered orally to 10 adult bobwhite quails (5 males and 5 females) at dose levels of 0, 1000 and 2000 mg product/kg bw. Birds were held at a temperature of 19 to 25 °C with a relative humidity of 27 to 62 % and 10 hours light per day. The birds were observed for 14 days for mortality and sublethal symptoms. Body weight and average feed consumption were measured for each dosage and control group.

The study fulfilled all validity criteria of OECD 223 guideline.

Signs of impairment of the digestive tract were seen in all test groups, mainly soft excrement, diarrhoea and excretion of uric acid. These effects occurred also occasionally in the control. They were most abundant in the days after the application while towards the end of the study the birds behaved normally or showed only a slight effect. No pathological changes were found at necropsy of the survivors and no mortality was observed. There were no treatment related effects on body weight compared to the control.

The acute oral LD_{50} for bobwhite quail was > 2000 mg product/kg bw and the LOED was > 2000 mg product/kg bw.

I. MATERIAL AND METHODS

Test item: FLU SC 500, specification No.: 102000018148; Batch ID.: 2007-011657; Sample identification: TOX 08109-00; analysed a.s. content: 42.2 % w/w (501 g/L).

Test design: Bobwhite quail (6 months old) were orally dosed with FLU SC 500 at 0, 1000 and 2000 mg product/kg bw. The test substance was placed in capsules based on treatment level and body weight and administered to the birds. Control birds received empty gelatine capsules. Each dosage group comprised 10 birds (5 female and 5 male birds) which were housed individually. Each cage had floor space that measured approximately 38 × 25 cm with a ceiling of 23 cm.

Birds were acclimatized for approximately 15 days prior to being randomized into test groups. At the start of acclimation, the quail were well developed and similar to birds from wild population. Only birds that appeared healthy were used for the study. 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 a temperature of 19 to 25 °C with a relative humidity of 27 to 62 %. The photoperiod was 10 hours of light per day during acclimation and throughout the test.

Observations for mortality and signs of intoxication including regurgitation were made continuously during the first hour and hourly on the day of dosing during the exposure period and at least once a day throughout the 14 days observation period. Body weights were recorded prior to test initiation (day -1), on day 7 and at test termination (day 14). Food consumption was determined by pen for each dosage group and control group for days 0-3, 3-7 and 7-14. Gross necropsies were carried out on all survivors.

Statistics: Since no bird died, it was not possible to calculate the LD_{50}, slope and confidence interval. Initially the data were analysed on homogeneous distribution (Kolmogoroff-Smirnov test, $p < 0.05$). In case the data were homogeneously distributed, they were subjected to an analysis of equal variances (Bartlett‘s test). In case of equal variances, subsequent analyses were conducted using parametric techniques (Dunnett’s test); otherwise the t-Test for inhomogeneous variances (Bonferroni Test) was used.

Dates of experimental work: September 29th to October 28th 2008.

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 %

Observations:

Mortality and clinical observations:

Signs of impairment of the digestive tract were seen in all treatment groups, mainly soft excrement, diarrhoea and excretion of uric acid. These effects occurred also occasionally in the control. They were most abundant in the days after the application while towards the end of the study the birds behaved normally or showed only a slight effect.

No mortalities were observed.

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	soft excrement, diarrhoea
1000	0	10	excretion of uric acid, soft excrement, diarrhoea, fluffed feathers
2000	0	10	soft excrement, diarrhoea, excretion of uric acid

Gross pathology:

No pathological changes were found at necropsy of the survivors.

Body weight and feed consumption:

There were no statistically significant differences for body weight development between treatment groups and control.

Table 10.1.1.1- 3: Mean body weight of surviving birds

Treatment level [mg product/ kg bw]	Mean body weight ± S.D. [g]					
	Females			Males		
	Day -1	Day 7	Day 14	Day -1	Day 7	Day 14
0 (control)	175.6 ± 15.9	190.8 ± 13.6	178.4 ± 12.2	183.6 ± 19.7	184.2 ± 15.9	190.6 ± 6.2
1000	184.0 ± 15.5	185.6 ± 9.9	188.2 ± 7.5	183.0 ± 28.1	189.0 ± 19.6	195.2 ± 18.7
2000	197.4 ± 16.6	177.0 ± 10.5	192.0 ± 13.2	188.2 ± 18.6	188.8 ± 8.4	185.0 ± 13.8

S.D.: Standard deviation

Food consumption was reduced between day 0-3 within the treatment groups (reduction of 15 % at 1000 mg prod./kg bw and 42 % at 2000 mg prod./kg bw). From day 3 on, the treatment groups consumed similar amounts of food as the control.

Table 10.1.1.1- 4: Mean food consumption

Treatment level [mg product/kg bw]	Mean food consumption [g/bird/d]		
	Days 0 - 3	Days 3 - 7	Days 7 - 14
Control	15.5	15.4	13.7
1000	13.3	15.9	13.7
2000	9.0	15.1	13.4

Biological findings:

Table 10.1.1.1- 5: Acute oral toxicity to Bobwhite Quail

Test substance	FLU SC 500
Test object	Bobwhite Quail (male, female)
LD_{50} [mg product/kg bw]	> 2000
Lowest observed effect dose (LOED) [mg product/kg bw]	> 2000

III. Conclusion

Based on this study the LD_{50} value for bobwhite quail exposed to FLU SC 500 was determined to be > 2000 mg product/kg bw. The LOED 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 10 dosed birds, the LD_{50} can be extrapolated to 3776 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.

Data Point:	KCP 10.1.1.2/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Attractiveness of tomato fields for herbivorous mammals and birds, field monitoring in Lombardia
Report No:	C042278
Document No:	M-232304-01-1
Guideline(s) followed in study:	Pesticides and Wildlife - Field Testings: Recommendations of an international workshop on terrestrial field testing of pesticides attached to Pesticide Effects on Terrestrial Wildlife, Somerville & Walker (ed.), Taylor & Francis, London 1990
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2007)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/02
Report Author:	[redacted]
Report Year:	2013
Report Title:	Generic field study on the foraging behaviour of Yellow Wagtails in tomato fields in Italy (2013)
Report No:	P12085-3
Document No:	M-487754-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009, EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No 3 to the DAR (rev 2017)
GLP/Officially recognised testing facilities:	Yes conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	No

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/03
Report Author:	[redacted]
Report Year:	2009
Report Title:	Generic field monitoring of birds in vegetable fields in Spain
Report No:	R07-199
Document No:	M-347259-01-1
Guideline(s) followed in study:	EU Council Directive 91/414/EEC
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/04
Report Author:	[redacted]
Report Year:	2009
Report Title:	Letter of access for generic behavioural ecology data - Study report Syngenta Limited document NA_11468 - Grouping: Vegetables, post emergence (foliar stages)
Report No:	M-347417-01-1
Document No:	M-347417-01-1
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

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/05
Report Author:	[redacted]
Report Year:	2008
Report Title:	An ecological study of the use of vineyards by birds in Southern France
Report No:	M-304340-01-2
Document No:	M-304340-01-2
Guideline(s) followed in study:	--
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the DAR (2011)
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	No

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/06
Report Author:	[redacted]
Report Year:	2007
Report Title:	The use of vineyards by birds in Southern France: An ecological study to refine the risk assessment for insecticide use
Report No:	ER-07-SCB-2[illegible]
Document No:	M-427241-01-1
Guideline(s) followed in study:	SANCO/4145/2000
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 2 to the DAR (2012)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	No

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/07
Report Author:	[redacted]
Report Year:	2012
Report Title:	Letter of access for generic behavioural ecology data - Study report ER-07-LPB-277 from DAS - The use of vineyards by birds in southern France: An ecological study to refine the risk assessment for insecticide use
Report No:	M-427251-01-1
Document No:	M-427251-01-1
Guideline(s) followed in study:	SANCO/4145/2000
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

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/08
Report Author:	[redacted]
Report Year:	2012
Report Title:	Foraging behaviour of the black redstart in vineyards in Germany
Report No:	423079
Document No:	M-487359-01-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	Yes, evaluated and accepted in the Addendum No 6 to the DAR (rev. 2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/09
Report Author:	[redacted]
Report Year:	2006
Report Title:	Feeding ecology of the relevant insectivorous bird species in strawberry fields in Germany
Report No:	RC06-054
Document No:	M-342897-01-1
Guideline(s) followed in study:	not applicable; the test was especially designed for the purpose of this study
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.2/1
Report Author:	[redacted]
Report Year:	2006
Report Title:	Letter of access for generic behavioural ecology data - study report R-20183 - Grouping: strawberry (foliar stages) - author, year: Moosmayer P, 2006
Report No:	M-347237-01-1
Document No:	M-347237-01-1
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

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/11
Report Author:	[redacted]
Report Year:	2016
Report Title:	Determination of residues of fluopyram in Poecilus cupreus (coleoptera: carabidae) using an extended laboratory test
Report No:	CW15/045
Document No:	M-545010-01-2
Guideline(s) followed in study:	Heimbach et al. (2000) modified US EPA OCSPP Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 4 to the DAR (rev. 2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	No

The document above was included here in this section for transparency reasons since it was part of the first listing process. It contains information relevant for the current active substance renewal process. The full study summary is presented under MCA 8.9/01 following the latest reporting and evaluation criteria. The location MCA was chosen for presenting the detailed study data to facilitate an easier and consistent review of all refinement data in one place, since the endpoints derived are relevant for several MCPs.

Data Point:	KCP 10.1.1.2/12
Report Author:	[redacted]
Report Year:	2013
Report Title:	Residue decline of fluopyram on arthropods after spray application in vines in Germany
Report No:	M-453376-01-2
Document No:	M-453376-01-2
Guideline(s) followed in study:	Regulation (EC) No 1107/2009, EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) US EPA OCSPP Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 4 to the DAR (rev. 2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was included here in this section for transparency reasons since it was part of the first listing process. It contains information relevant for the current active substance renewal process. The full study summary is presented under MCA 8.9/02 following the latest reporting and evaluation criteria. The location MCA was chosen for presenting the detailed study data to facilitate an easier and consistent review of all refinement data in one place, since the endpoints derived are relevant for several MCPs.

Data Point:	KCP 10.1.1.2/13
Report Author:	[redacted]
Report Year:	2016
Report Title:	Kinetic evaluation of fluopyram residues in foliage dwellers and flying insects in vines - Fluopyram (AE C656948)
Report No:	EnSa-15-0934
Document No:	M-544286-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 4 to the DAR (rev 2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was included here in this section for transparency reasons since it was part of the first listing process. It contains information relevant for the current active substance renewal process. The full study summary is presented under MCA 8.9/03 following the latest reporting and evaluation criteria. The location MCA was chosen for presenting the detailed study data to facilitate an easier and consistent review of all refinement data in one place, since the endpoints derived are relevant for several MCPs.

Data Point:	KCP 10.1.1.2/14
Report Author:	[redacted]
Report Year:	2015
Report Title:	Residue decline of fluopyram and prothioconazole on arthropods after spray application on oilseed rape fields in Western Germany
Report No:	P13067
Document No:	M-544190-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009, EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009)
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 4 to the DAR (rev 2017)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was included here in this section for transparency reasons since it was part of the first listing process. It contains information relevant for the current active substance renewal process. The full study summary is presented under MCA 8.9/04 following the latest reporting and evaluation criteria. The location MCA was chosen for presenting the detailed study data to facilitate an easier and consistent review of all refinement data in one place, since the endpoints derived are relevant for several MCPs.

Data Point:	KCP 10.1.1.2/15
Report Author:	[redacted]
Report Year:	2016
Report Title:	Kinetic evaluation of fluopyram residues in foliage dwellers and flying insects in oilseed rape
Report No:	EnSa-16-0035
Document No:	M-545077-01-1
Guideline(s) followed in study:	none
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 4 to the DAR (rev. 2017)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was included here in this section for transparency reasons since it was part of the first listing process. It contains information relevant for the current active substance renewal process. The full study summary is presented under MCA 8.9/05 following the latest reporting and evaluation criteria. The location MCA was chosen for presenting the detailed study data to facilitate an easier and consistent review of all refinement data in one place, since the endpoints derived are relevant for several MCPs.

Data Point:	KCP 10.1.1.2/16
Report Author:	[redacted]
Report Year:	2011
Report Title:	Fluopyram - Evaluation of OECD joint review dossier - WNL 6656 - Fluopyram - OECD-Joint Review/ EU-Wirkstoffpruefung zur Aufnahme von Wirkstoffen in Anhang I der Richtlinie 91/414/EWG
Report No:	M-409909-01-1
Document No:	M-409909-01-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.1.2/17
Report Author:	[redacted]
Report Year:	2012
Report Title:	Fluopyram - Peer review of new active substance - Request for additional information - Ecotoxicology - EFSA Letter Ref D(2012) HO/JS/al/620027 dated January 24, 2012
Report No:	M-428668-01-1
Document No:	M-428668-01-1
Guideline(s) followed in study:	Data Directive 91/414/EEC Bee Studies, not yet Peer Reviewed
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

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

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}
Orchards (apples)	Small herbivorous mammal	136.4	72.3

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.2- 3: Screening acute risk assessment for mammals

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}				
Orchards (apples)	Small herbivorous mammal	0.075	136.4	1.0	10.2	> 2000	> 196	10

The TER_A value is above the trigger of 10. Therefore, a Tier 1 risk assessment is not required.

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- 4: 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
Orchards (apples)	Fluopyram	232.1 [A]	75	> 2000	< 0.038	≤ 50	No concern

[A] Koc value 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) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg)*." This is the case for fluopyram. Therefore, the acute risk for mammals from drinking water that may contain residues from fluopyram is acceptable.

LONG-TERM REPRODUCTIVE ASSESSMENT

Table 10.1.2- 5: 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}				
Orchards (apples)	Small herbivorous mammal	0.075	72.3	1.0	0.53	2.87	14.5	5.05	5

The TER_{LT} value calculated in the chronic risk assessment on screening level exceeds the trigger of 5. Therefore, the long-term risk to mammals can be considered as acceptable.

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

Table 10.1.2- 6: 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
Orchards (apples)	Fluopyram	232.1 [A]	75	14.5	5.17	≤ 50	No concern

[A] Koc value 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- 7: 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- 8: 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

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- 9: Tier 1 long-term DDD and TER calculation for earthworm-eating mammals in apples

	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)	0.157 [B]	0.157 [B]
PEC_{worm} (mg/kg)	0.864	0.133
FIR/bw	1.28	1.28
DDD (mg/kg bw/d)	1.11	0.171
NO(A)EL (mg/kg bw/d)	14.5	14.5
TER_{LT}	13.1	84.9
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 (apples, 1 × 75 g a.s./ha) 21-day-TWA of 0.035 mg a.s./kg + plateau concentration (5 cm) of 0.022 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- 10: Tier 1 long-term DDD and TER calculation for fish-eating mammals in apples

	Fluopyram
BCF_{fish}	16 [A]
FOCUS Step 2 PEC_{sw} (twa, 21 d) (mg/L)	0.00626 [B]
PEC_{fish} (mg/kg)	0.100
FIR/bw	0.142
DDD (mg/kg bw/d)	0.014
NO(A)EL (mg/kg bw/d)	14.5
TER_{LT}	1020
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 (apples, 1 x 75 g (a.s./ha), FOCUS Step 2, Northern Europe, autumn application 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- 11: Mammalian toxicity data of the formulated product FLU SC 500

Test substance	Test design	Species	Endpoint	Reference
FLU SC 500	Acute	Rat	LD_{50} > 2000 mg prod./kg bw	[redacted] (2008) M-298203-01-1 KCA 7.1.1/02

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.

Data Point:	KCP 10.1.2.2/01
Report Author:	[redacted]
Report Year:	2003
Report Title:	Attractiveness of tomato fields for herbivorous mammals and birds, field monitoring in Lombardia
Report No:	C042278
Document No:	M-232304-01-1
Guideline(s) followed in study:	Pesticides and Wildlife - Field Testings: Recommendations of an international workshop on terrestrial field testing of pesticides attached to Pesticide Effects on Terrestrial Wildlife, Somerville & Walker (ed.), Taylor & Francis, London 1990
Deviations from current test guideline:	Current Guideline not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

Data Point:	KCP 10.1.2.2/02
Report Author:	[redacted]
Report Year:	2008
Report Title:	XDE-658948 50 SC - Magnitude of the residue in/on grass forage, fodder, and hay (crop group 17) and grass for seed
Report No:	RAGMP044
Document No:	M-300735-01-1
Guideline(s) followed in study:	EPA Ref. OPPTS 860.1500, Crop Field Trials
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It is considered superseded by the trials from EU countries submitted and analysed in the MCA point 8.9.

Data Point:	KCP 10.1.2.2/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	Foliar half-life calculation for AE C656948 in grass forage and hay
Report No:	EBGMP158
Document No:	M-300703-01-1
Guideline(s) followed in study:	850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the DAR (2011)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It is considered superseded by the trials from EU countries submitted and analysed in the MCA point 8.9.

Data Point:	KCP 10.1.2.2/04
Report Author:	[redacted]
Report Year:	2012
Report Title:	Statement on residue dissipation of fluopyram in treated foliage of dicotyledonous plants, kinetic evaluation
Report No:	EnSa-12-0018
Document No:	M-426930-02-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in the Addendum No. 2 to the DAR (2012)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It is considered superseded by the trials from EU countries submitted and analysed 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: Studies for Fluopyram and its metabolites and endpoints used in risk assessment for aquatic organisms

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
FLU SC 500	Fish, acute *Oncorhynchus mykiss*	96 h / static	LC_{50} > 236 mg prod./L (nom) (~ >100 mg a.s./L) A	[redacted] (2013) M-467867-01-1 KCP 10.2.1/05
	Fish, acute *Cyprinus carpio*	96 h / static	**LC_{50} > 200 mg prod./L (nom)** **(~ >84.0 mg a.s./L)**	[redacted] (2010) M-366324-01-1 KCP 10.2.1/06
	Invertebrate, acute *Daphnia magna*	0 - 48 h / static	**EC_{50} = 141 mg prod./L (nom)** **(~ = 59.2 mg a.s./L)**	[redacted] (2010) M-366819-01-1 KCP 10.2.1/07
	Green algae *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	E_rC_{50} = 16.1 mg prod./L (nom) (~ = 6.79 mg a.s./L) E_rC_{10} = 7.80 mg prod./L (nom) E_bC_{50} = 10.3 mg prod./L (nom) E_yC_{50} = 8.39 mg prod./L (nom)	[redacted] (2008) M-299910-01-1 KCP 10.2.1/08 Recalculation by [redacted] (2020) M-757704-01-1 KCP 10.2.1/09
	Green algae *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	**E_rC_{50} = 14.6 mg prod./L (nom)** **(~ = 6.13 mg a.s./L)** E_rC_{10} = 6.89 mg prod./L (nom) E_bC_{50} = 8 82 mg prod./L (nom) E_yC_{50} = 9.00 mg prod./L (nom)	[redacted] (2010) M-367124-03-1 KCP 10.2.1/10 Recalculation by [redacted] (2020) M-757717-01-1 KCP 10.2.1/11
	Aquatic macrophyte *Lemna gibba*	7 d / static	**E_rC_{50} = 16.2 mg prod./L (nom)** **(~ = 6.80 mg a.s./L)** E_rC_{10} = 4.78 mg prod./L (nom) E_yC_{50} = 11.9 mg prod./L (nom)	[redacted] (2020) M-758230-01-1 KCP 10.2.1/12
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	96 h /	LC_{50} > 4.95 mg a.s./L (mm) B	[redacted]

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Pimephales promelas	static		(2008) M-298918-01-1 KCA 8.2.1/03
	Fish, acute *Cyprinus carpio*	96 h / static	LC_{50} = 20.5 mg a.s./L (nom) [C]	[redacted] (2006) M-280108-01-1 KCA 8.2.1/04
	Fish, acute *Cyprinodon variegatus*	96 h / static	LC_{50} > 0.98 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 5 % lipid content) = [redacted]	[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} > 1.4 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**	
	Invertebrate, chronic *Daphnia magna*	21 d / static-renewal	**NOEC = 1.25 mg a.s./L (nom)** EC_{10}: not determined [D]	[redacted] (2008) 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)	54 d / static-renewal	**NOEC = 26 mg a.s./kg (mm)** EC_{10}: not determined [D]	[redacted] (2008) M-298809-01-1 KCA 8.2.5.3/01

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Chironomus tentans (spiked sediment)			Recalculation by [redacted] (2020) M-758550-01-1 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} = 0.54 mg a.s./L (nom)** EC_{20} = 1.37 mg a.s./L (nom) EC_{50} > 32 mg a.s./L (nom) [D]	[redacted] (2008) M-298266-01-1 KCA 8.2.5.3/01
	Sediment dweller, chronic *Leptocheirus plumulosus* (spiked sediment)	28 d Static renewal	NOEC = 25 mg a.s./kg (mm) LC_{50} > 25 mg a.s./kg (mm)	[redacted] (2008) M-298810-02-1 KCA 8.2.5.4/02
	Green algae *Pseudokirchneriella subcapitata* [E] (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] (2020) M-757659-01-1 KCA 8.2.6.1/03
	Freshwater diatom *Navicula pelliculosa*	0 - 72 h / static	E_rC_{50} = 9.66 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] (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] (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
Fluopyram-2 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* [E] (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)	

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	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)** [F]	[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)** [F]	[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)** [F] EC_{10}: not determined [D]	[redacted] (2010) M-615126-01-1 KCA 8.2.5.1/03
	Green algae *Pseudokirchneriella subcapitata* [E] (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)** [F] E_rC_{10} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [F] E_bC_{50} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [F] E_yC_{50} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [F]	[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
	Green algae *Pseudokirchneriella subcapitata* [E] (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) [F] E_rC_{10} = 2.239 mg p m./L (nom Na-TFA) = 1.881 mg p.m./L (nom TFA) [F] E_bC_{50} > 4.8 mg p m./L (nom Na-TFA) > 4.03 mg p.m./L (nom TFA) [F] E_yC_{50} = 4.190 mg p m./L (nom Na-TFA) = 3.52 mg p.m./L (nom TFA) [F]	[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) = 241.95 mg p.m./L (mm) E_rC_{10} = 5.59 mg p.m./L (nom) = 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)

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
				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) [F] **E_rC_{50} = >2016 mg p m./L (nom TFA) [F]** NOE_rC = 252 mg p m./L (nom TFA) [F] E_rC_{10} = 308 mg p m./L (nom TFA) [F]	[redacted] et al. (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 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, pm = pure metabolite, prod. = product
mm = mean measured; nom = nominal
Bold values used in risk assessment

A Justification on relevance of endpoint available in doc IICP FLUX SC 500, M-758755-02-1
B Practical limit of water solubility
C In all test levels precipitations were observable so the LC_{50} is clearly above the water solubility of the test item.
D Not determined due to mathematical reasons
E Formerly known as *Selenastrum capricornutum*
F 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 (2018) 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) $10\frac{M_{met}}{M_{ai}} LC_{50_{ai}}$	NA	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} > 1.01
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 apples.

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

Application in apples, 1 x 75 g a.s./ha

Table 10.2- 4: Initial max PEC_{sw} values – FOCUS Steps 1 and 2 – Single application in apples (autumn and summer, 1 x 75 g a.s./ha)

Compound	FOCUS Scenario	PECsw, max [µg/L]	
		Apples - Autumn (Oct. - Feb.) 1×75 g a.s./ha, BBCH 71-89	Apples – Summer (Jun. - Sep.) 1×75 g a.s./ha, BBCH 71-89
Fluopyram	STEP 1	33.0	23.0
	STEP 2 – North	**6.56**	4.57
	STEP 2 – South	5.90	**5.24**
Fluopyram-7-hydroxy	STEP 1	1.33	1.33
	STEP 2 – North	**0.232**	0.093
	STEP 2 – South	0.186	**0.139**
Trifluoroacetic acid (TFA)	STEP 1	1.06	1.06
	STEP 2 – North	**0.186**	0.074
	STEP 2 – South	0.149	**0.111**

Bold values used in risk assessment

Table 10.2- 5: Initial max PEC_{sed} values – FOCUS Steps 1 and 2 – Single application in apples (autumn and summer, 1 x 75 g a.s./ha)

Compound	FOCUS Scenario	PECsed, max [µg/kg]	
		Apples - Autumn (Oct. - Feb.) 1×75 g a.s./ha, BBCH 71-89	Apples – Summer (Jun. - Sep.) 1×75 g a.s./ha, BBCH 71-89
Fluopyram	STEP 1	51.2	51.2
	STEP 2 – North	**14.6**	10.0
	STEP 2 - South	13.1	**11.5**
Fluopyram-7-hydroxy	STEP 1	1.33	1.33
	STEP 2 – North	0.233	0.093
	STEP 2 - South	0.186	0.140
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

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

$RAC_{sw, ch} = EC_{50}$ / 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 apples, 1 x 75 g a.s./ha

Table 10.2- 6: Acute risk assessment based on FOCUS Step 2 for the application in apples (1 × 75 g a.s./ha)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
Autumn application					
FLU SC 500	Fish, acute *Cyprinus carpio*	LC_{50} >84000	>840	6.56	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 59200	592		Yes
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	6.56	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		Yes
Fluopyram-7-hydroxy	Fish, acute	LC_{50} 437 [A]	2.19 [B]	0.232	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >88700	>443.5 [B]		Yes
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.186	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 1008000	>10080		Yes
Summer application					
FLU SC 500	Fish, acute *Cyprinus carpio*	LC_{50} >84000	>840	5.24	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 59200	592		Yes
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	5.24	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		Yes
Fluopyram-7-hydroxy	Fish, acute	LC_{50} 437 [A]	2.19 [B]	0.139	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >88700	>443.5 [B]		Yes
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.111	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes

[A] Lower parent endpoint divided by 10

[B] 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 apples (autumn and summer application) at 75 g a.s./ha the acute trigger is met for all aquatic species for fluopyram and its metabolites as well as for the formulation.

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 apples, 1 x 75 g a.s./ha

Table 10.2- 7: Chronic risk assessment based on FOCUS Step 2 for the application in apples (1 × 75 g a.s./ha)

Compound	Species	Endpoint [µg/L]		RAC [µg/L]	$PEC_{(sw,max)}$ [µg/L]	RAC ≥ PEC_{sw}
Autumn application						
FLU SC 500	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	6130	613	6.56	Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	6800	680		Yes
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC	135	13.5	6.56	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 [A]	0.675 [B]	0.232	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC	125 [A]	6.25 [B]		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	20900	1045 [B]		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	9200	460 [B]		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC	13.5 [A]	1.35	0.186	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}~~ E_rC_{50}	~~924000~~ >2016000	~~92400~~ >201600		Yes

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
Summer application					
FLU SC 500	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} 6130	613	5.24	Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} 6800	680		Yes
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC 135	13.5	5.24	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 [A]	0.675 [B]	0.139	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC 125	6.25 [B]		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} 20900	1045 [B]		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} 9260	460 [B]		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC 13.5 [A]	1.35	0.111	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	~~92400~~ >201600		Yes

[A] 1st tier parent endpoint divided by 10

[B] 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.

Table 10.2- 8: Chronic risk assessment for sediment organisms based on FOCUS Step 2 for the application in apples (1 × 75 g a.s./ha)

Compound	Species	Endpoint [µg/kg]	RAC [µg/kg]	$PEC_{sed.max}$ [µg/kg]	$RAC \geq PEC_{sed}$
Autumn application					
Fluopyram tech.	Sediment dweller, *Chironomus tentans*	NOEC 26000	2600	14.6	Yes
Summer application					
Fluopyram tech.	Sediment dweller, *Chironomus tentans*	NOEC 26000	2600	14.5	Yes

For the application in apples (autumn and summer application) at 75 g a.s./ha the chronic trigger is met for all aquatic species for fluopyram and its metabolites as well as for the formulation.

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Data Point:	XCP 10.2/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Fluopyram - Evaluation of OECD joint review dossier - WNL 6656 - Fluopyram - OECD-Joint Review/ EU-Wirkstoffbriefumer zur Annahme von Wirkstoffen in Anhang I der Richtlinie 91/414/EWG
Report No:	M-409909-01-1
Document No:	M-409909-01-1
Guideline(s) followed in study:	not specified
Deviations from current test guideline:	current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

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 SC 500 G to fish (Oncorhynchus mykiss) under static conditions
Report No:	EBGMP066
Document No:	M-291039-01-1
Guideline(s) followed in study:	EPA-FIFRA § 72-1/SEP-ERS 540/9-85-006 (1982/1985) OPPTS 850.1075 (Public Draft, 1996) Directive 92/69/EEC, C.1 (1992) OECD No. 203 (rev. 1992)
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.2.1/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	Acute toxicity of AE C656948 SC 500 A G to the waterflea Daphnia magna in a static laboratory test system
Report No:	EBGMP067
Document No:	M-294507-01-1
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)
Deviations from current test guideline:	Current Guideline: 202 (2004) Deviations: Not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.2.1/03
Report Author:	[redacted]
Report Year:	2007
Report Title:	Pseudokirchneriella subcapitata growth inhibition test with Fluopyram SC 500
Report No:	EBGMP068
Document No:	M-292592-01-1
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: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.2.1/04
Report Author:	[redacted]
Report Year:	2008
Report Title:	Lemna gibba L. growth inhibition test with Fluopyram SC 500A G under static conditions
Report No:	EBGMP151
Document No:	M-297699-01-1
Guideline(s) followed in study:	OECD Guideline 221 (March 23, 2006); Equivalent to US EPA OPPTS Guideline No. 850.4400 (SUPP)
Deviations from current test guideline:	Current Guideline: 221 (2006) Deviations: Not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.2.1/05
Report Author:	[redacted]
Report Year:	2013
Report Title:	Fluopyram SC 500D G - Acute toxicity to fish (Oncorhynchus mykiss) under static conditions (limit test)
Report No:	EBGMN042
Document No:	M-467867-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC; Regulation (EC) No 1107/2009 (2009); US EPA OCSPP 850.1075
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The fish length at test start was 5.5 ± 0.6 cm and thus higher than the maximum 6 cm 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 static system. Juvenile fish were exposed to FLU SC 500 in groups of 30 (two replicates of 15 fish per test level) to an aqueous solution of the product at the single nominal concentration of 236 mg prod./L (corresponding to 100 mg a.s./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 equipped with an UV – detector on days 0, 2 and 4 for the test concentration and the control. Measured concentrations were in the 106 - 111 % range of nominal concentrations and no residues were found in the control samples above 0.139 mg a.s./L, which was used as the lowest standard concentration during the study. The mean measured concentration was 108 mg a.s./L.

The study fulfils all validity criteria of OECD 203 guideline.

In the controls no mortalities or sub-lethal effects were observed during the test. After 96 hours all 30 fish in the single test concentration of 236 mg prod./L showed symptoms like remaining for unusually long periods on the bottom of the aquarium, laying on their sides or backs, showing laboured respiration, turned turning dark in coloration or open mouth.

The endpoints based on nominal product concentrations of FLU SC 500 were: LC_{50} – 96 hours (95 % C.I.): > 236 mg prod./L (n.d.), LOEC – 96 hours: 236 mg prod./L and NOEC – 96 hours: < 236 mg prod./L.

I. MATERIALS AND METHODS

Test material	FLU SC 500 Specification No.: 102000022633 Batch No: 2010-008475 Content of a.s.: 42.4 % w/w fluopyram
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 the start of the study Mean body weight: 1.7 ± 0.7 g at the start of the study
Test solutions	Nominal concentration: 236 mg prod./L Nominal concentration fluopyram: 100 mg a.s./L Corresponding mean measured concentration: 108 mg a.s./L Control: water Evidence of undissolved material: During the whole exposure period an intensive turbidity caused by the test item was observed. Additionally, a homogeneous dispersion in the water and a turbidity were noticed observable after 4 hours till termination.
Replication	No. of vessels per concentration (replicates): 2 No. of vessels per control (replicates): 2
Organisms per replicate	No. of organisms per vessel: 15
Exposure	Static Total exposure duration: 96 hours
Test Vessel Loading	0.64 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 11.6 - 12.1 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: not reported pH: 7.1 - 7.8 Water hardness: 40 - 60 mg $CaCO_3$/L Dissolved oxygen: 69 - 95 % of saturation (aeration was added on study to reach oxygen saturation) Conductivity: < 0.2 mS/cm Alkalinity: not reported
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Discrete measurements of dissolved oxygen, pH and temperature were obtained at test initiation and after 24, 48, 72 and 96 hours. Temperature was 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. The chemical analyses were performed by using a High-performance liquid chromatograph (HPLC) equipped with an UV – detector.
Data analysis	Not needed as limit test.

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 %	69 - 95 %

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, day 2 and day 4 were between 106 and 111 % (see table below). Biological results are based on nominal concentrations of FLU SC 500.

No residues of fluopyram were found in the control samples above 0.139 mg a.s./L, which was used as the lowest standard concentration during the study.

Table 10.2.1- 2: Analytical results

Nominal concentration		Measured concentration [mg a.s./L] [A]			% of nominal [B]			Mean measured conc. [mg a.s./L] [A]	Mean % of nominal [A]
[mg prod./L]	[mg a.s./L]	Day 0	Day 2	Day 4	Day 0	Day 2	Day 4		
236	100	111	107	106	111	107	106	108	108

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

Biological results:

Observations:

In the controls no mortalities or sub-lethal findings were observed during the test.

After 96 hours all 30 fish in the single test concentration of 236 mg prod./L showed symptoms like remaining for unusually long periods on the bottom of the aquarium, laying on their sides or backs, showing laboured respiration, turning dark in coloration or open mouth.

Table 10.2.1- 3: Mortality

Nominal concentration [mg prod./L]	Dead fish [A] No. (%)				
	Exposure time				
	4 h	24 h	48 h	72 h	96 h
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
236	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

[A] Cumulative mortality of 2 replicates of each test level

III. CONCLUSION

The study meets the validity criteria according to OECD 203 (2019) and the endpoints based on nominal product concentrations of FLU SC 500 (mg prod./L) and nominal a.s. concentrations (mg a.s./L) were:

Endpoint	Nominal concentration	
	[mg prod./L]	[mg a.s./L]
LC_{50} – 96 hours (95 % C.I.):	**> 236** (n.d.)	**> 100** (n.d.)
LOEC – 96 hours: lowest concentration with an effect	236	100
NOEC – 96 hours: highest concentration without an effect (based on sublethal effects)	< 236	100
NOEC – 96 hours: highest concentration without an effect (based on mortality)	236	100

Assessment and conclusion by applicant:

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

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Data Point:	KCP 10.2.1/06
Report Author:	[redacted]
Report Year:	2010
Report Title:	Acute toxicity of fluopyram SC 500B to fish (Cyprinus carpio) under static conditions
Report No:	EBGMP260
Document No:	M-366324-01-1
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) JMAFF, 11 Nousan No. 6283 (Oct. 1999)
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The fish length at test start was 4.8 ± 1.0 cm and thus higher than the maximum 4 cm 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 Common carp (*Cyprinus carpio*) in a static system. Juvenile fish were exposed to FLU SC 500 in groups of 10 (one replicate of 10 fish per test level) to an aqueous solution of the product at nominal concentrations of 12.5, 25.0, 50, 100 and 200 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, 2 and 4 for each concentration and the control. Measured concentrations were in the 82 - 95 % range of nominal concentrations and no residues were found in the control samples above 0.188 mg a.s./L, which was used as the lowest standard concentration during the study. The mean measured concentrations were: 4.90, 8.91, 18.1, 36.9 and 76.6 mg a.s./L.

The study fulfils all validity criteria of OECD 203 guideline.

There were no behavioural abnormalities or mortalities of the fish in the controls. At test termination all fish in the two lowest test concentrations (12.5 and 25.0 mg prod./L) did not show any abnormal signs. However, in the three highest test concentrations (50.0, 100 and 200 mg prod./L) 8 to 10 fishes showed sub-lethal effects after 96 hours of exposure.

The endpoints based on nominal concentrations were: LC_{50} – 96 hours (95 % C.I.): > 200 mg prod./L (n.d.) and NOEC – 96 hours: 25 mg prod./L.

I. MATERIALS AND METHODS

Test material	FLU SC 500 Specification No.: 102000018148-01 Batch No: 2009-004770 Content of a.s.: 42.0 % w/w fluopyram
Guideline(s) adaptation	None specified
Test species	Common carp (*Cyprinus carpio*)

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: 48 ± 10 mm at test start Mean body weight: 1.9 ± 0.7 g at test start
Test solutions	Nominal concentrations: 12.5 – 25.0 – 50 – 100 – 200 mg prod./L Nominal concentrations fluopyram: 5.25 – 10.5 – 21.0 – 42.0 – 84.0 mg a.s./L Corresponding mean measured concentrations: 4.90 – 8.91 – 18.1 – 36.9 – 76.6 mg a.s./L Control: water Evidence of undissolved material: In the third highest test concentrations (50 mg prod./L) intensive turbidity caused by the test item was observed during the whole exposure period. The two highest test concentrations (100 and 200 mg prod./L) showed during the whole exposure period a homogeneous dispersion in the water, however, a turbidity was also observable.
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	0.48 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 21.5 - 22.9 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: not reported pH: 6.7 - 7.2 Water hardness: 40 - 60 mg $CaCO_3$/L Dissolved oxygen: 81 - 105 % of saturation (aeration was added on study to reach oxygen saturation) Conductivity: < 0.2 mS/cm Alkalinity: not reported
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Discrete measurements of dissolved oxygen and pH were obtained at test initiation and after 24, 48, 72 and 96 hours. Temperature was measured hourly by a calibrated data logger and daily by manual readings via a calibrated thermometer 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. The chemical analyses were performed by using a High-performance liquid chromatograph (HPLC – MS/MS).
Data analysis	Whenever possible, the LC_{50} values and the 95 %-confidence intervals were calculated every 24-hour using a computer program, which estimated the LC_{50} using one of three statistical techniques: moving average, logit analysis or probit analysis. The appropriate method was determined according to the data characteristics.

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 %	81 - 105 %

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, day 2 and day 4 were between 82 and 95 % (see table below). Biological results are based on nominal product concentrations of FLU SC 500.

No residues of fluopyram were found in the control samples above 0.188 mg a.s./L, which was used as the lowest standard concentration during the study.

Table 10.2.1- 5: Analytical results

Nominal concentration		Measured concentration [mg a.s./L]			% of nominal [A]			Mean measured concentration [mg a.s./L]	Mean % of nominal
[mg prod./L]	[mg a.s. /L]	Day 0	Day 2	Day 4	Day 0	Day 2	Day 4		
12.5	5.25	4.84	4.88	4.98	92	93	95	4.90	93
25.0	10.5	8.81	8.80	9.11	84	84	87	8.91	85
50.0	21.0	18.8 [B]	17.3	18.1	90	82	86	18.1	86
100	42.0	35.8	35.1	39.7	85	84	95	36.9	88
200	84.0	74.9 [B]	76.4 [B]	78.6 [B]	89	91	94	76.6	91

[A] Not given in report. Calculations based on measured concentrations on day 0 and 4.
[B] Average of duplicate water samples analysed.

Biological results:

Observations:

In the controls no mortalities or sub-lethal findings were observed during the test.

At test termination all fish in the two lowest test concentrations (12.5 and 25.0 mg prod./L) did not show any abnormal signs. However, in the three highest test concentrations (50.0, 100 and 200 mg prod./L) 8 to 10 fishes showed sub-lethal effects after 96 hours of exposure. In the third highest test concentration of 50.0 mg prod./L eight fish were inactive or displayed abnormally low activity. In the two highest test concentrations (100 and 200 mg prod./L) the fish showed the following behavioural observations: inactive or abnormally low activity, laboured respiration, remaining for unusually long periods on the bottom of the aquarium, loss of equilibrium with lateral deviation from their normal orientation or turning in a vertical position.

Table 10.2.1- 6: 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)
12.5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
25.0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
50.0	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
100	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
200	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

III. Conclusion

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

LC_{50} - 96 hours (95 % C.I.):	**> 200 mg prod./L** (not determined)
NOEC - 96 hours: highest concentration without an effect (based on mortality)	200 mg prod./L
NOEC - 96 hours: highest concentration without an effect (based on sublethal effects)	25.0 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) > 200 mg prod./L (corresponding to ~ > 84 mg a.s./L)

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Data Point:	KCP 10.2.1/07
Report Author:	[redacted]
Report Year:	2010
Report Title:	Acute toxicity of fluopyram SC 500B G to the waterflea Daphnia magna in a static laboratory test system
Report No:	EBGMP261
Document No:	M-366819-01-1
Guideline(s) followed in study:	OECD guideline 202,(2004); EEC Directive 92/69/EEC, part C.2 (1992)
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 SC 500 in groups of 30 (6 replicates of 5 organisms per test level) to the nominal concentrations of 9.53, 17.2, 30.9, 55.6 and 100 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 – UV on day 0 and 2. Recoveries on day 0 and 2 ranged in the three highest test concentrations (30.9, 55.6 and 100 mg prod./L, nominal concentrations) from 85.5 to 111 % of nominal values. The two lowest test concentrations (9.53 and 17.2 mg prod./L) revealed increased contents of fluopyram in aqueous solution with recoveries between 127 and 154 % of nominal values. Nevertheless, since these test concentrations caused no biological effects within 48 hours and since all biological-effects-concentrations (30.9 - 100 mg prod./L) were located well within the recommended 80-120 % of nominal, all results, including EC_{50}, are based on nominal test concentrations. No residues of fluopyram were found in the control samples higher than 0.207 mg 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. Immobility effects were observed after 24 hours at the 2 highest test concentrations (55.6 and 100 mg prod./L). After 48 hours daphnids were immobile at the 3 highest test concentrations (30.9, 55.6 and 100 mg prod./L)

The endpoint based on nominal concentrations was: EC_{50} – 48 hours (95 % C.I.): 141 mg prod./L (78.7 – 251 mg prod./L).

I. MATERIAL AND METHODS

Test material	FLU SC 500 Specification No.: 102000018148-01 Batch No.: 2009-004770 content of a.s.: 42.0 % w/w fluopyram (507 g a.s./L)
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: 9.53 – 17.2 – 30.9 – 55.6 – 100 mg prod./L (Mean measured recoveries based on a.s. content ranged from 85.5 to 154 % of nominal a.s. concentrations). Control: water Evidence of undissolved material: In the three lowest test concentrations (9.53, 17.2 and 30.9 mg prod./L), there were no remarkable observations and the test media were clear. In the two highest test concentrations (55.6 and 100 mg prod./L) the test item was lying on the bottom of the test beakers after 24 and 48 hours.
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: 20.1 - 20.4 °C Photoperiod: 16 hours light, 8 hours dark Light intensity: max. 1200 lux pH: 7.9 - 8.0 Water hardness: 249 mg/L as $CaCO_3$ (at test start) Dissolved oxygen: 7.8 - 8.3 mg/L (91.5 - 92.5 % of saturation) Conductivity: 566 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 preparation of test concentrations, 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 in media from the pooled replicates 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 exposure. Light intensity was measured at start of the study as „diffuse light“, immediately above the water surface of the test vessels.
Chemical analysis	Samples were taken at test start and at test end. The chemical analyses were performed by high-performance liquid chromatograph– UV (HPLC-UV).
Data analysis	For EC_{50} determination, a dose response relationship curve (displayed as sigmoid, shaped over the logarithm of the concentration) was modelled by Probit Analysis after Finney fitted by an iterative weighed linear regression according to the Maximum Likelihood principle which allows computation of EC_{50} and 95 % confidence limits for immobility rates if possible (mathematical limits based on quality of the dose-response pattern). The 95 % confidence intervals are calculated according to the method described and published by Fieller (1944) and Finney (1972). Calculations (mean and standard deviation) were performed using Microsoft Excel. The statistical analysis was carried out using the ToxRat Professional® Software (Vers.2.09, ToxRat Solutions GmbH, Germany).

II. RESULTS AND DISCUSSION

Table 10.2.1- 7: 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	7.9 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 in the three highest test concentrations (30.9, 55.6 and 100 mg prod./L, nominal concentrations) from 85.5 to 111 % of nominal values. The two lowest test concentrations (9.53 and 17.2 mg prod./L) revealed increased contents of fluopyram in aqueous solution with recoveries between 127 and 154 % of nominal values. Nevertheless, since these test concentrations caused no biological effects within 48 hours and since all biological-effects-concentrations (30.9 - 100 mg prod./L) were located well within the recommended 80-120 % of nominal, all results, including EC_{50}, are based on nominal test concentrations.

No residues of fluopyram were detected in the control samples higher than 0.207 mg a.s./L, which was used as the lowest standard concentration during this study.

Table 10.2.1- 8: Analytical results

Nominal concentration		Measured concentration [mg a.s./L]		% of nominal	
[mg prod./L]	[mg a.s./L]	Day 0 (New)	Day 2 (Aged)	Day 0 (New)	Day 2 (Aged)
9.53	2.63	3.82 [A]	4.05 [A]	145.2	154.0
17.2	5.25	6.67 [A]	6.89 [A]	127.0	131.1
30.9	10.5	11.7	11.4	111.4	108.6
55.6	21.0	19.1	20.0	91.0	95.2
100	42.0	36.0	35.9	85.7	85.5

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

Biological results:

Observations

No immobility or other effects on behaviour were observed in the control within 48 hours of exposure.

Immobility effects were observed after 24 hours in the 2 highest test concentrations (55.6 and 100 mg prod./L). After 48 hours daphnids were immobile in the 3 highest test concentrations (30.9, 55.6 and 100 mg prod./L).

Table 10.2.1- 9: Immobilisation of daphnids

Nominal concentration [mg prod./L]	No. of immobilized (cumulative %)	
	Exposure time	
	24 h	48 h
Control	0 (0)	0 (0)
9.53	0 (0)	0 (0)
17.2	0 (0)	0 (0)
30.9	0 (0)	1 (3.3)
55.6	2 (6.7)	6 (20)
100	3 (10)	10 (33.3)

III. CONCLUSION

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

EC_{50} – 48 hours (95 % C.I.):	**141 mg prod./L** (78.7 - 251 mg prod./L)
EC_{50} – 24 hours (95 % C.I.):	Not determined [A]

[A] Not determined due to minor effects (10 % immobilisation) at the highest test concentration (100 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) = 141 mg prod./L (corresponding to ~ = 59.2 mg a.s./L)

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Data Point:	KCP 10.2.1/08
Report Author:	[redacted]
Report Year:	2008
Report Title:	Pseudokirchneriella subcapitata growth inhibition test with fluopyram SC 500 G
Report No:	EBGMP159
Document No:	M-299910-01-1
Guideline(s) followed in study:	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006)
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 SC 500 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 0.954, 3.05, 9.77, 31.3, and 100 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 – UV on day 0 and 3 for each concentration and control. Measured concentrations were in the 86-98 % range of nominal concentrations and no residues were found in the control samples above the lowest standard concentration (0.0417 mg a.s./L). The biological results are based on nominal concentrations of FLU SC 500.

The study fulfils all validity criteria of OECD 201 guideline.

No physical abnormalities were observed in the controls or treatment groups during the study.

The 72 hour- endpoints based on nominal product concentrations were: 72 hour – E_rC_{50}: 16.1 mg prod./L, 72 hour – E_bC_{50}: 10.3 mg prod./L and 72 hour – E_yC_{50}: 8.39 mg prod./L.

I. MATERIAL AND METHODS

Test material	FLU SC 500 G Specification No.: 102000018148 Batch ID: 2007-011657 Content of a.s.: 42.2 % w/w (501 g fluopyram/L) Density: 1.188 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: 0.954 – 3.05 – 9.77 – 31.3 – 100 mg prod./L Mean measured recoveries based on a.s. content ranged from 86 to 98 % of nominal a.s. concentrations. 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: 21.5 - 22.0 °C Photoperiod: 24 hours light Light intensity: 6190 - 7570 lux Type of light: bank light containing cool white fluorescent lamps pH of control: 8.1 - 8.9 Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	The 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. Also, 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 HPLC – UV.
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- 10: 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	81.7
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 %	32.7 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	< 7 %	1.5 %

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 day 3 were in the range between 86 and 98 % of nominal concentrations (see table below). Therefore, biological results are based on nominal concentrations of FLU SC 500.

No residues of fluopyram were detected in the control samples in a concentration higher than 0.0417 mg a.s./L, which was used as the lowest standard concentration during the study.

Table 10.2.1- 11: Analytical results

Nominal concentration		Measured a.s. concentration [mg a.s./L]		% of nominal	
[mg prod./L]	[mg a.s./L]	Day 0	Day 3	Day 0	Day 3
0.954	0.403	0.390	0.397	97	98
3.05	1.29	1.21	1.23	94	96
9.77	4.12	3.58	4.00	87	97
31.3	13.2	14.4	11.9	86	90
100	42.2	37.5	36.4	89	86

Biological results:

Observations:

No physical abnormalities were observed in the controls or treatment groups during the study.

Table 10.2.1- 12: Cell density

Nominal product concentration [mg prod./L]	Mean cell density [x 10^4 cells/mL]		
	24 h	48 h	72 h
Control	3.0	14.3	81.7
0.954	3.2	17.7	84.0
3.05	2.0	16.5	79.3
9.77	2.3	11.8	35.8
31.3	2.2	1.7	1.8
100	1.2	1.5	0.7

Table 10.2.1- 13: Algae growth rate

Nominal product concentration [mg prod./L]	Mean growth rate [1/d]	% Inhibition [A]
	0 - 72 h	0 - 72 h
Control	1.467	-
0.954	1.474	-0.5
3.05	1.448	1.3
9.77	1.191 *	18.8
31.3	0.179 *	87.8
100	-0.154 *	111

A -% inhibition means increase in growth relative to the control

* Significantly (α=0.05, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure or Welch-t test for inhomogeneous variances with bonferroni adjustment

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.):	**16.1 mg prod./L** (15.6 – 16.7 mg prod./L)
E_rC_{20} -72 hours (95 % C.I.):	10.0 mg prod./L (9.58 – 10.5 mg prod./L)
E_rC_{10} -72 hours (95 % C.I.):	7.80 mg prod./L (7.36 -8.22 mg prod./L)
E_bC_{50} -72 hours (95 % C.I.):	10.3 mg prod./L (9.70 – 11.0 mg prod./L)
E_bC_{20} -72 hours (95 % C.I.):	6.22 mg prod./L (5.55 – 6.80 mg prod./L)
E_bC_{10} -72 hours (95 % C.I.):	4.76 mg prod./L (4.07 – 5.36 mg prod./L)
E_yC_{50} - 72 hours (95 % CI): [A]	**8.39 mg prod./L** (6.59 – 10.35 mg prod./L)
E_yC_{20} - 72 hours (95 % C.I.): [A]	4.49 mg prod./L (2.40 – 5.90 mg prod./L)
E_yC_{10} -72 hours (95 % C.I.): [A]	3.11 mg prod./L (1.25 – 4.49 mg prod./L)
LOE_rC - 72 hours: lowest concentration with an effect (based on growth rate)	9.77 mg prod./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate and yield [A])	3.05 mg prod./L

A Please refer to recalculation document M-757704-01-1

Reliability assessment (EPSA 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	7.8	7.36 – 8.22	0.110 (excellent)	$EC_{10} < EC_{20}$, low (high)
Yield	3.11	1.25 – 4.49	1.042 (poor)	EC_{20} low < EC_{10} < EC_{50}, low (medium)
Biomass	4.76	4.07 – 5.36	0.221 (good)	$EC_{10} < EC_{20}$, low (high)

Assessment and conclusion by applicant:

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

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Data Point:	KCP 10.2.1/09
Report Author:	[redacted]
Report Year:	2020
Report Title:	Statistical evaluation (non-GLP) of the study M-299910-01-1 (Dorgerloh, M., 2008, EBGMP159) on the chronic toxicity of fluopyram SC 500 G to Pseudokirchneriella subcapitata (currently known as: Raphidocelis subcapitata) under static conditions
Report No:	M-757704-01-1
Document No:	M-757704-01-1
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-299910-01-1 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 logit 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- 14: Re-calculated EC_{10}, EC_{20}, EC_{50} and NOEC values based on nominal concentrations

Endpoint	Fluopyram SC 500 G [mg product/L]	Fluopyram [mg a.s./L]
	Yield	Yield
72 hours - EC_{10} (95 % C.I.)	3.11 (1.25 – 4.49)	1.31 (0.53 – 1.90)
72 hours - EC_{20} (95 % C.I.)	4.49 (2.40 – 5.90)	1.89 (1.01 – 2.49)
72 hours - EC_{50} (95 % C.I.)	8.39 (6.59 – 10.35)	3.54 (2.78 – 4.36)
72 hours - NOEC	3.05	1.29

C.I.: Confidence interval

Assessment and conclusion by applicant:

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

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Data Point:	KCP 10.2.1/10
Report Author:	[redacted]
Report Year:	2010
Report Title:	Pseudokirchneriella subcapitata growth inhibition test with fluopyram SC 500B G
Report No:	EBGMP262
Document No:	M-367124-03-1
Guideline(s) followed in study:	OECD Guideline 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (March 23, 2006)
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 SC 500 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 0.960, 3.06, 9.80, 31.3 and 100 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 – UV on day 0 and 3 for each concentration and control. Measured concentrations were in the 92 - 105 % range of nominal concentrations and no residues were found in the control samples above the lowest standard concentration (0.0388 mg a.s./L). The biological results are based on nominal concentrations of FLU SC 500.

The study fulfils all validity criteria of OECD 201 guideline.

No morphological change in algae was observed in any test concentration and the control.

The 72 hour- endpoints based on nominal product concentrations were: 72 hour – E_rC_{50}: 14.6 mg prod./L, 72 hour – E_bC_{50}: 8.82 mg prod./L and 72 hour – E_yC_{50}: 9.00 mg prod./L.

I. MATERIAL AND METHODS

Test material	FLU SC 500 Specification No.: 102000018148 Batch ID: 2009-004770 Content of a.s.: 42.0 % w/w (501 g/L) Density: 1.193 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: 0.960 – 3.06 – 9.80 – 31.3 – 100 mg prod./L Mean measured recoveries based on a.s. content ranged from 92 to 105 % of nominal a.s. concentrations. 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: 21.3 - 22.0 °C Photoperiod: 24 hours light Light intensity: 7630 - 8320 lux Type of light: bank light containing cool white fluorescent lamps pH of control: 7.8 - 8.1 Conductivity: not reported Growth medium same as culture medium: Yes
Parameters Measured / Observations	pH values were measured at test start, daily afterwards. 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) – UV.
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	23.2
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 %	31.7 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	< 7 %	6.3 %

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 day 3 were in the range between 92 and 105 % of nominal concentrations (see table below). Therefore, biological results are based on nominal concentrations of FLU SC 500.

No residues of fluopyram were detected in the control samples above 0.0388 mg a.s./L, which was used as the lowest standard concentration during the study.

Table 10.2.1- 16: Analytical results

Nominal concentration		Measured a.s. concentration [mg a.s./L]		% of nominal	
[mg prod./L]	[mg a.s./L]	Day 0	Day 3	Day 0	Day 3
0.96	0.403	0.383	0.397	95	99
3.06	1.29	1.32	1.32	102	102
9.80	4.12	3.78	3.81	92	92
31.3	13.1	12.9	13.4	98	102
100	42.0	38.9	43.9	93	105

Biological results:

Observations:

No morphological change in algae was 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	3.1	9.1	23.4
0.96	4.5	10.0	26.2
3.06	4.2	6.0	25.2
9.80	3.0	3.3	10.5
31.3	1.5	1.5	1.7
100	0.8	1.2	1.0

Table 10.2.1- 18: Algae growth rate

Nominal concentration [mg prod./L]	Mean growth rate [1/d]	% Inhibition A
	0 - 72 h	0 - 72 h
Control	1.042	
0.96	1.083	-3.9
3.06	1.075	-3.1
9.80	0.784	24.8 *
31.3	0.202	80.2 *
100	0.000	100 *

A -% inhibition means increase in growth relative to the control

* Significantly (α=0.05, one-sided smaller) reduced, based on Williams multiple sequential t-test procedure or Welch-t test for inhomogeneous variances with bonferroni adjustment

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.):	**14.6 mg prod./L** (14.5 -14.8 mg prod./L)
E_rC_{20} -72 hours (95 % C.I.):	8.93 mg prod./L (8.83 - 9.03 mg prod./L)
E_rC_{10} -72 hours (95 % C.I.):	6.89 mg prod./L (6.78 - 6.99 mg prod./L)
E_bC_{50} -72 hours (95 % C.I.):	**8.82 mg prod./L** (7.85 - 9.82 mg prod./L)
E_bC_{20} -72 hours (95 % C.I.):	4.90 mg prod./L (3.88 - 5.73 mg prod./L)
E_bC_{10} -72 hours (95 % C.I.):	3.60 mg prod./L (2.61 - 4.42 mg prod./L)
E_yC_{50} - 72 hours (95 % CI): [A]	**9.00 mg prod./L** (8.13 – 9.52 mg prod./L)
E_yC_{20} - 72 hours (95 % CI): [A]	5.95 mg prod./L (3.77 – 7.01 mg prod./L)
E_yC_{10} - 72 hours (95 % CI): [A]	4.79 mg prod./L (2.50 – 6.05 mg prod./L)
LOEC - 72 hours: lowest concentration with an effect (based growth rate and biomass)	9.80 mg prod./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate, biomass and yield) [A]	3.06 mg prod./L

[A] Please refer to recalculation document M-787717-01-1

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	7.8	7.36 – 8.22	0.110 (excellent)	EC_{10} < EC_{20}, low (high)
Yield	3.11	1.25 – 4.49	1.042 (poor)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)
Biomass	4.76	4.07 – 5.36	0.271 (good)	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) = 14.6 mg prod./L (corresponding to ~ = 6.13 mg a.s./L)

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Data Point:	KCP 10.2.1/11
Report Author:	[redacted]
Report Year:	2020
Report Title:	Statistical evaluation (non-GLP) of the study M-367124-03-1 (Bruns, E., 2010, EBGMP262) on the chronic toxicity of fluopyram SC 500 G to Pseudokirchneriella subcapitata (currently known as: Raphidocelis subcapitata) under static conditions
Report No:	M-757717-01-1
Document No:	M-757717-01-1
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-367124-03-1 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	Fluopyram SC 500 [mg product/L]	Fluopyram [mg a.s./L]
	Yield	Yield
72 hours - EC_{10} (95 % C.I.)	4.79 (2.50 – 6.05)	2.02 (1.06 – 2.54)
72 hours - EC_{20} (95 % C.I.)	5.95 (3.77 – 7.01)	2.50 (1.60 – 2.95)
72 hours - EC_{50} (95 % C.I.)	9.00 (8.13 – 9.52)	3.78 (3.42 – 4.00)
72 hours - NOEC	3.06	1.29

C.I. Confidence interval

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) = 14.6 mg prod./L (corresponding to ~ = 6.13 mg a.s./L)

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Data Point:	KCP 10.2.1/12
Report Author:	[redacted]
Report Year:	2020
Report Title:	FLU SC500 - Toxicity to the aquatic plant Lemna gibba in a static growth inhibition test
Report No:	EBGM0677
Document No:	M-758230-01-1
Guideline(s) followed in study:	Commission Regulation (EC) No 761/2009, Annex, Part C, C.26.: "Lemna sp. Growth Inhibition Test", Official Journal of the European Union (EN), dated August 24, 2009 − EPA Guideline 712-C-008: OCSPP 850.4400, "Aquatic Plant Toxicity Test Using Lemna spp.", January 2012. − OECD Guidelines for the Testing of Chemicals, No. 221: "Lemna sp. Growth Inhibition Test", adopted March 23, 2006 − SANCO/3029/99 rev.4 11/07/00: Residues: Guidance for generating and reporting methods of analysis in support of pre-registration 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: OECD 221 (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 duckweed *Lemna gibba* was exposed to FLU SC 500 under static conditions for 7 days. 12 fronds per test vessel were used to test the nominal concentrations of 60, 19, 6.0, 1.9, 0.6 and 0.19 mg prod./L (corresponding to 25.38, 8.037, 2.538, 0.804, 0.2538 and 0.0804 mg a.s./L). The study design included 4 replicates for each test concentration and control. Observations and frond counts were done on days 3, 5 and at test termination (day 7). At test termination, frond density for each replicate treatment and control vessels were determined.

Concentrations of fluopyram were verified by LC-MS/MS on day 0 and day 7 for each concentration and control. Measured concentrations were in the 83 - 105 % range of nominal concentrations and no residues were found in the control samples above the limit of detection (LOD: 0.005 µg a.s./L). The biological results were based on nominal concentrations of the product.

The study fulfils all validity criteria of OECD 221 guideline.

No visual effects were observed in the three lowest test concentrations (0.19, 0.6 and 1.9 mg prod./L). At the three highest test concentrations the fronds showed deviations from the control replicates after 7 days; i.e. gibbous growth (6.0, 19 and 60 mg prod./L), slightly overlapping fronds (6.0 mg prod./L), smaller fronds (6.0, 19 and 60 mg test item/L), shortened roots (19 and 60 mg prod./L), necrosis (19 and 60 mg prod./L), chlorosis (19 and 60 mg prod./L)and separated fronds (19 and 60 mg prod./L).

Endpoints based on nominal concentrations of the product were: E_rC_{50} (based on frond numbers) (95 % C.I.): 16.2 mg prod./L (15.4 – 16.9 mg prod./L); E_rC_{50} (based on dry weight) (95 % C.I.): 35.3 mg prod./L (27.7 – 47.6 mg prod./L); E_yC_{50} (based on frond number) (95 % C.I.): 11.9 mg prod./L (11.3 – 12.3 mg prod./L) and E_yC_{50} (based on dry weight) (95 % C.I.): 12.5 mg prod./L (10.8 – 13.9 mg prod./L).

I. MATERIAL AND METHODS

Test material	FLU SC 500 Specification No.: 102000018148 Batch No.: EV57002782 Content of a.s.: 42.3 % w/w (502.7 g/L) Density: 1.189 g/mL
Guideline(s) adaptation	Not specified
Test species	Duckweed (*Lemna gibba*) strain G3
Acclimation	Inoculum pre-culture, preparation 7 days before the start of the main test. Cultivation under the same conditions as in main test
Culturing conditions	Growth medium: 20X AAP medium Culturing under test conditions
Test solutions	Nominal concentrations: 60 – 19 – 6.0 – 1.9 – 0.6 – 0.19 mg prod./L Corresponding nominal concentrations of fluopyram: 25.38 – 8.037 - 2.538 - 0.804 - 0.2538 - 0.0804 mg a.s./L Control: water Evidence of undissolved material: At the highest test concentration (60 mg prod./L) slightly pronounced colouration was observed at test start, and then slightly pronounced turbidity of the test medium was observed on days 3, 5 and 7.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Organisms per replicate	No. of fronds per vessel: 12
Exposure	Static Total exposure duration: 7 days.
Test conditions	Incubation chamber used: not specified Vessels: 250-mL glass dishes with 200 ml test solution Temperature: 23.2 – 24.8 °C Photoperiod: permanent light Light intensity: 6560 - 7900 lux (mean: 7117 lux) Type of light: fluorescent tubes pH of controls: 7.5 - 8.8 Water hardness: not reported Dissolved oxygen: not reported Conductivity: < 5 µS/cm Growth medium: 20X AAP
Parameters Measured / Observations	Determination of frond number was made on days 0, 3, 5 and 7. Visual observations of sub-lethal effects were performed on days 3, 5 and 7.

	Temperature was determined daily in one additional incubated glass vessel. The pH of each treatment was measured at test start and test end. The light was measured at least once during the test.
Sampling for chemical analysis	Duplicate samples were taken from all freshly prepared test media on day 0 including control. On day 7 duplicate samples were collected from all aged test levels including control by pouring together the contents of each treatment. Samples were analysed by using a LC-MS/MS.
Data analysis	ECx calculations were performed by probit analysis. LOEC and NOEC values were determined by the Williams t-test (frond number and dry weight) to detect significant differences between test concentrations and control. All statistical evaluations were done using the commercial program ToxRat Professional (version 3.3.0)

II. RESULTS AND DISCUSSION

Table 10.2.1- 20: Validity criteria

Validity criteria (OECD 221)	Required	Obtained
Doubling time	2.5 days	1.6 days

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 ranged between 99 and 105 % of nominal concentrations and on day 7 between 83 and 105 %. All biological results are based on nominal test concentrations of the formulation.

Fluopyram was not detected in the control samples above the limit of detection (LOD: 0.005 µg a.s./L).

Table 10.2.1- 21: Analytical results

Nominal concentration		Measured concentration [A] [µg a.s./L]		% of nominal	
[mg prod./L]	[µg a.s./L]	Day 0	Day 7	Day 0	Day 7
0.19	80.4	80.3	81.1	100	101
0.6	253.8	251.3	255.1	99	101
1.9	804	816.4	822.8	102	102
6.0	2538	2614.6	2652.8	103	105
19	8037	8132.5	8164.3	101	102
60	25380	26591.7	20994.5	105	83

[A] Not given in report. Calculations based on two measured samples.

Biological results:

Observations

No visual effects were observed in the three lowest test concentrations (0.19, 0.6 and 1.9 mg prod./L). At the higher test concentrations the fronds showed deviations from the control replicates after 7 days: i.e. gibbous growth (6.0, 19 and 60 mg prod./L), slightly overlapping fronds (6.0 mg prod./L), smaller fronds (6.0, 19 and 60 mg test item/L), shortened roots (19 and 60 mg prod./L), necrosis (19 and 60 mg prod./L), chlorosis (19 and 60 mg prod./L)and separated fronds (19 and 60 mg prod./L).

Table 10.2.1- 22: Results for frond number and corresponding growth rates and inhibitions

Nominal concentration		Mean [A] frond number Day 7	Mean [A] dry weight Day 7	Growth rate for frond number 0-7 days		Growth rate for dry weight 0-7 days	
[mg prod./L]	[µg a.s./L]			Mean [A] [1/d]	% Inhibition [B]	Mean [A] [1/d]	% Inhibition [B]
Control		241.0	32.8	0.429	-	0.461	
0.19	80.4	254.0	34.8	0.436	-1.7	0.469	-1.8
0.6	253.8	262.8	35.1	0.441	-2.9	0.471	-2.1
1.9	804	257.8	34.4	0.438	-2.1	0.467	-1.3
6.0	2538	237.3	31.3	0.426	0.6	0.454	1.5
19	8037	36.8	6.8	0.159	62.8 *	0.236	48.9 *
60	25380	14.0	3.4	0.022	94.9 *	0.203	56.0 *

A Mean value of 4 replicates
B -% inhibition means increase in growth relative to the control
* Significantly different from the control (based on Williams t-test, α= 0.05, one-sided smaller)

Table 10.2.1- 23: Results for yield based on frond number and corresponding % inhibitions

Nominal concentration		Yield based on frond number Day 7		Yield based on dry weight Day 7	
[mg prod./L]	[µg a.s./L]	Mean [A]	% Inhibition [B]	Mean [A]	% Inhibition [B]
Control		229.0	-	31.5	-
0.19	80.4	242.0	-5.7	33.5	-6.4
0.6	253.8	250.8	-9.7	33.8	-7.1
1.9	804	245.8	-7.3	33.1	-5.0
6.0	2538	225.3	1.6	30.0	4.9
19	8037	24.8	89.2	5.5	82.6 *
60	25380	2.0	99.1	4.1	87.0 *

A Mean value of 4 replicates
B -% inhibition means increase in yield relative to the control
* Significantly different from the control (based on Williams t-test, α= 0.05, one-sided smaller)

III. CONCLUSION

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

Endpoint (Day 0-7)	Effect on mean growth rate of frond number	Effect on mean growth rate of dry weight
E_rC_{50} (95 % C.I.):	**16.2 mg prod./L** (15.4 – 16.9 mg prod./L)	**35.3 mg prod./L** (27.7 – 47.6 mg prod./L)
E_rC_{20} (95 % C.I.)	10.7 mg prod./L 9.20 - 11 mg prod./L	9.49 mg prod./L (5.84 - 13.0 mg prod./L)
E_rC_{10} (95 % C.I.)	8.56 mg prod./L (7.00 - 9.80 mg prod./L)	4.78 mg prod./L (2.31 - 7.35 mg prod./L)
LOE_rC: lowest concentration with an effect	6.0 mg prod./L	6.0 mg prod./L
NOE_rC: highest concentration without an effect	19 mg prod./L	19 mg prod./L

Endpoint (Day 0 - 7)	Effect on mean yield of frond number	Effect on mean yield of dry weight
E_yC_{50} (95 % C.I.):	**11.9 mg prod./L** (11.3 – 12.3 mg prod./L)	**12.5 mg prod./L** (10.8 – 13.9 mg prod./L)
E_yC_{20} (95 % C.I.:	8.60 mg prod./L (8.05 – 9.11 mg prod./L)	8.34 mg prod./L (6.74 – 9.97 mg prod./L)
E_yC_{10} (95 % C.I.):	7.27 mg prod./L (6.72 – 7.78 mg prod./L)	7.01 mg prod./L (5.21 – 8.46 mg prod./L)
LOE_yC: lowest concentration with an effect	6.0 mg prod./L	6.0 mg prod./L
NOE_yC: highest concentration without an effect	19 mg prod./L	19 mg prod./L

Reliability assessment (EFSA 2015)

The following table provides reliability indicators for EC_{10} values for *Lemna gibba*.

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate, Frond Number	8.56	7.0 – 9.8	0.327 (good)	$EC_{10} < EC_{20}$, low (high)
Growth Rate, Dry Weight	4.78	2.31 – 7.35	1.054 (poor)	$EC_{10} < EC_{20}$, low (high)
Yield, Frond Number	7.27	6.72 – 7.78	0.146 (excellent)	$EC_{10} < EC_{20}$, low (high)
Yield, Dry Weight	7.01	5.21 – 8.46	0.464 (good)	EC_{20}, low < EC_{10} < EC_{50}, low (medium)

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) = 16.2 mg prod./L (corresponding to ~ = 6.88 mg a.s./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 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 (please refer to MCA, Section 8):

- 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)
- Chronic 10-day toxicity test with the solo formulation FLU SC 500 on adult honeybees under laboratory conditions (OECD 245; M-540072-01-1)
- Toxicity to honeybee larvae under laboratory conditions following repeated exposure (OECD guidance document 239; M-617279-01-1)
- One semi-field study using a special design to determine potential adverse acute, short-term and long-term effects on honey bees (*Apis mellifera* L.) and honey bee colonies, during and after continuous exposure exclusively to fluopyram-fortified carbohydrate and protein diet for a period of two complete honey bee brood cycles (6 weeks, 42 days) (this semi-field study is presented in KCA Section 8, Point 8.3.1.3/03, M-549350-01-2)
- One semi-field brood study following OECD guidance document 75 (using a more realistic spray scenario onto flowering *Phacelia* covering effects on mortality, foraging activity as well as general colony development) with the solo formulation FLU SC 500. This semi-field study is presented in KCP Section 10, Point 10.3.15/01, M-532474-01-1.
- One semi-field study following EPPO 170 with the solo formulation FLU 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 10.3.1.5/02, M-547034-01-1.

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

Test substance	Test species/ study type	Endpoint		References
Laboratory				
Fluopyram tech.	*Apis mellifera*, acute test	LD_{50} oral (48 h) LD_{50} contact (48 h)	**> 102.3 µg a.s./bee** **> 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

Test substance	Test species/ study type	Endpoint	References
	Bombus terrestris, acute test	LD_{50} contact (48 h) > 100 µg a.s./bumble bee	[redacted] (2015) M-510849-01-1 KCA 8.3.1.2/04
	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
	Apis mellifera, larva 22-day repeated feeding test	NOEC ≥ 520 mg a.s./kg diet NOED ≥ 80.1 µg a.s./larva EC_{10} 390 mg a.s./kg diet ED_{10} 60.1 µg a.s./larva EC_{20} 511 mg a.s./kg diet ED_{20} 78.7 µg a.s./larva EC_{50} > 520 mg a.s./kg diet ED_{50} > 80.1 µg a.s./larva	[redacted] (2018) M-617229-01-1 KCA 8.3.1.3/01
FLU SC 500	*Apis mellifera*, acute test	LD_{50} oral (48 h) **> 220 µg a.s./bee** LD_{50} contact (48 h) **> 200 µg a.s./bee**	[redacted] (2020) M-704653-01-1 KCP 10.3.1.1.1/02 KCP 10.3.1.1.2/02
	Bombus terrestris, acute test	LD_{50} oral (48 h) > 232.4 µg a.s./bumble bee LD_{50} contact (48 h) > 200 µg a.s./bumble bee	[redacted] (2020) M-690268-01-1 KCP 10.3.1.1.1/03 KCP 10.3.1.1.2/03
	Apis mellifera, 10-day oral feeding test	LDD_{50} > 81.4 µg a.s./bee/day NOEDD 81.4 µg a.s./bee/day LC_{50} > 3333 mg a.s./kg diet NOEC 3333 mg a.s./kg diet	[redacted] (2015) M-540072-01-1 KCA 8.3.1.2/01 KCP 10.3.1.2/01
Higher Tier			
Fluopyram tech.	*Apis mellifera*, Semi field honey bee feeding study with post-exposure field observation period	Overall, no adverse acute, short-term and long-term effects on mortality, colony strength and colony development, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality and colony health, as well as on overwintering performance after continuous exposure of honey bee colonies under confined conditions to a fluopyram-concentration of 10000 µg a.s./kg diet for a period of 6 consecutive weeks during springtime/early summer.	[redacted] (2016) M-549350-01-2 KCA 8.3.1.3/03
FLU SC 500	Semi-field honey bee brood study (according to OECD 75, forced exposure conditions)	Overall, no adverse effects on honey bee behaviour, brood development, colony strength and queen survival after one application of 250 g a.s./ha onto flowering *Phacelia tanacetifolia,* during active foraging of honeybees	[redacted] (2015) M-532474-01-1 KCP 10.3.1.5/01
	Honeybee colony development – Semi-Field (EPPO 170)	Overall, no adverse 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 after two sequential foliar applications at a rate of 250 g a.s./ha each onto blooming *Phacelia tanacetifolia.*	[redacted] (2016) M-547034-01-1 KCP 10.3.1.5/02

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

Risk assessment for bees

The risk assessment for bees for fluopyram is based on the application rates of one time 0.154 L product/ha corresponding to the maximum single application rate of 75 g a.s./ha for the application in apples using the endpoints (LD_{50} values) for the active substance fluopyram and the formulation FLU SC 500.

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./ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./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: $$Q_{HO} = \frac{\text{maximum application rate}}{LD_{50}\text{ oral}} = \frac{\text{[g a.s./ha or g product/ha]}}{\text{[µg a.s./bee or µg product/ bee]}}$$

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

Table 10.3.1- 2: Hazard quotients for bees for the application in apples, 1 x 75 g a.s./ha– oral exposure

Compound	Oral LD_{50} [µg a.s./bee]	Max. application rate [g/ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Fluopyram tech.	> 102.3	75	0.73	50	yes
FLU SC 500	> 220	75	< 0.34	50	yes

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 the application in apples, 1 x 75 g a.s./ha – contact exposure

Compound	Contact LD_{50} [µg a.s./bee]	Max. application rate [g a.s./ha]	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
Fluopyram tech.	> 100	75	< 0.75	50	yes
FLU SC 500	> 200	75	< 0.38	50	yes

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 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 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 apples 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 SC 500). Hence, the findings indicate that bumble bees do not exhibit greater sensitivity to FLU SC 500 or fluopyram tech. compared to the honey bee. The risk assessment for honeybees was therefore considered to be protective of other bees and to cover the exposure of non-*Apis* bees such as *Bombus terrestris*.

Chronic adult toxicity

A 10-day laboratory feeding study investigating the effects of fluopyram (administered as formulated product FLU SC 500) was conducted to assess chronic toxicity to honey bees in accordance with OECD Guideline No. 245. A solo SC formulation was chosen in place of technical material to enable chronic administration of fluopyram in a 50 % sugar solution and to overcome any solubility issues that may have occurred by using technical fluopyram with organic solvents.

The study concluded that continuous *ad libitum* feeding at 3333 mg a.s./kg diet over a period of 10 days led to 10 % mortality. The LDD_{50} was determined as > 81.4 µg a.s./bee/day. The NOEDD was identified at 81.4 µg a.s./bee/day. Daily dosing with 81.4 µg a.s./bee/day over 10 days (total dose = 814 µg a.s./bee) thus did not induce higher mortality compared to a single acute oral exposure at 102.3 µg a.s./bee. Therefore, study results do not indicate delayed or cumulative toxicity effects following chronic exposure to fluopyram compared with acute testing. Details of the study are presented together with the ecotoxicological endpoints in MCA, Section 8, Point 8.3.1.2/01, M-540072-01-1.

Chronic larval toxicity/effects on brood

A honey bee larval toxicity test assessing the effect of fluopyram 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 32.5, 65.0, 130, 260 and 520 mg a.s./kg diet. The corresponding cumulative doses were 5.01, 10.0, 20.0, 40.0 and 80.1 µg a.s./larva. The 22-day NOED (emergence) was determined to be ≥ 80.1 µg a.s./larva (corresponding NOEC of ≥ 520 mg a.s./kg diet), indicating no risk to honey bee development. Details of the study are presented together with the ecotoxicological endpoints in MCA, Section 8, Point 8.3.1.3/01, M-617279-01-1.

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 SC 500, further assessment of the chronic risk to adult bees and larvae is derived through findings from higher tier studies.

In order to investigate whether fluopyram would pose an acute, short-term and long-term risk on honey bees (*Apis mellifera* L.) and honey bee colonies, a semi-field study was conducted with fluopyram tech. by exposing honey bee colonies exclusively to fluopyram-fortified carbohydrate and protein diet (M-549350-01-2). Based on the results of the study no adverse acute, short-term and long-term effects on mortality, colony strength and colony development, brood development, food storage, honey bee

behaviour, queen survival, overall hive vitality and colony health, as well as on overwintering performance after continuous exposure of honey bee colonies under confined conditions to a fluopyram-concentration of 10000 µg a.s./kg diet. for a period of 6 consecutive weeks during springtime/early summer were detected.

Moreover, a semi-field honey bee study (according to the provisions of the OECD Guidance Document 75 in combination with the OEPP/EPPO Guideline No. 170(4) (2010)) was conducted with the representative formulation FLU SC 500 under forced/confined exposure conditions (KCP 10.3.1.5; M-532474-01-1) to clarify whether fluopyram poses a risk to honey bee brood and colony development under realistic worst-case conditions.

Furthermore, a semi-field honey bee study (according to OEPP/EPPO guideline No. 170(4)) with the representative formulation FLU SC 500 was conducted under forced/confined exposure conditions (KCP 10.3.1.5; M-547034-01-1). with two sequential foliar applications onto blooming *Phacelia tanacetifolia* at a rate of 250 g a.s./ha.

In both semi-field studies (KCP 10.3.1.5; M-532474-01-1 and KCP 10.3.1.5; M-547034-01-1) 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 after one or two applications of 250 g a.s./ha onto flowering *Phacelia tanacetifolia*.

It can be concluded from all higher tier studies (special design, OECD Guidance Document 75 and OEPP/EPPO guideline No. 170(4)) performed with fluopyram tech. and the representative formulation FLU SC 500, investigating side-effects on immature honey bee life stages, that fluopyram and the representative formulation FLU SC 500 are of low general intrinsic toxicity to honey bees.

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 SC 500A G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	34481035
Document No:	M-288186-01-1
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 not applicable.
Previous evaluation:	yes, evaluated and accepted in DAR (2014)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.3.1.1.1/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 (500 g/L): Effects (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	153531035
Document No:	M-704653-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 213 and 214 (1998)
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 214: An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion. The limit dose was 200 µg a.s./bee instead of the recommended 100 µg a.s./bee. These deviations are not expected to have impacted the study results. All validity criteria were met. Deviations from OECD Guideline 213: The limit dose was 200 µg a.s./bee instead of the recommended 100 µg a.s./bee. This deviation is 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 SC 500 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 a.s./bee by topical application (contact limit test) and to a single dose of 200.0 µg a.s./bee by feeding (oral limit test; actual dose based on the intake of the test item was 220.0 µg a.s./bee).

The contact test included a water control group (tap water with 0.5 % Adhäsit). 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.

In the contact toxicity test the LD_{50} value (48 h) of FLU SC 500 was > 200.0 µg a.s./bee. The oral LD_{50} value (48 h) of FLU SC 500 was > 220.0 µg a.s./bee.

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

I. Material and Methods

Test item: FLU SC 500, Specification No.: 102000018148, batch No.: EV57002782; TOX. No: TOX21459-00; analysed content of a.s.: 42.3 % w/w, Density: 1.189 g/mL (at 20°C).

Test species: Honey bee (*Apis mellifera* 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 a.s./bee by topical application (contact limit test) and to a single dose of 200.0 µg a.s./bee by feeding (oral limit test; actual dose based on the actual intake of the test item was 220.0 µg a.s./bee).

The controls used for the contact and oral tests were tap water containing 0.5 % Adhäsit) and 50 % w/v sucrose solution (500 g/L tap water), respectively. As a toxic reference dimethoate (D400.0 g/L nominal, 408 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 at nominal doses of 0.30, 0.15, 0.08 and 0.05 µg dimethoate/bee in the oral test.

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

Application in the contact test: In the contact toxicity test the test item was dissolved in tap water with 0.5 % Adhäsit and applied as one 5 µL droplet onto the dorsal thorax of bees using a calibrated pipette. For the control, one 5 µL droplet of tap water containing 0.5 % Adhäsit was used. The reference item was applied as one 5 µL droplet of dimethoate, dissolved in tap water with 0.5 % Adhäsit. A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item. Bees were shortly anaesthetized with CO_2 until they were immobilized immediately before application.

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 bees. This diet was offered in syringes which were weighed before and after introduction into the cages. After a maximum of 1 hour and 25 minutes the test item treated food was completely ingested by the bees and afterwards replaced by fresh, untreated food *ad libitum*.

Dose levels:

Nominal doses of the test item:	200.0 µg a.s./bee (contact limit test),
	200.0 µg a.s./bee (oral limit test)
Actual dose of the test item (oral test):	220.0 µg a.s./bee (based on the actual food intake)
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, 0.16, 0.08 and 0.06 µg dimethoate/bee

Test conditions: Temperature: 24 - 25 °C, relative humidity: 59 - 62 %; photoperiod: 24 h darkness (except during observations).

Statistics: Results obtained from the honey bees treated with the test item were compared to those obtained from the control in both the contact and oral tests. The contact and oral $LD_{50/20/10}$ values of the test item were estimated and not determined with any statistical methods since less than 10 % mortality occurred in the contact and oral tests. The contact and oral LD_{50} values of the reference item were determined with Probit Analysis (according to Finney 1971). The LD_{50} calculation of the reference item was carried out taking into account the mortality data corrected using Abbott's formula (1925). The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1 (ToxRat® Solutions GmbH).

Dates of work: May 04[th] to May 07[th], 2020

II. RESULTS AND DISCUSSION

Biological findings:

Contact Test:

In the contact toxicity test 6.0 % mortality was observed at 200.0 µg/bee after 48 hours. In the control 4.0 % mortality occurred. No test item related behavioural effects were observed at any time.

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	4.0	0.0
Test item [µg a.s./bee]						
200.0	0.0	0.0	0.0	0.0	6.0	0.0
Reference item [µg a.s./bee]						
0.10	0.0	0.0	4.0	0.0	4.0	0.0
0.15	10.0	4.0	26.0	0.0	36.0	0.0
0.20	10.0	12.0	42.0	0.0	50.0	0.0
0.30	24.0	30.0	72.0	0.0	78.0	4.0

Results are averages from 5 replicates (ten bees each) for the test item, control group and the reference item groups
Test item = FLU SC 500; reference item = dimethoate, control = CO_2/tap water control
Behav. abnorm. = behavioural abnormalities

Oral Test

In the oral toxicity test the maximum nominal test concentration of FLU SC 500 (200 µg/bee) corresponded to an actual intake of 220.0 µg/bee. This dose level resulted in 2.0 % mortality after 48 hours. In the control group (50 % w/v sucrose solution), 2.0 % mortality was observed. No test item induced 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 [%]	
Water control	0.0	2.0	2.0	0.0	2.0	0.0
Test item [µg a.s./bee]						
220.0	0.0	0.0	0.0	0.0	2.0	0.0
Reference item [µg a.s./bee]						
0.06	0.0	0.0	0.0	0.0	2.0	0.0
0.08	0.0	0.0	4.0	14.0	4.0	0.0
0.16	0.0	6.0	66.0	14.0	76.0	0.0
0.33	14.0	18.0	100.0	0.0	100.0	0.0

Results are averages from 5 replicates (ten bees each) for the test item, control group and the reference item groups
Test item = FLU SC500; reference item = dimethoate, control = tap water with sucrose solution
Behav. abnorm. = behavioural abnormalities

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 SC 500 to honey bees

Test item	FLU SC 500	
Test species	Honey bee *Apis mellifera* L.	
Exposure	**Contact**	**Oral**
Test duration	48 h	48 h
Dose rate [µg a.s./bee]	200.0	Nominal dose: 200.0 Actual dose: 220.0
LD_{50} [µg a.s./bee]	> 200.0	> 220.0

Reference item

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.22 µg a.s./bee and 0.14 µg a.s./bee, respectively. These values corresponded to the expected range cited in the OECD Guideline 213 (1998) and 214 (1998) and thus demonstrated the sensitivity of the test item.

Validity criteria:

The contact and oral toxicity 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		
	Control	≤ 10 %	4.0 %
	Oral Test		
	Control	≤ 10 %	2.0 %
LD_{50} of reference item (24 h)	Contact Test		
	Dimethoate	0.10 - 0.30 µg a.s./bee	0.22 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 SC 500 was tested in an acute contact and oral toxicity test on honey bees.

The LD_{50} (48 h) was determined to be > 200.0 µg a.s./bee in the contact toxicity test. The LD_{50} (48 h) was determined to be > 220.0 µg a.s./bee in the oral toxicity test.

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 a.s./bee

LD_{50} oral (48 hours) > 220.0 µg a.s./bee

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Bumble bees

Data Point:	KCP 10.3.1.1.1/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 (500 g/L): Effects (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory
Report No:	153531105
Document No:	M-690268-01-1
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 Guideline: OECD 246 and 247 (2017). Deviations from OECD Guideline 246: No information on bumble bee colonies concerning size, brood stages and number of bumble bees are reported. The limit dose was 200 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. Deviations from OECD Guideline 247: No information on bumble bee colonies concerning size, brood stages and number of bumble bees are reported. 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 SC 500 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 200 µg a.s./bumble bee by topical application (contact limit test) and to a single dose of 200 µg a.s./bumble bee by feeding (oral limit test; actual dose based on the intake of the test item was 232.4 µg a.s./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 30 % 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 ingredient fluopyram 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 SC 500 was estimated to be > 200 µg a.s./bumble bee. The contact NOED value (48 h) was calculated to be ≥ 200 µg a.s./bumble bee.

The oral LD_{50} value (48 h) of FLU SC 500 was > 232.4 µg a.s./bumble bee. The oral NOED value (48 h) was calculated to be ≥ 232.4 µg a.s./bumble bee.

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

I. Material and methods

Test item: FLU SC 500, Specification No.: 102000018148, origin Batch No.: EV57002782; TOX. No.: 21459-00; analysed content of a.s.: 502.7 g/L; Density: 1.189 g/cm^3.

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. Medium-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: 21 hours 39 minutes; oral test: 43 hours 25 minutes) with *ad libitum* access to untreated 50% w/v sucrose solution.

Test design: Acute contact toxicity of FLU SC 500 to adult bumble bees was assessed by exposing 50 worker bumble bees to 200 µg a.s./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) and reference item (10 µg dimethoate/bumble bee) treatment group, respectively.

Acute oral toxicity of FLU SC 500 to adult bumble bees was assessed by exposing 50 worker bumble bees to 200 µg a.s./bumble bee in 50 % w/v sucrose solution (oral limit test). This nominal treatment dose corresponded to a mean oral dose of 232.4 µg a.s./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) or reference item (mean oral dose of 4.9 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= 42, Water control: n= 43, Reference item: n= 20).

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 2 µL droplet onto the dorsal thorax of bumble bees using a calibrated pipette (Multipette ©, Eppendorf). The reference item was applied as one 2 µL droplet of dimethoate, dissolved in tap water with 0.1 % v/v Triton X-100. For the control, one 2 µL droplet of tap water containing 0.1 % v/v Triton X-100 was used.

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 untreated 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:	200 µg a.s./bumble bee (contact limit test),
	200 µg a.s./bumble bee (oral limit test)
Actual dose of the test item (oral test):	232.4 µg a.s./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): 4.9 µg dimethoate/bumble bee

Test conditions: Temperature: 24.8 – 25.3 °C; relative humidity: 43.5 – 63.5 %; 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 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: May 26th to May 29th, 2020 (biological phase)
June 18th to July 02nd, 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 and contact test the mean recoveries of the active ingredient fluopyram in the test item spiked solutions were 93 % and 89 %, respectively.

No residues of fluopyram were found in the control solution in the oral and contact test above the limit of quantification ($LOQ_{contact}$: 6.3 µg a.s./L; LOQ_{oral}: 7.6 µg a.s./L).

Table 10.3.1.1.1- 5: Analytical results

Test system	Nominal test concentration		Recovery
	[µg a.s./bumble bee]	[g a.s./kg feeding solution]	
Contact Test	200	100	89 % (mean value)
Oral Test	200	5	93 %

Biological findings:

Contact Test:

At the end of the contact toxicity test (48 hours after application) there was no mortality observed at 200 µg a.s./bumble bee. In the water control group (water with 0.1 % Triton X-100) 2.0 % mortality occurred. There were no test item related behavioural abnormalities 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	2.0	0.0
Test item [µg a.s./bumble bee]						
200	0.0	0.0	0.0	0.0	0.0	0.0
Reference item [µg a.s./bumble bee]						
10	0.0	76.7	96.7	100.0	96.7	100.0

Application volume: 2 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 mean. = mean of living individuals per treatment group
Test item = FLU SC 500; reference item = dimethoate; water control = tap water containing 0.1 % Triton X-100

Oral Test

At the end of the oral toxicity test (48 hours after application) 232.4 µg a.s./bumble bee resulted in 4.8 % mortality. No mortality occurred in the water control treatment group (50 % w/v sucrose solution). No test item induced behavioural abnormalities were detected at any time of the test.

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	0.0	0.0
Test item [µg a.s./bee]						
232.4	0.0	0.0	4.8	0.0	4.8	0.0
Reference item [µg a.s./bee]						
4.9	0.0	100.0	80.0	100.0	100.0	-

Application volume: 40 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
Mortality mean= mean of 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= 42, water control: n= 43, reference item: n=20)
Behav. Abnorm mean. = mean of living individuals per treatment group
Test item = FLU SC 500; 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 SC 500 to bumble bees

Test item	FLU SC 500			
Test species	Bumble bee *Bombus terrestris* L.			
Exposure	**Contact** [3] (tap water containing 0.1 % v/v Triton X-100)		**Oral** [3] (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 a.s./bumble bee]	200		200	
Actual dose rates [µg a.s./bumble bee]	200		232.4	
Test duration	24 h	48 h	24 h	48 h
$LD_{50, 20, 10}$ [µg a.s./bumble bee] [2]	> 200	> 200	> 232.4	> 232.4
NOED [µg a.s./bumble bee] [2, 4]	≥ 200	≥ 200	≥ 232.4	≥ 232.4
LOED [µg a.s./bumble bee] [2, 4]	> 200	> 200	> 232.4	> 232.4

1 For the 232.4 µg a.s./bumblebee test item treatment group 42 bumblebees were considered for the evaluation.
2 Results obtained from test item treated groups were compared to those obtained from the water control treatment group. 43 bumblebees of the water control group were considered for the evaluation.
3 As the test item treatment groups 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 4.9 µg dimethoate/bumble bee in the oral test. The reference item mortality of 96.7 % 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 %	2.0 %
	Oral Test		
	Control	≤ 10 %	0.0 %
LD_{50} of reference item (24 h)	Contact Test		
	Dimethoate	≥ 50 %	96.7 %
	Oral Test		
	Dimethoate	≥ 50 %	100.0 %

III. Conclusion

The toxicity of FLU SC 500 was tested in an acute contact and oral toxicity test on bumble bees.

The contact NOED value was calculated to be ≥ 200 µg a.s./bumble bee. The contact LD_{50} value was estimated to be > 200 µg a.s./bumble bee.

The oral NOED value was calculated to be ≥ 232.4 µg a.s./bumble bee. The contact LD_{50} value was estimated to be > 232.4 µg a.s./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} oral (48 hours) > 200 µg a.s./bumble bee

LD_{50} contact (48 hours) > 232.4 µg a.s./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 SC 500A4G (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	34481035
Document No:	M-288186-01-1
Guideline(s) followed in study:	OECD 213: OECD Guideline for the Testing of Chemicals, Honeybees, Acute Oral Toxicity Test, (adopted 23rd 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 not applicable.
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.3.1.1.2/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 (500 g/L): Effects (acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	153531035
Document No:	M-704653-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.3020, 850.supp. OECD 213 and 214 (1998)
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 214: An application volume of 5 µL was chosen in deviation to the guideline-specified value of 1 µL to ensure reliable dispersion. The limit dose was 200 µg a.s./bee instead of the recommended 100 µg a.s./bee. These deviations are not expected to have impacted the study results. All validity criteria were met. Deviations from OECD Guideline 213: The limit dose was 200 µg a.s./bee instead of the recommended 100 µg a.s./bee. This deviation is 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 the study summary on acute contact toxicity on honeybees please refer to Section CP 10.3.1.1.1/02.

Bumble bees

Data Point:	KCP 10.3.1.1.2/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 (500 g/L): Effects (acute contact and oral) on bumblebees (Bombus terrestris L.) in the laboratory
Report No:	153531105
Document No:	M-690268-01-1
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 Guideline: OECD 246 and 247 (2017) Deviations from OECD Guideline 246: No information on bumble bee colonies concerning size, brood stages and number of bumble bees are reported. The limit dose was 200 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. Deviations from OECD Guideline 247: No information on bumble bee colonies concerning size, brood stages and number of bumble bees are reported. 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 the study summary on acute contact toxicity on bumble bees please refer to Section CP 10.3.1.1.1/03.

CP 10.3.1.2 Chronic toxicity to bees

Data Point:	KCP 10.3.1.2/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Chronic oral toxicity test of fluopyram SC 500B G on the honey bee (Apis mellifera L.) in the laboratory
Report No:	87481136
Document No:	M-540072-01-1
Guideline(s) followed in study:	GLP compliant study based on OECD 213 (1998) and CEB No. 230 with modifications and current recommendations of the ring test group (2014) US EPA OCSPP Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 245 (2017) The test solution was not checked for possible evaporation from the feeders. The measured humidity (40-90 %) exceeded the recommended range of 50-70 %. These deviations are not expected to have impacted the study results. No further deviations to the current OECD Guideline 245 occurred. 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 please refer to MCA Section CA 8.3.1.2/01.

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

No studies are available for this formulated solo formulation. However, information on the toxicity of Fluopyram technical to honeybee larvae is presented in the active substance dossier MCA 8.3.1.3.

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:	2015
Report Title:	Assessment of side effects of fluopyram SC 500B G on the honey bee (Apis mellifera L.) in the semi-field after one application on Phacelia tanacetifolia in Germany 2014
Report No:	S14-00165
Document No:	M-532474-01-1
Guideline(s) followed in study:	OECD Guidance Document No. 75 (2007) and current recommendations of the AG Bienenschutz (Pistorius et al., 2012); OEPP/EPPO Guideline No. 170(4) (2010) US EPA OCSPP Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guidance Document OECD 75 (2007) Deviations: Minimum daytime temperature was below 15 °C, however, foraging activity was not adversely impacted and consistently above 10 bees/m² during and after application. No information about medication of bees prior to test start are given. No further deviation to the current Guidance Document 75 occurred. 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 potential side effects of FLU SC 500 on honey bee colonies (*Apis mellifera* L.), including brood development, after one application onto full-flowering *Phacelia tanacetifolia* in a semi-field study.

The test item FLU SC 500 was applied once at 250 g a.s./ha during full flowering of the crop *Phacelia tanacetifolia* (BBCH 65) while honey bees were actively foraging. The study included a control group (tap water) and reference item group (Insegar, active ingredient: fenoxycarb). For each treatment group (control, test item and reference item) 4 tunnels/replicates were set up, resulting in 12 tunnels in total, with each tunnel containing one honey bee colony.

No biologically relevant adverse effects of the test item on mortality of worker bees or pupae were observed. Foraging activity, behaviour, nectar- and pollen storage and bee brood development in individually marked brood cells were not affected.
No effects on colony strength or overall bee brood development were observed.

Based on the results of the study, it can be concluded that FLU SC 500 does not adversely affect honey bee mortality, flight intensity, behaviour, brood development, colony strength and queen survival when applied once at a rate of 250 g a.s./ha during honey bees actively foraging on a bee-attractive, flowering crop.

The study fulfils all validity criteria of OECD Guidance Document 75 (2007).

I. MATERIAL AND METHODS

Test item: FLU SC 500; Specification No.: 102000018148-01; Batch ID: EM4L011550; Sample Description: TOX10112-00; Analysed content of a.s.: 42.2 % w/w (501.4 g/L); Density: 1.188 g/mL.

Test species: Honey bees (*Apis mellifera* L.); small bee colonies, maintained according to normal beekeeping practice, consisting of a total of 11 combs containing 8 - 9 combs with honey and pollen and 7 - 9 brood combs containing eggs, larvae and capped cells. The preliminary brood check indicated healthy colonies with all brood stages present. The mean strength of the colonies per treatment group, one day before the application, was similar and ranged between 5161 and 5484 adult bees per colony.

Location of the field site: Germany

Test concentrations: Four control tunnels treated with 400 L tap water/ha, four test tunnels treated with the test item at 250 g a.s./ha in 400 L water/ha (corresponding to 498.6 mL product/ha), and four tunnels treated with the reference item Insegar at 1.2 kg product/ha in 400 L water/ha (corresponding to 300 g fenoxycarb/ha).

Test design: The aim of the study was to evaluate potential side effects of a spray application of FLU SC 500 on the honey bee (*Apis mellifera* L.) under confined semi-field conditions. A plot of *Phacelia tanacetifolia* with an effective crop area of ca. 65.12 m^2 (2 x 32.56 m^2) was prepared for each tunnel (16.0 m length x 5.0 m width x 3.5 m height) and each plot constituted one replicate. For each treatment group (control, test item and reference item), 4 tunnels/replicates were set up, resulting in 12 tunnels in total. One honey bee colony was moved into each tunnel. Commercial bee colonies were placed in the tunnels at early flowering of *Phacelia tanacetifolia* (BBCH 62) four days before the application. Applications of the test item FLU SC 500, control and reference item were conducted by spraying the whole area of *Phacelia* plants within the tunnel during full flowering of the crop (BBCH 65) with worker bees actively foraging.

The confined phase of the test started on 4DBA (DBA= days before the application) with the set-up of the colonies in the tunnels and ended with the removal of the colonies from the tunnels on 8DAA (DAA= days after the application). The monitoring phase started after removal of the colonies from the tunnels and ended after the last mortality and colony assessments on 26DAA.

After foliar (spray) application of the water (control), test item and reference item, ontogenesis of a defined number of honey bee eggs was observed for each colony of each treatment group. Mortality of adult bees and pupae/larvae as well as foraging activity of the adult bees were also assessed. The condition of the colonies was assessed in regular intervals until the end of the trial. Ontogenesis of the bees from egg to adult workers was observed for a period of 22 days following the daytime application (*i.e.* one complete honey bee brood cycle). On the Brood Area Fixing Day (BDF0), one day prior to the application, a digital picture of one or more brood comb(s) out of each hive per treatment group and replicate was taken, the file saved on a computer and a minimum of 209 eggs per colony marked. For each subsequent brood assessment (BFDn), the same comb(s) were taken out of the hives and digital photos were taken in order to investigate the progress of the brood development until day 22 following the daytime application (= BFD23 following BFD0).

The following parameters were assessed:

Mortality of adult bees and pupae: 4DBA - 26DAA (DBA: days before application; DDA: days after application);

Behavioural abnormalities and foraging activity (flight intensity): 4DBA - 7DAA (DBA: days before application; DDA: days after application);

Condition of the colonies (food stores, brood status and colony strength): 1 day before and 3, 9, 15, 22 and 26 days after the day of application (= end of the trial);

Bee brood development: 1 day before (= BFD0) and 3 (= BFD4), 9 (=BFD10), 15 (= BFD16), 22 (= BFD23) days after the day of application.

Test conditions: Natural field conditions. During the period of confinement, the daily precipitation was 0 mm on most days and 2 mm only on 5DAA. Temperatures were almost optimal. Accordingly, there was a good honey bee foraging activity on the crop within the tunnels. The temperature during the confinement period (day - 4 to day + 7) was between a min. of 5.7 - 13.0 °C and a max. of 19.3 - 27.7 °C.

After the exposure phase inside the tunnels rain occurred on 11 occasions until day 26. The temperature was between a min. of 11.0 - 18.4 °C and a max. of 15.9 – 26.1 °C.

Statistics: The data of mortality (adult, and pupae separately) and flight intensity in the pre- and post-application period of the test item group and the reference item group were compared to the control in a separate approach but in the same way. The data were tested for normality and homoscedasticity using the Shapiro-Wilk Test ($p > 0.05$) and folded F-Test ($p > 0.05$), respectively. Data were statistically compared using Student's t-Test (method pooled, one-sided, $p \leq 0.05$) in case of normality and homoscedasticity. In case of non homoscedascity but proven normality, t-Test was conducted with method Satterthwaite (one-sided, $p \leq 0.5$). In case of non-normality and non-homoscedasticity, Mann-Whitney exact test (one-sided, $p \leq 0.5$) was used. Log-transformation was conducted if necessary to achieve better fit to normality and homoscedasticity of data.

For the pre-application period, the test was performed as a two-sided test. During the exposure phase, right-sided tests were used for mortality of test item group and reference item group compared to control and left-sided tests were used for flight intensity of test item group and reference item group compared to control.

The data for brood indices, compensation indices and termination rates and colony condition were compared to the control using the same tests as the data of mortality and flight intensity with left-sided tests for brood and compensation indices as well as right-sided tests for the termination rates after the application and left- or right-sided tests for the colony condition.

All statistical analyses were conducted using SAS release Version 9.3.

Dates of work: June 13th to July 13th, 2014 (field phase)

II. RESULTS AND DISCUSSION

Application amounts:

The amount of test item applied was determined by measuring the prepared and the remaining spray solution and results confirmed that the spray solutions were prepared correctly and that the honey bees were adequately exposed to the test item. The accepted spray tolerance of +10% was met in all treatment groups. No rainfall occurred within at least 2 hours after the applications and wind speed was 0 m/s during all applications.

Table 10.3.1.5-1: Summary: fluopyram concentrations in spray solutions

Sample material	Day of sampling	Fluopyram		
		Concentration range (min – max) [g a.s./kg]	Mean concentration [g a.s./kg]	Mean deviation to target [%]
Spray solution	DAA0	250.5 – 261.0	256.7	2.74

DAA: Days after application

Biological results:

The influence of FLU SC 500 was evaluated by comparing the data of the assessments of the test item group to the reference item group and the control, and by comparing the pre-application data to the post-application data.

Mortality of the adult bees (worker bees)

Pre-application phase (4 to 0 days before application):

Mean mortality in the test item group (103.7 ± 29.6 dead bees/colony/day) was not statistically significantly different compared to the water control (107.2 ± 41.6 dead bees/colony/day) during the pre-application phase. Mortality in the reference item group was statistically significantly higher compared to the water control on day 4 before application (13.8 ± 6.4 dead bees/colony and 3.0 ± 1.6 dead bees/colony, respectively), however, this difference was not biologically relevant.

Exposure phase in the tunnels (day 0 after application to day 7):

On the application day the mortality in the test item and reference item groups (108.3 ± 55.9 and 100.0 ± 23.5 bees/colony/day, respectively) was lower compared to the control group (116.5 ± 42.8 bees/colony/day) but with no statistically significant difference.

A statistical evaluation of the mean mortality levels in the test item group of the post application period from day 0 after application to day 7 showed no statistically significant differences when compared to the control group. The average control mortality of adult bees during the exposure phase (day 0 to day 7 following the application) was 84.6 ± 23.1 dead bees/colony/day. The average mortality in the test item group was 75.7 ± 24.0 dead bees/colony/day. The reference item mortality was 62.5 ± 16.0 dead bees/colony/day.

Exposure and post-exposure phase (outside the tunnels) (day 0 after application to day 26):

The mean mortality of adult worker bees over the whole study period following application (0DAA - 26DAA) in the control, test item and reference item group were comparable and not statistically significantly different (35.7 ± 8.0, 31.1 ± 7.7 and 33.5 ± 6.9 dead bees/day/colony, respectively). A day wise comparison of mortality between test item treatment group and control indicated one statistically significant difference 26 days after application (10 ±7.4 and 2.8 ±1.5 dead bees/colony, respectively), however, this difference was not biologically relevant.

Mortality of larvae and pupae

Pre-application phase (4 to 0 days before application):

No statistical differences were found in the test item (0.2 ± 0.2 dead larvae+pupae/day/colony) and control group (0.0 ± 0.0 dead larvae+pupae /day/colony) when assessing larvae and pupae mortality.

There were no statistical differences in larval and pupal mortality between the reference item group and the control group.

Exposure phase in the tunnels (day 0 after application to day 7):

On the application day no mortality was observed in any treatment group.

From day 0 to day 7 after the application and during the exposure phase inside the tunnels, a mean of 0.3 ± 0.2 dead larvae+pupae/day/colony was found in the test item group which was not statistically significantly different compared to a mean of 0.1 ± 0.1 dead larvae+pupae/day/colony in the control group, respectively.

The reference item group indicated a mean of 0.1 ± 0.2 dead larvae+pupae/day/colony during this time. This value was not statistically significantly different compared to the control group.

Exposure and post-exposure phase (outside the tunnels) (day 0 after application to day 26):

The mean mortality of larvae and pupae over the whole after application period (0DAA - 26DAA) in the control and test item group were comparable with means of 0.2 ± 0.1 dead larvae+pupae/day/colony in both groups. The mean mortality in the reference item group of 7.2 ± 5.5 dead larvae+pupae/day/colony was statistically significantly higher compared to the control.

Thus, during the entire period after the applications (0DAA to 26DAA), the average sums of dead pupae per colony were similar for the control and test item treatment group, whereas in the reference item group, mortality was elevated by a factor of 36 compared to the control. Effects on pupae of Insegar are a well-known effect and the elevated mortality shows the efficacy of the reference treatment and the high sensitivity of the test system to detect adverse effects on brood and colony.

Table 10.3.1.5- 2: Daily mean mortality of dead worker bees, larvae and pupae per colony

		Treatment group		
Daily mean mortality	**Assessment day**	**Control**	**Test item**	**Reference item (R)**
Number of dead worker bees/colony ± SD	4DBA to 0DBA	107.2 ± 41.6	103.7 ± 29.6	120.2 ± 33.9
	0DAA	116.5 ± 42.8	108.3 ± 55.9	100.0 ± 23.5
	0DAA to 7DAA	84.6 ± 23.1	75.7 ± 24.0	62.5 ± 16.0
	0DAA to 26DAA	35.7 ± 8.0	31.1 ± 7.7	33.5 ± 6.9
Number of dead larvae+pupae/colony ± SD	4DBA to 0DBA	0.0 ± 0.0	0.2 ± 0.2	0.0 ± 0.0
	0DAA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	0DAA to 7DAA	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.2
	0DAA to 26DAA	0.2 ± 0.1	0.2 ± 0.1	7.2* ± 5.5

DAA: days after application, DBA: days before application;
SD: standard deviation;
* statistically significantly higher than control group

Overall no adverse treatment related effects of the test item on adult or juvenile mortality were observed.

Foraging Activity

Pre-application phase (4 to 0 days before application):

The mean foraging activity in the test item and reference item groups was comparable to the control group, resulting in overall daily mean values of 16.6 ± 1.4, 18.0 ± 3.6 and 16.3 ± 1.6 bees/m²/day in the control group, test item group and reference item group, respectively. No statistically significant differences were found between the three treatment groups.

Exposure phase in the tunnels (day 0 after application to day 7):

On the application day the foraging activities in the test item and reference item groups (26.5 ± 1.9 and 24.1 ± 3.7 bees/m²/day, respectively) were lower when compared to the control group (28.8 ± 3.5 bees/m²/day), but with no statistically significant differences.

From day 0 to day 7 the overall daily mean foraging activity in the test item group of 26.0 ± 0.8 bees/m²/day was higher compared to 25.8 ± 2.4 bees/m²/day in the control group but showed no statistically significant difference from the control.

The reference item resulted in a significant reduction of the foraging activity (21.6 ± 2.7 bees/m²/day) compared to the control.

Flight activity across treatments was similar when comparing the two phases with flight observation (pre-exposure and exposure phase), however a slight statistically significant reduction in foraging post-exposure was observed in the reference item group.

No significant test-item related adverse effects on flight intensity were observed.

Table 10.3.1.5- 3: Daily mean flight intensity as forager bees per m² per 15s

Daily mean flight intensity	Assessment day	Treatment group		
		Control	Test item	Reference Item
Bees/m² ± SD	4DBA to 0DBA	16.6 ± 1.4	18.0 ± 3.6	16.3 ± 1.6
	0DAA	28.8 ± 3.5	26.5 ± 1.9	24.1 ± 3.7
	0DAA to 7DAA	25.8 ± 2.4	26.0 ± 0.8	21.6* ± 2.7

DAA: days after application; DBA: days before application;
SD: standard deviation
*: statistically significant difference to control; p ≤ 0.05

Behavioural abnormalities

Differences in behaviour were observed in a small number of bees in the test item and reference item treatment groups on the day of application and on the day after application. However, these did not persist and are not considered to be biologically relevant.

Condition of the Colonies

The condition of the colonies was assessed over one complete brood cycle of the honey bees (*i.e.* 21 days).

At the beginning of the trial, the queen and all brood stages (eggs, larvae and closed brood) were found in all colonies as an indication of healthy colonies. The observed mean abundance of brood in the colonies (sum of cells containing eggs, larvae, and pupae) in the test item treatment group was similar to that in the control group.

From 3DAA onwards, the brood area in the reference item group was consistently lower than in the treatment group and statistically significantly different when compared to the control. This can be explained by the lethal effect of the reference item to brood and is consistent with the high brood termination rate in this treatment. Overall, honey bee brood development in the test item treatment group was not affected when compared to the control.

Besides the brood area, allocation of comb space to food (pollen and nectar) was also assessed. The proportion of pollen cells and nectar cells fluctuated with the needs of the colonies and showed no difference between treatment groups. The colonies were well provided during the course of the study.

Thus, no test-item related adverse effects on the development of the food storage area were observed.

Colony Strength

The mean number of honey bees per colony in the three treatment groups was similar one day before application and did not differ statistically (mean of 5161 to 5484 per colony).

Overall, the number of bees per colony developed similarly over the course of the study. A significant difference in the numbers of bees between the reference item group and the control group was observed only at the end of the monitoring phase (26DAA). This is most likely a first effect of the decreased hatching rates of young bees due to the pupal-disturbing effect of the reference item. The significantly stronger hives in the test item treatment group (compared to the control group) on day 9DAA could be explained by the larger number of brood cells before that time (i.e. on 3DAA).

The overall development of colony strength of all treatment groups showed fluctuations, which can be considered to be in a typical and normal range. The colony strength values of the test item group were on approximately the same level or even higher during the entire study than the corresponding values of the control group. Therefore, no test-item related adverse effects on colony strength were observed.

Table 10.3.1.5- 4: Colony strength: Mean numbers of bees

Treatment group	Mean numbers of bees ± SD					
	Day [1] -1	Day 3	Day 9	Day 15	Day 22	Day 26
Control	5484 ± 460	6595 ± 666	7988 ± 1137	9071 ± 828	6539 ± 398	7102 ± 306
Test Item	5161 ± 956	6216 ± 1134	11391** ± 1265	10983 ± 2003	6792 ± 748	7453 ± 660
Reference Item	5260 ± 706	6286 ± 596	9605 ± 1822	9591 ± 1720	6399 ± 1121	5316 * ± 1530

[1] In relation to the application
* Statistically significant difference (lower) to C, p≤0.05
** Statistically significant difference (higher) to C, p≤0.05

Development of Bee Brood

Brood Termination Rate:

The brood termination rate is the percentage of brood cells that do not successfully transition from egg to hatched worker bees. Based on this brood termination rate (BTR) the failure of individual eggs or larvae to develop was quantitatively assessed. If the expected brood stage was reached, a low BTR indicated successful development, while a high BTR indicated unsuccessful bee brood development.

Following the assessment of single cells from the egg stage to the successfully hatched worker bee, a mean termination rate of 32.06 % at BFD (Brood Fixing Day) 23 in the test item group was lower compared to the control group (42.63 %). The test item treatment group BTR was not statistically significantly different compared to the control group BTR.

Treatment with the reference item Insegar caused a clear decrease in brood development, resulting in a termination rate of 98.20 %. This decrease was statistically significantly different compared to the control group.

Brood Compensation Index:

The Brood Compensation Index is an indication for recovery and shows the development of the brood at each assessment. A continuous brood development was observed in the test item group as well as in the control group. The Brood Compensation Indices following the labelling of the egg stage up to day 22 after application (BFD+23) were higher in the test item group compared to the control group. Differences in the Brood Compensation Index between test item and control were not statistically significant.

The high brood termination rate of the marked cells after treatment of the crop with the reference item Insegar is also reflected by the statistically significantly lower Brood Compensation Indices in the reference item group when compared to the control, suggesting no recovery in the marked areas.

Table 10.3.1.5- 5: Results for Brood Compensation Index and Brood Termination Rate

Treatment Group	Compensation indices ± SD					Termination rate (BFD+23)
	BFD 0	BFD 4	BFD 10	BFD 16	BFD 23	[%]
Control	1.0 ± 0.0	1.66 ± 0.11	2.34 ± 0.53	2.34 ± 0.46	3.40 ± 0.53	42.63 ± 12.96
Test Item	1.0 ± 0.0	1.79 ± 0.14	2.79 ± 0.50	2.76 ± 0.48	3.85 ± 0.47	32.06 ± 12.39
Reference Item	1.0 ± 0.0	0.47* ± 0.46	0.20* ± 0.28	0.17* ± 0.18	0.59* ± 0.55	98.20* ± 2.16

BFD: Brood area fixing day;
SD: Standard deviation
* Statistically significant difference to the control, p < 0.05

Brood Index

The Brood Index is an additional indicator for the bee brood development and facilitates a comparison between the different treatments. Following the labelling of the egg stage, the Brood Indices of the test item group were higher compared to the control values. Differences in the Brood Index between test item group and the control group were not statistically significant. Following the labelling of the eggs after treatment with the reference item Insegar, the mean Brood Indices were statistically significant lower compared to the control Brood indices, indicating unfavourable brood development in the reference item group.

Table 10.3.1.5- 6: Results for Brood Index

Treatment Group	Brood indices at x days after brood area fixing day (BFD) ± SD				
	0	4	10	16	23
Control	1.0 ± 0.0	1.62 ± 0.15	2.32 ± 0.53	2.30 ± 0.52	2.87 ± 0.65
Test Item	1.0 ± 0.0	1.75 ± 0.13	2.78 ± 0.50	2.72 ± 0.50	3.40 ± 0.62
Reference Item	1.0 ± 0.0	0.45* ± 0.47	0.17* ± 0.28	0.07* ± 0.09	0.09* ± 0.11

BFD: Brood area fixing day;
SD: Standard deviation
* Statistically significant difference to the control, p < 0.05

Accordingly, no adverse effects of the test item on brood development were observed throughout the study following the labelling of the egg stage up to day 22 after application (BFD+23).

Validity criteria:

All validity criteria were met in this study.

Table 10.3.1.5- 7: Validity criteria

Validity criteria	Recommended		Obtained
Control mortality	Control	Should not be considerable (it is known that the method itself causes mortality due to the handling).	Daily mean control mortality from day 0 after application to day 7 varied between 12.3 and 143.5 dead bees per tunnel. The overall mean mortality after application until day 7 was 84.6 dead bees/tunnel/day.
Reference item mortality (bee brood termination rates)	Fenoxy-carb	There should be a high number of impacted bees	There was a high number of impacted bee brood, which resulted in 98.20 % termination of the initially observed eggs. This was statistically significant compared to the control. The mean number of dead pupae found in the reference item group was statistically significantly higher compared to the control. The effects on honey bee brood as observed in the reference item group demonstrated the sensitivity of the test system to detect effects on immature honey bee life stages.
Foraging activity shortly before and during daytime application	All groups	≥ 10 bees/m²	Mean flight densities shortly before (4 - 0 days) the application were: 16.6 ± 1.4 bees/m² in the control tunnels 18.0 ± 3.6 bees/m² in the test item tunnels 16.3 ± 1.6 bees/m² in the reference item tunnels Mean flight densities at daytime application were: 28.8 ± 3.5 bees/m² in the control tunnels 26.5 ± 1.9 bees/m² in the test item tunnels 24.1 ± 3.7 bees/m² in the reference item tunnels

III. CONCLUSION

In order to assess the potential effects of FLU SC 500 on honey bee colonies, including brood development, 250 g fluopyram in 400 L tap water/ha, tap water for the control and a reference item (Insegar, a.s. fenoxycarb) were applied to a full-flowering and highly bee-attractive crop (*Phacelia tanacetifolia*) under semi-field (tunnel) conditions during bee-flight.

No test-item related adverse effects on mortality or flight intensity were observed.

The quantitative assessments in individually marked brood cells revealed that FLU SC 500 did not cause any treatment-related adverse effects on honey bee brood development in individually marked cells.

The overall honey bee brood development in the test item treatment group T, measured as mean number of cells per colony containing the different types of brood, was not affected when compared to the control.

No test-item related adverse effects on colony strength or on the development of the food storage area were observed.

Some behavioural differences between control and test item were observed on days 0DAA and 1DAA but did not persist and were not considered to be biologically relevant.

Overall, FLU SC 500 applied at 250 g a.s./ha (target) to a flowering crop in the presence of foraging honey bees did not cause significant effects on mortality, flight intensity, behaviour, colony strength and brood development.

Assessment and conclusion by applicant:

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

FLU SC 500 applied at 250 g a.s./ha (target) to a flowering crop in the presence of foraging honey bees did not cause significant effects on mortality, flight intensity, behaviour, colony strength and brood development.

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Data Point:	KCP 10.3.1.5/02
Report Author:	[redacted]
Report Year:	2016
Report Title:	Semi-field tunnel study in Phacelia tanacetifolia, evaluating the effects of repeated foliar applications of Fluopyram SC 500 on honey bees Apis mellifera L. (Hymenoptera, Apidae) under confined conditions, followed by a post-exposure field observation period
Report No:	E 319 4528-2
Document No:	M-547034-01-1
Guideline(s) followed in study:	OEPP/EPPO Guideline No. 170 (4), 2010 (modified) US EPA OCSPP Guideline No. 850.SUPP
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 requested in the guideline. This deviation is 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 of repeated foliar applications of FLU SC 500 during full-bloom of the highly bee-attractive surrogate crop *Phacelia tanacetifolia*, on honey bees (*Apis mellifera* L.).

The study involved a full-factorial randomized block design, with five blocks and four treatment groups. Each block represented a tunnel containing one honey bee colony. The treatment groups consisted of a (negative) control group (C) - treated 2 × with tap water, two reference item groups (R1, R2) - treated with 1 x 400 g dimethoate a.s./ha, respectively, and the test item treatment group (T) - treated with 2 × 250 g fluopyram/ha, amounting to 20 tunnels in total. With foliar applications occurring in sequence, staggered seeding of the Phacelia crop was employed to ensure sufficient forage over the course of the 16 day exposure period. Mortality of bees, foraging activity, behaviour, brood development, colony strength, food stores, and colony health were assessed. After the confined semi-field exposure period, the colonies in the control and in the test item treatment group were released from confinement, to be repeatedly monitored under field conditions for the remainder of the season until overwintering, and were assessed for a final time after overwintering in the next spring.

The confined exposure of honey bee colonies to two repeated foliar applications with FLU SC 500 during full bloom did not result in adverse acute, 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.

Based on the results of this study, it can be concluded that FLU SC 500 does not adversely affect honey bee behaviour, brood development and colony strength when applied as two sequential foliar applications at a rate of 250 g a.s./ha, under the above described conditions.

I. MATERIAL AND METHODS

Test item: FLU SC 500, Specification No.: 102000018148-01, Batch-code: EZO1217101; Sample description: TOX No. 10066-00; Analysed content of a.s.: 41.6 % w/w (493.9 g/L).

Test species: Honey bees (*Apis mellifera* L.); small bee colonies, maintained according to normal beekeeping practice, each consisting of one laying sister queen and five occupied frames with a total of about 4000 honey bees; four of the five occupied frames were brood frames, one frame contained pollen and honey. The preliminary brood check indicated healthy colonies, with all brood stages present and a sufficient amount of pollen and honey to guarantee colony viability.

Location of the field site: Germany

Test concentrations:
Test item treatment: 250 g a.s./ha, corresponding to 602 g product/ha (based on an analytical content of 493.9 g a.s./L and a density of 1.188 g/mL).
Control: Tap water (concurrently to the test item applications).
Reference item treatment: 400 g dimethoate/ha, corresponding to 1031 g product/ha (based on an analytical content of 420.6 g a.s./L and a density of 1.084 g/mL).
A water volume of 300 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 SC 500 on honey bee colonies under semi-field conditions. A plot of *Phacelia tanacetifolia* with an effective crop size of 100 m² was prepared for each tunnel. For each treatment group (control, test item and two reference item groups), 5 tunnels/replicates were set up, resulting in 20 tunnels in total (biological assessment tunnels). One honey bee colony was placed into each tunnel. Colonies were placed into the tunnels three days before the 1st application of the test item. The 1st application was conducted when the *Phacelia* crop on one half of the confined exposure area had reached full bloom (BBCH 64-65). The 2nd test item application was carried out once the crop in the second half of the confined exposure area had reached full-bloom (BBCH 64-65). Applications of the test item FLU SC 500 involved spraying of the entire *Phacelia*-crop inside the tunnels during honey bees actively foraging on the crop. The colonies in the control group were concurrently exposed to two subsequent tap water applications. In contrast, the colonies in the reference item group R1 were exposed to a single foliar application of the reference item concurrently to the 1st, application in control and test item groups, whereas the colonies in reference item group R2 were exposed to a single application of the reference item at the same time as the 2nd water and test item applications in control and test item groups, respectively. Control and test item group colonies remained in their respective gauze tunnels for a period of 16 consecutive days following the 1st test item application. The entire confinement period (including acclimation prior to the 1st foliar applications) was 19 days.

The following parameters were assessed:

Mortality of bees (worker, drones, larvae and pupae) in dead bee traps and on polyethylene sheets laid out in front of the hives and along tunnel walls: -2DA1A (2 days before the 1st application) to 6DA2A (6 days after the 2nd application);

Foraging activity of the bees was assessed twice daily within an area of 1 m^2 at two different locations within each tunnel and with each count lasting 1 minute: -3DA1A to 6DA2A;

In addition, behavioural anomalies were recorded during foraging activity assessments and during colony assessments as part of the post-exposure monitoring period.

Assessments of colony strength (number of adult honey bees), brood development, presence of a healthy queen, food storage, and colony health were carried out once immediately before confinement, three times during the confined exposure period, and six times post-exposure, including one final assessment after overwintering.

Seven days after the 2nd test item application, all control and test item treated colonies were released from the tunnels and moved to a monitoring location. Reference item treated colonies (R1 and R2) were discarded. At the monitoring site, bees were allowed to forage freely under ambient field conditions. The development of the colonies and their overall health status were assessed in regular intervals of approximately 3 weeks until the end of the season and one last time after overwintering during springtime of the following year.

Test conditions: Climatic conditions were recorded by a weather station and by a data logger installed in one of the tunnels.

Statistics: The influence of the test item treatment was evaluated by comparing the data obtained for control group with the data obtained for the test item treatment group and with the data of the reference item group, respectively. Linear mixed effects models were used to evaluate the potential effect of test item, the effect of the different honey bee colonies (hives) was added as an error term. For mortality and foraging activity, additional linear mixed effects models for the comparison of control and reference item were calculated. Count data (i.e. mortality, colony parameter, estimates of bees at the tunnel/foraging bees) was log (decadic logarithm) transformed (log x + 1) in order to achieve normal distribution of residuals (homoscedasticity). ANOVA was performed for the fitted model in order to detect the influence of factors and interaction on the encountered variance. A significance level of $\alpha = 0.05$ was selected.

All statistical analyses were performed with the statistical software package R 3.1.3 (R Development Core Team, 2015).

Dates of work: June 21st, 2013 to March 10th, 2014.

II. RESULTS AND DISCUSSION

Biological results:

Mortality

During the confined exposure period, worker bee mortality in front of the hive and at the tunnel walls was low in both the control and the test item treatment group. In contrast, mortality in the reference item treatment was biologically and statistically significantly elevated following application.

Statistical analysis (linear mixed effects model) revealed no significant effect of the test item treatment on worker bee mortality as recorded in front of the hive ($p = 0.637$) or as recorded at the tunnel walls ($p = 0.299$). A significant effect of the date was found ($p = < 0.001$), the interaction of date and treatment was not significant ($p = 0.182$ for mortality at the front of the hive; $p = 0.128$ for mortality at the tunnel walls), indicating that mortality changed over time, but in the same manner for test item treatment and control. Furthermore, statistical analysis showed a highly significant ($p < 0.001$) effect of the reference item in comparison to the control. Thus, the honey bee colonies were sufficiently sensitive and the test system was adequate to detect effects on honey bee mortality.

Table 10.3.1.5- 8: Mean mortality ± SD of worker bees in front of the hives during confined exposure

Date	Control (Tap water)		Test Item Treatment (2 x 250 g FLU SC 500)		Reference Item R1 (1 x 400 g dimethoate/ha)		Reference Item R2 (1 x 400 g dimethoate/ha)	
	Mean count	±SD	Mean count	±SD	Mean count	±SD	Mean count	±SD
-2DB1A	3.8	5.2	8.4	9.9	1.4	1.9	4.8	3.8
-1DB1A	5	3.9	7.2	5.3	3.6	2.5	9.8	8.5
0DB1A	3	1.4	2.2	1.5	1.4	1.7	4.4	2.7
0DA1A	0.6	0.9	1.6	0.9	148.4	66.9	2.2	1.6
1DA1A	2.4	1.1	4.8	4.2	298	104.5	4.2	2.3
2DA1A	1.6	0.9	3	2.0	47.8	26.0	2.6	3.2
3DA1A	1.4	2.1	1.8	1.1	20.6	7.4	1.2	2.2
4DA1A	1.6	2.1	1.4	0.9	13.4	4.5	1	1.0
5DA1A	1.4	1.3	0.6	0.5	8.2	5.2	1.6	0.9
6DA1A	1.2	1.6	1.2	1.3	25.4	18.0	1	1.4
7DA1A	1.6	0.9	1.2	1.3	39	27.1	1.4	1.7
8DA1A	2	2.0	0.8	0.8	11	9.6	1.6	1.8
0DB2A	2.6	1.5	2	2.8	11.2	5.6	2.6	1.3
0DA2A	0.2	0.4	33.6	72.3	4.6	2.8	92.6	67.1
1DA2A	1.2	0.8	0	0.0	13	6.2	504.6	185.5
2DA2A	1.6	1.1	2.6	2.3	27.8	13.1	32.2	13.2
3DA2A	1.2	1.3	1	1.7	17.4	12.3	15.2	8.3
4DA2A	4	2.9	2	1.2	NA	NA	NA	NA
5DA2A	2.2	1.1	3	2.0	NA	NA	NA	NA
6DA2A	0.6	1.3	1.2	1.6	NA	NA	NA	NA

SD: Standard deviation

The number of dead drones recorded during the confined exposure period in front of the hive and at the tunnel walls was always low and much lower than the number of worker bees. Furthermore, there were several days where no drone mortality was recorded at all.

In both the control and the test item treatment group, no dead larvae or pupae were recorded at the tunnel walls. Mortality of immature honey bee life stages in front of the hive in both control and test item treatment, was very low and occurred only sporadically. Throughout the entire confined exposure period 32 dead immature life stages were found in the control group and 51 dead immature life stages were observed in the test item treatment group.

Thus, it can be concluded that sequential foliar spray applications of 2 × 250 g fluopyram/ha during flowering of a highly bee-attractive crop did not cause adverse effects on honey bee mortality.

Foraging Activity

Flight activity was very similar in the test item treatment group and in the control group. Honey bees accepted the flowering *Phacelia*-crop as foraging habitat and were therefore actively foraging on the crop in the tunnels. Fluctuations in foraging activity occurred simultaneously in the control and in the test item treatment group. Foraging activity was especially low on days with unfavourable weather conditions. Colonies experiencing a reference item treatment showed a temporary cessation of foraging activity following the respective application. Thus, the results showed a distinct effect of the reference item on foraging activity, indicating that the honey bee colonies were sufficiently sensitive and that the test system was adequate.

Table 10.3.1.5- 9: Mean Foraging Activity ± SD in the tunnels during confined exposure

Date	Timing	Control (Tap water)		Test Item Treatment (2 x 250 g FLU SC 500)		Reference Item R1 (1 x 400 g dimethoate/ha)		Reference Item R2 (1 x 400 g dimethoate/ha)	
		Mean count	±SD	Mean count	±SD	Mean count	±SD	Mean count	±SD
-2DB1A	AM	28	8.7	26.4	3.4	33.4	11.5	32	6.9
	PM	16.2	2.3	14.2	5.4	13.8	4.2	15.6	4.4
-1DB1A	AM	32.6	10.4	31.8	6.5	30	12.7	42.6	13.5
	PM	58.6	13.9	58.6	16.2	60.8	21.9	63	7.6
0DB1A	BA	42.4	2.7	40.6	5.9	40	2.3	43	10.1
0DA1A	AA1	45.8	6.3	42	6.9	0	0.0	46.6	3.0
	AA2	44.4	7.0	43.8	5.5	0	0.0	46.2	4.8
	AA3	53.6	4.1	49.6	9.4	0	0.0	53.4	5.4
1DA1A	AM	68.2	4.2	69.2	7.2	0.4	0.4	64.8	7.3
	PM	63.6	6.5	59.8	8.2	0.2	0.4	62.2	4.3
2DA1A	AM	5.8	9.8	11.4	12.8	0	0.0	8.2	8.2
	PM	0.6	1.3	0	0.0	0	0.0	0	0.0
3DA1A	AM	0	0.0	0	0.0	0	0.0	0	0.0
	PM	NA	NA	NA	NA	NA	NA	NA	NA
4DA1A	AM	5.2	8.3	5.8	7.8	0	0.0	8.8	14.7
	PM	58.8	11.6	50.2	13.3	0	0.0	41.8	11.5
5DA1A	AM	53	4.7	52.6	8.2	0	0.0	49.2	17.1
	PM	78.2	14.3	83.8	24.5	0.8	1.3	65.4	15.6
6DA1A	AM	76.2	10.3	78.4	10.0	0.6	1.3	71.6	12.7
	PM	90.8	5.6	93.2	13.7	0.8	0.8	87	9.1
7DA1A	AM	101	14.8	103.8	8.4	1.8	1.3	83.4	16.7
	PM	92	13.1	92.6	10.0	0.8	1.3	91.4	3.6
8DA1A	AM	19.8	6.7	21.8	0.8	0.6	1.3	18.4	5.7
	PM	43.6	2.3	39.2	4.1	0.2	0.4	40.4	6.2
0DB2A	BA	91.6	10.3	97	5.8	0	0.0	93.4	9.6
0DA2A	AA1	115	7.8	108.4	14.9	1.4	0.5	11.4	4.4
	AA2	96	12.0	86.2	14.6	1.2	1.6	1.8	2.0
	AA3	73	9.4	65.4	12.1	0.2	0.4	0.2	0.4
1DA2A	AM	87.6	7.8	88.4	5.4	0.6	0.9	0	0.0
	PM	102.4	14.8	98.6	12.0	6	10.4	0	0.0
2DA2A	AM	103.8	13.6	104.8	5.8	0.8	1.1	0	0.0
	PM	128.2	10.1	115.8	10.3	1	1.4	0	0.0
3DA2A	AM	76.6	17.0	79	16.4	7.4	11.8	0	0.0
	PM	121	12.0	103.8	13.1	1.8	4.0	0	0.0
4DA2A	AM	91.2	10.8	96.4	0.5	NA	NA	NA	NA
	PM	103.4	7.1	109	10.3	NA	NA	NA	NA
5DA2A	AM	91.4	6.9	96.4	12.5	NA	NA	NA	NA
	PM	104.6	13.3	102.6	9.5	NA	NA	NA	NA
6DA2A	AM	81	8.3	84	7.4	NA	NA	NA	NA
	PM	97.6	13.0	93	7.4	NA	NA	NA	NA

Means were calculated based on the sums of the two estimates (2 counts in separate 1 m^2 plots per tunnel) for each time point.
SD: Standard deviation
AM: Assessments before midday
PM: Assessments after midday
BA: Assessment before application
AA: Assessments after application
NA: Not assessed

Statistical analysis (linear mixed effects model) revealed no significant effect of the test item treatment on flight activity (p = 0.802). A significant effect of the date was found (p = < 0.001), the interaction of date and treatment was not significant (p = 0.999), indicating that flight activity varied over time, but in the same manner for treatment and control. Furthermore, statistical analysis showed a highly significant (p < 0.001) effect of the reference item in comparison to the control.

Thus, it can be concluded that sequential foliar spray applications of 2 × 250 g fluopyram/ha during flowering of a highly bee-attractive crop did not cause adverse effects on foraging activity.

Condition of the Colonies

The condition of the control and test item colonies was assessed four times immediately before and during the confinement period of the study and six times post exposure including one assessment after overwintering.

Colony Strength

During the confined exposure period (colony assessments 2 – 4), the colonies in control and test item group were able to grow, and the increase in colony strength during confinement indicated that the colonies adapted well to the confined conditions of the tunnels. Throughout the confined exposure period, colony strength in both groups was on a comparable level, with no distinct differences between the two exposure groups. After the release from the tunnels, colony strength increased during the summer in the control and test item group, as more resources became available when colonies were allowed to forage freely.

Table 10.3.1.5- 10: Colony Strength as mean number of adult worker bees

Colony Assessment Number	Control (Tap water)		Test Item Treatment (2 x 250 g FLU SC 500)		Reference Item R1 (1 x 400 g dimethoate/ha)		Reference Item R2 (1 x 400 g dimethoate/ha)	
	Mean count	±SD	Mean count	±SD	Mean count	±SD	Mean count	±SD
1	4000	0.0	4000	0.0	4000	0.0	4000	0.0
2	5840	1142.6	5817.5	1065.4	2645	1228.0	5197.5	870.5
3	6346.5	838.3	6989	891.1	2399.5	1083.0	2282.5	395.4
4	6602.5	773.4	6807.5	486.7	NA	NA	NA	NA
5	6791.5	1872.2	6255	1596.1	NA	NA	NA	NA
6	9696.5	4638.7	8294.5	3068.5	NA	NA	NA	NA
7	9754	4044.2	8791.5	3409.9	NA	NA	NA	NA
8	8768	2238.3	9400.5	3170.5	NA	NA	NA	NA
9	9000	1768.3	10037.5	4277.4	NA	NA	NA	NA
10	3284	970.1	4197	1975.1	NA	NA	NA	NA

SD: Standard deviation
NA: Not assessed

Overall, the development of the colonies as assessed by the mean number of (adult) worker bees was very homogenous throughout the entire study period with no distinct differences in colony strength between control and test item treatment. Statistical analysis (linear mixed effects model) revealed no significant effect of the test item treatment on the number of worker bees (colony strength; p = 0.854). A significant effect of the date was found (p = < 0.001), the interaction of date and treatment was not

significant (p = 0.987), indicating that the number of worker bees (colony strength) changed over time, but in the same manner for treatment and control. Thus, it can be concluded that sequential foliar spray applications of 2 × 250 g fluopyram/ha during flowering of a highly bee-attractive crop did neither cause adverse effects on colony strength.

Brood status/brood development

The honey bee colonies were equalised with regard to total brood (i.e. eggs + worker bee larvae + worker bee pupae).During the confined exposure period, the colonies in the control and test item treatment group were able to maintain their overall brood status and total bee brood was on a comparable level, with no distinct differences between the two exposure groups.

After the release from the tunnels, the average number of cells with brood initially increased in both control and test item group as more resources became available when colonies were allowed to forage freely. The brood status in the colonies in C and T reached their maximum at the beginning of August with an average of occupied brood cells of 15800 ± 10011 in the control and 17160 ± 2917 in the test item treatment group. Thereafter, the average number of cells with brood decreased, which reflected the development of honey bee colonies during the year. By end of October, at the last colony assessment before overwintering, numbers for total brood were 2848 ± 1897.5 in C and 4736 ± 912 in T, and as such the colonies entered the overwintering period on a comparable level.

By middle of March 2014, i.e. in spring of the following year, when the overwintering period had ended, the colonies were assessed for a final time. At this last colony assessment after overwintering, the average numbers of cells with brood in both control and test item group were slightly higher than the corresponding values prior to overwintering. Thus, it can be concluded that colonies in both treatment groups had already started breeding in response to the upcoming spring.

Statistical analysis (linear mixed effects model) revealed no significant effect of the test item treatment on the number of cells filled with brood (total brood; p = 0.153). A significant effect of the date was found (p = 0.002), the interaction of date and treatment was not significant (p = 0.627), indicating that the number of brood cells changed over time, but in the same manner for treatment and control.

Table 10.3.1.5- 11: Brood development as mean total brood cells (eggs+larvae+pupae)

Colony Assessment Number	Control (Tap water)		Test Item Treatment (2 x 250 g FLU SC 500)		Reference Item R1 (1 x 400 g dimethoate/ha)		Reference Item R2 (1 x 400 g dimethoate/ha)	
	Mean count	±SD	Mean count	±SD	Mean count	±SD	Mean count	±SD
1	8280	593.3	8080	178.9	7920	178.9	8000	489.9
2	9872	2078.2	10792	1936.9	1632	659.3	9520	1733.7
3	11040	1774.3	12120	1863.3	4320	3119.6	7320	1507.3
4	11368	1572.6	11720	2744.4	NA	NA	NA	NA
5	15800	10011.0	17160	2916.8	NA	NA	NA	NA
6	10800	4982.0	8720	4258.2	NA	NA	NA	NA
7	9280	1616.2	9480	3425.2	NA	NA	NA	NA
8	2272	1579.5	2840	1545.1	NA	NA	NA	NA
9	2848	1897.5	4736	912.0	NA	NA	NA	NA
10	5896	1792.8	6088	3652.2	NA	NA	NA	NA

SD: Standard deviation
NA: Not assessed

Thus, it can be concluded that sequential foliar spray applications of 2 × 250 g fluopyram/ha during flowering of a highly bee-attractive crop did not cause adverse effects on honey bee brood development.

Nectar and Pollen stores

Overall, the development of food stores (nectar/honey and pollen) of the colonies as assessed by the average number of cells with nectar/honey and pollen was very homogenous throughout the entire study period with no distinct differences between control and test item treatment. During the confined exposure period both the control and test item treated colonies experienced a slight decline in honey stores as a result of the limited foraging area inside the tunnels. Both treatment groups were supplemented with a small, identical quantity of ready-to-use syrup (Apiinvert®) at the end of confinement. Following release of the colonies at the monitoring site, the average number of cells with nectar/honey increased in both treatment groups as more resources became available.

Statistical analysis (linear mixed effects model) revealed no significant effect of the test item treatment on the number of cells filled with nectar/honey ($p = 0.628$) or with pollen ($p = 0.846$) within the colonies. A significant effect of the date was found ($p < 0.001$) for both honey/nectar and pollen cells. The interaction of date and treatment was not significant ($p = 0.387$ for honey/nectar; $p = 0.397$ for pollen), indicating that the number of honey/nectar and pollen cells changed over time, but in the same manner for treatment and control.

Colony health

The occurrence of the pathogens *Nosema* sp. and *Varroa* destructor, as well as of the viruses sacbrood virus and black queen cell virus occurred in both exposure groups to a similar extent, and their occurrence was therefore not linked to the absence or presence of the test item. In one single colony of the test item treatment group, on one occasion (before overwintering), deformed wing virus was detected. This finding is well in line with the slightly higher *Varroa* infestation level in the test item treatment group as measured via/on the *Varroa* boards (see below), however, an indication of a slightly higher *Varroa* infestation level in this group was already present before the confined exposure period.

Varroa-infestation levels, as measured via/on the *Varroa* boards (underneath the respective colonies), were very low during the entire experiment, in both the control and test item treatment group. There were no distinct differences between the control and the test item treatment group at any of the 16 *Varroa* assessments throughout the study.

No further pathogens (e.g. *Malpighamoeba mellificae*, *Paenibacillus larvae* spores, *Melissococcus plutonius*) or viruses (e.g. acute bee paralysis virus, chronic bee paralysis virus, Kashmir bee virus and Israel acute paralysis virus) were detected in any of the samples analysed.

Thus, it can be concluded that sequential foliar spray applications of 2 × 250 g fluopyram/ha during flowering of a highly bee-attractive crop did not cause adverse effects on colony health, as determined in terms of pathogen/bee disease and virus infestation as well as in terms of *Varroa*-infestation measured via the *Varroa*-mite-drop of the colonies, throughout the entire course of the study.

Behavioural abnormalities

In both the test item treatment group and control no acute symptoms of poisoning (e.g. cramping, agony) were observed after the corresponding applications. Also the cleaning behaviour of honey bees did not change after the test item applications and was similar to control. No repellent effect of the test item was observed after the applications, honey bees continued to forage on the treated crop. No aggressive or any other abnormal behaviour of the honey bees was noted, neither during the confinement period, nor during the monitoring period.

Thus, it can be concluded that sequential foliar spray applications of 2 × 250 g fluopyram/ha during flowering of a highly bee-attractive crop did not cause adverse effects on honey bee behaviour.

Validity criteria:

All validity criteria were met in this study.

Table 10.3.1.5- 12: Brood development

Validity Criteria		Recommended	Obtained
Foraging activity shortly before application	All groups	≥ 5 bees/m²	> 5 bees/m²
Control Mortality:	Control	Should not have been considerable.	0.6 ± 0.9 dead worker bees (after 1st application) 0.2 ± 0.4 dead worker bees (after 2nd application)
Reference Item Mortality:	Dimethoate	There should be a high number of impacted bees.	The results showed a distinct effect of the reference item on worker bee mortality.

III. CONCLUSION

In a special design study and in a full-factorial randomized block design, honey bee colonies were exposed to two sequential foliar applications of FLU SC 500 at a rate of 250 g a.s./ha each (i.e. 2 x 250 g a.s./ha), while maintained in a confined environment (gauze tunnels). The applications were made during full-flowering of the highly bee-attractive surrogate crop *Phacelia tanacetifolia*. After the confined semi-field exposure period, the colonies in the control and in the test item treatment group were released from confinement, to be monitored under field conditions for the remainder of the season until overwintering, and were assessed for a final time after overwintering in spring of the following year.

The exposure of honey bee colonies to two foliar applications with FLU SC 500 during full bloom did not result in adverse acute, short-term and long-term effects on mortality, colony strength, brood development, food storage, honey bee behaviour, queen survival, overall hive vitality and colony health, and overwintering performance.

Overall, the mortality and foraging activity were comparable to the control throughout the study duration and no effects of the test item on adult and immature honey bees were observed. Behaviour of the bees as well as nectar- and pollen storage were not affected. There were no observable effects on overall colony development, development of brood and colony strength.

Based on the results of this study, it can be concluded that FLU SC 500 does not adversely affect honey bee behaviour, brood development and colony strength when applied as two sequential foliar applications at a rate of 250 g a.s./ha, under the above described conditions.

Assessment and conclusion by applicant:

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

FLU SC 500 applied as two sequential foliar applications at a rate of 250 g a.s./ha does not adversely affect honey bee behaviour, brood development and colony strength.

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 SC 500 Tier 1 studies (laboratory studies on glass plates) with the standard test species *Aphidius rhopalosiphi* and *Typhlodromus pyri* 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 SC 500

Test species, Reference	Tested formulation, study type, exposure	Ecotoxicological endpoint	
Aphidius rhopalosiphi [redacted] (2008) M-298601-01-1 KCP 10.3.2.1/03	FLU SC 500 Laboratory, glass plates	LR_{50} > 2000 mL product/ha	
		Corr. Mortality [%]	Effects on reproduction [%]
	125 mL product/ha	0.0	1.4
	250 mL product/ha	0.0	5.5
	500 mL product/ha	3.6	1.4
	1000 mL product/ha	0.0	6.2
	2000 mL product/ha	3.6	4.1
Typhlodromus pyri [redacted] (2008) M-298616-01-1 KCP 10.3.2.1/04	FLU SC 500 Laboratory, glass plates	LR_{50} > 2000 mL product/ha	
		Corr. Mortality [%]	Effects on reproduction [%]
	125 mL product/ha	5.2	1.8
	250 mL product/ha	3.1	2.3
	500 mL product/ha	5.2	3.8
	1000 mL product/ha	5.2	4.7
	2000 mL product/ha	5.2	2.3

The exposure scenario is based on the use pattern as given in Table 10- 1. The formulation FLU SC 500 is intended to be applied at a rate of 0.15 L product/ha with 1 application in apple.

According to 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

[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

Application rate: 1 × 0.15 L product/ha

MAF (multiple application factor) = 1 (1 application)

Drift factor = 0.1573 (late), 90th percentile for 1 application

VDF = vegetation distribution factor = 5 (Tier 1; studies with 2D exposure system)

Correction factor = 10 (Tier 1)

The risk at Tier 1 is considered acceptable, if the calculated HQ is < 2

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

Crop	No. of appl.	Appl. rate [L prod./ha]	MAF	$PER_{in-field}$ [L prod./ha]
Apple	1	0.15	1.0	0.15

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	$PER_{off-field}$ [L prod./ha]
Apple	1	0.15	1.0	15.73	5	10	0.047

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

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]).

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

Table 10.3.2- 4: Tier 1 in-field risk assessment for non-target arthropods

Crop and application rate	Species	$PER_{in-field}$ [L prod./ha]	LR_{50} [L prod./ha]	HQ	Trigger
Apple 1 × 0.15 L prod./ha	*Aphidius rhopalosiphi*	0.15	> 2.0	< 0.075	2
	Typhlodromus pyri		> 2.0	< 0.075	2

PER: Predicted environmental rate; HQ: Hazard quotient

[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

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- 5: 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
Apple 1 × 0.15 L prod./ha	*Aphidius rhopalosiphi*	0.047	> 2.0	< 0.024	2
	Typhlodromus pyri		> 2.0	< 0.024	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.

Conclusion:

From the data and risk assessments presented above, it is concluded that unacceptable effects of FLU SC 500 on non-target arthropods in the in-field and off-field environment are not to be expected for the intended use of FLU 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 (ER50) of AE C656948 SC 500 to the parasitic wasp *Aphidius rhopalosiphi* (Desteffani-Perez) under laboratory conditions
Report No:	06/10148
Document No:	M-283320-01-1
Guideline(s) followed in study:	IOBC (MEAD-BRIGGS et al. 2000); Equivalent to US EPA OPPTS Guideline 850 (SUPP)
Deviations from current test guideline:	Current Guideline: IOBC, Mead-Briggs et al. (2000) Deviations: not applicable
Previous evaluation:	Yes, evaluated and accepted in DAR (2007)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.3.2.1/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	Dose-response toxicity (LR50) of AE C656948 SC 500 to the predatory mite Typhlodromus pyri (Scheuten) under laboratory conditions
Report No:	06 10 48 188
Document No:	M-283517-01-1
Guideline(s) followed in study:	IOBC (Bluemel et al. 2000); Equivalent to US EPA OPPTS Guideline No. 850.3400 (SUPP)
Deviations from current test guideline:	Current Guideline: BLÜMEL ET AL. (2000) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.3.2.1/03
Report Author:	[redacted]
Report Year:	2008
Report Title:	Dose-response toxicity (LR50) of Fluopyram SC 500G to the parasitic wasp Aphidius rhopalosiphi (Desteani-Perez) under laboratory conditions
Report No:	08 10 48 002 A
Document No:	M-298601-01-1
Guideline(s) followed in study:	IOBC Guideline (MEAD-BRIGGS et al. 2000) US EPA OCSPP 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 SC 500 on the parasitoid wasp, *Aphidius rhopalosiphi*, was investigated. Five product rates from 125 - 2000 mL product/ha were tested. Per test item rate 3 replicates of 10 wasps were exposed to FLU SC 500 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 3.6 % was found in the treatment rates. The LR_{50} was estimated to be greater than 2000 mL product/ha.

At 48 hours, 15 surviving females per treatment were confined individually for 24 hours over untreated wheat plants infested 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 product rates.

I. MATERIAL AND METHODS

Test item: FLU SC 500; specification no. 102000018148; batch ID 2007-011657; TOX08109-00; analysed content of a.s. = 501.0 g/L fluopyram; density 1.188 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 125, 250, 500, 1000 and 2000 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 thread.

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 39 - 81 % 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 3860 lux.

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

Dates of work: January 21, 2008 – February 04, 2008

II. RESULTS AND DISCUSSION

In a worst-case laboratory study, the effects of exposing the parasitoid wasp *Aphidius rhopalosiphi* to dried spray residues of FLU SC 500 on glass plates were determined.

Corrected mortalities of 0, 0, 3.6, 0 and 3.6 % were observed after 48 hours in the 125, 250, 500, 1000 and 2000 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 greater than 2000 mL product/ha.

There were no statistically significant effects on the reproductive performance of the surviving wasps in any of the product treatments.

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 SC 500
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		14.5	
Test item	125	0	14.3	1.4
	250	0	13.7	5.5
	500	3.6	14.3	1.4
	1000	0	13.6	6.2
	2000	3.6	13.9	4.1
Toxic ref.	0.3	100		
LR_{50} [5]	**> 2000 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)	6.7 %
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	14.5 mummies per female, no zero values.

III. Conclusion

The LR_{50} was estimated to be > 2000 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} > 2000 mL product/ha

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Data Point:	KCP 10.3.2.1/04
Report Author:	[redacted]
Report Year:	2008
Report Title:	Dose-response toxicity (LR50) of Fluopyram SC 500 G to the predatory mite Typhlodromus pyri (SCHEUTEN) under laboratory conditions
Report No:	08 10 48 003 A
Document No:	M-298616-01-1
Guideline(s) followed in study:	IOBC Guideline (BLÜMEL et al. 2000)
Deviations from current test guideline:	Current Guideline: BLÜMEL ET AL. (2000) Deviation: 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 SC 500 on the predatory mite, *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae), was investigated. Five test item rates from 125 - 2000 mL product/ha were tested. Per product rate 5 replicates of 20 mites were exposed to FLU SC 500 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 d no statistically significant differences compared to the control occurred and a corrected mortality up to 5.2 % was found in the treatment rates. The LR_{50} was estimated to be > 2000 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 SC 500; specification no. 102000018148; batch ID 2007-011657; TOX08109-00; analysed content of a.s. = 501.0 g/L fluopyram; density 1.188 g/mL.

Test design: Protonymphs of the predatory mite *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae; less than 24 h old, from a synchronised cohort) were exposed to dried spray residues of the product applied on glass plates at rates of 125, 250, 500, 1000 and 2000 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 49 - 71 % relative humidity with a photoperiod of 16 h and a light intensity of 2080 lux.

Statistics: Fisher's Exact 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: January 22, 2008 – February 05, 2008

II. RESULTS AND DISCUSSION

The effects of exposing the predatory mite *Typhlodromus pyri* to dried spray residues of FLU SC 500 on glass plates were determined.

Corrected mortalities of 5.2, 3.1, 5.2, 5.2 and 5.2 % were observed in the 125, 250, 500, 1000 and 2000 mL product/ha treatment rates of the product. None of the treatment rates differed significantly from the control (Fisher's Exact Binominal-test, $p \leq 0.05$) and the median lethal rate (LR_{50}) was estimated to be > 2000 mL product/ha, the highest rate tested.

At 125, 250, 500, 1000 and 2000 mL product/ha, the reproductive performance of the mites was reduced by 1.8, 2.3, 3.8, 4.7 and 2.3 % 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 SC 500 **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			6.13	
Test item	125	5.2	6.02	1.8
	250	3.1	5.99	2.3
	500	5.2	5.90	3.8
	1000	5.2	5.84	4.7
	2000	5.2	5.99	2.3
Toxic ref.	10	77.3	-	-
LR_{50} [5]	**> 2000 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 %	77.3 %
Mean cumulative number of eggs produced per female from 7 to 14 days in the control	≥ 4.0 per female	6.13 per female

III. CONCLUSION

The LR_{50} was estimated to be > 2000 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} > 2000 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:	Chronic dose response toxicity (ER50) of AE C656948 SC 500 to the rove beetle Aleochara bilineata GYLL. under extended laboratory conditions
Report No:	G 710 48/018 A
Document No:	M-295187-01-1
Guideline(s) followed in study:	IOBC Guideline (Grimm et al. 2000); Equivalent to US EPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: Grimm et al. (2000) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process.

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.

Table 10.4- 1: Maximum PECsoil values for fluopyram, its metabolites and FLU SC 500 in apples (for details see MCP Section 9, Point 9.1.3)

Compound	Apples		
	$PEC_{soil, initial}$ [mg/kg]	$PEC_{soil, plateau, 5 cm}$ [mg/kg]	$PEC_{soil, accu}$*
1 × 75 g a.s./ha			
Fluopyram	0.035	0.122	0.157
Fluopyram-7-hydroxy	0.002	<0.001	0.002
Trifluoroacetic acid (TFA)	0.001	<0.001	0.001
1 × 0.15 L prod./ha			
FLU SC 500	0.083 [1)]		

* $PEC_{soil, accu}$ means the sum of $PEC_{soil, initial}$ and $PEC_{soil, plateau}$

1) The PEC_{soil} value for the product FLU SC 500 is calculated based on the initial rate of the product (0.15 L/ha) in a single application, the portion reaching soil (BBCH 71-89, worst case interception of 65 % for apples late), the standard soil density (1.5 g/cm^3), the standard soil depth (5 cm) and the density of the formulation (1.191 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 SC 500 and Fluopyram metabolites

Test item	Test species, test design	Ecotoxicological endpoint	Reference
FLU SC 500	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 316 mg prod./kg dws $NOEC_{corr}$ = 158 mg prod./kg dws [A] NOEC = 134 mg a.s./kg dws [B] $NOEC_{corr}$ = 67 mg a.s./kg dws [A, B] EC_{10} = 277 mg prod./kg dws $EC_{10,corr}$ = 138.5 mg prod./kg dws [A] EC_{10} = 117.5 mg a.s./kg dws [A] $EC_{10,corr}$ = 59 mg a.s./kg dws [A, B]	[redacted] (2020) M-686776-01-1 KCA 8.4.1/02 KCP 10.4.1.1/02
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 [C]	[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 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] 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

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 fluopyram and its metabolites

Compound	Species, study type	Endpoint [mg/kg]	PEC_{soil} [mg/kg]	TER_{LT}	Trigger
Apples, 1 × 75 g a.s./ha					
Fluopyram	Earthworm, reproduction	NOEC = 67	0.157	427	5
Fluopyram-7-hydroxy	Earthworm, reproduction	NOEC = 9	0.002	2250 [A]	5
Trifluoroacetic acid (TFA)	Earthworm, reproduction	NOEC = 320	0.001	320000	5

[A] For the metabolite fluopyram-7-hydroxy the TER has been corrected according to an uncertainty factor of 2 in consideration of two enantiomers.

The TER values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on earthworms are to be expected from the intended use of FLU SC 500 in apples.

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Data Point:	KCP 10.4.1/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500: acute toxicity to earthworms (Eisenia fetida) tested in artificial soil with 5 percent peat
Report No:	LRT/RG-A-78/06
Document No:	M-284362-01-1
Guideline(s) followed in study:	OECD 207, OECD-Guideline for Testing of Chemicals, Earthworm, Acute Toxicity Tests (1984) Equivalent to US EPA OPPTS Guideline No. 850.6200 (SUB)
Deviations from current test guideline:	Current Guideline: OECD 207 (1984) Deviation: not applicable since test system is no longer a data requirement in EU
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. Furthermore, this study type is no longer a data requirement in EU. It is only shown for transparency reasons since it was part of the first listing process.

CP 10.4.1.1 Earthworms sub-lethal effects

Data Point:	KCP 10.4.1.1/01
Report Author:	[redacted]
Report Year:	2006
Report Title:	AE C656948 SC 500: Effects on survival, growth and reproduction on the earthworm Eisenia fetida tested in artificial soil with 5 percent peat
Report No:	LKC-RG-R-20/06
Document No:	M-268821-01-1
Guideline(s) followed in study:	ISO 11268-2: 1998 (E) and OECD 222: April 13, 2004; Equivalent to US EPA OPPTS Guideline No. 850.6200 (SUPP)
Deviations from current test guideline:	Current Guideline: OECD 222 (2016) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.4.1.1/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 (500 g/L): Effects on survival, growth and reproduction of the earthworm Eisenia fetida tested in artificial soil
Report No:	M-680776-01-1
Document No:	M-680776-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC, Regulation (EC) No. 1107/2009, US EPA OCSPP Not Applicable, ISO 11268-2: 1998 (E), OECD 222: July 29, 2016
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 SC 500 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. Eight test item rates from 18 to 1000 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 SC 500 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 offspring hatched from the cocoons per test vessel.

The study fulfilled all validity criteria of OECD 222 guideline.

The endpoints were: $NOEC_{mortality}$ ≥1000 mg prod./kg dry weight artificial soil, $LOEC_{mortality}$ >1000 mg prod./kg dry weight artificial soil, $NOEC_{growth}$ = 316 mg prod./kg dry weight artificial soil, $LOEC_{growth}$ = 562 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. The EC_{10} and EC_{20} related to reproduction are determined to be 277 and 356 mg prod./kg dry weight artificial soil, respectively.

I. MATERIALS AND METHODS

Test item: FLU SC 500, specification no.: 102000018148; Batch No.: EV57002782; TOX21406-00, analytical findings: 42.4 % w/w fluopyram equivalent to 504.9 g/L; density: 1.191 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 10 % 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): 70 % industrial quartz sand, 10 % Sphagnum peat (air dried and finely ground), 20 % Kaolinite clay.

After a period of 4 weeks the adult earthworms were removed from the test vessels, 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 four weeks. After this additional test period the reproduction was determined by counting the number of offspring hatched from the cocoons per test vessel.

Climatic conditions: The climatic conditions were in the temperature range 20 ± 2 °C with a photoperiod of 16 hours light and a light intensity of 400 - 800 lux.

Statistics: Data for mortality were not statistically evaluated because no mortality occurred. Data of growth were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's -Test and Leven's -Test (α = 0.05) respectively. Data of growth were normally distributed and homogeneity of variances was given. Therefore William's t-test, one-sided-smaller, α = 0.05 was used to determine NOEC and LOEC values. Data of reproduction were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's-Test and Leven's -Test (α = 0.05) respectively. Data of reproduction were normally distributed and homogeneity of variances was given. Therefore William's t-test, one-sided-smaller, α = 0.05 was used to determine NOEC and LOEC values. The calculation of EC_{10}/EC_{20} and their 95% confidence limits was based on the model which provides the best fit out of different suitable regression models.

Dates of work: September 09, 2019 – November 11, 2019

II. RESULTS AND DISCUSSION

Table 10.4.1.1- 1: Effects of FLU SC 500 on *Eisenia fetida*

Parameter	Treatment [mg product/kg dry weight artificial soil]								
	Control	**18**	**32**	**56**	**100**	**178**	**316**	**562**	**1000**
Mortality of adult earthworms [%] after 28 days	0	0	0	0	0	0	0	0	0
Significance (Mortality)*	-	-	-	-	-	-	-	-	-
Mean change of body fresh weight of the adults from day 0 to day 28 [%]	20.99	28.53	24.04	25.45	27.61	28.24	19.35	7.09	6.43
Standard deviation	5.39	3.03	4.05	7.27	7.20	1.35	2.46	2.60	2.93
Significance (body fresh weight)*	-	-	-	-	-	-	-	+	+
Mean number of off-spring per test vessel after 56 days	297.1	267.0	327.8	303.3	271.5	302.3	266.8	142.0	58.3
Standard deviation	33.3	11.2	53.8	35.7	35.3	36.0	6.8	14.4	11.5
Coefficient of variance (%)	11.2	4.2	16.4	11.8	13.0	11.9	2.6	10.2	19.8
% of control	-	89.9	110.3	102.1	91.4	101.7	89.8	47.8	19.6
Significance (reproduction)**	-	-	-	-	-	-	-	+	+

	Adult Mortality	**Growth**	**Reproduction**
NOEC [mg prod./kg dry weight soil]	**>1000**	**316**	**316**
LOEC [mg prod./kg dry weight soil]	**>1000**	**562**	**562**
EC_{10} (mg prod./kg dry weight artificial soil) ***			**277**
95 % confidence limits			(222 - 356)
EC_{20} (mg prod./kg dry weight artificial soil) ***			**356**
95 % confidence limits			(303 - 400)

* Dunnett`s Multiple t-test, two-sided, α = 0.05), + = significant, - = not significant

** William's t-test, one-sided smaller, α = 0.05), + significant, - not significant

*** EC_{10}, EC_{20} (Probit analysis using linear likelihood regression)

Mortality:

After 28 days of exposure, no mortality in the control group was observed. Therefore, no statistical evaluation was conducted. Therefore, the endpoints related to mortality were: NOEC: ≥1000 mg prod./kg dry weight artificial soil and LOEC: >1000 mg prod./kg dry weight artificial soil.

Effects on growth:

No statistically significant effects for the growth relative to the control were in the test item concentrations up to and including 316 mg test item/kg dry weight artificial soil (Dunnett`s Multiple t-

test, two-sided, α = 0.05). Therefore, the endpoints related to growth were: NOEC: 316 mg prod./kg dry weight artificial soil and LOEC: 562 mg prod./kg dry weight artificial soil.

Effects on reproduction:

No statistically significant differences concerning the number of juveniles relative to the control were observed in the test item concentrations up to and including 316 mg test item /kg dry weight artificial soil (William's t-test, one-sided smaller, α = 0.05).

Therefore, the endpoints related to reproduction were: NOEC: 316 mg prod./kg dry weight artificial soil and LOEC: 562 mg prod./kg dry weight artificial soil. The EC_{10} (C.I.) and EC_{20} (C.I.) values were calculated to be 277 (222 - 322) mg prod./kg soil dry weight and 356 (303 - 400) 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	236 - 342
Coefficient of variance of reprodiction in the control	≤ 30 %	11.2 %

Reference test:

The corresponding toxic standard reference test, with the reference test item mixed into the artificial soil, was performed from 2019-02-04 to 2019-04-05 (Rg-R-Ref 32/19; NON-GLP). Effects on mortality and growth of the adults after an exposure period of 28 days and the number of offspring after 56 days were determined.

No mortality of the adult earthworms was observed 28 days after application. The change of body weight of the adult earthworms of the test concentration of 5.0 mg a.s./kg dry weight soil was statistically significant reduced in comparison to the control (results of a Dunnett's multiple sequential t-test, two-sided, α = 0.05).

The number of juveniles per test vessel of the test concentrations 2.5 and 5.0 mg a.s./kg dry weight soil were statistically significant reduced in comparison to the control (results of a Welch t-test after Bonferroni-Holm, one-sided smaller, α = 0.05).

According to the guideline significant effects should be observed between 1 and 5 mg a.s./kg dry weight artificial soil. Thus, the results of this reference test indicated that the test system was sensitive to the reference test item.

III. Conclusion

All validity criteria were met. The endpoints were:

$NOEC_{mortality}$: ≥1000 mg prod./kg dry weight artificial soil

$LOEC_{mortality}$: >1000 mg prod./kg dry weight artificial soil

$NOEC_{growth}$: = 316 mg prod./kg dry weight artificial soil
$LOEC_{growth}$: = 562 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
$EC_{10reproduction}$: = 277 mg prod./kg dry weight artificial soil
$EC_{20reproduction}$: = 356 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 = 316 mg prod./kg dws or 134 mg a.s./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 FLU SC 500 and fluopyram metabolites

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
Springtails, reproduction			
FLU SC 500	*Folsomia candida* reproduction 28 d, mixed	NOEC = 178 mg prod./kg dws $NOEC_{corr}$ = 89 mg prod./kg dws [A] NOEC = 75.5 mg a.s./kg dws [B] $NOEC_{corr}$ = 37.8 mg a.s./kg dws [A, B] EC_{10} = 240 mg prod./kg dws $EC_{10,corr}$ = 120 mg prod./kg dws [A] 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
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) [C] EC_{10} calculation not possible	[redacted] (2012) M-436127-01-1 KCA 8.4.2.1/05
Soil mites, reproduction			
FLU SC 500	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 1000 mg prod./kg dws $NOEC_{corr}$ ≥ 500 mg prod./kg dws [A] 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
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
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) [C] EC_{10} calculation not possible	[redacted] (2012) M-436326-01-1 KCA 8.4.2.1/05

dws = dry weight soil, 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] 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)

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 fluopyram and its metabolites

Compound	Species, study type	Endpoint [mg/kg]	PEC_{soil} [mg/kg]	TER_{LT}	Trigger
Apples, 1 × 75 g a.s./ha					
Fluopyram	*Folsomia candida*	NOEC ≥ 37.8	0.157	241	5
Fluopyram	*Hypoaspis aculeifer*	NOEC ≥ 212	0.157	1350	5
Fluopyram-7-hydroxy	*Folsomia candida*	NOEC ≥ 281	0.002	70250 [A]	5
Fluopyram-7-hydroxy	*Hypoaspis aculeifer*	NOEC ≥ 500	0.002	≥ 125000 [A]	5
Trifluoroacetic acid (TFA)	*Folsomia candida*	NOEC ≥ 84	0.001	≥ 84000	5
Trifluoroacetic acid (TFA)	*Hypoaspis aculeifer*	NOEC ≥ 84	0.001	≥ 84000	5

[A] 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 SC 500 in apples.

CP 10.4.2.1 Species level testing

Data Point:	KCP 10.4.2.1/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500A G: Influence on the reproduction of the collembola species Folsomia candida tested in artificial soil with 5 % peat
Report No:	FRM-COLL-50/07
Document No:	M-288904-01-1
Guideline(s) followed in study:	ISO 11267 (1999) Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) Deviation: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.4.2.1/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	Fluopyram SC 500: Influence on mortality and reproduction on the soil mite species Hypoaspis aculeifer tested in artificial soil with 5 % peat
Report No:	KRed-HR-3[illegible]
Document No:	M-287030-01-1
Guideline(s) followed in study:	Recommendations of the Hypoaspis Ring-test group (HASTE), Final Meeting on January 15, 2007 in Utrecht; Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.4.2.1/03
Report Author:	[redacted]
Report Year:	2019
Report Title:	Fluopyram SC 500 g/L: Influence on mortality and reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	E 314 05458-0
Document No:	M-675002-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 232 US EPA OCSPP Not Applicable
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 SC 500 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 SC 500 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 mortality of the adult test organisms was statistically significant different in the three highest test item rates (316, 562 and 1000 mg prod./kg dry weight soil) compared to the control. No statistically significant differences in mortality between control up to and including 178 mg prod./kg dry weight artificial soil occurred.

Therefore, the No-Observed-Effect-Concentration (NOEC) for mortality is 178 mg prod./kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for mortality is 316 mg prod./kg dry weight artificial soil. The LC_{10} and LC_{20} values for mortality were calculated to be 240 and 488 mg prod./kg soil dry weight.

Concerning the number of juveniles statistical analysis revealed no significant difference between control up to and including 562 mg prod./kg dry weight artificial soil. At the highest test item rate (1000 mg prod./kg dry weight artificial soil) a statistically significant difference in reproduction compared to the control occurred.

Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is 562 mg prod./kg dry weight artificial soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 1000 mg prod./kg dry weight artificial soil. The EC_{10} and EC_{20} values for reproduction were calculated to be 794 and 991mg prod./kg soil dry weight.

I. MATERIALS AND METHODS

Test item: FLU SC 500; specification no.: 102000018148; Batch No.: EV57002782; TOX21406-00; analytical findings: 42.4 % w/w fluopyram equivalent to 504.9 g a.s./L; density: 1.191 g/mL (20 °C).

Test design: Ten collembolans (10 - 12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control and treatment. 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 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 William's t-test (one-sided smaller, $\alpha = 0.05$) was applied to reproduction data. The LLCs values were calculated with Logit analysis and the ECx values with 3-param. normal CDF analysis.

Dates of work: September 23, 2019 – October 25, 2019

II. Results and Discussion

Table 10.4.2.1- 1: Effects on mortality and reproduction of *Folsomia candida* after treatment with FLU SC 500

Test concentration [mg prod./kg dry weight artificial soil]	Adult mortality [%]	Significance (*)	Mean number of juveniles per test vessel ± SD	Reproduction [% of control]	Significance (**)
Control	2.5		900.3 ± 133.2		--
18	2.5	-	923.5 ± 105.4	102.6	-
32	5.0	-	883.8 ± 117.5	98.2	-
56	5.0	-	873.8 ± 64.0	97.1	-
100	7.5	-	954.3 ± 46.2	106.0	-
178	3.3	-	989.0 ± 186.3	109.9	-
316	20.0	+	857.8 ± 55.4	95.3	-
562	17.5	+	891.5 ± 165.1	99.0	-
1000	42.5	+	721.5 ± 134.3	80.1	+

Endpoints	Mortality	Reproduction
NOEC [mg prod/kg dry weight artificial soil]	178	562
LOEC [mg prod./kg dry weight artificial soil]	316	1000
LC_{10}/ EC_{10} [mg prod./kg dry weight artificial soil]	240 (139 - 341)	795 (634 - 860)
LC_{20}/ EC_{20} [mg prod./kg dry weight artificial soil]	488 (344 - 714)	990 (917 - 1012)

Calculations were done with unrounded values
n.d.: Not determined
SD: Standard deviation
LLCs: Logit analysis; ECx = 3-param. normal CDF analysis
* Fisher's exact Binominal Test with Bonferroni correction, one-sided greater, $\alpha = 0.05$, "-": non-significant; "+": significant
** William's t-test, one-sided smaller, $\alpha = 0.05$, "-": 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.

Concerning the mortality of the adult test organisms statistical analysis (Fisher's Exact Binominal Test with Bonferroni Correction, one-sided-greater, $\alpha = 0.05$) revealed no significant difference between control up to and including 178 mg prod./kg dry weight artificial soil.

Therefore, the NOEC for mortality is 178 mg prod./kg dry weight artificial soil. The LOEC for mortality is 316 mg prod./kg dry weight artificial soil.

The LC_{10} and LC_{20} values for mortality were calculated to be 240 mg prod./kg soil dry weight (95% confidence limits: 139 - 341) and 488 mg prod./kg soil dry weight (95% confidence limits: 344 - 714), respectively (Logit analysis).

Reproduction:

Concerning the number of juveniles statistical analysis (William's t-test, one-sided smaller, $\alpha = 0.05$) revealed no significant difference between control up to and including 562 mg /kg dry weight artificial soil.

Therefore, the NOEC for reproduction is 562 mg prod./kg dry weight artificial soil. The LOEC for reproduction is 1000 mg prod./kg dry weight artificial soil.

The EC_{10} and EC_{20} values for reproduction were calculated to be 794 mg prod./kg soil dry weight (95 % confidence limits: 659 - 777) and 991 mg prod./kg soil dry weight (95 % confidence limits: 925 - 1005), respectively (3-param. normal CDF analysis analysis).

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 %	2.5 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	900
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	14.8 %

All validity criteria of the OECD 232 guideline were fulfilled.

Reference test:

The most recent non-GLP-test (LAR-Coll-Ref-36/19) with the reference item Boric acid was performed at test concentrations 44, 67, 100, 150 and 225 mg Boric acid/kg dry weight artificial soil.

The $NOEC_{reproduction}$ was calculated to be 44 mg Boric acid/kg dry weight artificial soil and accordingly the $LOEC_{reproduction}$ is 67 mg Boric acid/kg dry weight artificial soil according Williams t-test, $\alpha = 0.05$, one-sided smaller.

Boric acid showed an EC_{50} of 137 mg test item/kg dry weight artificial soil (95 % confidence limits from 123 mg to 152 mg Boric acid/kg dry weight artificial soil) for reproduction according Probit analysis using linear maximum likelihood regression. The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial soil dry weight).

III. CONCLUSION

All validity criteria were met. The endpoints were:

$NOEC_{mortality}$: 178 mg prod./kg dry weight artificial soil

$LOEC_{mortality}$: 316 mg prod./kg dry weight artificial soil

$LC_{10\ mortality}$: 240 (139 - 341) mg prod./kg dry weight artificial soil

$LC_{20\ mortality}$: 488 (344 - 714) 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

$EC_{10\ reproduction}$: 795 (634 - 860) mg prod./kg dry weight artificial soil

$EC_{20\ reproduction}$: 990 (917 - 1012) mg prod./kg dry weight artificial soil

According to EFSA (2015) the level of protection for the LC_{10} is classified as "high". The normalised width of confidence interval (NW) rating for the EC_{10} is "fair".

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: $NOEC_{mortality}$ = 178 mg product/kg dws or 75.5 mg a.s./kg dws

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Data Point:	KCP 10.4.2.1/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 g/L: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 05448-5
Document No:	M-678468-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 OECD Guideline 226 (2016) 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 SC 500 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. 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 soil mites each were exposed to FLU SC 500 mixed into artificial soil. Mortality of the soil mites was assessed after 14 days.

The study fulfilled all validity criteria of OECD 226 guideline.

No statistically significant differences in mortality 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. Due to the lack of a concentration-response relationship, no LC_x values could be calculated.

The reproduction rate of the soil mites was assessed after 14 days. No statistically significant differences compared to the control occurred. Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is ≥1000 mg prod./kg dws. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is >1000 mg prod./kg dws. Due to the lack of a concentration-response relationship, no EC_x values could be calculated.

I. MATERIALS AND METHODS

Test item: FLU SC 500; specification no.: 102000018148; Batch No.: EV57002782; TOX21406-00; analytical findings: 42.4 % w/w fluopyram equivalent to 504.9 g/L; density: 1.191 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 18, 32, 56, 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 3, 7 and 10 days after test start. 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, α = 0.05) was applied to mortality data and Williams t-test (one-sided smaller, α = 0.05) was applied to reproduction data.

Dates of work: October 04, 2019 – October 24, 2019

II. RESULTS AND DISCUSSION

Table 10.4.2.1- 3: Effects on mortality and reproduction of *Hypoaspis aculeifer* after treatment with FLU SC 500

Test concentration [mg prod./kg dry weight artificial soil]	Adult mortality [%]	Significance (*)	Mean number of juveniles per test vessel ± SD	Reproduction [% of control]	Significance (**)
Control	2.5		313.5 ± 7.8	-	
18	5.0	-	356.5 ± 29.7	107.3	-
32	5.0	-	356.3 ± 12.4	113.6	-
56	2.5	-	359.5 ± 14.7	114.7	-
100	7.5	-	349.8 ± 22.4	111.6	-
178	5.0	-	374.0 ± 10.9	119.3	-
316	2.5	-	327.8 ± 19.9	104.5	-
562	0.0	-	344.3 ± 22.4	109.8	-
1000	7.5	-	363.3 ± 21.6	115.9	-
Endpoints				**Adult mortality**	**Reproduction**
NOEC [mg prod./kg dry weight artificial soil]				≥1000	≥1000
LOEC [mg prod./kg dry weight artificial soil]				>1000	>1000
EC_{10} [mg prod./kg dry weight artificial soil]				-	n.d.
EC_{50} [mg prod./kg dry weight artificial soil]				-	n.d.

Calculations were done with unrounded values.
n.d.: Not determined
SD: Standard deviation
(*) 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 2.5 % 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 group and any treatment group up to and including 1000 mg prod./kg dry weight artificial soil.

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 (Williams t-test, one-sided smaller, α = 0.05) revealed no significant difference between control group and any treatment group up to and including 1000 mg prod./kg dry weight artificial soil.

Therefore, the NOEC for reproduction is ≥ 1000 mg prod./kg dry weight artificial soil. The LOEC for reproduction is > 1000 mg prod./kg dry weight artificial soil.

Due to the lack of a concentration-response relationship no reliable EC_x-calculation is possible. Therefore no EC_{10}/EC_{20}-value can be reported.

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 %	2.5 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	313.5
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	2.5 %

Reference test:

The corresponding non-GLP-test (LAR-HR-Ref-28/19) 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 EC 400 G showed a LC_{50} of 3.5 mg a.s./kg for mortality of the adult mites according Logit analysis using maximum likelihood regression (confidence limits from 3.3 mg a.s./kg to 3.8 mg a.s./kg).

The reproduction of the soil mites was not significantly reduced in comparison to the control up to and including 3.2 mg a.s./kg dry weight artificial soil. Therefore, the NOEC is calculated to be 3.2 mg a.s./kg dry weight artificial soil and accordingly the LOEC is 5.6 mg a.s./kg dry weight artificial soil. Since variances of the data were homogenious, Williams t-test α = 0.05, one-sided smaller was used.

Dimethoate EC 400 G showed an EC_{50} of 6.7 mg a.s./kg dry weight artificial soil (95 % confidence limits from 6.5 mg a.s./kg to 7.0 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

$NOEC_{reproduction}$: ≥1000 mg prod./kg dry weight artificial soil

$LOEC_{reproduction}$: >1000 mg prod./kg dry weight artificial soil

Due to the lack of a concentration-response relationship no reliable EC-calculation was possible.

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 ≥ 1000 mg product/kg dws or ≥ 424 mg a.s./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.

Data Point:	KCP 10.4.2.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500: Effects on soil litter degradation
Report No:	LR/SLD-32/07
Document No:	M-290278-01-1
Guideline(s) followed in study:	Effects of Plant Protection Products on Functional Endpoints in Soil (EPFES, Lisbon 2002, Guidance Document Joerg Roembke et al.; Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted (RAR 2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	yes

The study above was performed with an outdated formulation and the study type is no longer a data requirement in the EU. However, it is shown for transparency reasons since it was part of the first listing process.

CP 10.5 Effects on soil nitrogen transformation

Table 10.5- 1: Studies on nitrogen transformation with FLU SC 500, Fluopyram and its metabolites

Test substance	Test species, test design	Ecotoxicological endpoint		Reference
N-transformation				
FLU SC 500	Study duration 28 d	No unacceptable effects at an appl. rate of:	15.88 mg prod./kg dws 6.73 mg a.s./kg dws	[redacted] (2013) M-674874-01-1 KCP 10.5/03
Fluopyram	Study duration 28 d	No unacceptable effects at an appl. rate of:	**3.33 mg a.s./kg dws**	[redacted] (2006) M-281477-01-1 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)** [A]	[redacted] (2013) M-444423-01-1 KCA 8.5/04

dws = dry weight soil, a.s. = active substance; p m = pure metabolite, prod. = product

[A] 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

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 SC 500 for nitrogen transformation

Compound	Species	Endpoint [mg prod./kg]	$PEC_{soil,\ max}$ [mg prod./kg]	Refinement required
Apples, 1 × 0.15 L prod./ha				
FLU SC 500	Soil micro-organisms	15.88	0.083	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
Apples, 1 × 75 g a.s./ha				
Fluopyram	Soil micro-organisms	3.33	0.157	No
Fluopyram-7-hydroxy	Soil micro-organisms	10	0.002	No [A]
Trifluoroacetic acid (TFA)	Soil micro-organisms	1.344	0.001	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.

Study summaries

Data Point:	KCP 10.5/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500A G: Determination of effects on nitrogen transformation in soil
Report No:	LRT-N-82/07
Document No:	M-289207-01-1
Guideline(s) followed in study:	OECD 216; adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Micro organisms: Nitrogen Transformation Test; US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 216 (2000) Deviation: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

Data Point:	KCP 10.5/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500A G : Determination of effects on carbon transformation in soil
Report No:	LRT-C-70/07
Document No:	M-289388-01-1
Guideline(s) followed in study:	OECD 217, Adopted January 21, 2000, OECD Guideline for the Testing of Chemicals, Soil Micro organisms: Carbon Transformation Test; US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: OECD 217 (2000), Deviations: not applicable since test system is no longer a data requirement in EU
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. Furthermore, this study type is no longer a data requirement in the EU. It is only shown for transparency reasons since it was part of the first listing process.

Data Point:	KCP 10.5/03
Report Author:	[redacted]
Report Year:	2019
Report Title:	Fluopyram SC 500 (500 g/L): Effects on the activity of soil microflora (Nitrogen transformation test)
Report No:	19 48 SMN 0050
Document No:	M-674874-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP Not Applicable OECD 216 (2000)
Deviations from current test guideline:	Current Guideline: OECD 216 (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 effect of FLU SC 500 on the activity of soil microflora with regard to nitrogen transformation was tested during an exposure of 28 days in a silty-loamy sand soil by comparing control and treatment. Two test item rates of 1.59 and 15.88 mg product/kg dry weight soil (equivalent to 1 and 10 L prod./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 and 28 days. No adverse effects of fluopyram SC 500 on nitrogen transformation in soil (in terms of Nitrate-N in mg/kg soil dry weight/time interval/day) were observed at both test concentrations at the end of the test (time interval 14 - 28 days after application).

I. Materials and Methods

Test item: FLU SC 500, Specification No.: 102000018148, BCS-code: BCS-AR83685; batch no.: EV57002782; Sample description: TOX21406-00; analytical content: 42.4 % w/w (504.9 g/L) fluopyram, density 1.191 g/mL.

Test design: A silty-loamy sand soil (DIN 4220) with pH 6.0, 3.68 % C_{org}, and with the water holding capacity of 37.37 g/100 g dry soil was exposed for 28 days to 1.59 mg prod./kg soil dry weight and 15.88 mg prod./kg soil dry weight. Application rates were equivalent to 1 L prod./ha and 10 L prod./ha. 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 was mixed with 0.5 % (i.e. 1.0 g/200 g soil d.w.) lucerne meal. One additional soil sample (without Lucerne meal) was used for determination of the initial NO_3-N-content. The initial NO_3-N-content was 6.55 mg /100 g soil d.w.

The soil of each treatment was tested as a series of 3 replicates. 200 g soil dry weight (= one sub-sample) per test vessel was weighted. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14 and 28 days after application and the NH_4-N-, NO_3-N- and NO_2-N-contents were determined.

Climatic conditions: The test vessels were kept in darkness in a climatic room and the temperature ranged between 19.1 -20.5 °C during the test. The water content of the soil ranged between 42.80 - 43.85 % of WHC. The pH value of the soil ranged between 6.0 and 6.1.

Statistics: A statistical evaluation of the test results was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5 % significance level). The statistical analysis was performed with the software ToxRat Professional 3.3.0 (Ratte 2018).

Dates of work: October 10, 2019 – November 07, 2019

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 4.9 % 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 SC 500

Time interval [days]	Control			1.59 mg prod./kg soil dry weight equivalent to 1 L prod./ha				15.88 mg prod./kg soil dry weight equivalent to 10 L prod./ha			
	Nitrate-N[1)]			Nitrate-N[1)]			% difference to control	Nitrate-N[1)]			% difference to control
0-7	5.14	±	0.32	5.50	±	0.13	+7.0 [ns]	5.46	±	0.37	+6.2 [ns]
7-14	1.93	±	0.08	1.91	±	0.07	-0.7 [ns]	2.42	±	0.48	+25.4 [ns]
14-28	1.29	±	0.35	0.99	±	0.24	-23.2 [ns]	1.22	±	0.03	-5.2 [ns]

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

ns No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

The product FLU SC 500 caused a temporary stimulation of the daily nitrate rate at the tested concentration of 15.88 mg prod./kg dry soil at time interval 7 - 14 days after application.

However, no adverse effects of FLU SC 500 on nitrogen transformation in soil could be observed at both tested concentrations (1.59 mg prod. and 15.88 mg prod./kg dry soil) at the end of the test, 28 days after application (time interval 14 - 28). Differences from the control of - 23.2 % (test concentration 1.59 mg prod./kg dry soil) and - 5.2 % (test concentration 15.88 mg prod./kg dry soil) were measured at the end of the 28-day incubation period (time interval 14 - 28).

Reference test:

In the most recent test with the toxic standard (conducted from 2019-01-10 to 2019-02-07), Dinoterb caused an effect of + 62.7 % and + 120.9 % (required ≥ 25 %) on the nitrogen transformation in a field soil at the tested concentrations of 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, 28 days after application (time interval 14 - 28) and thus demonstrates the sensitivity of the test system.

III. CONCLUSION

FLU SC 500 caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 15.88 mg prod./kg soil dry weight, which are equivalent to application rates up to 10 L prod./ha or 6.73.mg a.s./kg dws.

Assessment and conclusion by applicant:

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

The endpoint is: 15.88 mg product/kg dws or 6.73 mg a.s./kg dws

CP 10.6 Effects on terrestrial non-target higher plants

For the formulation FLU SC 500 (representative formulation) two single dose studies (testing 11 and 10 species) and one dose response study (testing 3 species) on terrestrial plant vegetative vigour, as well as one single dose study (testing 10 species) and two dose response studies (testing 3 species and one species) on terrestrial plant seedling emergence were conducted to determine possible effects on terrestrial non-target higher plants.

Table 10.6- 1: Effect values relevant for the risk assessment for non-target terrestrial plants for FLU 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] *Lolium perenne* [m] *Zea mays* [m]	Vegetative vigour; Tier 1, single dose, 21 days	ER_{50} > 250 g a.s./ha (all species)	[redacted] (2008) M-301453-01-1 KCP 10.6.2/05
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]	Vegetative vigour; Tier 1, single dose, 21 days	ER_{50} > 500 g a.s./ha (all species)	[redacted] (2020) M-688439-01-1 KCP 10.6.2/06
Fagopyrum esculentum [d] *Allium cepa* [m] *Avena sativa* [m]	Vegetative vigour; Tier 2, dose response, 21 days	ER_{50} > 500 g a.s./ha (all species)	[redacted] (2020) M-696933-02-1 KCP 10.6.2/07
Fagopyrum esculentum [d]	Seedling emergence; Tier 2, dose response, 21 days	ER_{50} > 500 g a.s./ha	[redacted] (2012) M-428356-01-1 KCP 10.6.2/03
Beta vulgaris [d] *Brassica napus* [d] *Cucumis sativus* [d] *Fagopyrum esculentum* [d] *Glycine max* [d] *Helianthus annuus* [d] *Allium cepa* [m] *Avena sativa* [m] *Lolium perenne* [m] *Zea mays* [m]	Seedling emergence; Tier 1, single dose, 21 days	ER_{50} > 500 g a.s./ha (all species)	[redacted] (2020) M-688440-01-1 KCP 10.6.2/08
Beta vulgaris [d] *Allium cepa* [m] *Lolium perenne* [m]	Seedling emergence; Tier 2, dose response, 21 days	ER_{50} > 500 g a.s./ha (all species)	[redacted] (2020) M-696931-01-1 KCP 10.6.2/09

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 75 g a.s./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.

Deterministic risk assessment

Table 10.6-2: Deterministic assessment of the risk for non-target plants due to the use of FLU SC 500 in apple

Intended use	Apple, 1 × 75 g a.s./ha, BBCH 71-89			
product	FLU SC 500			
Application rate (g a.s./ha)	1 × 75			
Test species	**ER_{50} (g a.s./ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**TER criterion: TER ≥ 5***
All species -seedling emergence	>500	15.73	11.8	>42.4
All species -vegetative vigour	>500	15.73	11.8	>42.4

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}

Conclusion:
From the information presented above it is concluded that the use of FLU 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).

Data Point:	KCP 10.6.1/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	Evaluation of the pre-emergence (PPI) biological activity of AE C656948 SC 500
Report No:	PPI-07002
Document No:	M-297136-01-1
Guideline(s) followed in study:	Equivalent to US EPA OPPTS Guideline No. 850.SUPP
Deviations from current test guideline:	Current Guideline: not applicable Deviations: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2012)
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

CP 10.6.2 Testing on non-target plants

Data Point:	KCP 10.6.2/01
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500A G - Effect on the vegetative vigour of ten species of non-target terrestrial plants (Tier 1)
Report No:	VV07/038
Document No:	M-295544-01-1
Guideline(s) followed in study:	US EPA 123-1, described by Horst and Ellwanger (1982) and OECD 227 (July 2006, adopted); Equivalent to US EPA OPPTS Guideline No. 850.4150
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

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Data Point:	KCP 10.6.2/02
Report Author:	[redacted]
Report Year:	2007
Report Title:	AE C656948 SC 500A G effect on seedling emergence and seedling growth test of ten species of non-target terrestrial plants (Tier 1 and 2)
Report No:	SE07/037
Document No:	M-295406-01-1
Guideline(s) followed in study:	US EPA 123-1, described by Horst and Ellwanger (1982) and OECD 208 (July 2006, adopted); Equivalent to US EPA OPPTS Guideline No. 850.4100
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted in DAR (2011)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

The study above was performed with an outdated formulation. It is only shown for transparency reasons since it was part of the first listing process. New data has been generated with the representative formulation for the active substance renewal process, which is presented in this section further below.

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Data Point:	KCP 10.6.2/03
Report Author:	[redacted]
Report Year:	2012
Report Title:	FLUOPYRAM SC 500B G - Effects on the seedling emergence and growth of one species of non-target terrestrial plants (Tier 2)
Report No:	SE12/006
Document No:	M-428356-01-1
Guideline(s) followed in study:	OPPTS 850.4225, US EPA Ecological Effect Test Guideline, April 1996, Seedling emergence, Tier II and OECD 208 Guidelines for the testing of chemicals, Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
Deviations from current test guideline:	Current Guideline: OECD 208 (2006) Deviations: Temporary deviation from climate condition (light). The range of light intensity was not reported. However, 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). Deviation from recommended plant density. All validity criteria were met. The deviation listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	yes, evaluated and accepted in Addendum 2 to the DAR (2012)
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 SC 500 on the seedling emergence and growth of non-target terrestrial plant *Fagopyrum esculentum* (buckwheat), following a pre-emergence application of the product to the soil surface. Planting density included 5 seeds per pot, with 8 replicate pots, respectively, for a total of 40 seeds per treatment level. The sown seeds of the plant species were treated with 5 application rates and a water control. The application rates were: 31.3; 62.5; 125; 250 and 500 g a.s./ha. The application was done at a volume rate of 100 L/ha. Assessments were made 7, 14 and 21 days after application against the deionised water treated controls. On day 0, 7 and 14, only plant emergence, survival and phytotoxicity were recorded. The study was terminated 21 days after application. Final assessments were made for emergence, plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight.

The study fulfils all validity criteria of OECD 208 guideline.

No visual phytotoxicity in none of the treatment groups were observed on day 21 in this study. The NOER and LOER values (based on emergence, survival, shoot height and shoot dry weight) were determined to be 500 g a.s./ha and > 500 g a.s./ha, respectively. The ER_{50} value (based on emergence, survival, shoot height and shoot dry weight) was > 500 g a.s./ha.

I. Material and Methods

Test item: FLU SC 500, specification no.: 102000018148 - 01; batch ID.: 2010-008479, Sample description: FAR01554-00; active substance (analysed content): fluopyram: 42.2 % w/w (502.4 g/L); density: 1.191 g/mL).

Test design: *Fagopyrum esculentum* (buckwheat) was tested in this seedling emergence and growth test. The germination rate of the seeds used in this study, observed in an annual germination tests, was ≥ 70 %. The seeds were sown one day prior to application of the product to the soil surface in 10.5 cm plastic pots. The used soil was a sandy-silt loam with washed sand.

Planting density included 5 seeds per pot, with 8 replicate pots, respectively, for a total of 40 seeds per

treatment level. The sown seeds of the plant species were treated with 5 application rates and a water control. The application rates were: 31.3; 62.5; 125; 250 and 500 g a.s./ha. The test solutions were applied at a volume rate of 100 L/ha. Control pots were sprayed with 100 L/ha of deionized water.

Assessments were made 7, 14 and 21 days after application against the deionised water treated controls. On day 0, 7 and 14, only plant emergence, survival and phytotoxicity were recorded. The study was terminated 21 days after application. Final assessments were made for emergence, plant survival, visual phytotoxicity, plant growth stage, shoot length and shoot dry weight. Phytotoxicity (e.g. chlorosis, necrosis, bleaching, wilting, leaf deformation, stunting) was recorded from the living plants at each assessment time following a 0 - 90 % rating system in 10 % steps. Any plant considered as being dead was not rated for visual phytotoxicity and removed from the pot.

Climatic conditions: Following application, the pots with plants were maintained under greenhouse conditions with natural daylight supplemented by artificial lighting. 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 regulated to maintain 70 ± 30 %.

Statistical analysis of data was performed to obtain NOER, LOER, LR/ER$_{25}$ and LR/ER$_{50}$ values for emergence, survival, shoot length and shoot dry weight, using ToxRat statistical software.

Dates of work: February 15, 2012 – March 14, 2012

II. RESULTS AND DISCUSSION

Validity criteria:

The validity criteria of OECD 208 were fulfilled.

The seedling emergence of control plants was ≥ 70 % (actually 92.5 %). The mean survival of emerged control seedlings was ≥ 90 % (actually 100 %) 21 days after at least 50 % emergence in the control.

There was no visual phytotoxicity of control seedlings and control plants exhibited normal growth. The environmental conditions during the test time were kept identical. The pots used for this study were filled in equal manner with the same soil.

Analytical results:

The analysis of fluopyram in the solution of the highest tested application rate (500 g a.s./L) revealed measured concentrations of 99.4 % of nominal concentration.

Biological findings:

No visual phytotoxicity in none of the treatment groups were observed on day 21 in this study.

Table 10.6.2-1: Effects on phytotoxicity of *Fagopyrum esculentum* at the final assessment on day 21

Plant species	Phytotoxicity summary [mean damage in %] [g a.s./ha]					
	Control	31.3	62.5	125	250	500
Fagopyrum esculentum	0	0	0	0	0	0

Table 10.6.2- 2: Effects on growth stages (BBCH) of Fagopyrum esculentum at the final assessment on day 21

Plant species	Growth stage (BBCH) Min - Max at test item rates [g a.s./ha]					
	Control	31.3	62.5	125	250	500
Fagopyrum esculentum	51 - 55	51 - 55	51 - 55	51 - 55	51 - 55	51 - 55

The NOER, LOER, LR_{25}/ER_{25} and LR_{50}/ER_{50} for survival, emergence, shoot length and shoot dry weight expressed in g a.s./ha for *Fagopyrum esculentum* are summarised in the following table for the final assessment (on day 21 after application).

Table 10.6.2- 3: Effects of FLU SC 500 on survival, emergence, shoot height and shoot dry weight of *Fagopyrum esculentum*

Endpoints	Survival	Emergence	Shoot length	Shoot dry weight
LR_{50} / ER_{50} [g a.s./ha]	> 500 [B] (n.d.)	> 500 [A] (n.d.)	> 500 [A] (n.d.)	> 500 [A] (n.d.)
LR_{25} / ER_{25} (95 % C.I.) [g a.s./ha]	> 500 [B] (n.d.)	> 500 [A] (n.d.)	> 500 [A] (n.d.)	> 500 [A] (n.d.)
LOER [g a.s./ha]	> 500 [B]	> 500	> 500	> 500
NOER [g a.s./ha]	500 [B]	500	500	500

[A] Calculated values were outside the range tested or not determined
[B] No computations performed due to no effects observed

III. Conclusion

In this seedling emergence and growth study, FLU SC 500 was tested under greenhouse conditions for the effects on the seedling emergence, survival, growth and shoot dry weight of the single species *Fagopyrum esculentum*, 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 occurred. Therefore the ER_{50} (based on emergence, survival, shoot height and shoot dry weight) was determined to be > 500 g a.s./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} > 500 g a.s./ha

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Data Point:	KCP 10.6.2/04
Report Author:	[redacted]
Report Year:	2012
Report Title:	Fluopyram - Peer review of new active substance - Request for additional information - Ecotoxicology - EFSA Letter Ref D(2012) HD/JS/al/620027 dated January 24, 2012
Report No:	M-428668-01-1
Document No:	M-428668-01-1
Guideline(s) followed in study:	Data Directive 91/414/EEC Bee Studies, not yet Peer Reviewed
Deviations from current test guideline:	Current Guideline: not applicable
Previous evaluation:	yes, evaluated and accepted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

The document above was only included for transparency reasons since it was part of the first listing process. It does not contain information relevant for the current active substance renewal process.

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Data Point:	KCP 10.6.2/05
Report Author:	[redacted]
Report Year:	2008
Report Title:	Non-target terrestrial plants: an evaluation of the effects of Fluopyram SC500G in the vegetative vigour test (Tier 1)
Report No:	VV08/01
Document No:	M-301453-01-1
Guideline(s) followed in study:	OECD 227 (July 2006): vegetative vigour test (Tier I)
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. Deviation from recommended plant density. 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 SC 500 on the vegetative vigour of eleven 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 eleven species, 7 dicotyledonous and 4 monocotyledonous species from 9 plant families were tested in this vegetative vigour test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Lycopersicon esculentum* (tomato), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn), *Fagopyrum esculentum* (buckwheat). Planting density included 4 plants per pot with 8 replicate pots, respectively, for a total of 32 plants per treatment level. The plant species were treated at the single application rate of 500 mL prod./ha (equivalent to nominal 250 g a.s./ha) and a water control. The application was done at a volume rate of 100 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 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.

There were no adverse effects of the single treatment at 500 mL prod./ha on the survival and dry weight of the 11 species tested and no visible phytotoxicity was observed in any of the treated crops. Therefore the ER_{50} (based on survival and shoot dry weight) was 500 mL prod./ha (corresponding to 250 g a.s./ha).

I. Material and methods

Test item: FLU SC 500, specification no.: 102000018148; batch ID.: 2007-011657, Sample description: TOX 08109-00; active substance (analysed content): fluopyram: 42.2 % w/w (501 g/L); density: 1.188 g/mL)

Test design: A total of eleven species, 7 dicotyledonous and 4 monocotyledonous species from 9 plant families were tested in this vegetative vigour test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Lycopersicon esculentum* (tomato), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn), *Fagopyrum esculentum* (buckwheat). 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 10.5 and 13 cm plastic pots. The used soil was a sandy-silt loam.

Planting density included 4 plants per pot with 8 replicate pots, respectively, for a total of 32 plants per treatment level. The plant species were treated at the 2 - 4 leaf stage at an single application rate of 500 mL product/ha (corresponding to 250 g a.s./ha) and a water control. The test solutions were applied onto the foliage of plants and above-ground portions at a volume rate of 100 L/ha. Control pots were sprayed with 100 L/ha of deionized water.

Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and phytotoxicity were recorded. Final assessments were made for plant survival, visual phytotoxicity, plant growth stage and shoot dry weight. 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 symptoms of phytotoxicity and removed from the pot.

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 10 to 31 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was not reported.

Statistical analysis was carried out for sugar beet, oat and ryegrass using the Pair wise Mann-Whitney-U-test (one sided smaller), using the ToxRat statistical software.

Dates of work: January 18, 2008 – February 13, 2008

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 OECD guideline (OECD 227), there was no visible phytotoxicity in control plants. Normal growth occurred in the controls of the eleven species tested. The control plants of each species showed normal variation in growth, plant development and morphology. The environmental conditions during the test time were kept identical within each species. The pots used for all species of this study were filled in equal manner with the same soil.

The germination rate of the seeds used in this study was not reported. However, as routine germination tests were carried out on the seeds to ensure their viability, the germination rate was considered to be in the acceptable range.

Analytical results:

No chemical analysis of the initial test item stock solution was performed.

Biological findings:

No visual phytotoxicity was observed at the final assessment (on day 21 after application) in this vegetative vigour study for the control and the treatments.

There was no adverse effect of the treatment at 500 mL prod./ha on the survival and dry weight of the 11 species tested.

The survival, shoot dry weight, phytotoxicity and plant growth stages 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- 4: Effects of Fluopyram SC 500 on survival and shoot dry weight

Plant species	Survival					Shoot dry weight		
	Control		500 mL prod./ha			Control	500 mL prod./ha	
	No. plants	% Survival	No. plants	% Survival	% Inhibition	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Beta vulgaris	32	100	32	100	0	1.904	1.729	9.2
Brassica napus	32	100	32	100	0	2.883	2.975	- 3.2
Cucumis sativus	32	100	32	100	0	2.205	2.186	0.9
F. esculentum	32	100	32	100	0	2.842	2.744	3.4
Glycine max	32	100	32	100	0	2.705	2.564	5.2
Helianthus annuus	32	100	32	100	0	2.838	2.626	7.5
L. esculentum	32	100	32	100	0	1.898	1.866	1.7
Allium cepa	32	100	32	100	0	0.066	0.071	- 7.6
Avena sativa	32	100	32	100	0	0.924	0.848	8.2
Lolium perenne	32	100	32	100	0	0.337	0.309	8.1
Zea mays	32	100	32	100	0	2.628	3.029	- 15.3

[A] Negative figures indicate that there was an increase in biomass (dry weight) when compared to the untreated 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- 5: Summary of phytotoxicity and growth stages (BBCH) following exposure to FLU SC 500 at the final assessment on day 21

Plant species	Phytotoxicity [A]		BBCH growth stages	
	Control	500 mL prod./ha	Control	500 mL prod./ha
Beta vulgaris	0	0	16 - 18	16 - 18
Brassica napus	0	0	16 - 18	16 - 18
Cucumis sativus	0	0	63 - 65	63 - 65
Fagopyrum esculentum	0	0	61	61
Glycine max	0	0	69	69
Helianthus annuus	0	0	23 - 26	24 - 26
Lycopersicon esculentum	0	0	51 - 60	60
Allium cepa	0	0	12 - 14	12 - 14
Avena sativa	0	0	21	21 - 22
Lolium perenne	0	0	26 - 27	26 - 27
Zea mays	0	0	15 - 16	15 - 16

[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 study, in which the effect of FLU SC 500 on 11 non-target terrestrial plant species was tested under greenhouse conditions, no adverse effects of the single treatment at 500 mL prod./ha on the survival and dry weight of the 11 species were determined compared to the control. Therefore the ER_{50} (based on survival and dry weight) was determined to be > 500 mL prod./ha (corresponding to 250 g a.s./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} > 250 g a.s./ha

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Data Point:	KCP 10.6.2/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 g/L: Effects on the vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions (Tier I)
Report No:	S19-22934
Document No:	M-688439-01-1
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 SC 500 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 - 3 leaf stage. A total of ten species, 6 dicotyledonous and 4 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), *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 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 0.995 L product/ha (equivalent to nominal 500 g a.s./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 phytotoxicity were recorded. Final assessments were made for plant survival, phytotoxicity, plant growth stage and shoot dry weight.

The study fulfils all validity criteria of OECD 227 guideline.

Symptoms of phytotoxicity (mean effect of 14 % compared to the control) only occurred in *Fagopyrum esculentum*. There were no adverse effects of the single treatment at 500 mL prod./ha on survival, shoot height and shoot dry weight above the 50 % effect level. Therefore the ER_{50} (based on survival, shoot height and shoot dry weight) was > 0.995 L product/ha (corresponding to > 500 g a.s./ha).

I. MATERIAL AND METHODS

Test item: FLU SC 500; specification no.: 102000018148; supplier batch no.: EV57002782; Sample description: TOX 21459-00; active substance (analysed content): fluopyram: 42.3 % w/w (502.7 g/L); density: 1.189 g/mL)

Test design: A total of ten species, 6 dicotyledonous and 4 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), *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 germination rates of the seeds used in this study, observed in a seedling emergence test, were 87 - 99 %. The plants were grown in a greenhouse in commercial non porous 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 0.995 L product/ha (equivalent to nominal 500 g a.s./ha) and a water control. The test solution was applied onto the middle of plant height 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. 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/m^2/s. The pots were set up sorted per treatment group after application. All pots were repositioned on the first and second assessment day to compensate for potential variability in growth conditions.

Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and phytotoxicity were recorded. Final assessments were made for plant survival, phytotoxicity, plant growth stage and shoot dry weight. Phytotoxicity was recorded from the living plants at each assessment time following a 0 - 90 % rating system in 10 % steps, to describe the extent of the visual symptoms in comparison to the control, taking into account necrosis, deformation and change in colour. Any plant considered as being dead was not rated for visual phytotoxicity and removed from the pot.

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. 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 α = 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 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 analysed concentration of fluopyram in the product solution corresponded to 101.9 % of the target concentration.

Biological findings:

Visual phytotoxicity observed at the final assessment (on day 21 after application) in this vegetative vigour study occurred in *Fagopyrum esculentum* and included chlorosis and necrosis with a mean effect of 14 % compared to the control.

All plants survived until test end.

There were statistically significant reductions of shoot height for the plant species *Fagopyrum esculentum* (27.8 %), *Helianthus annuus* (9.7 %), *Avena sativa* (6.6%) and *Lolium perenne* (7.2 %) at the single treatment rate of 500 mL 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- 6: Summary of phytotoxicity and growth stages (BBCH) following exposure to FLU SC 500 at the final assessment on day 21

Plant species	Phytotoxicity [A]		BBCH growth stages	
	Control	500 g a.s./ha	Control	500 g a.s./ha
Beta vulgaris	0	0 / -	15 - 15	15 - 15
Brassica napus	0	0 / -	15 - 15	15 - 15
Cucumis sativus	0	0 / -	63 - 63	63 - 63
Fagopyrum esculentum	0	14 / NE, CC	67 - 67	65 - 65
Glycine max	0	0 / -	62 - 62	62 - 62
Helianthus annuus	0	0 / -	18 - 18	18 - 18
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

Table 10.6.2- 7: Effects of Fluopyram SC 500 on shoot height and shoot dry weight

Plant species	Shoot height			Shoot dry weight		
	Control	500 g a.s./ha		Control	500 g a.s./ha	
	Mean [cm]	Mean [cm]	% Inhibition [A]	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Beta vulgaris	14.3	14.9	- 4.2	1.454	1.429	1.7
Brassica napus	14.5	14.8	- 2.1	0.910	0.902	0.9
Cucumis sativus	61.8	73.6	- 19.1	4.134	3.950	4.5
Fagopyrum esculentum	122.2	88.2	27.8 *	2.234	2.379	- 6.5
Glycine max	37.5	34.1	9.1	2.651	2.809	- 6.0
Helianthus annuus	18.6	16.8	9.7 *	2.012	1.688	16.1 *
Allium cepa	42.0	41.8	0.5	0.737	0.548	25.6 *
Avena sativa	57.4	53.6	6.6 *	1.103	0.783	29.0 *
Lolium perenne	43.3	40.2	7.2 *	0.866	0.759	12.4 *
Zea mays	82.2	79.1	3.8	3.591	3.302	8.0

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, $\alpha = 0.05$)

III. CONCLUSION

As a result of this vegetative vigour and growth study, in which the effect of FLU SC 500 on 10 non-target terrestrial plant species was tested under greenhouse conditions, no adverse effects of the single treatment at 500 mL prod./ha on the survival, shoot height and shoot dry weight of the 10 species above the 50 % effect level occurred. Therefore the ER_{50} (based on survival, shoot height and shoot dry weight) was determined to be > 0.995 L product/ha (corresponding to > 500 g a.s./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} > 500 g a.s./ha

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Data Point:	KCP 10.6.2/07
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 g/L: Effects on the vegetative vigour of three non-target terrestrial plant species under greenhouse conditions (Tier 2)
Report No:	S20-01148
Document No:	M-696933-02-1
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 deviation 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 SC 500 on the vegetative vigour of three non-target terrestrial plant species at 31.3, 62.5, 125, 250 and 500 g a.s./ha, following a post-emergence application of the active substance onto the foliage of plants at the 2 leaf stage. A total of three species, 1 dicotyledonous and 2 monocotyledonous species from 2 plant families were tested in this vegetative vigour test: *Fagopyrum esculentum* (buckwheat), *Allium cepa* (onion), *Avena sativa* (common oat). 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 application rates of 31.3, 62.5, 125, 250 and 500 g a.s./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 phytotoxicity were recorded. Final assessments were made for plant survival, phytotoxicity, plant growth stage and shoot dry weight.

The study fulfils all validity criteria of OECD 227 guideline.

No mortality and no differences in the growth stage of the plants compared to the control occurred. Necrosis with a mean effect of 12 % and 9 % occurred in *Fagopyrum esculentum* at 500 and 250 g a.s./ha, respectively. Regarding shoot height a LOER of 250 g a.s./ha and a NOER of 125 g a.s./ha was determined for *Allium cepa*. The ER_{25} and ER_{50} could not be estimated for any of the plant species tested due to a lack of inhibition equal to or above 25% and is reported as > 500 g a.s./ha, respectively. For shoot dry weight a LOER of 500 g a.s./ha and a NOER of 250 g a.s./ha was determined for *Fagopyrum esculentum*. The ER_{25} and ER_{50} could not be estimated for any of the plant species tested due to a lack of inhibition equal to or above 25% and is reported as > 500 g a.s./ha, respectively.

I. Material and Methods

Test item: FLU SC 500; specification no.: 102000018148; supplier batch no.: EV57002782; Sample description: TOX 21459-00; active substance (analysed content): fluopyram: 42.3 % w/w (502.7 g/L); density: 1.189 g/mL)

Test design: A total of three species, 1 dicotyledonous and 2 monocotyledonous species from 2 plant families were tested in this vegetative vigour test: *Fagopyrum esculentum* (buckwheat), *Allium cepa* (onion), *Avena sativa* (common oat). The germination rates of the seeds used in this study, observed in a seedling emergence test, were 91 - 98 %. The plants were grown in a greenhouse in commercial non porous 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 leaf stage with a single application rates of 31.3, 62.5, 125, 250 and 500 g a.s./ha and a water control. The test solution was applied onto the middle of plant height 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. An independent set of LED lamps above each cultivation table ensured an appropriate exposure to light. The light intensity was in the range of 350-390 µmol/m²/s. The pots were set up sorted per treatment group after application. All pots were repositioned on the first and second assessment day to compensate for potential variability in growth conditions.

Assessments were made 7, 14 and 21 days after application. On day 7 and 14, only plant survival and phytotoxicity were recorded. Final assessments were made for plant survival, phytotoxicity, plant growth stage and shoot dry weight. Phytotoxicity was recorded from the living plants at each assessment time following a 0 - 90 % rating system in 10 % steps, to describe the extent of the visual symptoms in comparison to the control, taking into account necrosis, deformation and change in colour. Any plant considered as being dead was not rated for visual phytotoxicity and removed from the pot.

Analysis of the product solution and the control solution were conducted by HPLC – UV.

Climatic conditions: Following application, the pots with plants were maintained under greenhouse conditions. The temperature was 15 to 31 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was 26 to 71 %.

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 followed by a William`s test in case that both requirements were fulfilled and the trend analysis by contrast was significant. If the trend analysis by contrast was not significant, the Dunnetts t-test was conducted. The multiple Welch`s t-test with Bonferroni-Holm adjustment was conducted in case that the data were non-homogenous. 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. In case of an increase in the test item groups compared to the control group for shoot dry weight, no statistical evaluation was conducted. Statistical analysis was performed using the program ToxRat Professional Version 3.3.0.

Dates of work: March 31, 2020 – April 23 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 three 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 analysed concentration of fluopyram in the product solution corresponded to 99 % of the target concentration.

Biological findings:

No mortality and no differences in the growth stage of the plants compared to the control occurred after treatment with the test item at any rate tested.

Visual injuries occurred in *Fagopyrum esculentum*. The observed symptom was necrosis with a mean effect of 12% at 500 g a.s./ha and 9% at 250 g a.s./ha.

An application of the test item resulted in a statistically significant reduction of the shoot height of *Allium cepa* at the two highest application rates (250 and 500 g a.s./ha) and a statistically significant reduction of the shoot dry weight of *Fagopyrum esculentum* at the highest application rate (500 g a.s./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- 8: Summary of endpoints of FLU SC 500 for mortality at the final assessment

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Fagopyrum esculentum	-	500	> 500	> 500
Monocotyledonous species				
Allium cepa	-	500	> 500	> 500
Avena sativa	-	500	> 500	> 500

Table 10.6.2- 9: Summary of endpoints of FLU SC 500 for shoot height at the final assessment

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Fagopyrum esculentum	-	500	> 500	> 500
Monocotyledonous species				
Allium cepa	250 [a]	125	> 500	> 500
Avena sativa	-	500	> 500	> 500

LOER determined with: [a] Williams` test; one-sided smaller, α = 0.05

Table 10.6.2- 10: Summary of endpoints of FLU SC 500 for shoot dry weight at the final assessment

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Fagopyrum esculentum	500 [a]	250	> 500	> 500
Monocotyledonous species				
Allium cepa	-	500	> 500	> 500
Avena sativa	-	500	> 500	> 500

LOER determined with: [a] Williams` test; one-sided smaller, α = 0.05

III. CONCLUSION

In a vegetative vigour study, FLU SC 500 was tested under greenhouse conditions for effects on survival, growth, plant height and shoot dry weight of three non-target terrestrial plant species, following a post-emergence application of the test item to the soil surface. The ER_{25} and ER_{50} could not be estimated for any of the plant species tested due to a lack of inhibition equal to or above 25% and is reported as > 500 g a.s./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} > 500 g a.s./ha

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Data Point:	KCP 10.6.2/08
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 g/L: Effects on the seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions (Tier 1)
Report No:	S19-22933
Document No:	M-688440-01-1
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 deviation 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 SC 500 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), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn), *Fagopyrum esculentum* (buckwheat). 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 0.995 L product/ha (equivalent to nominal 500 g a.s./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 plant emergence, survival and phytotoxicity were recorded. Final assessments were made for emergence, plant survival, phytotoxicity, plant growth stage and shoot dry weight 21 days post emergence of 50 % of the control seedlings.

The study fulfils all validity criteria of OECD 208 guideline.

Phytotoxicity observed at the final assessment (day 21 after 50 % control seedling emergence) included chlorosis, necrosis and deformation of the seedlings. The mean effect was in all species below 10 % 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 500 mL prod./ha. Therefore the ER_{50} (based on emergence, survival, shoot height and shoot dry weight) was > 0.995 L product/ha (corresponding to > 500 g a.s./ha).

I. MATERIAL AND METHODS

Test item: FLU SC 500; specification no.: 102000018148; supplier batch no.: EV57002782; Sample description: TOX 21459-00; active substance (analysed content): fluopyram: 42.3 % w/w (502.7 g/L); density: 1.189 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), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn), *Fagopyrum esculentum* (buckwheat). 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 non porous 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 0.995 L product/ha (equivalent to nominal 500 g a.s./ha) and a water control. The test solutions were 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 and placed on the tables. The pots were set up sorted per treatment group after application. All pots were repositioned on the first and second assessment day to compensate for potential variability in growth conditions.

The control pots of each species were observed daily for the number of seedlings emerged until 50 % of the seedlings had 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 plant emergence, survival and phytotoxicity were recorded. Final assessments were made for emergence, plant survival, phytotoxicity, BBCH growth stage and shoot dry weight 21 days post emergence of 50 % of the control seedlings. Phytotoxicity ratings referred to the whole plants within a replicate at a range from 10 to 90 %, to describe the extent of the visual symptoms in comparison to the control, taking into account necrosis, deformation and change in colour. Any plant considered as being dead was not rated for phytotoxicity.

Analysis of the product 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 the range of 260-400 µmol/m²/s . 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 %.

Statistical analysis of seedling emergence and post-emergence mortality data were tested with Fisher`s exact test. 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 between 94 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.

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 99.6 % 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 and deformation of the plants. Symptoms of phytotoxicity with a mean effect ≤ 10 % compared to the control occurred in *Beta vulgaris*, *Cucumis sativus*, *Fagopyrum esculentum* and *Lolium perenne*.

No statistically significant effects on the parameter seedling emergence were observed for any of the plant species tested. The highest inhibition occurred for *Avena sativa* with 21.1 % compared to the control group. Post-emergence mortality of 5.0 % was observed only for *Brassica napus* in the single treatment at 500 mL prod./ha.

Statistically significant reductions in shoot height compared to the control group were observed for *Beta vulgaris, Brassica napus*, *Cucumis sativus*, *Glycine max* and *Lolium perenne*.

Statistically significant reductions in shoot dry weight compared to the control group were observed for *Beta vulgaris*, *Cucumis sativus*, *Fagopyrum esculentum*, *Allium cepa* and *Lolium perenne*.

The growth stage, symptoms of phytotoxicity, shoot height and dry weight as well as emergence and 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- 11: Summary of growth stages (BBCH) and phytotoxicity following exposure to FLU SC 500 at the final assessment on day 21

Plant species	BBCH growth stage Min - Max		Phytotoxicity: mean % / Symptoms [A]	
	Control	500 g a.s./ha	Control	500 g a.s./ha
Beta vulgaris	13 - 13	13 - 13	0	2 / DE
Brassica napus	13 - 13	13 - 13	0	0 / -
Cucumis sativus	12 - 12	12 - 12	0	0 / DE
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 - 21	0	0 / -
Lolium perenne	21 - 21	21 - 21	0	5 / CC, NE
Zea mays	15 - 15	15 - 15	0	0 / -

[A] Symptoms of phytotoxicity: None (-), CC = change in colour, DE = deformation, NE = necrosis

Table 10.6.2- 12: Summary of shoot height and shoot dry weight and corresponding percent inhibitions following exposure to FLU SC 500 at the final assessment on day 21

Plant species	Shoot height			Shoot dry weight		
	Control	500 g a.s./ha		Control	500 g a.s./ha	
	Mean [cm]	Mean [cm]	% inhibition [A]	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Beta vulgaris	12.3	10.2	17.1 *	0.369	0.258	30.1 *
Brassica napus	14.7	13.4	8.8 *	0.830	0.909	- 9.5
Cucumis sativus	16.1	14.4	10.6 *	0.641	0.492	23.2 *
Fagopyrum esculentum	41.3	38.5	6.8	0.783	0.687	12.3 *
Glycine max	22.9	19.9	13.1 *	0.845	0.810	4.1
Helianthus annuus	6.7	6.6	1.5	0.695	0.770	- 10.8
Allium cepa	22.0	21.6	1.8	0.068	0.051	25.0 *
Avena sativa	39.8	43.7	- 9.8	0.343	0.376	- 9.6
Lolium perenne	31.9	28.4	11.0 *	0.143	0.087	39.2 *
Zea mays	46.7	49.1	- 5.1	0.533	0.721	- 35.3

* Statistically significantly different compared to the control (Student's t-test; one-sided smaller, $\alpha = 0.05$)

[A] Negative values indicate that there was an increase compared to the control

Table 10.6.2- 13: Summary of emergence and cumulative mortality following exposure to FLU SC 500 at the final assessment on day 21

Plant species	Seedling emergence			Cumulative mortality	
	Control	500 g a.s./ha		Control	500 g a.s./ha
	% emerged	% emerged	% Inhibition	% mortality	% mortality
Beta vulgaris	100	100	0	0	0
Brassica napus	95	95	0	0	5.0
Cucumis sativus	95	100	- 5.3	0	0
Fagopyrum esculentum	95	100	- 5.3	0	0
Glycine max	95	100	- 5.3	0	0
Helianthus annuus	100	100	0	0	0
Allium cepa	95	95	0	0	0
Avena sativa	95	75	21.1	0	0
Lolium perenne	90	85	5.6	0	0
Zea mays	100	95	5.0	0	0

III. CONCLUSION

In a seedling emergence and growth study, FLU SC 500 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 test item 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 > 0.995 L product/ha (corresponding to > 500 g a.s./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} > 500 g a.s./ha.

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Data Point:	KCP 10.6.2/09
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram SC 500 g/L: Effects on the seedling emergence and seedling growth of three non-target terrestrial plant species under greenhouse conditions (Tier 2)
Report No:	S20-01146
Document No:	M-696931-01-1
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 (humidity). All validity criteria were met. The deviation 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 study objective was to determine the effects of FLU SC 500 on seedling emergence and early growth of three non-target terrestrial plant species as these plant species had shown relevant effects in a preceding limit test and are therefore investigated in this rate-response study. A total of three species, 1 dicotyledonous and 2 monocotyledonous species from 2 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* (sugar beet), *Allium cepa* (onion), *Lolium perenne* (ryegrass). 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 5 application rate of 31.1, 62.5, 125, 250, 500 g a.s./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 plant emergence, survival and phytotoxicity were recorded. Final assessments were made for emergence, plant survival, phytotoxicity, plant growth stage and shoot dry weight 21 days post emergence of 50 % of the control seedlings.

The study fulfils all validity criteria of OECD 208 guideline.

No visual injury occurred in any of the plant species tested until the last assessment day.

There were no adverse effects on emergence, post-emergence mortality, shoot height and shoot dry weight above the 50 % effect level. Therefore the ER_{50} was determined to be > 500 g a.s./ha.

Statistically significant differences in shoot dry weight compared to the control group were observed for *Beta vulgaris*. A LOER of 125 g a.s./ha and a NOER of 62.5 g a.s./ha was determined for this species.

I. Material and Methods

Test item: FLU SC 500; specification no.: 102000018148; supplier batch no.: EV57002782; Sample description: TOX 21459-00; active substance (analysed content): fluopyram: 42.3 % w/w (502.7 g/L); density: 1.189 g/mL.

Test design: A total of three species, 1 dicotyledonous and 2 monocotyledonous species from 2 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* (sugar beet), *Allium cepa*

(onion), *Lolium perenne* (ryegrass). The seeds were sown on the day of application of the product to the soil surface in commercial non porous 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 5 application rate of 31.1, 62.5, 125, 250, 500 g a.s./ha and a water control. The test solutions were applied at a volume rate of 200 L/ha. Control pots were sprayed with 200 L/ha of deionized water.

The pots were set up sorted per treatment group after application. All pots were repositioned on the first and second assessment day to compensate for potential variability in growth conditions.

The control pots of each species were observed daily for the number of seedlings emerged until 50 % of the seedlings had 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 plant emergence, survival and phytotoxicity were recorded. Final assessments were made for emergence, plant survival, phytotoxicity, BBCH growth stage and shoot dry weight 21 days post emergence of 50 % of the control seedlings. Phytotoxicity ratings referred to the whole plants within a replicate at a range from 10 to 90 %, to describe the extent of the visual symptoms in comparison to the control, taking into account necrosis, deformation and change in colour. Any plant considered as being dead was not rated for phytotoxicity.

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 and natural daylight was supplemented by artificial lighting. The light intensity was in the range of 360 - 390 $\mu mol/m^2/s$. The temperature was regulated to maintain 15 to 32 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was regulated to maintain 24 to 79 %.

Statistical analysis of seedling emergence and post-emergence mortality was done with Fisher`s exact test. The data of shoot height and shoot dry weight were tested for normality and homoscedasticity using Shapiro-Wilk`s Test and Levene-Test followed by Dunnett`s t-test in case that both requirements were fulfilled and the trend analysis by contrast was not significant. The Jonckheere-Terpstra test was conducted in case the data were not normal distributed but homogenous and the trend analysis was significant. If the trend analysis by contrast was not significant, the multiple Mann-Whitney U-test with Bonferroni-Holm adjustment was conducted. 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 31,2020 – April 29, 2020

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 95 %) 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 100 % of the nominal concentration.

Biological findings:

No statistically significant effects on the parameter seedling emergence were observed for any of the plant species tested. Inhibitions below 10 % occurred in each of the three plant species tested.

Post-emergence mortality of 5.0 % and 5.9 % occurred in *Allium cepa* and *Lolium perenne*, respectively. The post-emergence mortality was not statistically significant.

No visual injuries occurred in any of the plant species tested.

No differences in the growth stages between the test item groups and the control group of all three plant species tested were observed.

No statistically significant differences in shoot height compared to the control group were observed for any of the plant species tested.

Statistically significant differences in shoot dry weight compared to the control group were observed for *Beta vulgaris*.

The growth stage, shoot height and dry weight as well as emergence and survival 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- 14: Summary of endpoints of FLU SC 500 for seedling emergence at the final assessment

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Beta vulgaris	-	500	> 500	> 500
Monocotyledonous species				
Allium cepa	-	500	> 500	> 500
Lolium perenne	-	500	> 500	> 500

Table 10.6.2- 15: Summary of endpoints of FLU SC 500 for post-emergence mortality at the final assessment

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Beta vulgaris	-	500	> 500	> 500
Monocotyledonous species				
Allium cepa	-	500	> 500	> 500
Lolium perenne	-	500	> 500	> 500

Table 10.6.2- 16: Summary of endpoints of FLU SC 500 for shoot height at the final assessment

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Beta vulgaris	-	500	> 500	> 500
Monocotyledonous species				
Allium cepa	-	500	> 500	> 500
Lolium perenne	-	500	> 500	> 500

Table 10.6.2- 17: Summary of endpoints of FLU SC 500 for shoot dry weight at the final assessment

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Beta vulgaris	125 [a]	62.5	> 500	> 500
Monocotyledonous species				
Allium cepa	-	500	> 500	> 500
Lolium perenne	-	500	> 500	> 500

LOER determined with: [a] Jonckheere-Terpstra test; one-sided smaller, $\alpha = 0.05$

III. CONCLUSION

In a Tier 2 seedling emergence and growth study, FLU SC 500 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 test item to the soil surface.

No adverse effects on emergence, post-emergence mortality, shoot height and shoot dry weight above the 25/50 % effect level occurred. Therefore the ER_{25}/ER_{50} was determined to be > 500 g a.s./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} > 500 g a.s./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.