Document Title

1st amendment of

Summary of the ecotoxicological studies
Bixafen + Fluopyram + Prothioconazole EC 260 (65+65+130 g/L)

Data Requirement(s)

Regulation (EC) No 1107/2009 & Regulation (EC) 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

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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-766073-01-1
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-766073-02-1

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

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CP 10 ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Fluopyram was included in Annex I to Council Directive 91/414/EEC in 2013 (Regulation (EU) 802/2013 into Force on August 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 Bixafen + Fluopyram + Prothioconazole EC 260 (65+65+130 g/L) abbreviation BIX + FLU + PTZ EC 260, is an emulsifiable concentrate formulation containing 65 g/L of Bixafen, 65 g/L Fluopyram and 130 g/L Prothioconazole. This formulation is registered throughout Europe under trade names such as Ascra Xpro EC 260. BIX+FLU+PTZ EC 260 was not already a representative formulation of Bayer AG for the Annex I inclusion of Prothioconazole under Council Directive 91/414/EEC.

BIX + FLU + PTZ EC 260 is an end use product proposed for use in the field on cereals (barley) 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
Barley	BBCH 30-61	1	-	0.6	0.039
Barley	BBCH 30-61	1	-	1.2	0.078

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], E., 2011	Pulses and oilseeds (P/O)	Bean	Foliar	[UL-^{14}C-phenyl]
M-299067-01-1	[redacted] 2008				[2,6-^{14}C-pyridyl]
M-298790-01-1	[redacted] 2008	Fruit (F)	Bell pepper	Drip irrigation	[UL-^{14}C-phenyl]
M-298741-01-1	[redacted], 2008				[2,6-^{14}C-pyridyl]
M-345948-01-1	[redacted] 2009	Cereal/Grass crops (C/G)	Wheat	Seed treatment	[UL-^{14}C-phenyl] & [2,6-^{14}C-pyridyl]
M-615284-01-1	[redacted] 2018	Miscellaneous	Rice	Foliar	[UL-^{14}C-phenyl]
M-615282-01-1*	[redacted] 2018				[2,6-^{14}C-pyridyl]

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

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

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

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

EFSA Journal 2013;11(4):3052: "After foliar applications, fluopyram constitutes the major component of the radioactive residues, accounting for more than 85% TRR in grape, potato leaves and bean leaves, collected 4 to 51 days after the last application. Fluopyram was however observed in lower proportions in potato tubers and bean seeds, representing 5% to 21% TRR. In these matrices the residues were mostly composed of the metabolites resulting from the cleavage of the parent molecule; fluopyram-benzamide (M25), fluopyram-PAA (M40) and fluopyram-PCA (M43).

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

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

RMS comment:

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

Applicant response:

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

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

Metabolite	Overall Maximum Concentration (foliar and drip)		
	%TRR	mg parent eq./kg	Comment
Fluopyram-7-hydroxy AE C656948-7-hydroxy / M08 / BCS-AA10065	1.0	0.43	Grape leaves
	0.3	0.20	Grape (Summer Cut: leaves BBCH71)
	1.6	0.60	Bean foliage
	1.1	0.20	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	0.327	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	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.

AE F148815: BCS-AA10014 / M25			

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 barley 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.

From the 32 trials conducted for winter and spring barley, 20 analysed the decline of residues in green material. The application was performed at BBCH 61 and grain and straw were only sampled at harvest. AE C656948 was found to continuously decline from the samplings from day 0, through days 7, 14 and 21 (only available in eight trials) to day 28 after treatment. The residue levels of AE C656948-benzamide were typically below the LOQ (<0.01 mg/kg), with exception of 11 trials, in which the residue levels either constantly increased or showed a short upturn followed by a decrease to values below the LOQ.

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 use on barley. 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, 2007c), 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	6.4	0.312	0.155	0.0	0.049	<0.051	36.6	1.812
	Wheat Grain	29	93	-	-	-	3.6	0.006	0.003	13.1	0.021	0.022	27.3	0.043
	Wheat Straw	29	93	13.6	1.844	0.990	3.4	0.461	0.228	-	-	-	23.1	3.132
133	Lettuce	133	216	-	-	-	60.9	0.070	0.035	-	-	-	26.6	0.031
	Radish Tops	133	196	-	-	-	77.3	0.186	0.092	-	-	-	15.1	0.036
	Radish Roots	133	196	-	-	-	54.9	0.013	0.006	-	-	-	28.2	0.006
	Wheat Forage	133	281	28.9	0.065	0.035	5.1	0.011	0.006	-	-	-	23.3	0.052
	Wheat Grain	133	335	23.3	0.004	0.003	19.0	0.003	0.003	-	-	-	7.0	0.001
	Wheat Straw	133	335	14.6	0.123	0.067	25.5	0.215	0.107	-	-	-	15.5	0.131
365	Lettuce	365	421	-	-	-	87.0	0.539	0.267	-	-	-	2.1	0.013
	Radish Tops	365	421	-	-	-	87.5	1.755	0.869	-	-	-	3.8	0.076
	Radish Roots	365	421	-	-	-	60.9	0.022	0.011	-	-	-	24.2	0.009
	Wheat Forage	365	410	59.3	0.513	0.276	14.8	0.128	0.063	-	-	-	4.8	0.042
	Wheat Grain	365	449	24.5	0.013	0.007	17.9	0.010	0.005	-	-	-	7.3	0.004
	Wheat Straw	365	449	28.0	0.063	0.356	5.1	0.121	0.060	-	-	-	7.2	0.172

Pyridyl label- metabolites

Plot	Crop Part	PBI (days)	DALA (days)	M-08			M-05			M-02			M-09			M-06			Fluopyram	
				%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg a.s. equivs	mg/kg	%TRR	mg/kg
29	Lettuce	29	83	-	-	-	13.0	0.039	0.026	17.4	0.053	0.031	5.3	0.016	0.008	-	-	-	35.8	0.108
	Radish Tops	29	71	-	-	-	3.3	0.069	0.046	10.4	0.217	0.128	4.8	0.100	0.052	-	-	-	51.1	1.072
	Radish Roots	29	71	-	-	-	9.6	0.011	0.007	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	-	-	-	14	0.060	0.063	33.7	1.445
	Wheat Grain	29	93	-	-	-	13.1	0.341	0 225	69.6	1.809	1.064	-	-	-	-	-	-	1.8	0.046
	Wheat Straw	29	93	-	-	-	7.7	0.544	0.359	7.0	0.494	0.291	-	-	-	-	-	-	34.9	2.462
133	Lettuce	133	217	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	79.9	0.027
	Radish Tops	133	197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	72.2	0.171
	Radish Roots	133	197	-	-	-	2.0	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	9.4	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	-	-	-	5.1	0.022	0.015	27.1	0.114	0.067	6.0	0.025	0.013	-	-	-	25.2	0.106
	Radish Roots	365	421	9.5	0.003	0.002	5.3	0.002	0.001	10.0	0.003	0.002	-	-	-	-	-	-	55.8	0.018
	Wheat Forage	365	410	6.3	0.015	0.009	18.3	0.045	0.030	8 2	0.020	0.012	9.9	0.024	0.012	-	-	-	27.8	0.068
	Wheat Grain	365	449	-	-	-	64.9	0.116	0.077	14.2	0.025	0.015	-	-	-	-	-	-	2.9	0.005
	Wheat Straw	365	449	4.8	0.048	0.028	14.2	0.143	0.094	4 1	0.042	0.025	-	-	-	-	-	-	27.5	0.277

Appendix 2

Summary of the residue decline trials for fluopyram treated barley

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948	AE C656948-benzamide
Report No RA- 12-2130 (M-736932-01-1) Trial No 12-2130-01	1 x 0.078 kg a.s./ha at BBCH 61	green material	0	1.0	<0.01
		green material	7	0.23	<0.01
		green material	14	0.16	<0.01
		green material	28	0.035	<0.01
		grain	64	0.015	<0.01
		straw	64	0.054	<0.01
Report No RA- 12-2130 (M-736932-01-1) Trial No 12-2130-02	1 x 0.078 kg a.s./ha at BBCH 61	green material	0	1.3	<0.01
		grain	47	0.025	<0.01
		straw	47	0.058	<0.01
Report No RA- 12-2130 (M-736932-01-1) Trial No 12-2130-03	1 x 0.078 kg a.s./ha at BBCH 61	green material	0	1.7	<0.01
		green material	7	0.86	<0.01
		green material	14	0.28	<0.01
		green material	27	0.08	<0.01
Report No RA- 12-2130 (M-736932-01-1) Trial No 12-2130-04	1 x 0.078 kg a.s./ha at BBCH 61	green material	0	1.8	<0.01
		grain	69	<0.01	<0.01
		straw	69	0.024	0.17
Report No RA- 12-2163 (M-736963-01-1) Trial No 12-2163-01	1 x 0.125 kg a.s./ha at BBCH 61	green material	0	1.8	<0.01
		green material	7	0.26	<0.01
		green material	14	0.19	<0.01
		green material	28	0.043	<0.01
		grain	64	0.018	<0.01
		straw	64	0.14	0.13
Report No RA- 12-2163 (M-736963-01-1) Trial No 12-2163-02	1 x 0.125 kg a.s./ha at BBCH 61	green material	0	1.8	<0.01
		green material	7	0.95	<0.01
		green material	14	0.31	<0.01
		green material	28	0.14	<0.01
		grain	62	0.025	<0.01
		straw	62	0.066	<0.01
Report No RA- 12-2163 (M-736963-01-1) Trial No 12-2163-03	1 x 0.125 kg a.s./ha at BBCH 61	green material	0	2.6	<0.01
		green material	7	0.62	<0.01
		green material	14	0.3	<0.01
		green material	28	0.062	<0.01
		grain	54	0.027	<0.01
		straw	54	0.057	<0.01
Report No RA- 12-2163 (M-736963-01-1) Trial No 12-2163-04	1 x 0.125 kg a.s./ha at BBCH 61	green material	0	2.1	<0.01
		green material	7	0.25	<0.01
		green material	14	0.088	<0.01
		green material	28	0.03	<0.01
		grain	56	0.014	<0.01
		straw	56	0.025	<0.01
Report No RA- 12-2163 (M-736963-01-1) Trial No 12-2163-05	1 x 0.125 kg a.s./ha at BBCH 61	green material	0	1.9	<0.01
		grain	53	0.018	<0.01
		straw	53	0.014	<0.01
Report No RA- 12-2163 (M-736963-01-1) Trial No 12-2163-06	1 x 0.125 kg a.s./ha at BBCH 61	green material	0	2.2	<0.01
		grain	47	0.026	<0.01
		straw	47	0.081	<0.01

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948	AE C656948-benzamide
Report No RA- 12-2163 (M-736963-01-1) Trial No 12-2163-07	1 x 0.125 kg a.s./ha at BBCH 61	green material grain straw	0 46 46	2.4 0.033 0.13	<0.01 0.013 0.037
Report No RA- 12-2163 (M-736963-01-1) Trial No 12-2163-08	1 x 0.125 kg a.s./ha at BBCH 61	green material grain straw	0 69 69	2.6 0.016 0.11	0.01 <0.01 0.029
Report No RA- 17-2071 (M-673920-01-1) Trial No 17-2071-01	1 x 0.101 kg a.s./ha at BBCH 61	green material green material green material green material green material grain straw	0 7 14 22 30 55 55	3.9 1.2 1.1 0.19 <0.01 0.099 0.12	<0.01 0.052 0.016 <0.01 <0.01 <0.01 0.013
Report No RA- 17-2071 (M-673920-01-1) Trial No 17-2071-02	1 x 0.101 kg a.s./ha at BBCH 61	green material green material green material green material green material grain straw	0 7 9 15 21 28 50 50	1.8 0.59 0.23 0.19 0.11 0.077 0.04 0.065	<0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01 <0.01
Report No RA- 17-2071 (M-673920-01-1) Trial No 17-2071-03	1 x 0.101 kg a.s./ha at BBCH 61	green material green material green material green material green material grain straw	0 7 13 21 27 50 50	3.9 1.4 0.76 0.22 0.15 0.024 0.096	<0.01 0.029 0.035 <0.01 <0.01 <0.01 0.011
Report No RA- 17-2071 (M-673920-01-1) Trial No 17-2071-04	1 x 0.101 kg a.s./ha at BBCH 61	green material green material green material green material green material grain straw	0 7 14 20 28 41 41	3.2 1.1 0.51 0.52 0.22 0.049 0.34	<0.01 0.018 0.012 0.021 0.011 <0.01 0.025
Report No RA- 12-2132 (M-736934-01-1) Trial No 12-2132-01	1 x 0.078 kg a.s./ha at BBCH 61	green material green material green material green material grain straw	0 7 14 28 49 49	1.3 0.33 0.11 0.042 0.012 0.097	<0.01 <0.01 <0.01 <0.01 <0.01 0.019
Report No RA- 12-2132 (M-736934-01-1) Trial No 12-2132-02	1 x 0.078 kg a.s./ha at BBCH 61	green material grain straw	0 57 57	1.5 <0.01 0.025	<0.01 <0.01 <0.01
Report No RA- 12-2132 (M-736934-01-1) Trial No 12-2132-03	1 x 0.078 kg a.s./ha at BBCH 61	green material green material green material green material grain straw	0 7 15 28 42 42	2 0.58 0.17 0.1 0.028 0.18	<0.01 0.01 0.01 <0.01 <0.01 0.015

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948	AE C656948-benzamide
Report No RA- 12-2132 (M-736934-01-1) Trial No 12-2132-04	1 x 0.078 kg a.s./ha at BBCH 61	green material	0	2	<0.01
		green material	7	0.71	0.01
		green material	14	0.55	0.016
		green material	28	0.32	0.01
		grain	42	0.034	<0.01
		straw	42	0.77	0.028
Report No RA- 12-2132 (M-736934-01-1) Trial No 12-2132-05	1 x 0.078 kg a.s./ha at BBCH 61	green material	0	2	<0.01
		green material	7	0.89	<0.01
		green material	15	0.58	0.019
		green material	28	0.62	0.019
		grain	56	0.079	0.011
		straw	56	1.1	0.061
+Report No RA- 12-2132 (M-736934-01-1) Trial No 12-2132-07	1 x 0.078 kg a.s./ha at BBCH 61	green material	0	2.1	<0.01
		grain	55	<0.01	<0.01
		straw	55	0.095	0.021
Report No RA- 12-2132 (M-736934-01-1) Trial No 12-2132-08	1 x 0.078 kg a.s./ha at BBCH 61	green material	0	2	<0.01
		grain	35	0.11	0.01
		straw	35	0.4	0.02
Report No RA- 17-2018 (M-656993-01-1) Trial No 17-2018-01	1 x 0.101 kg a.s./ha at BBCH 61	green material	0	2.7	<0.01
		green material	7	0.58	<0.01
		green material	14	0.58	0.015
		green material	22	0.37	0.011
		green material	28	0.31	0.011
		grain	38	0.036	<0.01
		straw	38	0.46	0.029
Report No RA- 17-2018 (M-656993-01-1) Trial No 17-2018-02	1 x 0.101 kg a.s./ha at BBCH 61	green material	0	4.1	0.01
		green material	7	0.63	0.017
		green material	14	0.3	<0.01
		green material	22	0.19	0.01
		green material	28	0.19	0.014
		grain	44	<0.01	<0.01
		straw	44	<0.01	<0.01
Report No RA- 17-2018 (M-656993-01-1) Trial No 17-2018-03	1 x 0.101 kg a.s./ha at BBCH 61	green material	0	2.4	<0.01
		green material	8	1.5	<0.01
		green material	14	1.1	0.02
		green material	18	1.1	0.021
		green material	20	0.94	0.022
		green material	27	0.86	0.029
		grain	48	0.041	<0.01
		straw	48	1.2	0.034
Report No RA- 17-2018 (M-656993-01-1) Trial No 17-2018-04	1 x 0.101 kg a.s./ha at BBCH 61	green material	0	6.8	<0.01
		green material	7	2.4	0.31
		green material	14	2.2	0.04
		green material	21	1.7	0.016
		green material	23	1.8	0.017
		green material	27	1.6	0.011
		grain	43	0.019	<0.01
		straw	43	0.38	<0.01
Report No RA- 18-2101 (M-668264-01-1) Trial No 18-2101-01	1 x 0.1 kg a.s./ha at BBCH 61	grain	57	0.032	0.014
		straw	57	0.14	0.091

Report No. (Document No.) Trial No.	Application	Portion analysed	PHI (days)	Residues (mg/kg)	
				AE C656948	AE C656948-benzamide
Report No RA- 18-2101 (M-668264-01-1) Trial No 18-2101-02	1 x 0.1 kg a.s./ha at BBCH 61	green material	0	3.6	<0.01
		green material	7	1.1	0.011
		green material	14	0.35	<0.01
		green material	21	0.16	<0.01
		green material	28	0.074	<0.01
		grain	48	0.028	<0.01
		straw	48	0.046	<0.01
Report No RA- 18-2101 (M-668264-01-1) Trial No 18-2101-03	1 x 0.1 kg a.s./ha at BBCH 61	grain	44	0.038	<0.01
		straw	44	0.14	0.017
Report No RA- 18-2101 (M-668264-01-1) Trial No 18-2101-04	1 x 0.1 kg a.s./ha at BBCH 61	green material	0	1.3	<0.01
		green material	7	0.41	<0.01
		green material	14	0.16	<0.01
		green material	21	0.047	<0.01
		green material	28	0.027	<0.01
		grain	42	0.014	<0.01
		straw	42	0.024	0.02

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%	41%	**% 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 0722/9 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-302101-01-1	Cabbage	N	SFO	1.979	1	marked decline, unlikely to be influenced by very little early rainfall	EnSa-20-0832
R 2006 0544/7 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	1.286	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	1.674	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	4.984	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

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
R 2006 0469/6 M-292082-01-1	Leek	S	SFO	7.054	2	frequent irrigation and occasional rainfall may have markedly influenced residue dissipation, although this is not discernible in the decline pattern	EnSa-20-0832
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

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
E19RP102-04 M-758824-01-1	Young cereals	N	FOMC	4.319	2	moderate rain d2, slight influence	EnSa-20-0834
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

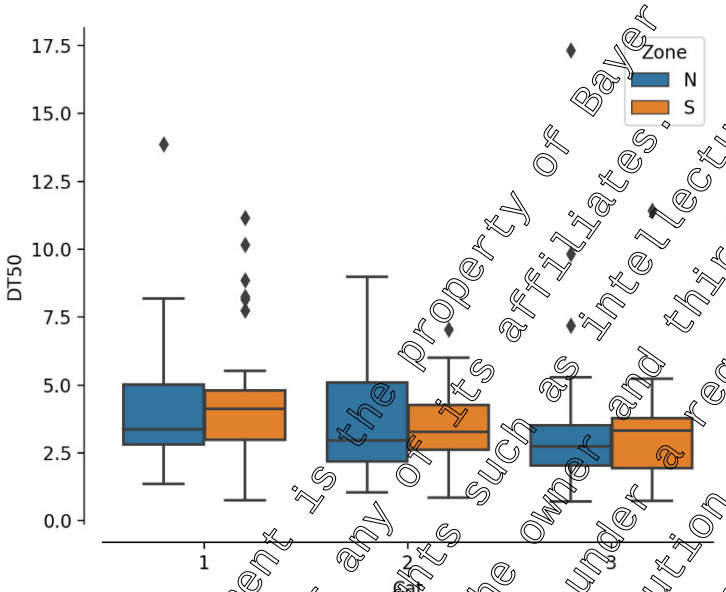
Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
R 2007 0249/3 M-304276-01-1	Leek	N	HS	3.392	3	early rainfall coincides with marked drop (influence likely)	EnSa-20-0832
R 2007 0569/7 M-304288-01-1	Leek	N	FOMC	3.551	3	early rainfall coincides with 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

Trial Edition no.	Crop	Zone	Kinetic model	DT_{50} mod	Cat	Influence rain and/or irrigation	Source DT_{50}
R 2007 0553/0 M-298639-01-1	Peas	N	HS	3.401	3	Rain on day 2, influence likely	Ensa-20-8030
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	1.747	3	early rain, marked decline	EnSa-20-0834
13-2950-02 M-471216-01-1	Young cereals	N	HS	2.03	3	rainfall day0, marked decline	EnSa-17-0484
13-2950-03 M-471216-01-1	Young cereals	N	HS	1.2	3	early rainfall, marked decline	EnSa-17-0484
13-2950-04 M-471216-01-1	Young cereals	N	SFO	1.25	3	early rainfall, marked decline	EnSa-17-0484
15-2953-01 M-566828-01-1	Young cereals	N	HS	3.48	3	early rain, marked decline	EnSa-17-0484
18-2954-01 M-675129-02-1	Young cereals	S	SFO	4.201	3	heavy rain d4, marked decline	EnSa-20-0834
18-2954-02 M-675129-02-1	Young cereals	S	SFO	3.599	3	heavy rain d4, marked decline	EnSa-20-0834
E19RP087-03 M-758649-01-1	Young cereals	S	SFO	3.415	3	heavy rain d3, marked decline	EnSa-20-0834

Influence of the residue zone on foliage DT_{50}

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

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



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

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

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

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

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

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

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

Applicant assessment on effects on biodiversity

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

The risk assessments for bird and mammals result in acceptable outcomes at screening or tier 1 level.

The risk assessment for aquatic organisms is acceptable when considering FOCUS step 2 PECsw or maximum product 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 2 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 BIX+FLU+PTZ EC 260 has low potential to cause unacceptable effects on biodiversity and the ecosystem via trophic interactions. To the best of our knowledge and with the presented safety profile of the active substance fluopyram and the representative lead formulation, the applicant does not foresee any effects on biodiversity and the ecosystem.

CP 10.1 Effects on birds and other terrestrial vertebrates

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

CP 10.1.1 Effects on birds

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

Test substance	Test design	Test species	Endpoint		Reference
Fluopyram tech.	Acute oral toxicity	Bobwhite quail (*Colinus virginianus*)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2011) M-263049-04-1 KCA 8.1.1.1/01
	Acute oral toxicity	Zebra finch (*Taeniopygia guttata*)	LD_{50}	> 2000 mg a.s./kg bw	[redacted] (2008) M-307871-02-1 KCA 8.1.1.1/02
			LD_{50}	= 3036 mg a.s./kg bw	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 > 1536.4 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] [redacted] (2008) M-299245-02-1 KCA 8.1.1.3/01
	22-weeks feeding chronic, reproduction	Bobwhite quail (*Colinus virginianus*)	NOAEC 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 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}	Bobwhite quail (*Colinus virginianus*) –	Lowest EC_{10}	7.8 mg a.s./kg bw/d (14-day survivors per eggs set)	[redacted] [redacted] (2019) M-667209-01-1

Test substance	Test design	Test species	Endpoint		Reference
	calculation	both chronic studies combined			KCA 8.1.1.3/04
	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-667214-01-1 KCA 8.1.1.3/05
BIX + FLU+ PTZ EC 260	Acute	Bobwhite quail	LD_{50} (mix)	> 2000 mg total a.s./kg bw	Table 10.1.1- 6

Note:
Studies referring to KCA are filed in the dossier for the active substance
Studies written in grey type are referring to studies in the corresponding Baseline-dossier, whereas studies in black type are studies of the Supplemental dossier
a.s. = active substance
[A] Factor 1.518 for 10 birds/dose level with a single mortality (study result: 12 individuals and 1 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
Cereals (barley)	Small omnivorous bird	158.8	64.8

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

Crop	Generic focal species	Shortcut value (SV)	
		Acute RA based on RUD_{90}	Long-term RA based on RUD_m
Cereals (barley) BBCH 30-61	Small omnivorous bird "lark" BBCH 30-39	Not needed	5.4 [1]
	Small omnivorous bird "lark" BBCH ≥ 40	Not needed	3.3

[1] Shortcut value for BBCH 30-39 is used in the risk assessment as worst case covering scenario BBCH ≥ 40

ACUTE DIETARY RISK ASSESSMENT

Table 10.1.1- 4: Screening acute risk assessment for birds (fluopyram)

Crop	Indicator species	DDD			DDD	LD_{50} [mg a.s./ kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
Cereals (barley) 1 × 0.039 kg a.s./ha	Small omnivorous bird	0.039	158.8	1.0	6.19	> 2000	> 323	10
Cereals (barley) 1 × 0.078 kg a.s./ha	Small omnivorous bird	0.078	158.8	1.0	12.4	> 2000	> 161	10

Table 10.1.1- 5: Screening acute risk assessment for birds (bixafen + fluopyram + prothioconazole)

Crop	Indicator species	DDD			DDD	LD_{50} (mix) [mg total a.s./ kg bw]	TER_A	Trigger
		Appl. rate [kg total a.s./ha]	SV_{90}	MAF_{90}				
Cereals (barley) 1 × 0.156 kg total a.s./ha	Small omnivorous bird	0.156	158.8	1.0	24.8	> 2000	> 80.7	10
Cereals (barley) 1 × 0.312 kg total a.s./ha	Small omnivorous bird	0.312	158.8	1.0	49.5	> 2000	> 40.4	10

For fluopyram and the predicted endpoint of BIX+FLU+PTZ EC 260 the TER_A values are above the trigger of 10. Therefore, a Tier 1 risk assessment is not required.

Combined toxicity risk assessment

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

For the assessment of acute effects (mortality), a surrogate LD_{50} (mix) can be calculated for the mixture risk assessment. The EFSA GD 2009 indicates that the following equation should be used for deriving a surrogate LD_{50} (mix) for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

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

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

The active substance content of the formulation BIX+FLU+PTZ EC 260 addressed in this dossier is 65 g bixafen/L product, 65 g fluopyram/L product and 130 g prothioconazole/L product, making up a total of 260 g a.s./L product.

The table below shows the calculation of the predicted LD_{50} (mix) of bixafen, fluopyram and prothioconazole when mixed in these proportions (step 1 in Appendix B of EFSA GD 2009).

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

Table 10.1.1- 6: Avian LD_{50} (mix) for bixafen, fluopyram and prothioconazole when combined as BIX+FLU+PTZ EC 260 (step 1 in Appendix B of EFSA GD 2009)

	Fluopyram	Bixafen	Prothioconazole
Content of a.s. in product [g a.s./L prod.]	65	65	130
Fraction in the a.s. mixture	0.25	0.25	0.50
LD_{50} of a.s. [mg a.s./kg bw]	> 2000	> 2000 [A]	> 2000 [B]
Fraction / LD_{50}	< 0.000125	< 0.000125	< 0.00025
Sum	< 0.00050		
1/sum = predicted LD_{50} (mix) [mg total a.s./kg bw]	> 2000		

[A] LD_{50} for Bobwhite quail given in EFSA Journal 2012;10(11):2917

[B] LD_{50} for Bobwhite quail given in EFSA Scientific Report (2007) 106, 1-98

It is obvious from the comparison of the (low) acute oral toxicity of the active substances, and their relative proportions within the formulated product BIX+FLU+PTZ EC 260, that neither fluopyram nor bixafen nor prothioconazole contributes to more than 90 % to the predicted acute mixture toxicity (see next table). Consequently, according to EFSA GD 2009 the acute risk assessment should be performed using the predicted LD_{50} (mix). This risk assessment is presented in Table 10.1.1- 5.

Table 10.1.1- 7: Avian "tox per fraction" for BIX+FLU+PTZ EC 260 (step 1 in Appendix B of EFSA GD 2009)

	Fluopyram	Bixafen	Prothioconazole	"Mix"
Content of a.s. in product [g a.s./L prod.]	65	65	130	260
Fraction in the a.s. mixture	0.25	0.25	0.50	1
LD_{50} of a.s. [mg a.s./kg bw]	> 2000	> 2000 [A]	> 2000 [B]	> 2000
Tox per fraction	8000	> 8000	> 4000	> 20000
Contribution to predicted toxicity	25 %	25 %	50 %	100 %

[A] LD_{50} for Bobwhite quail given in EFSA Journal 2012;10(11):2917

[B] LD_{50} for Bobwhite quail given in EFSA Scientific Report (2007) 106, 1-98

EFSA GD 2009 recommends as next step (2a and 2b in Appendix B) to check the predicted toxicity against measured toxicity from LD_{50} studies conducted with the formulation. However, no study with the formulation was conducted. Therefore, steps 2a and 2b cannot be conducted.

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

For the use in crops under assessment in this evaluation (barley) the leaf scenario is not considered relevant according to the EFSA GD 2009.

Acute risk assessment for birds drinking contaminated water from puddles

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

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

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

Crop	Compound	K_{oc} [L/kg]	AR_{eff} (Appl. rate × MAF_m) [g a.s./ha]	LD_{50} [mg a.s./kg bw]	Ratio (AR_{eff}/ LD_{50})	"Escape clause" No concern if ratio	Conclusion
Cereals (barley) 1 × 0.039 kg a.s./ha	Fluopyram	232.1 [A]	39	> 2000	< 0.020	≤ 50	No concern
Cereals (barley) 1 × 0.156 kg total a.s./ha	Bixafen + fluopyram + prothioconazole	232.1 [B]	156	> 2000	< 0.078	≤ 50	No concern
Cereals (barley) 1 × 0.078 kg a.s./ha	Fluopyram	232.1 [A]	78	> 2000	< 0.039	≤ 50	No concern
Cereals (barley) 1 × 0.312 kg total a.s./ha	Bixafen + fluopyram + prothioconazole	232.1 [B]	312	> 2000	< 0.156	≤ 50	No concern

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

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

LONG-TERM REPRODUCTIVE RISK ASSESSMENT

EFSA GD 2009 recommends not to conduct a combined reproductive risk assessment for compounds not sharing the same mode of action (step 3). Therefore, no combined reproductive risk assessment is required for BIX+FLU+PTZ EC 260 in this AIR-evaluation but may be conducted post-AIR according to the respective zonal guidance.

Screening step

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

Crop	Indicator species	DDD				DDD	NOEL [mg a.s./kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
Cereals (barley) 1 × 0.039 kg a.s./ha	Small omnivorous bird	0.039	64.8	1.0	0.53	1.34	4.5	**3.36**	5
Cereals (barley) 1 × 0.078 kg a.s./ha	Small omnivorous bird	0.078	64.8	1.0	0.53	2.68	4.5	**1.68**	5

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

Tier 1

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

Crop	Generic focal species	DDD				DDD	NOEL [mg a.s./ kg bw/d]	TER_{LT}	Trigger
		Appl. rate [kg a.s./ha]	SV_m	MAF_m	f_{TWA}				
Cereals (barley) 1 × 0.039 kg a.s./ha	Small omnivorous bird "lark" BBCH 30-39	0.039	5.4	1.0	0.53	0.112	4.5	40.3	5
Cereals (barley) 1 × 0.078 kg a.s./ha	Small omnivorous bird "lark" BBCH 30-39	0.078	5.4	1.0	0.53	0.223	4.5	20.2	5

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

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

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

Crop	Compound	Koc [L/kg]	AR_{eff} (Appl. rate × MAF_m) [g a.s./ha]	NO(A)EL [mg a.s./ kg bw/d]	Ratio (AR_{eff}/ NOEL)	"Escape clause" No concern if ratio	Conclusion
Cereals (barley) 1 × 0.039 kg a.s./ha	Fluopyram	232.1 [A]	39	4.5	8.67	≤ 50	No concern
Cereals (barley) 1 × 0.078 kg a.s./ha	Fluopyram	232.1 [A]	78	4.5	17.3	≤ 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- 12: Log Pow value of fluopyram

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

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

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

Generic avian indicator species	Body weight (g)	Example	FIR/bw
Earthworm eater	100	Thrush	1.05
Fish eater	1000	Heron	0.159

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

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

Table 10.1.1- 14: Tier 1 long-term DDD and TER calculation for earthworm-eating birds in barley (1 × 0.039 kg a.s./ha)

	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)	0.019 [B]	0.019 [B]
PEC_{worm} (mg/kg)	0.105	0.016
FIR/bw	1.05	1.05
DDD (mg/kg bw/d)	0.110	0.017
NO(A)EL (mg/kg bw/d)	4.5	4.5
TER_{LT}	41.0	265
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 (cereals, 1 × 39 g a.s./ha): 21-day-TWA of 0.010 mg a.s./kg + plateau concentration (20 cm) of 0.009 mg a.s./kg
[C] Measured BCF resulting from a bioaccumulation study in earthworms, please refer to MCA 8.1.3

Table 10.1.1- 15: Tier 1 long-term DDD and TER calculation for earthworm-eating birds in barley (1 × 0.078 kg a.s./ha)

	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)	0.039 [B]	0.039 [B]
PEC_{worm} (mg/kg)	0.215	0.033
FIR/bw	1.05	1.05
DDD (mg/kg bw/d)	0.226	0.035
NO(A)EL (mg/kg bw/d)	4.5	4.5
TER_{LT}	20.0	129
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- 6 (cereals, 1 × 78 g a.s./ha): 21-day-TWA of 0.021 mg a.s./kg + plateau concentration (20 cm) of 0.018 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- 16: Tier 1 long-term DDD and TER calculation for fish-eating birds in barley (1 × 0.039 kg a.s./ha)

	Fluopyram
BCF_{fish}	16 [A]
FOCUS Step 2 PEC_{SW} (twa, 21 d) (mg/L)	0.00417 [B]
PEC_{fish} (mg/kg)	0.067
FIR/bw	0.159
DDD (mg/kg bw/d)	0.011
NO(A)EL (mg/kg bw/d)	4.5
TER_{LT}	424
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- 10 (winter cereals, 1 × 39 g a.s./ha), FOCUS Step 2, Northern Europe, autumn application as worst case

Table 10.1.1- 17: Tier 1 long-term DDD and TER calculation for fish-eating birds in barley (1 × 0.078 kg a.s./ha)

	Fluopyram
BCF_{fish}	16 [A]
FOCUS Step 2 PEC_{SW} (twa, 21 d) (mg/L)	0.00835 [B]
PEC_{fish} (mg/kg)	0.134
FIR/bw	0.159
DDD (mg/kg bw/d)	0.021
NO(A)EL (mg/kg bw/d)	4.5
TER_{LT}	212
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- 15 (winter cereals, 1 × 78 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

For animal welfare reasons, no acute oral toxicity study with the preparation was performed in birds.

CP 10.1.1.2 Higher tier data on birds

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

CP 10.1.2 Effects on terrestrial vertebrates other than birds

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

Test substance	Test design	Test species	Endpoint	Reference
Fluopyram	Acute oral	Rat	LD_{50} > 2000 mg a.s./kg bw	[redacted] (2005) M-259398-01-1 KCA 5.2.1/01
	Two-generation study	Rat	NOAEL = 14.5 mg a.s./kg bw/d	[redacted] (2008) M-299334-01-1 KCA 5.6.1/02
BIX+FLU+PTZ EC 260	Acute	Rat	LD_{50} > 1420 mg prod./kg bw	[redacted] (2013) M-463048-01-1 KCA 7.1.1/01
BIX+FLU+PTZ EC 260	Acute	Rat	LD_{50} (mix) > 3912 mg total a.s./kg bw	Table 10.1.2- 7

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
Cereals (barley)	Small herbivorous mammal	118.4	48.3

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

Crop	Generic focal species	Shortcut value (SV)	
		Acute RA based on RUD_{90}	Long-term RA based on RUD_m
Cereals (barley) BBCH 30-61	Small insectivorous mammal "shrew" BBCH ≥ 20	5.4	Not needed
	Small herbivorous mammal "vole" BBCH ≥ 40	40.9	Not needed
	Small omnivorous mammal "mouse" BBCH 30 – 39	8.6 [A]	Not needed
	Small omnivorous mammal "mouse" BBCH ≥ 40	5.2	Not needed

[A] Shortcut value for BBCH 30 – 39 is used in the risk assessment as worst case covering scenario BBCH ≥ 40

ACUTE DIETARY RISK ASSESSMENT

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

Crop	Indicator species	DDD			DDD	LD_{50} [mg a.s./kg bw]	TER_A	Trigger
		Appl. rate [kg a.s./ha]	SV_{90}	MAF_{90}				
Cereals (barley) 1 × 0.039 kg a.s./ha	Small herbivorous mammal	0.039	118.4	1.0	4.62	> 2000	> 433	10
Cereals (barley) 1 × 0.078 kg a.s./ha	Small herbivorous mammal	0.078	118.4	1.0	9.24	> 2000	> 217	10

Table 10.1.2- 5: Screening acute risk assessment for mammals (BIX+FLU+PTZ EC 260)

Crop	Indicator species	DDD			DDD	$LD_{50\ mix}$ [mg total a.s./kg bw]	TER_A	Trigger
		Appl. rate [kg total a.s./ha]	SV_{90}	MAF_{90}				
Cereals (barley) 1 × 0.156 kg total a.s./ha	Small herbivorous mammal	0.156	118.4	1.0	18.5	> 3912	> 211	10
Cereals (barley) 1 × 0.312 kg total a.s./ha	Small herbivorous mammal	0.312	118.4	1.0	36.9	> 3912	> 106	10

For fluopyram and the $LD_{50\ mix}$ the TER_A values are above the trigger of 10 already at screening level. whereas the TER_A values for the product (with the estimated LD_{50} of 1420 mg prod./kg bw/d) would require a Tier 1 assessment.

Table 10.1.2- 6: First-tier acute risk assessment for mammals (BIX+FLU+PTZ EC 260)

Crop	Generic focal species	DDD			DDD	LD_{50} [mg prod./ kg bw]	TER_A	Trigger
		Appl. rate [kg prod./ha]	SV_{90}	MAF_{90}				
Cereals (barley) 1 × 0.6 L prod/ha	Small insectivorous mammal "shrew" BBCH ≥ 20	0.607	5.4	1.0	3.3	1420	434	10
	Small herbivorous mammal "vole" BBCH ≥ 40	0.607	40.9	1.0	24.8	1420	57	10
	Small omnivorous mammal "mouse" BBCH 30 – 39	0.607	8.6	1.0	5.2	1420	450	10
Cereals (barley) 1 × 1.2 L prod/ha	Small insectivorous mammal "shrew" BBCH ≥ 20	1.213	5.4	1.0	6.6	1420	217	10
	Small herbivorous mammal "vole" BBCH ≥ 40	1.213	40.9	1.0	49.6	1420	29	10
	Small omnivorous mammal "mouse" BBCH 30 – 39	1.213	8.6	1.0	10.4	1420	136	10

The Tier 1 TER_A values calculated in the acute risk assessment with the estimated LD_{50} of 1420 mg prod./kg bw exceed the a-priori-acceptability trigger of 10 indicating an acceptable acute risk to mammals for the intended use of the product in cereals.

There is moderate uncertainty about the precision of the LD_{50} estimate with non-linear interpolation, but this is compensated by

- the extra margin of safety of about factor 3 for the most critical scenario (voles) at 1.2 L/ha (all other scenarios provide higher extra margins of safety)
- potential to demonstrate further increased margins of safety for the most critical scenario (voles at BBCH ≥ 40) when considering that interception of cereals at BBCH can be considered to be 90% instead of the 70% included in the Tier 1 short-cut value

Therefore the acute risk to mammals from the use of the representative formulation can be considered as low.

Combined toxicity risk assessment

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

For the assessment of acute effects (mortality), a surrogate LD_{50} (mix) can be calculated for the mixture risk assessment. The EFSA GD 2009 indicates that the following equation should be used for deriving a surrogate LD_{50} (mix) for a mixture of active substances with known toxicity assuming dose additivity:

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

where:

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

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

The active substance content of the formulation BIX+FLU+PTZ EC 260 addressed in this dossier is 65 g bixafen/L product, 65 g fluopyram/L product and 130 g prothioconazole/L product, making up a total of 260 g a.s./L product.

The table below shows the calculation of the predicted LD_{50} (mix) of bixafen, fluopyram and prothioconazole when mixed in these proportions (step 1 in Appendix B of EFSA GD 2009).

Table 10.1.2- 7: Mammalian LD_{50} (mix) for bixafen, fluopyram and prothioconazole when combined as BIX+FLU+PTZ EC 260 (step 1 in Appendix B of EFSA GD 2009)

	Fluopyram	Bixafen	Prothioconazole
Content of a.s. in product [g a.s./L prod.]	65	65	130
Fraction in the a.s. mixture	0.25	0.25	0.50
LD_{50} of a.s. [mg a.s./kg bw]	2000	> 5000 [A]	> 6200 [B]
Fraction / LD_{50}	0.000125	< 0.00005	< 0.00008
Sum	< 0.00026		
1/sum = predicted LD_{50} (mix) [mg total a.s./kg bw]	> 3912		

[A] LD_{50} for rat given in EFSA Journal 2012;10(11):2917

[B] LD_{50} for rat given in EFSA Scientific Report (2007) 106, 1-98

It is obvious from the comparison of the (low) acute oral toxicity of the active substances, and their relative proportions within the formulated product BIX+FLU+PTZ EC 260, that neither bixafen nor fluopyram nor prothioconazole contributes to more than 90 % to the predicted acute mixture toxicity (see next table).

Table 10.1.2- 8: Mammalian "tox per fraction" for BIX+FLU+PTZ EC 260 (step 1 in Appendix B of EFSA GD 2009)

	Fluopyram	Bixafen	Prothioconazole	"Mix"
Content of a.s. in product [g a.s./L prod.]	65	65	130	260
Fraction in the a.s. mixture	0.25	0.25	0.50	1
LD_{50} of a.s. [mg a.s./kg bw]	> 2000	> 5000 [A]	> 6200 [B]	> 3912
Tox per fraction	> 8000	> 20000	> 12400	> 40400
Contribution to predicted toxicity	19.8 %	49.5 %	30.7 %	100 %

[A] LD_{50} for rat given in EFSA Journal 2012;10(11):2917
[B] LD_{50} for rat given in EFSA Scientific Report (2007) 106, 1-98

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

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

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

With:

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

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

LD_{50} (mix) = Measured acute toxicity value for the mixture

A greater value on the right side of the equation indicates that the formulation is more toxic than predicted from the toxicity of the individual components (active substances and co-formulants of known toxicity). This may be due to, e.g. further toxic co-formulants, toxicokinetic interaction or synergism/potentiation of effect. It may also reflect the inherent variability of toxicity testing. In all these cases, the use of the LD_{50} for the formulation is recommended for the first-tier assessment, because it cannot be excluded that such effects would also occur after exposure of animals to residues in the environment. Dismissing the LD_{50} of the formulation from the risk assessment would only be acceptable at a higher tier if any observed greater toxicity in the test could be clearly and unambiguously ascribed to a factor that would not be relevant under environmental exposure conditions.

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

Left side of the equation:

$$\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._{i.})} = \frac{0.25}{\frac{> 2000\ mg\ a.s}{kg\ bw}} + \frac{0.25}{\frac{> 5000\ mg\ a.s}{kg\ bw}} + \frac{0.5}{\frac{> 6200\ mg\ a.s}{kg\ bw}} =< 0.00026$$

Right side of the equation: $\frac{1}{LD_{50}(\text{mix})} = \frac{1}{\frac{> 76.62 \text{ mg total a.s.}}{\text{kg bw}}} =< 0.0033$

0.00026 < 0.013

The greater value on the right side indicates that the formulation BIX+FLU+PTZ EC 260 is more toxic than predicted from the toxicity of the individual components bixafen, fluopyram and prothioconazole. Therefore, the use of the LD_{50} for the formulation is recommended for the risk assessment (please refer to Table 10.1.2- 5 (screening step) and Table 10.1.2- 6 (Tier 1)).

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- 9: 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)	LD_{50}	Ratio (AR_{eff}/ LD_{50})	"Escape clause" No concern if ratio	Conclusion
Cereals (barley) 1 × 0.039 kg a.s./ha	Fluopyram	232.1 A	39 g a.s./ha	> 2000 mg a.s./kg bw	< 0.020	≤ 50	No concern
Cereals (barley) 1 × 0.607 kg prod./ha	Bixafen + fluopyram + prothioconazole	232.1 B	607 g prod./ha	1420 mg prod./kg bw	0.427	≤ 50	No concern
Cereals (barley) 1 × 0.078 kg a.s./ha	Fluopyram	232.1 A	78 g a.s./ha	> 2000 mg a.s./kg bw	< 0.039	≤ 50	No concern
Cereals (barley) 1 × 1.213 kg prod./ha	Bixafen + fluopyram + prothioconazole	232.1 B	1213 g prod./ha	1420 mg prod./kg bw	0.854	≤ 50	No concern

A K_{OC} value given in MCP 9.2.4.1 (Table 9.2.4-1)
B K_{oc} of fluopyram

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

LONG-TERM REPRODUCTIVE ASSESSMENT

EFSA GD 2009 recommends not to conduct a combined reproductive risk assessment for compounds not sharing the same mode of action (step 3). Therefore, no combined reproductive risk assessment is required for BIX+FLU+PTZ EC 260 in this AIR-evaluation but may be conducted post-AIR according to the respective zonal guidance.

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

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}				
Cereals (barley) 1 × 0.039 kg a.s./ha	Small herbivorous mammal	0.039	48.3	1.0	0.53	0.998	14.5	14.5	5
Cereals (barley) 1 × 0.078 kg a.s./ha	Small herbivorous mammal	0.078	48.3	1.0	0.53	2.00	14.5	7.26	5

The screening level TER_{LT} value exceeds the trigger of 5. Therefore, a risk assessment at Tier 1 is not required as the risk to mammals is considered acceptable.

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

Table 10.1.2- 11: Evaluation of potential concern for exposure of mammals from drinking water (long-term, escape clause)

Crop	Compound	Koc [L/kg]	AR_{eff} (Appl. rate × MAF_m) [g a.s./ha]	NOAEL [mg a.s./kg bw/d]	Ratio (AR_{eff}/ NOAEL)	"Escape clause" No concern if ratio	Conclusion
Cereals (barley) 1 × 0.039 kg a.s./ha	Fluopyram	232.1 [A]	39	14.5	2.69	≤ 50	No concern
Cereals (barley) 1 × 0.078 kg a.s./ha	Fluopyram	232.1 [A]	78	14.5	5.38	≤ 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 mammals from drinking water that may contain residues from fluopyram is acceptable.

RISK ASSESSMENT OF SECONDARY POISONING

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

Table 10.1.2- 12: Log Pow value of fluopyram

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

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

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

Generic mammalian indicator species	Body weight (g)	Example	FIR/bw
Earthworm eater	100	Common shrew	1.28
Fish eater	1000	Otter	0.142

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

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

Table 10.1.2- 14: Tier 1 long-term DDD and TER calculation for earthworm-eating mammals in barley (1 × 0.039 kg a.s./ha)

	Fluopyram	
	Tier 1	Refinement
Kow	2060	2060
KOC [mL/g]	232.1 [A]	232.1 [A]
foc	0.02	0.02
BCF_{worm}	5.51	0.85 [C]
$PEC_{soil, accu}$ (mg/kg)	0.019 [B]	0.019 [B]
PEC_{worm} (mg/kg)	0.105	0.016
FIR/bw	1.28	1.28
DDD (mg/kg bw/d)	0.134	0.021
NO(A)EL (mg/kg bw/d)	14.5	14.5
TER_{LT}	108	701
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 (cereals, 1 × 39 g a.s./ha): 21-day-TWA of 0.010 mg a.s./kg +

plateau concentration (20 cm) of 0.009 mg a.s./kg
C Measured BCF resulting from a bioaccumulation study in earthworms, please refer to MCA 8.1.3

Table 10.1.2- 15: Tier 1 long-term DDD and TER calculation for earthworm-eating mammals in barley (1 × 0.078 kg a.s./ha)

	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)	0.039 [B]	0.039 [B]
PEC_{worm} (mg/kg)	0.215	0.033
FIR/bw	1.28	1.28
DDD (mg/kg bw/d)	0.275	0.042
NO(A)EL (mg/kg bw/d)	14.5	14.5
TER_{LT}	52.7	342
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- 6 (cereals, 1 × 78 g a.s./ha): 21-day-TWA of 0.021 mg a.s./kg + plateau concentration (20 cm) of 0.018 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- 16: Tier 1 long-term DDD and TER calculation for fish-eating mammals in barley (1 × 0.039 kg a.s./ha)

	Fluopyram
BCF_{fish}	16 [A]
FOCUS Step 2 PEC_{SW} (twa, 21 d) (mg/L)	0.00417 [B]
PEC_{fish} (mg/kg)	0.067
FIR/bw	0.142
DDD (mg/kg bw/d)	0.0095
NO(A)EL (mg/kg bw/d)	14.5
TER_{LT}	1531
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- 10 (winter cereals, 1 × 39 g a.s./ha), FOCUS Step 2, Northern Europe, autumn application as worst case

Table 10.1.2- 17: Tier 1 long-term DDD and TER calculation for fish-eating mammals in barley (1 × 0.078 kg a.s./ha)

	Fluopyram
BCF_{fish}	16 [A]
FOCUS Step 2 PEC_{SW} (twa, 21 d) (mg/L)	0.00835 [B]
PEC_{fish} (mg/kg)	0.134
FIR/bw	0.142
DDD (mg/kg bw/d)	0.0190
NO(A)EL (mg/kg bw/d)	14.5
TER_{LT}	764
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- 15 (winter cereals, 1 × 78 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

An LD_{50} study according to OECD 423 (toxic class method) has been conducted with the formulation BIX + FLU + PTZ EC 260, including two dose levels (6 animals treated with 300 mg prod./kg bw, 3 animals treated with 2000 mg prod./kg bw).

The results from this study are not suited to calculate a proper LD_{50} (0 of 6 animals died after dosing with 300 mg/kg, 2 of 3 animals died after dosing with 2000 mg/kg bw). Employing non-linear interpolation would suggest 1420 mg prod./kg bw as LD_{50} estimate.

For the generic mammal focal species scenarios of relevance for the risk assessment conducted in this dossier, i.e. with 90th percentile short-cut values ranging 5.4 – 42.1 for the acute risk assessment, TER_A values with that LD_{50} estimate (1420 mg prod./kg bw), would range between 29 – 217 taking the intended application pattern into account (1.2 L product/ha, MAF_{90} = 1.0, product density: 1.011 kg/L). Thus, a quantitative risk assessment based on the product would not change the conclusion derived from the risk assessment based on the individual active substances. Furthermore, wild mammals will not be exposed to the liquid formulation, rather the dried residues of the active substance on/in foliage, seeds or arthropods.

Table 10.1.2- 18: Mammalian toxicity data of the formulated product BIX+FLU+PTZ EC 260

Test substance	Test design	Species	Endpoint		Reference
BIX+FLU+PTZ EC 260	Acute	Rat	LD_{50}	> 300 - < 2000 mg prod./kg bw ~ 1420 mg prod./kg bw	[redacted] (2013) M-463048-01-1 KCA 7.1.1/01

CP 10.1.2.2 Higher tier data on mammals

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

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

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

CP 10.2 Effects on aquatic organisms

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

Table 10.2- 1: 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
BIX+ FLU+ PTZ EC 260	Fish, acute *Oncorhynchus mykiss*	96 h / static	**LC_{50} = 1.77 mg prod./L (nom)**	[redacted] (2013) M-475973-01-1 KCP 10.2.1/01
	Invertebrate, acute *Daphnia magna*	0 - 48 h / static	**EC_{50} = 3.39 mg prod./L (nom)**	[redacted] (2013) M-476467-01-1 KCP 10.2.1/02
	Green algae *Pseudokirchneriella subcapitata* (currently known as *Raphidocelis subcapitata*)	0-72 h / static	**E_rC_{50} = 2.97 mg prod./L (nom)** E_rC_{10} = 1.48 mg prod./L (nom) E_bC_{50} = 1.91 mg prod./L (nom) E_bC_{50} = 1.80 mg prod./L (nom)	[redacted] (2013) M-475997-01-1 KCP 10.2.1/03
Fluopyram tech.	Fish, acute *Oncorhynchus mykiss*	96 h / static	LC_{50} > 1.09 mg a.s./L (nom)	[redacted] (2008) M-277770-02-1 KCA 8.2.1/01
	Fish, acute *Lepomis macrochirus*	96 h / static	LC_{50} > 5.68 mg a.s./L (nom)	[redacted] (2008) M-278441-02-1 KCA 8.2.1/02
	Fish, acute *Pimephales promelas*	96 h / static	LC_{50} > 4.95 mg a.s./L (mm) [A]	[redacted] (2008) M-298918-01-1 KCA 8.2.1/03
	Fish, acute *Cyprinus carpio*	96 h / static	LC_{50} = 30.5 mg a.s./L (mm) [B]	[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 (mm)	[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

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Fish, BCF flow-through *Lepomis macrochirus*	28 d exposure + 14 d depuration / flow-through	BCF (whole fish, wet weight) = 18 BCF (whole fish, normalized to 6 % lipid content) = 16	[redacted] (2008) M-298506-01-1 KCA 8.2.2.3/03
	Invertebrate, acute *Daphnia magna*	48 h / static	EC_{50} > 20 mg a.s./L (nom)	[redacted] (2008) 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-297351-01-1 KCA 8.2.4.2/01
	Invertebrate, acute *Crassostrea virginica*	96 h / flow-through	LC_{50} > 1.44 mg a.s./L (mm) (shell deposition and mortality)	[redacted] (2006) M-282691-01-1 KCA 8.2.4.2/02
	Invertebrate, acute *Americamysis bahia*	96 h / flow-through	LC_{50} > 0.50 mg a.s./L (mm)	[redacted] (2007) M-282839-01-2 KCA 8.2.4.2/03
	Invertebrate, acute Geometric mean	-	Geometric mean **EC_{50} = 1.638 mg a.s./L**	
	Invertebrate, chronic *Daphnia magna*	21 d / static-renewal	**NOEC = 1.25 mg a.s./L (nom)** EC_{10}: not determined [C]	[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) *Chironomus tentans* (spiked sediment)	21 d / static-renewal	**NOEC = 26 mg a.s./kg (mm)** EC_{10}: not determined [C]	[redacted] (2008) M-298809-01-1 KCA 8.2.5.3/01 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_{15} = 1.37 mg a.s./L (nom) EC_{50} > 32 mg a.s./L (nom) [C]	[redacted] (2008) M-298266-01-1 KCA 8.2.5.4/01
	Sediment dweller, chronic *Leptocheirus plumulosus* (spiked sediment)	28 d / Static-renewal	NOEC = 38 mg a.s./kg (mm) LC_{50} > 94 mg a.s./kg (mm)	[redacted] (2008) M-298810-02-1 KCA 8.2.5.4/02

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 - 72 h / static	E_rC_{50} = 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 8.2.1.1/01 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} = 7.08 mg a.s./L (mm) E_rC_{10} = 5.23 mg a.s./L (mm) E_bC_{50} = 5.42 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
	Amphibia, *Xenopus laevis*	48 h / static	LC_{50} > 5.0 mg a.s./L (nom)	[redacted] (2010) M-395416-01-1 KCA 8.2.8/01
Fluopyram-7-hydroxy	Invertebrate, acute *Daphnia magna*	0 - 48 h / static	**EC_{50} > 88.7 mg p m./L (nom)**	[redacted] (2020) M-759029-01-1 KCA 8.2.4.1/02
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	**E_rC_{50} = 20.9 mg p m./L (nom)** E_rC_{10} = 20.2 mg p m./L (nom) E_yC_{50} = 13.0 mg p m./L (nom)	[redacted] (2020) M-758708-01-1 KCA 8.2.6.1/05
		0 -96 h / static	E_rC_{50} = 21.1 mg p m./L (nom) E_rC_{10} = 20.4 mg p m./L (nom) E_yC_{50} = 13.7 mg p m./L (nom) E_bC_{50} = 12.6 mg p m./L (nom)	
	Aquatic macrophyte *Lemna gibba*	7 d / static	**E_rC_{50} = 9.2 mg p m./L (mm)** E_rC_{10} = 5.2 mg p m./L (mm) E_yC_{50} = 7.1 mg p m./L (mm)	[redacted] (2020) M-759030-01-1 KCA 8.2.7/02
Trifluoro-acetic acid (TFA)	Fish, acute *Brachydanio rerio*	96 h / static	LC_{50} > 1200 mg p m./L (nom Na-TFA) **> 1008 mg p m./L (nom TFA)** [E]	[redacted] (1992) M-247889-01-1 KCA 8.2.1/06

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Invertebrate, acute *Daphnia magna*	0 - 48 h / static	**EC_{50}** > 1200 mg p m./L (nom Na-TFA) **> 1008 mg p m./L (nom TFA)** [E]	[redacted] (1992) M-247890-01-1 KCA 8.2.4.1/03
	Invertebrate, chronic *Daphnia magna*	21 d / Semi-static	**NOEC** = 30 mg p.m./L (nom Na-TFA) **≥ 25.2 mg p.m./L (nom TFA)** [E] EC_{10}: not determined	[redacted] (2010) M-615126-01-1 KCA 8.2.5.1/05
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	**E_rC_{50}** > 1.2 mg p.m./L (nom Na-TFA) **> 1.01 mg p.m./L (nom TFA)** [E] E_rC_{10} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E] E_bC_{50} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E] E_yC_{50} > 1.2 mg p m./L (nom Na-TFA) > 1.01 mg p.m./L (nom TFA) [E]	[redacted] (1993) M-247818-02-1 KCA 8.2.6.1/06 Re-evaluation by [redacted] (2021) M-762268-02-1 KCA 8.2.6.1/07
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 -72 h / static	E_rC_{50} = 169 mg p m./L (nom Na-TFA) = 134.4 mg p.m./L (nom TFA) [E] E_rC_{10} = 2.239 mg p m./L (nom Na-TFA) = 1.881 mg p.m./L (nom TFA) [E] E_bC_{50} > 4.8 mg p m./L (nom Na-TFA) > 4.03 mg p.m./L (nom TFA) [E] E_yC_{50} = 4.190 mg p m./L (nom Na-TFA) = 3.52 mg p.m./L (nom TFA) [E]	[redacted] (1992) M-247820-01-1 KCA 8.2.6.1/08 Re-evaluation by [redacted] (2021) M-762208-02-1 KCA 8.2.6.1/09
	Green algae *Pseudokirchneriella subcapitata* [D] (currently known as *Raphidocelis subcapitata*)	0 -72 h / Static	E_rC_{50} = 237.07 mg p.m./L (nom) E_rC_{50} = 241.95 mg p.m./L (mm) E_rC_{10} = 5.59 mg p.m./L (nom) E_rC_{10} = 5.80 mg p.m./L (mm) E_bC_{50} = 26.866 mg p m./L (mm) E_yC_{50} = 18.956 mg p m./L (mm)	[redacted] (2017) M-615180-01-1 KCA 8.2.6.1/12 Re-evaluation by [redacted] (2021) M-762267-01-1 KCA 8.2.6.1/13

Test substance	Test species	Time scale/ Study type	Endpoint	Reference
	Aquatic macrophyte *Lemna gibba*	7 d / static	E_yC_{50} = 1100 mg p.m./L (nom Na-TFA) = 924 mg p m./L (nom TFA) [E] **E_rC_{50} > 2016 mg p m./L (nom TFA) [E]** NOE$_rC$ = 252 mg p m./L (nom TFA) [E] E_rC_{10} = 308 mg p m./L (nom TFA) [E]	[redacted] (1993) M-247900-01-1 KCA 8.2.7/03 Endpoint recalculation by [redacted] (2021) M-768038-01-1 KCA 8.2.7/06

Note:
Studies referring to KCA are filed in the dossier for the active substance
Studies written in grey 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 Practical limit of water solubility
B In all test levels precipitations were observable so the LC_{50} is clearly above the water solubility of the test item.
C Not determined due to mathematical reasons
D Formerly known as *Selenastrum capricornutum*
E As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to Trifluoroacetic acid with factor 0.84.

Metabolites

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

Step 1: Are the studies with the active substance adequate for assessing the potential effect of the metabolites?
No: Go to step 3.

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

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

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

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

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

Application in barley, 1 x 78 g a.s./ha

Table 10.2- 4: Initial max PEC_{sw} values – FOCUS Steps 1 and 2 – Single application in barley (Spring cereals, spring / summer, 1 x 78 g a.s./ha)

Compound	FOCUS Scenario	PECsw, max [µg/L]	
		Barley (Spring cereals - Spring, Mar. – May) 1 × 78 g a.s./ha, BBCH 30-61	Barley (Spring cereals – Summer, Jun. - Sep.) 1 × 78 g a.s./ha, BBCH 30-61
Fluopyram	STEP 1	20.6	20.6
	STEP 2 – North	3.74	3.74
	STEP 2 - South	**6.89**	5.31
Fluopyram-7-hydroxy	STEP 1	1.38	1.38
	STEP 2 - North	0.221	0.221
	STEP 2 - South	**0.442**	0.331
Trifluoroacetic acid (TFA)	STEP 1	1.11	1.11
	STEP 2 – North	0.177	0.177
	STEP 2 - South	**0.353**	0.265

Bold values used in risk assessment

Table 10.2- 5: Initial max PEC_{sw} values – FOCUS Steps 1 and 2 – Single application in barley (Winter cereals, autumn / spring / summer, 1 x 78 g a.s./ha)

Compound	FOCUS Scenario	PECsw, max [µg/L]		
		Barley (Winter cereals, - Autumn, Oct. - Feb.) 1 × 78 g a.s./ha, BBCH 30-61	Barley (Winter cereals, – Spring, Mar. - May) 1 × 78 g a.s./ha, BBCH 30-61	Barley (Winter cereals, - Summer, Jun. - Sep.) 1 × 78 g a.s./ha, BBCH 30-61
Fluopyram	STEP 1	20.6	20.6	20.6
	STEP 2 – North	**8.40**	3.74	3.74
	STEP 2 - South	6.89	6.89	5.31
Fluopyram-7-hydroxy	STEP 1	1.38	1.38	1.38
	STEP 2 – North	**0.552**	0.221	0.221
	STEP 2 - South	0.442	0.442	0.331
Trifluoroacetic acid (TFA)	STEP 1	1.11	1.11	1.11
	STEP 2 – North	**0.441**	0.177	0.177
	STEP 2 - South	0.353	0.353	0.265

Bold values used in risk assessment

Table 10.2- 6: Initial max PEC_{sed} values – FOCUS Steps 1 and 2 – Single application in barley (Spring cereals, spring / summer, 1 x 78 g a.s./ha)

Compound	FOCUS Scenario	PECsed, max [µg/L]	
		Barley (Spring cereals - Spring, Mar. - May) 1 × 78 g a.s./ha, BBCH 30-61	Barley (Spring cereals – Summer, Jun. - Sep.) 1 × 78 g a.s./ha, BBCH 30-61
Fluopyram	STEP 1	47.3	47.3
	STEP 2 – North	8.57	8.57
	STEP 2 - South	**15.9**	12.2
Fluopyram-7-hydroxy	STEP 1	1.39	1.39
	STEP 2 – North	0.221	0.221
	STEP 2 - South	0.443	0.332
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

Table 10.2- 7: Initial max PEC_{sed} values – FOCUS Steps 1 and 2 – Single application in barley (Winter cereals, autumn / spring / summer, 1 x 78 g a.s./ha)

Compound	FOCUS Scenario	PECsed, max [µg/L]		
		Barley (Winter cereals - Autumn, Oct. - Feb.) 1 × 78 g a.s./ha, BBCH 30-61	**Barley (Winter cereals – Spring, Mar. - May) 1 × 78 g a.s./ha, BBCH 30-61**	**Barley (Winter cereals, Summer, Jun. - Sep.) 1 × 78 g a.s./ha, BBCH 30-61**
Fluopyram	STEP 1	47.3	47.3	47.3
	STEP 2 – North	**19.5**	8.57	8.57
	STEP 2 - South	15.9	15.9	12.2
Fluopyram-7-hydroxy	STEP 1	1.39	1.39	1.39
	STEP 2 – North	0.353	0.221	0.221
	STEP 2 - South	0.443	0.443	0.332
Trifluoroacetic acid (TFA)	STEP 1	<0.001	<0.001	<0.001
	STEP 2 – North	<0.001	<0.001	<0.001
	STEP 2 - South	<0.001	<0.001	<0.001

Bold values used in risk assessment

Application in barley, 1 x 39 g a.s./ha

Table 10.2- 8: Initial max PEC_{sw} values – FOCUS Steps 1 and 2 – Single application in barley (Spring cereals, spring / summer, 1 x 39 g a.s./ha)

Compound	FOCUS Scenario	PECsw, max [µg/L]	
		Barley (Spring cereals - Spring, Mar. - May) 1 × 39 g a.s./ha, BBCH 30-61	**Barley (Spring cereals – Summer, Jun. - Sep.) 1 × 39 g a.s./ha, BBCH 30-61**
Fluopyram	STEP 1	10.3	10.3
	STEP 2 – North	1.87	1.87
	STEP 2 - South	**3.44**	2.66
Fluopyram-7-hydroxy	STEP 1	0.692	0.692
	STEP 2 – North	0.110	0.110
	STEP 2 - South	**0.221**	0.166
Trifluoroacetic acid (TFA)	STEP 1	0.553	0.553
	STEP 2 – North	0.088	0.088
	STEP 2 - South	**0.177**	0.132

Bold values used in risk assessment

Table 10.2- 9: Initial max PEC_{sw} values – FOCUS Steps 1 and 2 – Single application in barley (Winter cereals, autumn/ spring / summer, 1 x 39 g a.s./ha)

Compound	FOCUS Scenario	PECsw, max [µg/L]		
		Barley (Winter cereals - Autumn, Oct. - Feb.) 1 × 39 g a.s./ha, BBCH 30-61	Barley (Winter cereals – Spring, Mar. - May) 1 × 39 g a.s./ha, BBCH 30-61	Barley (Winter cereals – Summer, Jun. - Sep.) 1 × 39 g a.s./ha, BCH 30-61
Fluopyram	STEP 1	10.3	10.3	10.3
	STEP 2 – North	**4.23**	1.87	1.87
	STEP 2 - South	3.44	3.44	2.66
Fluopyram-7-hydroxy	STEP 1	0.692	0.692	0.692
	STEP 2 – North	**0.276**	0.110	0.110
	STEP 2 - South	0.224	0.221	0.166
Trifluoroacetic acid (TFA)	STEP 1	0.553	0.553	0.553
	STEP 2 – North	**0.221**	0.088	0.088
	STEP 2 - South	0.177	0.177	0.132

Bold values used in risk assessment

Table 10.2- 10: Initial max PEC_{sed} values – FOCUS Steps 1 and 2 – Single application in barley (Spring cereals, spring / summer, 1 x 39 g a.s./ha)

Compound	FOCUS Scenario	PECsed, max [µg/L]	
		Barley (Spring cereals - Spring, Mar. - May) 1 × 39 g a.s./ha, BBCH 30-61	Barley (Spring cereals – Summer, Jun. - Sep.) 1 × 39 g a.s./ha, BBCH 30-61
Fluopyram	STEP 1	23.7	23.7
	STEP 2 – North	4.28	4.28
	STEP 2 - South	**7.93**	6.11
Fluopyram-7-hydroxy	STEP 1	0.693	0.693
	STEP 2 – North	0.111	0.111
	STEP 2 - South	0.221	0.166
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

Table 10.2- 11: Initial max PEC$_{sed}$ values – FOCUS Steps 1 and 2 – Single application in barley (winter cereals, autumn/ spring / summer, 1 x 39 g a.s./ha)

Compound	FOCUS Scenario	PECsed, max [µg/L]		
		Barley (Winter cereals - Autumn, Oct. - Feb.) 1 × 39 g a.s./ha, BBCH 30-61	Barley (Winter cereals - Spring, Mar. - May) 1 × 39 g a.s./ha, BBCH 30-61	Barley (Winter cereals – Summer, Jun. - Sep.) 1 × 39 g a.s./ha, BBCH 30-61
Fluopyram	STEP 1	23.7	23.7	23.7
	STEP 2 – North	**9.76**	4.28	4.28
	STEP 2 - South	7.93	7.93	6.11
Fluopyram-7-hydroxy	STEP 1	0.693	0.693	0.693
	STEP 2 – North	0.277	0.111	0.111
	STEP 2 - South	0.221	0.221	0.166
Trifluoroacetic acid (TFA)	STEP 1	<0.001	<0.001	<0.001
	STEP 2 – North	<0.001	<0.001	<0.001
	STEP 2 - South	<0.001	<0.001	<0.001

Bold values used in risk assessment

Predicted environmental concentrations for the formulation BIX+FLU+PTZ EC 260

Application in barley, 1 x 1.2 L prod./ha (78 g a.s./ha)

Table 10.2- 12: Initial PEC$_{sw}$ values for the formulation BIX+FLU+PTZ EC 260 following the single application in barely – 1 × 1.2 L prod./ha

Compound	Maximum use rate	No spray buffer (Drift)	Drift reducing nozzles	PECsw [A] [µg product/ha]
BIX+FLU+PTZ EC 260	1.2 L prod./ha	0 m	0 %	**11.202**

Bold values used in risk assessment

[A] Calculation based on Rautmann drift values for arable crops assuming a specific density of 1.011 g/mL (please refer to study M-475973-01-1).

Application in barley, 1 x 1.2 L prod./ha (78 g a.s./ha)

Table 10.2- 13: Initial PEC$_{sw}$ values for the formulation BIX+FLU+PTZ EC 260 following the single application in barely – 1 × 0.6 L prod./ha

Compound	Maximum use rate	No spray buffer (Drift)	Drift reducing nozzles	PECsw [A] [µg product/ha]
BIX+FLU+PTZ EC 260	0.6 L prod./ha	0 m	0 %	**5.601**

Bold values used in risk assessment

[A] Calculation based on Rautmann drift values for arable crops assuming a specific density of 1.011 g/mL (please refer to study M-475973-01-1).

Risk assessment for aquatic organisms

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

Acute risk assessment:

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

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

Chronic risk assessment:

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

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

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

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

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

ACUTE RISK ASSESSMENT FOR AQUATIC ORGANISMS

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

Application in barley, 1 x 78 g a.s./ha

Table 10.2-14: Acute risk assessment based on FOCUS Step 2 for the application in barley (1 × 78 g a.s./ha)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
		Spring cereals			
BIX+ FLU+ PTZ EC 260	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1770	17.7	11.202 [A]	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 3390	33.9		Yes
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	6.89	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		Yes

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
Fluopyram-7-hydroxy	Fish, acute	LC_{50} 437 [B]	2.19 [C]	0.442	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >88700	>443.5 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.353	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes
Winter cereals					
BIX+ FLU+ PTZ EC 260	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1770	17.7	11.202 [A]	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 3390	33.9		Yes
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	8.46	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		Yes
Fluopyram-7-hydroxy	Fish, acute	LC_{50} 437 [B]	2.19 [C]	0.552	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >88700	>443.5 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.441	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes

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

For the application in barely (surrogate crops: spring and winter cereals) at 78 g a.s./ha the acute trigger is met for all aquatic species for fluopyram and its metabolites as well as for the formulation.

Application in barley, 1 x 39 g a.s./ha

Table 10.2- 15: Acute risk assessment based on FOCUS Step 2 for the application in barley (1 × 39 g a.s./ha)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
Spring cereals					
BIX+ FLU+ PTZ EC 260	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1770	17.7	5.601 [A]	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 3390	33.9		Yes
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	43.7	3.44	Yes

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		Yes
Fluopyram-7-hydroxy	Fish, acute	LC_{50} 437 [B]	2.19 [C]	0.221	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >88700	>443.5 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.177	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes
Winter cereals					
BIX+ FLU+ PTZ EC 260	Fish, acute *Oncorhynchus mykiss*	LC_{50} 1770	177	5.601 [A]	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 3390	33.9		Yes
Fluopyram tech.	Fish, acute Geometric mean	LC_{50} 4370	437	4.23	Yes
	Invertebrate, acute Geometric mean	EC_{50} 1638	16.38		Yes
Fluopyram-7-hydroxy	Fish, acute	LC_{50} 437 [B]	2.19 [C]	0.276	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >88700	>443.5 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, acute *Oncorhynchus mykiss*	LC_{50} >1008000	>10080	0.221	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} >1008000	>10080		Yes

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

For the application in barley (surrogate crops: spring and winter cereals) at 39 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 barley, 1 x 78 g a.s./ha

Table 10.2- 16: Chronic risk assessment based on FOCUS Step 2 for the application in barley (1 x 78 g a.s./ha)

Compound	Species	Endpoint [µg/L]		RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC / PEC_{sw}
Spring cereals						
BIX+ FLU+ PTZ EC 260	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	2970	297	11.202	Yes
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC	135	13.5	6.89	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC	1250	125		Yes
	Invertebrate, chronic *Chironomus riparius*	EC_{10}	540	54		Yes
	Algae *Skeletonema costatum*	E_rC_{50}	>1130	>113		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	2510	251		Yes
Fluopyram-7-hydroxy	Fish, chronic *Pimephales promelas*	NOEC	13.5 [B]	0.675 [C]	0.442	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC	125 [B]	6.25 [C]		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	20900	1045 [C]		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	9200	460 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC	13.5 [B]	1.35	0.353	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}~~ E_rC_{50}	~~924000~~ >2016000	~~92400~~ >201600		Yes
Winter cereals						
BIX+ FLU+ PTZ EC 260	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	2970	297	11.202 [A]	Yes
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC	135	13.5	8.46	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

Compound	Species	Endpoint [µg/L]		RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
Fluopyram-7-hydroxy	Fish, chronic *Pimephales promelas*	NOEC	13.5 [B]	0.675 [C]	0.552	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC	125 [B]	6.25 [C]		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	20900	1045 [C]		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	9200	460 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC	13.5 [B]	1.35	0.441	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

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

Table 10.2- 17: Chronic risk assessment for sediment organisms based on FOCUS Step 2 for the application in barley (1 × 78 g a.s./ha)

Compound	Species	Endpoint [µg/kg]	RAC [µg/kg]	$PEC_{sed.max}$ [µg/kg]	RAC ≥ PEC_{sed}
Spring cereals					
Fluopyram tech.	Sediment dweller *Chironomus tentans*	NOEC 26000	2600	15.9	Yes
Winter cereals					
Fluopyram tech.	Sediment dweller *Chironomus tentans*	NOEC 26000	2600	19.5	Yes

For the application in barley (surrogate crops: spring and winter cereals) at 78 g a.s./ha the chronic trigger is met for all aquatic species for fluopyram and its metabolites as well as for the formulation.

Application in barley, 1 x 39 g a.s./ha

Table 10.2- 18: Chronic risk assessment based on FOCUS Step 2 for the application in barley (1 x 39 g a.s./ha)

Compound	Species	Endpoint [µg/L]		RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC > PEC_{sw}
Spring cereals						
BIX+ FLU+ PTZ EC 260	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	2970	297	5.601 [A]	Yes
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC	135	13.5	3.44	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC	1250	125		Yes
	Invertebrate, chronic *Chironomus riparius*	EC_{10}	540	54		Yes
	Algae *Skeletonema costatum*	E_rC_{50}	>1130	>113		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	2510	251		Yes
Fluopyram-7-hydroxy	Fish, chronic *Pimephales promelas*	NOEC	13.5 [B]	0.675 [C]	0.221	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC	125 [B]	6.25 [C]		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	20900	1045 [C]		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	9200	460 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC	13.5 [B]	1.35	0.177	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}~~ E_rC_{50}	~~924000~~ >2016000	~~92400~~ >201600		Yes
Winter cereals						
BIX+ FLU+ PTZ EC 260	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	2970	297	5.601 [A]	Yes
Fluopyram tech.	Fish, chronic *Pimephales promelas*	NOEC	135	13.5	4.23	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

Compound	Species	Endpoint [µg/L]		RAC [µg/L]	PEC$_{sw.max}$ [µg/L]	RAC ≥ PEC$_{sw}$
Fluopyram-7-hydroxy	Fish, chronic *Pimephales promelas*	NOEC	13.5 [B]	0.675 [C]	0.276	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC	125 [B]	6.25 [C]		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50}	20900	1045 [C]		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	9200	460 [C]		Yes
Trifluoroacetic acid (TFA)	Fish, chronic *Pimephales promelas*	NOEC	13.5 [B]	1.35	0.221	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}~~ E_rC_{50}	~~924000~~ >2016000	~~92400~~ >201600		Yes

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

Table 10.2- 19: Chronic risk assessment for sediment organisms based on FOCUS Step 2 for the application in barley (1 × 39 g a.s./ha)

Compound	Species	Endpoint [µg/kg]	RAC [µg/kg]	PEC$_{sed.max}$ [µg/kg]	RAC ≥ PEC$_{sed}$
Spring cereals					
Fluopyram tech.	Sediment dweller *Chironomus tentans*	NOEC 26000	2600	7.93	Yes
Winter cereals					
Fluopyram tech.	Sediment dweller *Chironomus tentans*	NOEC 26000	2600	9.76	Yes

For the application in barley (surrogate crops: spring and winter cereals) at 39 g a.s./ha the chronic trigger is met for all aquatic species for fluopyram and its metabolites as well as for the formulation.

Combined toxicity risk assessment

According to the EFSA "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters" (EFSA Journal 2013;11(7):3290; chapter 10.3.11), for products containing more than one active substances, the mixture toxicity shall be addressed via the Concentration Addition (CA) Model. And, following the recommendations of the EFSA "Outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology" (EFSA Supporting publication 2019: EN-1673), it is necessary to consider whether the formulation is more or less toxic than the parents. When the endpoint of the PPP (expressed in terms of fluopyram) is at least three times lower than the equivalent endpoint for the active substance, it should be considered to be more toxic.

The measured toxicity data (ECx) available for the given endpoint is shown in the table below for the formulated product (PPP) BIX+ FLU+ PTZ EC 260 and the active substances bixafen, fluopyram and prothioconazole.

Is the formulation three times more toxic than fluopyram?

Table 10.2- 20: Comparison of endpoints available for the formulated product (PPP) BIX+FLU+PTZ EC 260 and the active substance fluopyram

Test species	Endpoint and test system	Measured toxicity of PPP [mg prod./L]	Fluopyram [mg a.s./L]	Formulation endpoint recalculated for fluopyram [A] [mg a.s./L]	Fluopyram endpoint / Recalculated formulation endpoint
O. mykiss	LC$_{50}$, acute, 96 h	1.77	> 1.89	not calculated	-
D. magna	EC$_{50}$, acute, 48 h	3.39	> 20	not calculated	-
Pseudokirchneriella subcapitata	E_rC_{50}, short-term, 72 h	2.97	8.9	0.06	142

[A] Amount of fluopyram in the test item used in formulation studies: 6.35 % (please refer to study M-475973-01-1)

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

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

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

MDR calculation

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

Table 10.2- 21: Overview of endpoints available for the formulated product (PPP) BIX+FLU+PTZ EC 260 and the active substances fluopyram, bixafen and prothioconazole

Test species	Endpoint and test system	Measured toxicity of PPP [mg prod./L]	FLU [mg a.s./L]	BIX [A] [mg a.s./L]	PTZ [B] [mg a.s./L]
O. mykiss (PPP / PTZ / BIX) *C. variegatus* (FLU)	LC_{50}, acute, 96 h	1.77	> 0.98	0.095	1.83
D. magna (PPP / PTZ / BIX) *C. virginica* (FLU)	EC_{50}, acute, 48 h	3.39	> 0.44	1.2	1.3

[A] Please refer for endpoints to EFSA Journal 2012;10(11):2917: Peer review of the pesticide risk assessment of the active substance bixafen

[B] Please refer for endpoints to EFSA Scientific Report (2007) 106, 1-98: Conclusion regarding the peer review of the pesticide risk assessment of the active substance prothioconazole

Table 10.2- 22: Summary of results obtained in the studies with the formulated product (PPP) BIX+FLU+PTZ EC 260 and comparison of calculated and measured mixture toxicity

Test species	Endpoint and test system	Measured toxicity of PPP (converted to be a.s. based) ($LC_{50\ PPP}$ or $EC_{50\ PPP}$) [mg total a.s./L]	Calculated mixture toxicity [A] (a.s. in product) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$ [mg total a.s./L]	Model deviation ratio (MDR = $EC_{50\ mix\text{-}CA}$ / $EC_{50\ PPP}$)
Fish	LC_{50}, acute, 96 h	0.227	0.313	1.38
Aquatic invertebrates	EC_{50}, acute, 48 h	0.435	0.863	1.98

[A] The mixture toxicity of the formulation was re-calculated based on the measured contents of fluopyram (64.21 g/L), of bixafen (65.61 g/L) and prothioconazole (128.5 g/L) within the formulation and the product density (1.011 g/cm^3) (please refer to study M-475973-01-1).

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

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

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

Data Point:	KCP 10.2.1/01
Report Author:	[redacted]
Report Year:	2013
Report Title:	Acute toxicity of bixafen + fluopyram + prothioconazole EC 260 (65+65+130 g/L) to the rainbow trout (Oncorhynchus mykiss) under static conditions
Report No:	EBDRR001
Document No:	M-475973-01-1
Guideline(s) followed in study:	FIFRA 72-1 [9], OCSPP Guideline 850.1075, OECD Guideline 203 [4]
Deviations from current test guideline:	Current Guideline: 203 (2019) Deviations: The fish length at test start was not reported. The missing information was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

An acute toxicity test was performed with the Rainbow Trout (*Oncorhynchus mykiss)* in a static system. Juvenile fish were exposed to BIX + FLU + PTZ EC 260 (65+65+130 g/L) in groups of 10 (one replicate of 10 fish per test level) to an aqueous solution of the product at nominal concentrations of 0.313, 0.625, 1.25, 2.50, and 5.00 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 LC-MS/MS on days 0 and 4 for each concentration and the control. Measured concentrations were in the 88 - 102 % range of nominal concentrations and no residues were found in the control samples above the LOQ (1.0 µg a.s./L).

The study fulfils all validity criteria of OECD 203 guideline.

In the control and in the two lowest test concentrations (0.313 and 0.625 mg prod./L), all fish survived until the end of the experiment and no signs of intoxication occurred. All fish in the third highest test concentration (1.25 mg prod./L) showed sublethal effects like loss of equilibrium, laying on bottom, laying on side, remaining at surface, laboured respiration, quiescent behaviour or darkened colouration. In the two highest test concentrations (2.50 and 5.0 mg prod./L) all fish were dead after 24 hours.

The endpoints based on nominal concentrations were: LC_{50} - 96 hours (95 % C.I.): 1.77 mg prod./L (1.25 - 2.50 mg prod./L), LOEC - 96 hours: 1.25 mg prod./L and NOEC - 96 hours (based on sublethal effects): 0.625 mg prod./L.

I. MATERIALS AND METHODS

Test material	BIX + FLU + PTZ EC 260 (65+65+130 g/L) Specification No.: 102000027828 Batch No: 2013-002135 Content of a.s.: 6.35 % w/w fluopyram 6.49 % w/w bixafen 12.7 % w/w prothioconazole Density: 1.011 g/mL
Guideline(s) adaptation	None specified
Test species	Rainbow Trout (*Oncorhynchus mykiss*)
Acclimation	At least 14 days to test conditions. Health during acclimation: no mortalities for 7 days prior to testing, no treatments for disease.
Organism age/size	Mean length: 57.6 ± 2.5 mm at the start of the study Mean body weight: 1.57 ± 0.29 g at the start of the study
Test solutions	Nominal concentrations: 0.313 - 0.625 - 1.25 - 2.50 - 5.00 mg prod./L Mean measured recoveries based on a.s. content for fluopyram ranged from 88 and 102 % of nominal concentrations Control: water Evidence of undissolved material: No precipitates were observed during the exposure period.
Replication	No. of vessels per concentration (replicates): 1 No. of vessels per control (replicates): 1
Organisms per replicate	No. of organisms per vessel: 10
Exposure	Static Total exposure duration: 96 hours
Test Vessel Loading	0.52 g fish/L test medium
Feeding during test	None
Test conditions	Temperature: 11.6 - 11.9 °C Photoperiod: 16 hours light / 8 hours dark; with 30 minute transition periods Light intensity: 582 - 864 lux pH: 7.2 - 7.9 Water hardness: 42 - 48 mg $CaCO_3$/L Dissolved oxygen: 66 - 103 % of saturation Conductivity: 133 - 150 µmhos/cm Alkalinity: 36 - 40 mg/L
Parameters Measured / Observations	Fish were observed for mortalities and sub-lethal behavioural effects after 4, 24, 48, 72 and 96 hours. Measurements of dissolved oxygen and pH were obtained at test initiation and once daily in all test levels with surviving fish. Alkalinity, hardness, and conductivity were determined on day 0 and 4. Temperature was measured continuously throughout the exposure.

Sampling for Chemical analysis	Samples of test solutions were taken at test initiation (0 hour) and at test termination (96 hours) for analysis of the active substance fluopyram. The chemical analyses were performed by Liquid Chromatograph/ Tandem Mass Spectrometry system (LC-MS/MS).
Data analysis	The 96-hour LC_{50} value and the 95% confidence intervals were calculated by a LC_{50} computer program developed by Stephan et al. using binomial probability, moving average or probit statistical methods. Based on the pattern of the data across the range of concentrations tested, the slope of the dose response curve could not be calculated. The NOEC, NOLEC and LOEC were empirically determined based on mortalities and sublethal effects.

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 %	66 - 103 %

Analytical results:

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

Recoveries for fluopyram on day 0 and day 4 were between 88 and 102 % of nominal a.s. concentrations (see table below). Biological results are based on nominal product concentrations of BIX + FLU + PTZ EC 260 (65+65+130 g/L).

No residues of fluopyram were found in the control samples above the limit of quantification (LOQ: 1.0 µg a.s./L).

Table 10.2.1- 2: Analytical results for fluopyram

Nominal concentration		Measured concentration [µg a.s./L]		% of nominal		Mean measured concentration [µg a.s./L]	Mean % of nominal
[mg prod./L]	[µg a.s./L] [A]	Day 0	Day 4	Day 0	Day 4		
0.313	19.9	18.6	17.6	93	88	18.1	91
0.625	39.7	40.2	38.0	101	96	39.1	98
1.25	79.4	79.0	79.1	99	100	79.0	100
2.50	159	162	159	102	100	160	101
5.00	318	321	320	101	101	321	101

[A] Considering a purity of 6.35 % of the active substance.

Biological results:

Observations:

In the control and in the two lowest test concentrations (0.313 and 0.625 mg prod./L), all fish survived until the end of the experiment and no signs of intoxication occurred. All fish in the third highest test concentration (1.25 mg prod./L) showed sublethal effects like loss of equilibrium, laying on bottom, laying on side, remaining at surface, laboured respiration, quiescent behaviour or darkened coloration. In the two highest test concentrations (2.50 and 5.0 mg prod./L) all fish were dead after 24 hours.

Table 10.2.1- 3: Mortality

Nominal concentration [mg prod./L]	Dead fish No. (%) Exposure time				
	4 h	24 h	48 h	72 h	96 h
Control	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.313	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
0.625	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
1.25	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2.50	0 (0)	10 (100)	-	-	-
5.00	1 (10)	10 (100)	-	-	-

- No observations, all fish dead.

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.):	1.77 mg prod./L (1.25 - 2.50 mg prod./L)
NOEC - 96 hours: highest concentration without an effect (based on mortality)	1.25 mg prod./L
NOEC - 96 hours: highest concentration without an effect (based on sublethal effects)	0.625 mg prod./L
LOEC - 96 hours: lowest concentration with an effect	1.25 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} = 1.77 mg prod./L

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Data Point:	KCP 10.2.1/02
Report Author:	[redacted]
Report Year:	2013
Report Title:	Acute toxicity of Bixafen + Fluopyram + Prothioconazole EC 260 (65+65+130 g/L) Daphnia magna under static conditions
Report No:	M-476467-01-1
Document No:	M-476467-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 FIFRA 72-2 (1982) OCSPP Guideline 850.1010 (1996) OECD Guideline 202 (2004)
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 BIX+FLU+PTZ EC 260 (65+65+130 g/L) in groups of 20 (4 replicates of 5 organisms per test level) to the nominal concentrations of 0.177, 0.389, 0.855, 1.88, 4.13, 9.09, and 20.0 mg prod./L Additionally, a control was included. Immobilisation and sub-lethal behavioural effects were determined after 4, 24 and 48 hours.

Concentrations of fluopyram were verified by LC-MS/MS on day 0 and 2. Measured concentrations were in the 96 - 110 % range of nominal concentrations and no residues were found in the control samples above the LOQ (1.0 µg a.s./L).

The study fulfils all validity criteria of OECD 202 guideline.

All control daphnids were in good health. Daphnids in the lowest test concentration (0.177 mg prod./L) were normal. Sublethal effects and/or immobilisation were observed in all other test levels.

The endpoints based on nominal concentrations were: EC_{50} – 48 hours (95 % C.I.): 3.39 mg prod./L (1.88 - 9.09 mg prod./L), LOEC: 0.389 mg prod./L and NOEC: 0.177 mg prod./L.

I. MATERIAL AND METHODS

Test material	BIX+FLU+PTZ EC 260 (65+65+130 g/L) Specification No.: 102000027828-01 Batch No.: 2013-002135 Content of a.s.: 6.35 % w/w fluopyram (64.21 g/L) 6.49 % w/w bixafen (65.61 g/L) 12.7 % w/w prothioconazole (128.5 g/L) Density: 1.011 g/mL
Guideline(s) adaptation	None specified
Test species	Water flea (*Daphnia magna*)

Organism age/size at study initiation	First instar neonates, less than 24 hours old
Test solutions	Nominal concentrations: 0.177 – 0.389 – 0.855 – 1.88 – 4.13 – 9.09 – 20.0 mg prod./L Corresponding mean measured concentrations: not relevant Control: water Evidence of undissolved material: No precipitate was observed during exposure.
Replication	No. of vessels per concentration (replicates): 4 No. of vessels per control (replicates): 4
Organisms per replicate	No. of organisms per vessel: 5
Exposure	Static Total exposure duration: 48 hours
Feeding during test	None
Test conditions	Temperature: 19.6 - 19.8 °C Photoperiod: 16 hours light, 8 hours dark; 30min. transition periods Light intensity: 581 - 662 lux pH: 8.3 - 8.5 Water hardness: 164 - 174 mg/L as $CaCO_3$ Dissolved oxygen: 8.0 - 8.5 mg/L (88 - 94 % of saturation) Conductivity: 400 - 430 µS/cm Alkalinity: 133 - 136 $CaCO_3$/L
Parameters Measured / Observations	Observations for immobility and sub-lethal behavioural effects were made after 4, 24 and 48 hours of exposure. The dissolved oxygen, conductivity, hardness, alkalinity and pH values were measured at test start solutions and at test end. Additionally, water temperatures were recorded continuously throughout the exposure. The light intensity was measured at test start as close as possible to the surface of the test solution.
Chemical analysis	Samples were taken at test start from freshly prepared batch solutions and at test end from composite solutions from all replicate samples. The chemical analyses were performed by Liquid Chromatograph/ Tandem Mass Spectrometry system (LC-MS/MS).
Data analysis	The 24 and 48-hour EC_{50} value was calculated by a LC_{50} computer program developed by Stephan et al. using binomial probability, moving average or probit statistical methods. The NOEC and LOEC were empirically determined based upon observation data including lethal and sublethal effects.

II. Results and Discussion

Table 10.2.1-4: Validity criteria

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

Analytical results:

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

Recoveries on day 0 and 2 ranged from 96 to 110 % of nominal values. The biological results are based on nominal product concentrations.

Given that the toxicity cannot be attributed to any one of the a.s. compounds but to the formulation as a whole, all results were based on nominal concentrations of BIX+FLU+PTZ EC 260 (65+65+130 g/L).

The other active ingredients prothioconazole and bixafen were not analysed since it is present in the added formulation in a fixed ratio to the analysed active ingredient.

No residues of fluopyram were detected in the control samples above the limit of quantification (LOQ: 1.0 µg a.s./L).

Table 10.2.1- 5: Analytical results for fluopyram

Nominal concentration		Measured concentration [µg a.s./L]		% of nominal	
[mg prod./L]	[µg a.s./L]	Day 0 (New)	Day 2 (Aged)	Day 0 (New)	Day 2 (Aged)
0.177	11.2	10.8	10.8	96	96
0.389	24.7	24.9	24.6	101	100
0.855	54.3	54.2	55.0	100	101
1.88	119	120	119	101	100
4.13	262	269	269	103	103
9.09	577	624	613	108	106
20.0	1270	1399	1385	110	109

Biological results:

Observations

All control daphnids were in good health. Daphnids in the lowest test concentration (0.177 mg prod./L) were normal. Sublethal effects and/or immobilisation were observed in all other test concentrations.

Table 10.2.1- 6: Immobilisation of daphnids

Nominal concentration [mg prod./L]	No. of immobilized (cumulative %)		
	Exposure time		
	4h	24 h	48 h
Control	0 (0)	0 (0)	0 (0)
0.177	0 (0)	0 (0)	0 (0)
0.389	0 (0)	0 (0)	2 (10)
0.855	0 (0)	0 (0)	1 (5)
1.88	0 (0)	0 (0)	2 (10)
4.13	0 (0)	0 (0)	13 (65)
9.09	1 (5)	5 (25)	20 (100)
20.0	0 (0)	10 (50)	20 (100)

III. Conclusion

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

EC_{50} – 48 hours (95 % C.I.):	3.39 mg prod./L (1.88 - 9.09 mg prod./L)
EC_{50} – 24 hours (95 % C.I.):	18.4 mg prod./L (13.6 – 33.9 mg prod./L)
LOEC – 48 hours: lowest concentration with an effect	0.389 mg prod./L
NOEC – 48 hours: highest concentration without an effect	0.177 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} = 3.39 mg prod./L

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Data Point:	KCP 10.2.1/03
Report Author:	[redacted]
Report Year:	2013
Report Title:	Toxicity of bixafen + fluopyram + prothioconazole EC 260 (65+65+130 g/L) to the green algae Pseudokirchneriella subcapitata during a 72 hour exposure
Report No:	EBDRR003
Document No:	M-475997-01-1
Guideline(s) followed in study:	FIFRA Guideline 123-2 (1982) [9], OCSPP Guideline 850.4500 (2012) [13], OECD Guideline 201 (2006) [6]
Deviations from current test guideline:	Current Guideline: OECD 201 (2006) Deviations: The pH increase in the control was 1.6 units and thus higher than the maximum 1.5 units as recommended in OECD 201. This deviation was not expected to have impacted the study results. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The green alga *Pseudokirchneriella subcapitata* were exposed to BIX+FLU+PTZ EC 260 (65+65+130 g/L) under static conditions for 72 hours. Algal cultures with an initial nominal cell count of approximately 1.0 × 10^4 cells/mL were used to test the nominal concentrations of 0.375, 0.750, 1.50, 3.00, and 6.00 mg prod./L. The study design included 3 replicates for each test concentration and 3 replicates for the control. At 24 hour-intervals, the cell density (cells/mL) of each culture was counted.

Concentrations of fluopyram were verified by LC–MS/MS on day 0 and 3 for each concentration and control. Measured concentrations were in the 96 - 106 % range of nominal concentrations and no residues were found in the control samples above the LOQ (1.0 µg a.s./L). Therefore, and since the toxicity has to be attributed to the tested formulation as a whole, all biological results were related to nominal concentrations of the formulation BIX+FLU+PTZ EC 260 (65+65+130 g/L).

The study fulfils all validity criteria of OECD 201 guideline.

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

The 72 hour- endpoints based on nominal product concentrations were: 72 hour – E_rC_{50}: 2.97 mg prod./L (2.91 - 3.02 mg prod./L); 72 hour – E_bC_{50}: 1.91 mg prod./L (1.77 - 2.00 mg prod./L) and 72 hour – E_yC_{50}: 1.80 mg prod./L (1.67 - 1.89 mg prod./L).

I. MATERIAL AND METHODS

Test material	BIX + FLU + PTZ EC 260 (65+65+130 g/L) Specification No.: 102000027828-01 Batch ID: 2013-002135 Content of a.s.: 6.35 % w/w fluopyram 6.49 % w/w bixafen 12.7 % w/w prothioconazole Density: 1.011 g/mL
Guidelines adaptation	Not specified.
Test species	Freshwater Green alga *Pseudokirchneriella subcapitata*

Culturing conditions	In-house 4-day old pre-culture held under test conditions.
Test solutions	Nominal product concentrations: 0.375 – 0.750 – 1.50 – 3.00 and 6.00 mg prod./L Corresponding mean measured concentrations: not relevant Control: untreated medium Evidence of undissolved material: No precipitates were observed during exposure.
Replication	No. of vessels per concentration (replicates): 3 No. of vessels per control (replicates): 3
Exposure	Static Total exposure duration: 72 hours
Initial cell density	1×10^4 cells/mL in each test group
Test conditions	Temperature: 22.6 - 23.8 °C Photoperiod: 24 hours light Light intensity: 5580 - 5910 lux Type of light: bank light containing cool white fluorescent lamps pH of control: 7.6 - 9.2 Conductivity: 84.5 - 94.8 µmhos/cm Growth medium same as culture medium: Yes
Parameters Measured / Observations	pH- and conductivity values were measured at test start and at test end (72 hours). Temperature was determined by a continuous measurement in one additional incubated glass vessel. Cell density measurements were daily done manual counts via light microscope and hemocytometer slide. Cellular observations were done with visual inspection via light microscope.
Sampling for chemical analysis	Samples of test solutions were taken at test initiation (0 hour) from batch preparation for each level. At test termination (72 hours) samples were collected from composite samples of all replicates for each test concentration. Samples were analysed by using a Liquid Chromatograph/ Tandem Mass Spectrometry system (LC) – MS/MS.
Data analysis	Raw or transformed data from treatment groups were compared to controls for normality and homogeneity of variance using the Shapiro-Wilks test and Bartlett Equality of Variances, respectively. If normality and homogeneity of variance were demonstrated for the raw or transformed values, then parametric analyses were conducted using analysis of variance (ANOVA) followed by Dunnett's test. If normality and/or homogeneity of variance were not demonstrated on raw or transformed values, nonparametric procedures were used. The $EC_{10, 20, 50}$ values, and the respective 95 % confidence intervals, were calculated using linear interpolation for cell yield, cumulative biomass, and growth rate. Statistical evaluations were done with the software CETIS (Tidepool Scientific Software, version 1.8.7.4).

II. RESULTS AND DISCUSSION

Table 10.2.1- 7: 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	84
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 %	17 %
The coefficient of variation of average specific growth rates during the 72-hour test period in replicate control cultures must not exceed 7 %.	7 %	0.4 %

Analytical results:

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

Recoveries for fluopyram on day 0 and day 3 were between 96 and 106 % of nominal concentrations (see table below). Therefore, and since the toxicity has to be attributed to the tested formulation as a whole, all calculated results were related to nominal concentrations of the formulation BIX+FLU+PTZ EC 260 (65+65+130 g/L).

No residues of fluopyram were detected in the control samples above the limit of quantification (LOQ: 1.0 µg a.s./L).

Table 10.2.1- 8: Analytical results

Nominal concentration		Measured a.s. concentration [µg a.s./L]		% of nominal	
[mg prod./L]	[µg a.s./L]	Day 0	Day 3	Day 0	Day 3
0.375	23.8	23.7	23.0	99	97
0.750	47.6	47.5	45.6	100	96
1.50	95.3	94.5	94.7	99	99
3.00	191	193	200	101	105
6.00	381	381	405	100	106

Biological results:

Observations:

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

Table 10.2.1- 9: Algae growth rate

Nominal concentration [mg prod./L]	Mean growth rate [A] [1/h]	% Inhibition [B]
	0 - 72 h	0 - 72 h
Control	0.061647	
0.375	0.062324	-1.1
0.750	0.062632	-1.6
1.50	0.055865	9.4 *
3.00	0.030671	50.2 *
6.00	0.002564	95.8 *

A Growth rate [1/h] is calculated from the cell density data.
B % Inhibition=100-((Treatment group parameter mean/control parameter mean)*100).
* * Statistically significant from control (Dunnett's multiple comparison test; $p \leq 0.05$).

Table 10.2.1- 10: Biomass

Nominal concentration [mg prod./L]	Cumulative biomass [A]	% Inhibition of cumulative biomass [B]
	0 - 72 h	0 - 72 h
Control	1544.2	-
0.375	1637.3	-6.0
0.750	1613.8	-4.5
1.50	1067.2	30.9 *
3.00	229.2	85.2 *
6.00	23.3	98.5 *

A Cumulative biomass is equal to the area under the growth curve.
B % Inhibition=100-((Treatment group parameter mean/control parameter mean)*100).
* Statistically significant from control (Dunnett's multiple comparison test; $p \leq 0.05$).

Table 10.2.1- 11: Yield

Nominal concentration [mg prod./L]	Mean yield [A] [x 10^4 cells/mL]			% Inhibition of yield
	0 - 24 h	0 - 48 h	0 - 72 h	0 - 72 h
Control	2.84	19.67	83.67	-
0.375	3.22	21.00	88.00	-5.2
0.750	3.38	18.78	90.17	-7.8
1.50	2.61	14.44	54.83	34.5 *
3.00	1.59	3.91	8.10	90.3 *
6.00	0.32	0.55	0.20	99.8 *

[A] Yield is determined from cell densities collected approximately every 24 hours throughout the duration of the test.
* Statistically significant from control (Dunnett's multiple comparison test; $p \leq 0.05$).

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.):	**2.97 mg prod./L** (2.91 - 3.02 mg prod./L)
E_rC_{20} -72 hours (95 % C.I.):	1.80 mg prod./L (1.75 - 1.84 mg prod./L)
E_rC_{10} -72 hours (95 % C.I.):	1.48 mg prod./L (1.34 - 1.55 mg prod./L)
E_bC_{50} -72 hours (95 % C.I.):	**1.91 mg prod./L** (1.77 - 2.00 mg prod./L)
E_bC_{20} -72 hours (95 % C.I.):	1.17 mg prod./L (0.984 - 1.26 mg prod./L)
E_bC_{10} -72 hours (95 % C.I.):	0.948 mg prod./L (0.719 - 0.982 mg prod./L)
E_yC_{50} - 72 hours (95 % C.I.):	**1.80 mg prod./L** (1.67 - 1.89 mg prod./L)
E_yC_{20} -72 hours (95 % C.I.):	1.12 mg prod./L (1.04 - 1.18 prod./L)
E_yC_{10} -72 hours (95 % C.I.):	0.926 prod./L (0.833 - 0.951 prod./L)
LOEC - 72 hours: lowest concentration with an effect (based on growth rate, yield and biomass)	1.50 mg prod./L
NOEC - 72 hours: highest concentration without an effect (based on growth rate, yield and biomass)	0.750 mg prod./L

Reliability assessment (EFSA 2015)

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

Biological endpoints	EC_{10} [mg a.s./L]	95% CL	NW	Relationship $EC_{10}/EC_{20/50}$
Growth Rate	1.48	1.34 – 1.55	0.142 (excellent)	$EC_{10} < EC_{20}$, low (high)
Yield	0.926	0.833 – 0.951	0.127 (excellent)	$EC_{10} < EC_{20}$, low (high)
Biomass	0.948	0.719 – 0.982	0.277 (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} = 2.97 mg prod./L

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

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

CP 10.2.3 Further testing on aquatic organisms

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

CP 10.3 Effects on arthropods

CP 10.3.1 Effects on bees

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

Where bees are likely to be exposed, Commission Regulations (EU) 283/2013 and 284/2013 require testing of both acute (oral and contact) and chronic toxicity, including sub-lethal effects. As there are no current testing requirements for any bee other than for the honey bee within Regulation EU 1107/2009, and as the target crop (cereals) is non-nectariferous and considered to be unattractive to bees for pollen collection, the risk assessment for honey bees is considered to be protective of bees in general and no additional studies on bumble bees have been carried out.

In addition to the standard toxicity studies performed with adult honey bees (OECD 213 and 214) the following studies are also provided:

- Chronic 10-day toxicity test with the representative formulation BIX + FLU + PTZ EC 260 on adult honeybees under laboratory conditions (OECD 245; M-688335-01-1)
- Toxicity to honeybee larvae under laboratory conditions following repeated exposure (OECD guidance document 239; M-704601-01-1)

Table 10.3.1- 1: Ecotoxicological endpoints relevant for the risk assessment for bees for BIX + FLU + PTZ EC 260

Test substance	Test species/ study type	Endpoint		References
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
BIX+ FLU+ PTZ EC 260	*Apis mellifera*, acute test	LD_{50} oral (48 h) LD_{50} contact (48 h)	**> 312 µg prod./bee** **> 200 µg prod./bee**	[redacted] (2013) M-469774-01-1 KCP 10.3.1.1.1/01 KCP 10.3.1.1.2/01
	Apis mellifera, 10-day oral feeding test	LDD_{50} NOEDD LC_{50} NOEC	> 50.1 µg prod./bee/day 20.7 µg prod./bee/day > 2538 mg prod./kg diet 634 mg prod./kg diet	[redacted] (2020) M-688355-01-1 KCP 10.3.1.2/01
	Apis mellifera, larva 22-day repeated feeding test	NOEC NOED EC_{10} ED_{10} EC_{20} ED_{20} EC_{50} ED_{50}	417 mg prod./kg diet 66.6 µg prod./larva 548 mg prod./kg diet 86.6 µg prod./larva 650 mg prod./kg diet 102.7 µg prod./larva 842 mg prod./kg diet 133.1 µg prod./larva	[redacted] (2020) M-704601-01-1 KCP 10.3.1.3/01

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

Risk assessment for bees

The risk assessment for bees for fluopyram is based on the one-time foliar application of the formulated product BIX+ FLU+ PTZ EC 260 to barley at rates of 1.2 L product/ha (corresponding to an application rate for fluopyram of 78 g a.s./ha), and 0.6 L product/ha (corresponding to an application rate for fluopyram of 39 g a.s./ha). The acute toxicity endpoints (LD_{50} values) for both the active substance fluopyram and the formulation BIX+ FLU+ PTZ EC 260 are used as part of this risk assessment.

Hazard Quotients

The risk assessment is based on Hazard Quotient approach (Q_H) by calculating the ratio between the application rate (expressed in g a.s. or product per ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s. or product per bee).

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

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

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

Table 10.3.1- 2: Hazard quotients for bees for application in barley, 1 x 78 g a.s./ha – oral exposure

Compound	Oral LD_{50} [µg/bee]	Max. application rate [g/ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Fluopyram tech.	> 102.3	78	< 0.76	50	yes
BIX+ FLU+ PTZ EC 260	> 312	1213.2 [A]	< 3.89	50	yes

[A] Based on an application rate of 1200 mL prod./ha and a product density of 1.011 g/mL

Table 10.3.1- 3: Hazard quotients for bees for application in barley, 1 x 39 g a.s./ha – oral exposure

Compound	Oral LD_{50} [µ/bee]	Max. application rate [g/ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
Fluopyram tech.	> 102.3	39	< 0.38	50	yes
BIX+ FLU+ PTZ EC 260	> 312	606.6 [A]	< 1.94	50	yes

[A] Based on an application rate of 600 mL prod./ha and a product density of 1.011 g/mL

The hazard quotients for oral exposure for both single application rates at 78 and 39 g a.s./ha corresponding to 1213.2 and 606.6 g product/ha, respectively, are below the validated trigger value for higher tier testing (i.e. $Q_{HO} < 50$).

Table 10.3.1- 4: Hazard quotients for bees for application in barley, 1 x 78 g a.s./ha – contact exposure

Compound	Contact LD_{50} [µg/bee]	Max. application rate [g/ha]	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
Fluopyram tech.	> 100	78	< 0.78	50	yes
BIX+ FLU+ PTZ EC 260	> 200	1213.2 [A]	< 6.07	50	yes

[A] Based on an application rate of 1200 mL prod./ha and a product density of 1.011 g/mL

Table 10.3.1- 5: Hazard quotients for bees for application in barley, 1 x 39 g a.s./ha – contact exposure

Compound	Contact LD_{50} [µg/bee]	Max. application rate [g/ha]	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
Fluopyram tech.	> 100	39	< 0.39	50	yes
BIX+ FLU+ PTZ EC 260	> 200	606.6 [A]	< 3.03	50	yes

[A] Based on an application rate of 600 mL prod./ha and a product density of 1.011 g/mL

The hazard quotients for contact exposure for both single application rates at 78 and 39 g a.s./ha, corresponding to 1213.2 and 606.6 g product/ha, respectively, are below the validated trigger value for higher tier testing (i.e. $Q_{HO} < 50$).

Further considerations regarding the risk to bees

Both the active substance fluopyram and the formulated product BIX+FLU+PTZ EC 260 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 BIX+FLU+PTZ EC 260 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 barley for both the active substance and the formulated product are considerably lower than the levels regarded to indicate a risk to bees. As per the GAP, a maximum of one foliar spray application of the formulated product is intended in cereals between BBCH 30 and 61. Cereals are non-nectariferous and generally considered unattractive to bees for pollen collection, thus rendering exposure to the formulated product unlikely.

Although the probability of chronic exposure to the formulated product for either honey bee adults or larvae is considered to be low, the applicant recognizes the new requirements for chronic effects data as stipulated by Commission Regulation (EU) No. 284/2013. As such, a chronic oral toxicity test (10-day feeding) as per OECD Guideline No. 245 as well as a chronic larvae laboratory study (repeated exposure) as per OECD Guidance Document No. 239 with the formulated product BIX+FLU+PTZ EC 260 were carried out. These studies will address potential chronic toxicity to honey bees and effects on honey bee development and other honey bee life stages, respectively, in accordance with the data requirements as set out in Commission Regulation (EU) No. 284/2013.

Chronic adult toxicity

A 10-day laboratory feeding study investigating the effects of BIX+FLU+PTZ EC 260 was conducted to assess chronic toxicity to honey bees in accordance with OECD Guideline No. 245.

The study concluded that continuous *ad libitum* feeding at 2538 mg product/kg diet over a period of 10 days led to 40 % mortality. The LDD_{50} was determined as > 50.1 µg product/bee/day. The NOEDD was identified at 20.7 µg product/bee/day. Daily dosing with 20.7 µg product/bee/day of over 10 days (total dose = 207 µg product/bee) thus did not induce higher mortality compared to a single acute oral exposure at 104.4 µg product/bee, which represents the acute oral NOED as identified by KCP 10.3.1.1.1/01, M-469774-01-1. Therefore, study results do not indicate delayed or cumulative toxicity effects following chronic exposure to BIX+FLU+PTZ EC 260 compared with acute testing. Details of the study are presented in KCP 10.3.1.2/01, M-688355-01-1.

Chronic larval toxicity/effects on brood

A honey bee larval toxicity test assessing the effect of BIX+FLU+PTZ EC 260 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 108, 237, 522, 1147 and 2522 mg product/kg diet (corresponding to actual concentrations of 88, 199, 417, 937, and 1993 mg product/kg diet). The corresponding nominal cumulative doses were 17.0, 37.5, 82.6, 182 and 399 µg product/larva (with actual consumed doses of 13.9, 31.5, 66.0, 148 and 315 µg product/larva). The 22-day NOED (emergence) was identified at 66.0 µg product/larva (with corresponding NOEC of 417 mg product/kg diet), indicating no risk to honey bee development. Details of the study are presented in KCP 10.3.1.3/01, M-704601-01-1.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

Data Point:	KCP 10.3.1.1.1/01
Report Author:	[redacted]
Report Year:	2013
Report Title:	Effects of bixafen + fluopyram + prothioconazole EC 260 (65+65+130) G (Acute Contact and Oral) on Honey Bees (Apis mellifera L.) in the Laboratory
Report No:	81781035
Document No:	M-469774-01-1
Guideline(s) followed in study:	OECD 213 and 214, 1998
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 213: No deviations to the current OECD Guideline 213 occurred. All validity criteria were met. 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. 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 BIX + FLU + PTZ EC 260 (65+65+130 g/L) to the honey bee (*Apis mellifera* L.). Mortality of bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

Under laboratory conditions, 50 worker bees were exposed for 48 hours to a single dose of 200.0 µg product/bee by topical application (contact limit test) and 30 worker bees were exposed for 48 hours to doses of 312.0, 165.7, 104.5, 53.9 and 26.6 µg product/bee by feeding (oral dose response test, values based on the actual intake of the test item).

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

The contact LD_{50} value (48 h) was determined to be > 200.0 µg product/bee. The oral LD_{50} value was determined to be >312.0 µg product/bee.

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

I. MATERIAL AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); Specification number: 102000027828 – 01; Batch identification: 2013-002135, Sample description: TOX10113-00; analytical value: bixafen 6.49 % w/w, 65.61 g/L, fluopyram 6.35 % w/w, 64.21 g/L, prothioconazole 12.7 % w/w, 128.5 g/L; density: 1.011 g/mL;

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 product/bee by topical application (contact limit test) and 30 worker bees were exposed for 48 hours to doses of 312.0, 165.7, 104.5, 53.9 and 26.6 µg product/bee by feeding (oral dose response test, value based on the actual intake of the test item).

The control used for the contact test was tap water with 0.5 % Adhäsit (improves spreading of the test droplet on the water-repellent hairs on the thorax of bees) (water control group). In the oral test, bees in the control group were exposed to 50 % w/w aqueous sugar solution. The toxic reference dimethoate (Perfekthion EC 400, 400.0 g/L nominal, 411.7 g/L analytical) was applied at nominal dose levels of 0.30, 0.20, 0.15 and 0.10 µg dimethoate/bee in the contact test and 0.30, 0.15, 0.08 and 0.05 µg in the oral test.

In the contact toxicity test each treatment group (test item, controls and reference item) comprised 5 replicates including 10 bees each. In the oral toxicity test each treatment group (test item, controls and reference item) comprised 3 replicates including 10 bees each.

Application in the contact test: A single 5 µL droplet of the control, test item and toxic standard (vehicle: 0.5 % Adhäsit) was placed on the dorsal bee thorax using a Burkard – Applicator, following anaesthetization of the bees with CO_2. Based on practical experience, a 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 uL droplet, since a higher volume ensured a more reliable dispersion of the test item.

Application in the oral test: The test item and reference item were applied in 50 % w/w sugar syrup (30 % Saccharose, 31 % Glucose, 39 % Fructose). Untreated sugar solution was offered to bees in the control group. The treated food was offered in syringes, which were weighed before and after introduction into the cages (duration of feeding between 65 minutes and 6 hours). After a maximum of 6 hours, the syringes containing the treated food were removed, weighed and replaced by ones containing fresh, untreated food (50 % w/w sugar solution).

Dose levels:

Nominal doses of the test item: 200.0 µg product/bee (contact limit test)

400.0, 200.0, 100.0, 50.0 and 25.0 µg product/bee (oral does response test)

Actual dose of the test item (oral test): 312.0, 165.7, 104.5, 53.9 and 26.6 µg product/bee

Nominal doses of the reference item: 0.30, 0.20, 0.15 and 0.10 µg dimethoate per bee (contact test)

0.30, 0.15, 0.08 and 0.05 µg dimethoate per bee (oral test)

Actual doses of the reference item (oral test): 0.32, 0.16, 0.08 and 0.06 µg dimethoate/bee

Test conditions: Temperature: 24 - 25 °C; relative humidity: 52 - 82 %; photoperiod: 24 h darkness (except during observations).

Statistics: Results obtained with the bees treated with the test item and the reference item were compared to those obtained with the control in both the contact and oral tests. The oral $LD_{50, 20, 10}$ values of the test item were estimated with Weibull Analysis. The contact and oral LD_{50} values of the reference item were estimated using the binomial distribution (according to STEPHAN, 1977). It was not necessary to correct

the test item and the reference item mortality, since no control mortality occurred in either the contact or oral toxicity tests, respectively. The NOED of the test item was estimated using Fisher Exact Test (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 2.10.05 (® ToxRat Solutions GmbH).

Dates of experimental work: June 3rd to August 7th , 2013

II. RESULTS AND DISCUSSION

Biological findings:

Contact test

At the end of the contact toxicity test (48 hours after application) 4.0 % mortality occurred at 200.0 µg product/bee. There was no mortality in the control group (water + 0.5 % Adhäsit).

During the first assessment (four hours after application) 58 % of the bees were behaving abnormal (moving coordination problems and apathy). After 24 and 48 hours of the application one bee was apathetic.

Since 4.0 % mortality occurred in the 200.0 µg product/bee group, the contact LD_{50} can be considered as > 200.0 µg product/bee.

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

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg product/bee]						
200.0	0.0	58.0	2.0	2.0	4.0	2.0
Reference item [µg a.s./bee]						
0.10	2.0	0.0	8.0	0.0	12.0	2.0
0.15	4.0	0.0	10.0	12.0	40.0	4.0
0.20	2.0	42.0	52.0	2.0	60.0	0.0
0.30	4.0	72.0	74.0	12.0	84.0	10.0

Results are mean values of 5 replicates (control, test item and reference item) containing 10 bees each
Behav. abnorm. = behavioural abnormalities
Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L) , reference item: dimethoate, water control = tap water

Oral test

The maximum nominal dose levels of the test item (400.0 and 200.0 µg product/bee) could not be achieved because the bees did not ingest the full volume of treated sugar solution even when offered over a period of six hours. Actual oral doses of 312.0, 165.7, 104.5 and 26.6 µg product/bee resulted in mortality levels of 30.0, 26.7, 3.3 and 3.3 %, respectively (48 hours after application).

No mortality occurred in the 53.9 µg product/bee treatment group as well as in the control group (50 % aqueous sugar solution).

During the first assessment (four hours after application) moving coordination problems and/or apathy occurred in 40.0, 63.3, 40.0 and 13.3 % of the bees in the 312.0, 165.7, 104.5 and 53.9 µg product/bee

treatment groups, respectively. During the 24 and 48 hours assessments, behavioural abnormalities were only observed in the 312.0 µg product/bee treatment group.

Since 30.0 % mortality occurred in the 312.0 µg product/bee group, the oral LD_{50} can be considered as > 312.0 µg product/bee.

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

Treatment group	After 4 h		After 24 h		After 48 h	
	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.	Mortality	Behav. abnorm.
	Mean [%]		Mean [%]		Mean [%]	
Control	0.0	0.0	0.0	0.0	0.0	0.0
Test item [µg product/bee]						
26.6	0.0	0.0	0.0	0.0	3.3	0.0
53.9	0.0	13.3	0.0	0.0	0.0	0.0
104.5	0.0	40.0	3.3	0.0	3.3	0.0
165.7	0.0	63.3	26.7	0.0	26.7	0.0
312.0	6.7	40.0	20.0	23.3	30.0	6.7
Reference item [µg a.s./bee]						
0.06	0.0	0.0	6.7	0.0	10.0	0.0
0.08	0.0	13.3	10.0	3.3	26.7	3.3
0.16	0.0	36.7	46.7	16.7	66.7	0.0
0.32	6.7	60.0	76.7	13.3	83.3	3.3

Results are mean values of 3 replicates (control, test item and reference item) containing 10 bees each
Behav. abnorm. = behavioural abnormalities;
Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L), reference item: dimethoate, control = 50 % w/w sugar solution

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

Table 10.3.1.1.1- 3: Contact and oral toxicity of BIX + FLU + PTZ EC 260 (65+65+130 g/L) to honey bees

Test item	BIX + FLU + PTZ EC 260 (65+65+130 g/L)	
Test species	Honey bee *Apis mellifera* L.	
Exposure	**Contact** (solution in Adhäsit (0.5 %)/ water)	**Oral** (in 50% w/w aqueous sugar solution)
Test duration	48 h	48 h
Dose rate [µg product/bee]	200.0	Nominal dose: 400.0 – 200.0 – 100.0 – 50.0 – 25.0 Actual dose: 312.0 – 165.7 – 104.5 – 53.9 – 26.6
LD_{50} [µg product/bee]	> 200.0	> 312.0
LD_{20} [µg product/bee]	> 200.0	200.2
LD_{10} [µg product/bee]	> 200.0	116.6
NOED [µg product/bee]	≥ 200.0	104.5

Reference item

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.20 and 0.17 µg a.s./bee, respectively.

Validity criteria:

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

Table 10.3.1.1.1- 4: Validity criteria

Validity Criteria	**Recommended**		**Obtained**
Control mortality	Contact Test		
	Water control	≤ 10 %	0 %
	Oral Test		
	Control	≤ 10 %	0 %
LD_{50} of reference item (24 h)	Contact Test		
	Dimethoate	0.10 - 0.30 µg a.s./bee	0.20 µg a.s./bee
	Oral Test		
	Dimethoate	0.10 - 0.35 µg a.s./bee	0.17 µg a.s./bee

III. CONCLUSION

The toxicity of BIX + FLU + PTZ EC 260 (65+65+130 g/L) was tested in both, an acute contact limit test and an acute oral toxicity dose response test on honey bees.

The contact LD_{50} value (24 and 48 h) was > 200.0 µg product/bee, respectively. The oral LD_{50} value (24 and 48 h) was > 312.0 µg product/bee.

Assessment and conclusion by applicant:

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

The endpoints are:

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

LD_{50} oral (48 hours) > 312.0 µg product/bee

CP 10.3.1.1.2 Acute contact toxicity to bees

Data Point:	KCP 10.3.1.1.2/01
Report Author:	[redacted]
Report Year:	2013
Report Title:	Effects of bixafen + fluopyram + prothioconazole EC 260 (65+65+130) G (Acute Contact and Oral) on Honey Bees (Apis mellifera L.) in the Laboratory
Report No:	81781053
Document No:	M-469774-01-1
Guideline(s) followed in study:	OECD 213 and 214, 1998
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) Deviations from OECD Guideline 213: No deviations to the current OECD Guideline 213 occurred. All validity criteria were met. 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. 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 oral and contact toxicity of BIX + FLU + PTZ EC 260 to honey bees, please refer to section CP 10.3.1.1/01.

CP 10.3.1.2 Chronic toxicity to bees

Data Point:	KCP 10.3.1.2/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Bixafen + fluopyram + prothioconazole EC 260 (65+65+130 g/L): Chronic toxicity to the honey bee Apis mellifera L. under laboratory conditions
Report No:	19 48 BAC 0033
Document No:	M-688355-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No 1107/2009 (2009) US EPA OCSPP 850.SUPP OECD 245 (adopted 9 October 2017)
Deviations from current test guideline:	Current Guideline: OECD 245 (2017) Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the chronic oral toxicity ($LDD_{50/20/10}$/$LC_{50/20/10}$ and NOEDD/NOEC) of BIX + FLU + PTZ EC 260 (65+65+130 g/L) applied on 10 consecutive days to young adults of 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.

Worker honey bees (*Apis mellifera* L.) aged two days or less were orally exposed to a daily application of BIX + FLU + PTZ EC 260 (65+65+130 g/L) diluted in the bee food (50 % w/v aqueous sucrose solution) at nominal concentrations of 2538, 1269, 634, 317 and 159 mg product/kg feeding solution, corresponding to 50.1, 35.3, 20.7, 10.2 and 5.88 µg product/bee/day (actual doses) for 10 consecutive days. An untreated control (50 % w/v aqueous sucrose solution) and a reference item (dimethoate) were included in this study.

Mortality and behavioural abnormalities were assessed daily. The concentration of the test item in the feeding solutions was verified analytically.

The LDD_{50} and LC_{50} were determined to be > 50.1 µg product/bee/day and > 2538 mg product/kg feeding solution, respectively. The LDD_{20} and LC_{20} were determined to be 37.5 µg product/bee/day and 1457 mg product/kg food, respectively. The LDD_{10} and LC_{10} were determined to be 31.1 µg product/bee/day and 1016 mg product/kg feeding solution, respectively.

The NOEDD and NOEC were determined to be 20.7 µg product/bee/day and 634 mg product/kg feeding solution, respectively.

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

I. MATERIAL AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L), Specification No.: 102000027828; Batch No.: EM4L025147; Sample Description: TOX21235-00; analysed content of active substance: bixafen 65 g/L (nominal), 6.44% (w/w) corresponding to 65.10 g/L (analysed), fluopyram 65 g/L (nominal), 6.56%

(w/w) corresponding to 66.33 g/L (analysed) and prothioconazole 130 g/L (nominal), 12.5% (w/w) corresponding to 126.7 g/L (analysed), density: 1.011 g/mL.

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

Test concentrations and dose levels:

Test item concentrations: 2538, 1269, 634, 317 and 159 mg product/kg feeding solution

Nominal test item doses (calculated based on mean expected uptake of feeding solution of 33 mg/bee/day): 100.0, 49.8, 24.9, 12.5 and 6.23 µg product/bee/day

Actual test item doses: 50.1, 35.3, 20.7, 10.2 and 5.88 µg product/bee/day

Reference item concentration: 0.696 mg dimethoate/kg feeding solution

Nominal reference item dose (calculated based on mean expected uptake of feeding solution of 33 mg/bee/day): 0.0273 µg a.s./bee/day

Actual reference item dose: 0.0148 µg a.s./bee/day

Control group: 50 % w/v sucrose solution

Each group (test item, controls and reference item) comprised 3 replicates containing 10 bees each.

Test design: Worker honey bees (*Apis mellifera* L., < 2 days old) were orally exposed to a daily application of BIX + FLU + PTZ EC 260 (65+65+130 g/L) diluted in the bee food (50 % w/v aqueous sucrose solution) for 10 consecutive days.

Brood combs with capped cells were taken from outside hives and different colonies. Sufficient food supply was ensured either by honey and pollen which was on the same brood comb or by an additional comb containing food. These frames were placed without adult worker bees in a "five comb hive body" and incubated under controlled environmental conditions in a climatic chamber at 33 ± 2 °C in darkness for one day. Afterwards, the newly hatched worker bees were transferred into the test cages in groups of 10 bees per cage.

The exposure took place for a period of 10 days. Daily dose rates were based on a theoretical food consumption of 33 mg/bee/day. Test item solutions were prepared freshly every day. The reference item feeding solutions was prepared once for the whole feeding period and stored in the refrigerator at about 6 °C. The respective feeding solutions (test item, control and reference item) were provided *ad libitum* in a plastic syringe, which had been weighed before application. The feeders remained in the cages for about 24 h (± 2 h). The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units. Any unconsumed food was discarded. The evaporation of test solutions from the feeders was investigated in additional test cages which were set up with the main test and was subtracted from the calculated food consumption to give the corrected food consumption accounting for the loss by evaporation. The food consumption per bee was calculated by the number of surviving bees per assessment and the amount of food consumed on the following assessment day.

Mortality and behavioural abnormalities were assessed daily.

Test conditions: Temperature: 31.3 – 33.6 °C; Relative humidity: 55.0 – 62.8 %; Photoperiod: 24 h darkness (except during observation).

Statistics: Stepdown Cochran-Armitage Test Procedure was used for mortality data (one-sided greater, $\alpha = 0.05$) and determination of NOEDD/NOEC (no observed effect dietary dose/concentration). Mortality data were arcsine-transformed for determination of LDD_X and LC_X (lethal dietary doses/concentrations) by Probit analysis using linear maximum likelihood regression. The statistical calculations were performed with the computer program ToxRat Professional 3.2.1 (® ToxRat Solutions GmbH.).

Analytics: For verification of the exposure concentration, all test item solutions as well as control solution were sampled in duplicate directly after preparation on day 0 to day 9.The chemical analysis was performed by using High Performance Liquid Chromatograph (HPLC) with mass spectrometric (MS-MS) detection.

Dates of work: August 06th to August 16th, 2019; March 03rd 2020 (analytical phase completion)

II. RESULTS AND DISCUSSION

Analytical results:

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

The concentrations of the active ingredients bixafen, fluopyram and prothioconazole were determined in feeding solutions of all treatment groups. The mean recoveries ranged between 98 % and 101 % for bixafen, between 98 % and 103 % for fluopyram and between 90 % and 95 % for prothioconazole.

None of the active substances bixafen, fluopyram and prothioconazole have been detected in the control feeding solutions above 30 % of the Limit of Quantification (Bixafen LOQ: 0.00530 mg a.s./kg diet, fluopyram LOQ: 0.00540 mg a.s./kg diet, prothioconazole LOQ: 0.00103 mg a.s./kg.

Table 10.3.1.2- 1: Analytical results for bixafen, fluopyram and prothioconazole

Treatment group [A] [mg product/kg diet]	Nominal concentration [mg a.s./kg diet]	Mean measured concentration [mg a.s./kg diet]	Mean recovery from target [%]
Bixafen			
Control	0	< 30 % of LOQ	-
2538	163	160	97.7
1269	81-7	80.3	98.3
634	40.9	40.1	98.0
317	20.4	20.1	98.2
159	10.2	10.3	101
Fluopyram			
Control	0	< 30 % of LOQ	-
2538	166	163	98.0
1269	83.2	85.6	103
634	41.6	42.2	102
317	20.8	21.0	101
159	10.4	10.7	103
Prothioconazole			
Control	0	< 30 % of LOQ	-
2538	317	286	90.1
1269	159	146	91.9
634	79.3	73.0	92.0
317	39.7	36.9	92.9
159	19.8	18.9	95.4

LOQ: Limit of Quantification: bixafen = 0.00530 mg a.s./kg diet, fluopyram = 0.00540 mg a.s./kg diet, prothioconazole = 0.00103 mg a.s./kg.

Biological results:

Summary of mean mortality and toxicity of BIX + FLU + PTZ EC 260 (65+65+130 g/L) to adult honey bees after 10 days of chronic exposure:

Table 10.3.1.2- 2: 10-day chronic oral toxicity test with BIX + FLU + PTZ EC 260 (65+65+130 g/L) to young honey bees

Treatment group	Daily dose		Concentration	At day 10		
	Nominal	Consumed [A]		Mean mortality	Number of bees with behavioural abnormalities [D]	Mean behavioural abnormalities
	[µg product/bee/day]		[mg product/ kg diet]	[%]	[No.]	[%]
Control	-	-	-	0.0	0 out of 30	0.0
Test item	100	50.1	2538	40.0 *	0 out of 18	0.0
	49.8	35.3	1269	10.0 *	0 out of 27	0.0
	24.9	20.7	634	0.0	0 out of 30	0.0
	12.5	10.2	317	3.3	0 out of 29	0.0
	6.23	5.88	159	0.0	0 out of 30	0.0
Reference Item	[µg a.s./bee/day]		[mg a.s./kg diet]			
	0.0273	0.0148	0.696	90.0	0 out of 3	0.0

Endpoints		10 d
Test item doses	LDD_{50} [µg product/bee/day]	> 50.1
	LDD_{20} [µg product/bee/day] [B] (95 % C.I.)	37.5 (30.8 – 41.1)
	LDD_{10} [µg product/bee/day] [B] (95 % C.I.)	31.1 (21.9 – 35.6)
	NOEDD [µg product/bee/day] [C]	20.7
Test item concentrations	LC_{50} [mg product/kg feeding solution]	> 2538
	LC_{20} [mg product/kg feeding solution] [B] (95 % C.I.)	1457 (1067 – 1721)
	LC_{10} [mg product/kg feeding solution] [B] (95 % C.I.)	1016 (589 – 1295)
	NOEC [mg product/kg feeding solution] [C]	634

Results are mean values of 3 replicates, containing 10 bees each, Calculations are performed with non-rounded values and corrected for evaporation
Corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947), negative values are treated as "0", due to 0.0% mortality in the control group no correction needed
C.I.: Confidence interval
A Mean dose per bee per day; dose measured based on consumed feeding solution
B Lethal dietary dose/concentration (95 %-C.I. lower/upper) were calculated by Probit analysis using linear max. likelihood regression after arcsine-transformation of the mortality data
C No observed effect dietary dose/concentration (NOEC/NOEDD) was calculated using Step-down Cochran-Armitage Procedure; (one-sided greater, $\alpha = 0.05$)
D Number of bees with behavioural abnormalities referring to number of remaining bees
* Statistically significant difference in pairwise comparison between test item treatment and untreated control group AC (Step-down Cochran-Armitage Test Procedure; $\alpha = 0.05$; one-sided greater)

Taking into account the actual food uptake the bees consumed doses of 50.1, 35.3, 20.7, 10.2 and 5.88 µg product/bee/day, which caused mortalities of 40.0, 10.0, 0.0, 3.3, and 0.0 %, respectively, after 10 days. No mortality occurred in the control group. The obtained mortalities in the two highest test

levels (50.1 and 35.3 µg product/bee/day) were statistically significantly increased compared to the control group.

Behavioural abnormalities were observed at the two highest test levels. Single bees were described as being affected in terms of uncoordinated movements or moribund on D3, D5, D6 and D8. No treatment related abnormal behaviour was observed in any of the test item groups in the final assessment on the last day of the test.

Reference item:

The reference item (dimethoate) was administered in one dosage of 0.0273 µg product/bee/day (actual average intake based on food consumption was 0.0148 µg a.s./bee/day) which caused a continuously increasing mortality leading to 90 % mortality at day 10.

Validity criteria:

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

Table 10.3.1.2- 3: Validity criteria

Validity criteria according to OECD GD 245 (2017)	Recommended		Obtained
Mortality after 10 days of exposure	Control	≤ 15 %	0.0 %
Mortality after 10 days of exposure	Dimethoate	≥ 50 %	90 %

III. Conclusion

The chronic oral toxicity of BIX + FLU + PTZ EC 260 (65+65+130 g/L) was tested on young adult honey bees (*Apis mellifera* L.) in a 10-day feeding study under laboratory conditions.

The LDD_{50} and LC_{50} were determined to be > 50.1 µg product/bee/day and > 2538 mg product/kg food, respectively. The LDD_{20} and LC_{20} were determined to be 37.5 µg product/bee/day and 1457 mg product/kg feeding solution, respectively. The LDD_{10} and LC_{10} were determined to be 31.1 µg product/bee/day and 1016 mg product/kg feeding solution, respectively.

The NOEDD and NOEC were determined to be 20.7 µg product/bee/day and 634 mg product/kg feeding solution, respectively.

Assessment and conclusion by applicant:

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

The endpoints are:

LDD_{50} oral (10 days) > 50.1 µg product/bee/day

LC_{50} oral (10 days) > 2538 mg product/kg diet

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

Data Point:	KCP 10.3.1.3/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Bixafen + fluopyram + prothioconazole EC 260 (65+65+130 g/L) - Repeated exposure to honey bee larvae (Apis mellifera L.) under laboratory conditions
Report No:	19 48 BLC 0046
Document No:	M-704601-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009 (2009) Directive 2003-01 (CANADA/PMRA) US EPA OCSPP 850.SUPP OECD Guidance Document 239 (2016)
Deviations from current test guideline:	Current Guidance Document: OECD 239 (2016) Deviations: The relative humidity between day 8 and day 15 was 50-70 % and thus lower than the recommended range of 80 ± 5 % due to a malfunction of the climate chamber. 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 chronic toxicity ($ED_{50/20/10}$, $EC_{50/20/10}$, NOED/NOEC for adult emergence at day 22) of BIX + FLU + PTZ EC 260 (65+65+130 g/L) applied to honey bee, *Apis mellifera* L., larvae in an *in vitro* test after repeated exposure.

First instar honey bee larvae of *Apis mellifera* L. were transferred from brood combs of 3 hives to polystyrene grafting cells in 48-well cell culture plates 2 days before the start of the exposure period (D1, grafting). Larvae were exposed to 5 concentrations of BIX + FLU + PTZ EC 260 (65+65+130 g/L) (nominal 2522, 1147, 522, 237 and 108 mg product/kg diet, corresponding to nominal cumulative doses of 399, 182, 82.6, 37.5 and 17.0 mg product/larva) via the larval diet on 4 consecutive days (day 3 to day 6). No additional feeding of the larvae took place after day 6.

A reference item (dimethoate tech. at a cumulative dose of 7.6 µg a.s./larva) and an untreated control were included in the experimental design.

The larval mortality was recorded daily from day 4 to day 8. Additionally, other observations such as small body size or unconsumed diet on D8 were noted. Pupal mortality was evaluated at day 15 and the adult emergence rate was assessed on day 22.

In the analytical phase of the study, the concentration of the active ingredients bixafen, fluopyram and prothioconazole in the larval diet of each day of the exposure period was determined. The mean recoveries ranged between 82.8 – 91.2 % for bixafen, 86.3 – 97.5 % for fluopyram and 57.2 – 70.0 % for prothioconazole in the final diets.

The NOED and LOED were determined to be 66.0 µg and 148 µg product/larva (based on adult emergence), respectively. The NOEC and LOEC were 417 and 937 mg product/kg diet, respectively.

The ED_{50}, ED_{20} and ED_{10} values (based on adult emergence) were determined to be 133.1 µg, 102.7 µg and 86.6 µg product/larva, respectively. The EC_{50}, EC_{20} and EC_{10} values were determined to be 842 mg, 650 mg and 548 mg product/kg diet, respectively.

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

I. MATERIAL AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); Specification No.: 102000027828; Batch No.: EM4L025147; TOX No.: 21235-00, content of bixafen: 65 g/L (nominal), 6.44% w/w corresponding to 65.10 g/L (analysed), fluopyram: 65 g/L (nominal), 6.56% w/w corresponding to 66.33 g/L (analysed) and prothioconazole: 130 g/L (nominal), 12.5% w/w corresponding to 126.7 g/L (analysed); density: 1.011 g/mL

Test species: Honey bee (*Apis mellifera* L., subspecies *Buckfast*), synchronized first instar (L1, one day old) larvae originating from three adequately fed, healthy (free of clinical symptoms) queen-right bee colonies. The larvae were taken from hives that had not received treatments with chemical substances for at least one month.

Test concentrations and dose levels:

5 test item groups; concentrations (nominal): 2522, 1147, 522, 237 and 108 mg product/kg diet

Cumulative doses (nominal): 399, 182, 82.6, 37.5 and 17.0 µg product/larva

One reference item group exposed to a cumulative dose of 7.6 µg dimethoate/larva (concentration of dimethoate: 48.0 mg a.s./kg diet).

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

Each treatment group (test item, control, reference item) comprised 3 replicates, with 12 larvae each (each colony represented a replicate).

Test design: First instar honey bee larvae of *Apis mellifera* L. were transferred from brood combs of 3 hives to polystyrene grafting cells in 48-well cell culture plates 2 days before the start of the exposure period (D1, grafting). From day 3 until day 6 of the test, 5 different concentrations of BIX + FLU + PTZ EC 260 (65+65+130 g/L) mixed into the larval diet (aqueous yeast/sugar solution mixed with royal jelly 1:1 (w/w)) were fed to larvae of the test item groups. One single concentration of the reference item dimethoate mixed into the larval diet was fed to the larvae of the reference item group. A blank control (larval diet with water) was included in the experimental design. The volumes and contents of diets are presented in the table below.

Table 10.3.1.3- 1: Feeding scheme

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

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

The daily feeding volume increased from 20 µL to 50 µL diet per larva over the application period. After the applications, no additional feeding of the larvae took place.

The cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva and the density of the diet (diet B/C:1.13 g/cm³) were considered for the calculation of the cumulative doses per larva.

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

Test conditions: Temperature: 34.0 – 35.0 °C; relative humidity: day 1 to 8: 90 - 100 %. day 8 to 15: 30 - 70 %. day 15 to 22: 56 - 64 %; photoperiod: 24 h darkness (except during handling and assessments).

Statistics: For each concentration the cumulative mortalities were corrected for control mortality according to the formula of ABBOTT (1925), modified by SCHNEIDER-ORELLI (1947).

The Step-down Cochran-Armitage Test was used for statistical analysis of the adult emergence data and the estimation of the NOEC/NOED and LOEC/LOED as the data showed a monotonic trend. The accepted significance level was α = 0.05 (one-sided greater). The ED/EC$_{10/20/50}$ values were determined with the Weibull analysis using linear maximum likelihood regression. The statistical calculations were performed with the statistical program ToxRat Professional version 3.3.0.

Analytics: All final diets of the control and test item treatment group were sampled in duplicate as analysis and retain samples directly from the prepared diet. The chemical analysis was performed by using Reversed Phase High Performance Liquid Chromatograph (RP-HPLC) with MS/MS detection.

Dates of work: September 02nd to September 23th, 2019

II. RESULTS AND DISCUSSION

Analytical results:

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

The analytical dose verification of the larval diet from day 3 until day 6 resulted in mean measured recoveries of 82.8 – 91.2 % for bixafen, 86.3 – 97.5 % for fluopyram and 57.2 – 70.0 % for prothioconazole.

In the control samples, the concentration was below 30 % of the Limit of quantification for all active substances (LOQ bixafen: 0.00502 mg a.s./kg, LOQ fluopyram: 0.00512 mg a.s./kg, and LOQ prothioconazole: 0.00972 mg a.s./kg).

Table 10.3.1.3- 2: Analytical results for bixafen

Treatment group	Nominal conc. of bixafen [mg a.s./kg diet]	Sampling Time	Measured conc. of bixafen [mg a.s./kg diet]	Recovery from target [%]	Mean recovery from target [%]
Control	0.00	D 3	< 30 % of LOQ	-	-
		D 4	< 30 % of LOQ	-	
		D 5	< 30 % of LOQ	-	
		D 6	< 30 % of LOQ	-	
Test item	162	D 3	132	81.3	82.8
		D 4	141	86.8	
		D 5	132	81.4	
		D 6	133	81.9	
	73.9	D 3	65.2	88.2	86.2
		D 4	66.3	89.7	
		D 5	60.8	82.3	
		D 6	59.6	80.6	
	33.6	D 3	29.6	87.9	85.8
		D 4	29.5	87.9	
		D 5	29.0	86.2	
		D 6	27.3	81.3	
	15.3	D 3	14.4	94.6	91.2
		D 4	14.5	95.0	
		D 5	13.9	90.9	
		D 6	12.9	84.3	
	6.93	D 3	6.53	94.2	90.0
		D 4	6.28	90.6	
		D 5	6.27	90.4	
		D 6	5.89	85.0	

LOQ: Limit of Quantification: 0.00502 mg bixafen/kg

Table 10.3.1.3- 3: Analytical results for fluopyram

Treatment group	Nominal conc. of fluopyram [mg a.s./kg diet]	Sampling Time	Measured conc. of fluopyram [mg a.s./kg diet]	Recovery from target [%]	Mean recovery from target [%]
Control	0.00	D 3	< 30 % of LOQ	-	-
		D 4	< 30 % of LOQ	-	
		D 5	< 30 % of LOQ	-	
		D 6	< 30 % of LOQ	-	
Test item	165	D 3	143	86.2	86.3
		D 4	154	92.8	
		D 5	135	81.6	
		D 6	140	84.8	
	75.3	D 3	70.4	93.6	89.7
		D 4	71.6	95.2	
		D 5	65.3	86.8	
		D 6	62.8	83.4	
	34.2	D 3	32.0	93.3	92.0
		D 4	32.1	93.8	
		D 5	31.3	91.3	
		D 6	29.3	85.6	
	15.6	D 3	15.7	101	97.5
		D 4	16.0	103	
		D 5	15.1	97.3	
		D 6	13.8	88.8	
	7.06	D 3	7.15	101	97.4
		D 4	6.99	99.0	
		D 5	6.99	99.0	
		D 6	6.39	90.5	

LOQ: Limit of Quantification: 0.00512 mg fluopyram/kg

Table 10.3.1.3- 4: Analytical results for prothioconazole

Treatment group	Nominal conc. of prothioconazole [mg a.s./kg diet]	Sampling Time	Measured conc. of prothioconazole [mg a.s./kg diet]	Recovery from target [%]	Mean recovery from target [%]
Control	0.00	D 3	< 30 % of LOQ	-	-
		D 4	< 30 % of LOQ	-	
		D 5	< 30 % of LOQ	-	
		D 6	< 30 % of LOQ	-	
Test item	315	D 3	214	68.0	68.0
		D 4	224	71.0	
		D 5	209	66.2	
		D 6	210	66.7	
	143	D 3	106	73.8	70.0
		D 4	106	73.8	
		D 5	101	70.6	
		D 6	88.7	61.9	
	65.3	D 3	41.7	63.9	63.1
		D 4	42.9	65.8	
		D 5	41.6	63.7	
		D 6	38.4	58.8	
	29.6	D 3	18.9	63.6	63.3
		D 4	18.9	63.9	
		D 5	19.0	64.0	
		D 6	18.3	61.8	
	13.5	D 3	8.35	62.0	57.2
		D 4	7.82	58.1	
		D 5	7.27	54.0	
		D 6	7.39	54.9	

LOQ: Limit of Quantification: 0.00972 mg prothioconazole/kg

<u>Biological results:</u>

On day 8, larval mortality was 2.8 % in the control group and 88.9 % in the reference item group. In the test item group larval mortalities on day 8 were 100.0 %, 41.7 %, 8.3 %, 0.0 % and 0.0 % following a treatment with 399, 182, 82.6, 37.5 and 17.0 µg product/larva, respectively.

In the final assessment on day 22, an adult emergence rate of 77.8 % was determined for the honey bees in the control group. In the test item treated group, the adult honey bees emerged at rates of 0.0 %, 25.0 %, 77.8 %, 80.6 % and 91.7 % exposed to a cumulative dose 399, 182, 82.6, 37.5 and 17.0 µg product/larva, respectively, during the larval stages. Due to lower recovery rates of prothioconazole the doses were corrected for the actual rates resulting in doses of 315, 148, 66.0, 31.5 and 13.9 µg product/larva. On day 22, larvae treated with 399 or 182 µg product/larva (corrected dose rate of 315 and 148 µg product/larva), showed an emergence rate, which was statistically significantly different compared to the control. During the assessments of mortality and emergence no other test item related observations such as deviating sizes, appearances and malformations of the test organisms were made.

On day 8, 5.6 % of the remaining larvae treated with the second top dose of the test item (182 µg product/larva), were observed to have food left. They did not emerge successfully until day 22.

Table 10.3.1.3- 5: Mortality and other observations of larvae and adult emergence in the repeated exposure toxicity test

Treatment group	Cumulative dose		Concentration		Day 8			Day 22		
					Larval mortality D3 - D8		Mean OO	Total mortality Day 3 - 22		Adult emergence rate
	nom.	corr.[1]	nom.	corr.[1]	abs.	corr.		abs.	corr.	actual
	[µg product/ larva]		[mg product/kg diet]		[%]		[%]	[%]		[%]
Control	-	-	-	-	2.8	0.0	0.0	22.2	0.0	77.8
Test item (BIX + FLU + PTZ EC 260 (65+65+130 g/L))	399	315	2522	1933	100.0	100.0	-	100.0	100.0	0.0 *
	182	148	1147	937	41.7	40.0	5.6	75.0	67.9	25.0 *
	82.6	66.0	522	417	8.3	5.7	0.0	22.2	0.0	77.8
	37.5	31.5	237	199	0.0	0.0	0.0	19.4	0.0	80.6
	17.0	13.9	108	88	0.0	0.0	0.0	8.3	0.0	91.7
Reference Item (Dimethoate)	[µg a.s./larva]		[µg a.s./kg diet]							
	7.6	-	48	-	88.9	88.6	33.3	97.2	96.4	2.8

Results are averages based on 3 replicates (hives) containing 12 larvae each; nom.: nominal; corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947); mortality in test and reference item treated groups were corrected by the mortality of the control (ACY; abs.: absolute mortality as counted from the results; calculation were performed with non-rounded values; OO: Other observations (e.g. remaining food); negative values were set to "0"

* Statistically significant difference compared to control (Step-down Cochran-Armitage Test; p=0.05; one sided greater)

1 Values were corrected for actual recovery rates (mean recoveries of all active substances)

Table 10.3.1.3- 6: Calculated endpoints of the repeated exposure larvae toxicity test

Treatment	Endpoint: Adult emergence at day 22	
Test item cumulative doses [µg product/larva]	ED_{50} (95 % C.I.) [2]	133.1 (121.2 – 146.0)
	ED_{20} (95 % C.I.) [2]	102.7 (90.9 – 116.2)
	ED_{10} (95 % C.I.) [2]	86.6 (73.7 – 101.8)
	LOED [1]	148
	NOED [1]	66.0
Test item concentrations [mg product/kg diet]	EC_{50} (95 % C.I.) [2]	842 (767 – 924)
	EC_{20} (95 % C.I.) [2]	650 (575 - 735)
	EC_{10} (95 % C.I.) [2]	548 (466 - 644)
	LOEC [1]	937
	NOEC [1]	417

C.I.: Confidence interval

1 Step-down Cochran-Armitage Test; α= 0.05; one-sided greater

2 Weibull analysis using linear maximum likelihood regression

3 Based on corrected values

Validity criteria:

All validity criteria of the OECD Guidance Document 239 (2016) were met.

Table 10.3.1.3- 7: Validity criteria

Validity criteria acc. to OECD TG 239 (2016)	Recommended		Obtained
Larval mortality between day 3 and day 8 in the control group (across all replicates)	Control	≤ 15 %	2.8 %
Adult emergence rate until day 22 in the control group (across all replicates)	Control	≥ 70 %	77.8 %
Larval mortality between day 3 and day 8 in the reference group (across all replicates)	Dimethoate	≥ 50 %	88.9 %

III. CONCLUSION

In a repeated exposure larval toxicity study performed in a dose-response design with BIX+ FLU+ PTZ EC 260 (65+65+130 g/L), the NOED and LOED were determined to be 66.0 µg and 148 µg product/larva (based on adult emergence), respectively. The NOEC and LOEC were 417 and 937 mg product/kg diet, respectively.

The ED_{50}, ED_{20} and ED_{10} values (based on adult emergence) were determined to be 133.1 µg, 102.7 µg and 86.6 µg product/larva, respectively. The EC_{50}, EC_{20} and EC_{10} values were determined to be 842 mg, 650 mg and 548 mg product/kg diet, respectively.

Assessment and conclusion by applicant:

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

The endpoints are:

NOED - emergence (22 days) = 66.0 µg product/larva

NOEC - emergence (22 days) = 417 mg product/kg diet

CP 10.3.1.4 Sub-lethal effects

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

CP 10.3.1.5 Cage and tunnel tests

Further testing was not necessary when considering the outcome of the risk assessment and the results of the lower-tier studies.

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 BIX+FLU+PTZ EC 260 Tier 2 studies (extended laboratory studies) with *Aphidius rhopalosiphi, Typhlodromus pyri, Chrysoperla carnea* and *Coccinella septempunctata* and an aged residues study with *Typhlodromus pyri,* the most sensitive species tested in Tier 2, 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 BIX+FLU+PTZ EC 260

Test species, **Reference**	**Tested formulation, study type, exposure**	**Ecotoxicological endpoint**		
Aphidius rhopalosiphi [redacted] (2014) M-480611-01-1 KCP 10.3.2.2/01	BIX + FLU + PTZ EC 260 Extended Lab., exposure on potted barley plants	LR_{50} > 2550 mL product/ha ER_{50} > 2550 mL product/ha		
		Corr. Mortality [%] [A]	Effects on Reproduction [%] [B]	
	Control			
	255 mL prod./ha	0.0	-6.8	
	453 mL prod./ha	0.0	-15.6	
	806 mL prod./ha	0.0	7.2	
	1434 mL prod./ha	0.0	-17.0	
	2550 mL prod./ha	0.0	-32.8	
Typhlodromus pyri [redacted] (2014) M-480613-01-1 KCP 10.3.2.2/02	BIX + FLU + PTZ EC 260 Extended laboratory, exposure on detached bean leaves	LR_{50} = 1191 mL prod./ha ER_{50} > 675 mL prod./ha		
		Corr. Mortality [%] [A]	Effect on Reproduction [%] [B]	
	Control			
	120 mL prod./ha	4.5	23.3	
	213 mL prod./ha	9.0	26.3	
	379 mL prod./ha	6.7	37.9	
	675 mL prod./ha	11.2	40.4	
	1200 mL prod./ha	59.6	n.a.	
Typhlodromus pyri [redacted] (2015) M-535877-01-1 KCP 10.3.2.2/03	BIX + FLU + PTZ EC 260 Extended laboratory, exposure to aged residues on potted apple seedlings	Corr. Mortality [%] [A]	Eggs/Female/ Day	Effect on Reproduction [%] [B]
	2 x 1.5 L product/ha (interval of 14 days)	15.7 * at 0 DA(L)T	1.2 at 0 DA(L)T	71.6 ** at 0 DA(L)T
		7.2 at 14 DA(L)T	4.1 at 14 DA(L)T	19.7 at 14 DA(L)T
		2.3 at 28 DA(L)T	5.3 at 28 DA(L)T	- 16.0 at 28 DA(L)T

Test species, Reference	Tested formulation, study type, exposure	Ecotoxicological endpoint
Chrysoperla carnea [redacted] (2013) M-476030-01-1 KCP 10.3.2.2/04	BIX + FLU + PTZ EC 260 Extended laboratory., exposure on detached bean leaves	LR_{50} > 2550 mL prod./ha Corr. Mortality [%] [A] / Eggs/Female/ Day / Hatching [%] Control: - / 28.0 / 89.4 255 mL prod./ha: 8.3 / 31.4 / 91.6 453 mL prod./ha: -2.8 / 20.8 / 88.4 806 mL prod./ha: 5.6 / 37.8 / 89.2 1434 mL prod./ha: 25.0 / 37.5 / 89.2 2550 mL prod./ha: 41.7 / 17.9 / 86.5
Coccinella septempunctata [redacted] (2013) M-476172-01-1 KCP 10.3.2.2/05	BIX + FLU + PTZ EC 260 Extended laboratory, exposure on detached bean leaves	LR_{50} > 2550 mL prod./ha Corr. Mortality [%] [A] / Eggs/Female/ Day / Hatching [%] Control: - / 13.4 / 82.7 255 mL prod./ha: -2 / 12.3 / 86.9 453 mL prod./ha: 2.7 / 7.9 / 85.5 806 mL prod./ha: 18.1 / 13.2 / 87.1 1434 mL prod./ha: 35.1 / 12.9 / 84.8 2550 mL prod./ha: 13.5 / 10.5 / 86.4

DA(L)T: Days after last application; n.a. not assessed

[A] Positive values indicate increased mortality compared to the control; Negative values indicate better survivorship compared to the control

[B] Positive values indicate reduced reproduction compared to the control; Negative values indicate better performance compared to the control

* Statistically significantly different compared to control (Fisher's Exact test, one-sided, $\alpha = 0.05$)

** Statistically significantly different compared to control (Welch test, $\alpha = 0.05$)

The exposure scenario is based on the use pattern as given in Table 10- 1. The product BIX + FLU + PTZ EC 260 is intended to be applied at a rate of 0.6 L product/ha (1 application in barley) or 1.2 L product/ha (1 application in barley).

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: Max. single application rate × MAF

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

Application rate: 0.6 L product/ha or 1.2 L product/ha

[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

MAF (multiple application factor) = 1.0 (1 application)

Drift factor = 0.0277, 90th percentile for one application (according to Ganzelmeier)

VDF = vegetation distribution factor = 5 (Tier 2; studies with 2D exposure system) and 1 (Tier 2; studies with 3D exposure system)

Correction factor = 5 (Tier 2)

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

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

Crop	No. of appl.	Appl. rate [L prod./ha]	MAF	$PER_{in\text{-}field}$ [L prod./ha]
Barley	1	0.6	1.0	0.6
Barley	1	1.2	1.0	1.2

MAF: Multiple application factor; PER: Predicted environmental rate

Table 10.3.2- 3: Exposure calculation for the off-field scenario (Tier 2)

Crop	No. of appl.	Appl. rate [L prod./ha]	MAF	Drift [%]	Test system	VDF	Correction factor	$PER_{off\text{-}field}$ [L prod./ha]
Barley	1	0.6	1.0	2.77	3D [A]	1	5	0.083
					2D [B]	5	5	0.017
Barley	1	1.2	1.0	2.77	3D [A]		5	0.166
					2D [B]	5	5	0.033

MAF: Multiple application factor; VDF: Vegetation distribution factor; PER: Predicted environmental rate
[A] Relevant for the extended lab study with *Aphidius rhopalosiphi* (M-480611-01-1)
[B] Relevant for the extended lab studies with *Typhlodromus pyri* (M-480613-01-1), *Chrysoperla carnea* (M-476030-01-1) and *Coccinella septempunctata* (M-476172-01-1)

Risk assessment for non-target arthropods

The risk assessment was performed according to the Guidance Document on Terrestrial Ecotoxicology (SANCO/10329/2002 rev. 2 final, 2002) and to the Guidance Document on regulatory testing and risk assessment procedures for plant protection products with non-target arthropods (ESCORT 2, Candolfi *et al*. 2000[16]).

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

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

Table 10.3.2- 4: Tier 2 in-field risk assessment for non-target arthropods

Crop and application rate	Species	$PER_{in\text{-}field}$ [mL prod./ha]	LR_{50}/ER_{50} [mL prod./ha]	$PER_{in\text{-}field}$ below rate with ≤ 50% effect
Barley 1 × 0.6 L prod./ha	*Aphidius rhopalosiphi*	600	> 2550	Yes
	Typhlodromus pyri		> 675	Yes
	Chrysoperla carnea		> 2550	Yes
	Coccinella septempunctata		> 2550	Yes
Barley 1 × 1.2 L prod./ha	*Aphidius rhopalosiphi*	1200	> 2550	Yes
	Typhlodromus pyri		> 675	No
	Chrysoperla carnea		> 2550	Yes
	Coccinella septempunctata		> 2550	Yes

PER: Predicted environmental rate

The $PER_{in\text{-}field}$ is below the rate with ≤ 50% effect for the species *Aphidius rhopalosiphi, Typhlodromus pyri, Chrysoperla carnea* and *Coccinella septempunctata* for the application of 1 × 0.6 L prod./ha. For the application rate of 1 × 1.2 L prod./ha in barley, the $PER_{in\text{-}field}$ is below the rate with ≤ 50% effect for the species *Aphidius rhopalosiphi, Chrysoperla carnea* and *Coccinella septempunctata,* but not for *Typhlodromus pyri.*

Hence, an aged residue study with *Typhlodromus pyri* is presented to demonstrate the potential for recovery of in-field non-target arthropod populations and thus acceptable risk.

Aged residue study with *Typhlodromus pyri*

An extended aged residue laboratory study was performed with the most sensitive species *Typhlodromus pyri.* BIX + FLU + PTZ EC 260 was applied to potted apple seedlings at a rate of 2 × 1.5 L product/ha. The exposure of the test organisms to fresh residues directly after the second application of the test item (0 DA(L)T) resulted in a corrected mortality of 15.7 %. After 14 days (14 DA(L)T) 7.2 % corrected mortality occurred and 2.3 % corrected mortality was detected in the final bioassay started four weeks after the second application of the test item (28 DA(L)T). In the first bioassay (0 DA(L)T) a reduction in reproductive success relative to the control of 71.6 % could be detected. The effect on reproduction decreased to 19.7 % reduction compared to the control after two weeks of aging (14 DA(L)T). In the third bioassay (28 DA(L)T), no reduction was found anymore.

Since effects on mortality and reproduction were no longer statistically significant after aging of the residues for 14 days, it can be concluded that the potential for recovery is given within two weeks after the application. Therefore, the results of this study further support the conclusion that no unacceptable effects on non-target arthropods in the in-field area are expected from the intended use of BIX + FLU + PTZ EC 260.

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

Table 10.3.2- 5: Tier 2 off-field risk assessment for non-target arthropods

Crop and application rate	Species	Off-field $PER_{max.}$ [mL prod./ha]	LR_{50}/ER_{50} [mL prod./ha]	$PER_{off\text{-}field}$ below rate with ≤ 50% effect
Barley 1 × 0.6 L prod./ha	*Aphidius rhopalosiphi*	83	> 2550	Yes
	Typhlodromus pyri	17	> 675	Yes
	Chrysoperla carnea	17	> 2550	Yes
	Coccinella septempunctata	7	> 2550	Yes
Barley 1 × 1.2 L prod./ha	*Aphidius rhopalosiphi*	166	> 2550	Yes
	Typhlodromus pyri	33	> 675	Yes
	Chrysoperla carnea	33	> 2550	Yes
	Coccinella septempunctata	33	> 2550	Yes

PER: Predicted environmental rate

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

Conclusion:

From the data and risk assessments presented above, it is concluded that unacceptable effects of BIX + FLU + PTZ EC 260 on non-target arthropods in the in-field and off-field environment are not to be expected for the intended uses of BIX + FLU + PTZ EC 260.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Standard laboratory studies were not performed for non-target arthropods since extended laboratory studies have been performed, which are shown under CP 10.3.2.2.

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:	2014
Report Title:	Effects of bixafen + fluopyram + prothioconazole EC 260 (65 + 65 + 130 g/L) on the parasitoid Aphidius rhopalosiphi, extended laboratory study - Dose response test
Report No:	83871002
Document No:	M-480611-01-1
Guideline(s) followed in study:	Mead-Briggs et al. 2010
Deviations from current test guideline:	Current Guideline: Mead-Briggs et al. (2010) Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In an extended laboratory test the effects on the survival and reproduction of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on adults of *Aphidius rhopalosiphi* were investigated. Five product rates from 255 – 2550 mL product/ha were tested. Per product rate 6 replicates of 5 female parasitoids were exposed to BIX + FLU + PTZ EC 260 (65+65+130 g/L) on treated barley plants. Mortality was assessed after 48 hours of exposure by counting the number of dead and moribund wasps. Number of aphid mummies was counted 10 – 11 days after the 24 hours parasitisation period in all replicates where the females were alive after the 24 hour parasitisation period.

All of the validity criteria were met according to Mead-Briggs *et al.* (2010).

No mortality was observed in the control as well as in the product groups. The settling rate of the parasitoids on the plants was 46.7 % at 255 mL product/ha, 56.0 % at 453 mL product/ha, 67.3 % at 806 mL product/ha, 52.0 % at 1434 mL product/ha and 44.7 % at 2550 mL product/ha compared to the control with 76.0 % parasitoids on the plants during the first 3 hours of the study.

The reproductive capacity of *A. rhopalosiphi* was tested at all product rates. The effect on reproduction was - 6.8 % at 255 mL product/ha, - 15.6 % at 453 mL product/ha, 7.2 % at 806 mL product/ha, - 17.0 % at 1434 mL product/ha and - 32.8 % at 2550 mL product/ha, respectively, compared to the control.

The LR_{50} for mortality effects of the product was estimated to be > 2550 mL product/ha. The ER_{50} for reproduction effects of the product was estimated to be > 2550 mL product/ha.

I. Material and Methods

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L), Spec. no: 102000031262, Batch No: 2013-002135, TOX10113-00, analysed content of active substance: 6.49 % w/w (65.61 g/L) bixafen, 6.35 % w/w (64.21 g/L) fluopyram and 12.7 % w/w (128.5 g/L) prothioconazole, density: 1.011 g/mL.

Test design: Under extended laboratory conditions adults of *Aphidius rhopalosiphi*, not older than 48 hours, were exposed to dried spray deposits of 255, 453, 806, 1434 and 2550 mL product/ha (diluted in 400 L deionised water/ha) on treated barley plants (*Hordeum vulgare*) (6 replicates each containing 5

female parasitoids per treatment group). Deionised water was used as a control treatment and Dimethoate (Perfekthion: 10.0 mL product/ha diluted in 400 L deionised water/ha) as a reference treatment. Survival of the parasitoids was assessed after 2, 24 and 48 hours. At 48 hours, for treatment groups with < 50 % corrected mortality survived females were removed and their reproductive capacity was assessed by confining them individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The adult parasitoids were removed after 24 hours and the aphid-infested plants left for further 10 - 11 days before the numbers of aphid mummies that had developed were assessed. The number of aphid mummies obtained from maximum 20 replicates per treatment group was used to calculate the mean aphid mummies production per female within the 24 hours parasitisation period. No fecundity assessment was performed for the reference item.

Test conditions: Temperature: 19 - 21 °C; relative humidity: 62 - 75 % (acclimatisation and exposure period), 72 - 85 % (post-exposure period, within the test units); photoperiod: 16 h light : 8 h dark; light intensity: 620 - 780 lux (acclimatisation and exposure period), 1810 - 2180 lux (parasitisation period), 9500 - 16480 lux (post-parasitisation period).

Statistics: Mortality data were analysed for significance using the Fisher's Exact Test. Settling data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.05$) and the Levene's test ($\alpha = 0.05$). Because settling data were normally distributed and homogenous the Dunnett's t-test (multiple comparison, one-sided, $\alpha = 0.05$) was used for calculating the mean value of parasitoids were settled on the plants with product residues. For calculating the mean value of parasitoids were settled on the plants with reference item, the Student t-test (pair wise comparison, one-sided, $\alpha = 0.05$) was used, because settling data were normally distributed and homogenous, too.

Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.05$) and the Levene's test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogenous the Dunnett's t-test (multiple comparison, one-sided, $\alpha = 0.05$) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Dates of work: January 21, 2014 - February 04, 2014

II. Results and Discussion

In this extended laboratory test the effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) residues on the survival of *Aphidius rhopalosiphi* were determined at 255, 453, 806, 1434 and 2550 mL product/ha, applied to treated barley plants (*Hordeum vulgare*).

The corrected mortality in all test item rates was 0 %. At 255, 453, 1434 and 2550 mL product/ha the settling rate of 46.7 %, 56.0 %, 52.0 % and 44.7 % parasitoids on the plants was statistically significantly lower compared to the control with 76.0 % parasitoids on the plants during the first 3 hours of the study. At the product rate of 806 mL product/ha a settling rate of 67.3 % was found, which was not statistically significant. In the reference item group the settling rate was 51.3 %, which was statistically significantly lower compared to the control.

The reproductive capacity of *A. rhopalosiphi* was tested at all product rates. The effect on reproduction was - 6.8 % at 255 mL product/ha, - 15.6 % at 453 mL product/ha, 7.2 % at 806 mL product/ha, - 17.0 % at 1434 mL product/ha and - 32.8 % at 2550 mL product/ha, respectively, compared to the control. All product rates except oft 806 mL product/ha were statistically significant.

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

Table 10.3.2.2- 1: Effects of dried spray residues on treated barley plants on the parasitic wasp, *Aphidius rhopalosiphi* (Hymenoptera, Braconidae) in an extended laboratory study

Test item	BIX + FLU + PTZ EC 260 (65+65+130 g/L)					
Test organism	*Aphidius rhopalosiphi*					
Exposure on	Barley plants					
		Mortality after 48 h [%]		**Reproduction**		**Settling rate (first 3 h) 4)**
Treatment	**Rate 1) [mL product/ha]**	**Uncorrected 2)**	**Corrected 3)**	**Rate [mummies per female] 5)**	**Reduction relative to control [%] 6)**	**% Wasps on plant**
Control	-	0.0	-	47.7	-	76.0
Test item	255	0.0	0.0	51.0	- 6.8	46.7 *
	453	0.0	0.0	55.2	- 15.6	56.0 *
	806	0.0	0.0	44.3	7.2	67.3
	1434	0.0	0.0	55.8	- 17.0	52.0 *
	2550	0.0	0.0	63.4	- 32.8	44.7 *
Toxic ref.	10.0	90.0 *	90.0	-	-	51.3 *
LR_{50}: > 2550 mL product/ha **ER_{50}: > 2550 mL product/ha**						

1) Application rate in 400 L water/ha
2) Mortality: after 48 hours of exposure to spray residues on plant surfaces (Fisher's Exact Test, α = 0.05, * = significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Settling rate: mean value of parasitoids settled on the plants from 5 consecutive observations during the first 3 hours of the study (test item: Dunnett's t-test, α = 0.05; * = significant, reference item: Student t-test, α = 0.05; * = significant)
5) Reproduction: mean number of parasitized aphids/female (Dunnett's t-test, one sided, α = 0.05)
6) Calculated on the exact raw data; negative values indicate better performance compared to the control

<u>Validity criteria:</u>

All of the validity criteria were met (according to Mead-Briggs *et al.*,2010).

Table 10.3.2.2- 2: Validity criteria

Validity criteria	Required	Obtained
Mortality in water control	≤ 10 %	0 %
Corrected mortality reference item	≥ 50 %	90 %
Mean reproduction per female in water control	≥ 5	47.7
Number of wasps in the water control producing zero values for reproduction	≤ 2	0

III. CONCLUSION

The LR_{50} for mortality effects of the product was estimated to be > 2550 mL product/ha.

The ER_{50} for reproduction effects of the product was estimated to be > 2550 mL product/ha.

The figures obtained fulfil the validity criteria of the laboratory method for exposure on plants according to according to Mead-Briggs *et al.*(2010).

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} > 2550 mL product/ha

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Data Point:	KCP 10.3.2.2/02
Report Author:	[redacted]
Report Year:	2014
Report Title:	Effects of bixafen + fluopyram + prothioconazole EC 260 (65 + 65 + 130 g/L) on the predatory mite Typhlodromus pyri, extended laboratory study - Dose response test
Report No:	83872062
Document No:	M-480613-01-1
Guideline(s) followed in study:	Bluemel et al. 2000 and Oomen 1988
Deviations from current test guideline:	Current Guideline: Bluemel et al. (2000) and Oomen (1988) Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In an extended laboratory test the effects on the survival and reproduction of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the predatory mite *Typhlodromus pyri* were investigated. Five test item rates from 120 - 1200 mL product/ha were tested. Per product rate 10 replicates of 10 individuals were exposed to BIX + FLU + PTZ EC 260 (65+65+130 g/L) on treated bean leaves. Survival of the mites was assessed after 3 and 7 days. For the reproduction assessment surviving mites from the control and from all test item groups where the corrected mortality was < 50% were sexed and the number of eggs per females was recorded on 3 assessment days within one week.

All validity criteria according to Blümel *et al.* (2000) were met.

The mortality of two highest product rates 675 and 1200 mL product/ha is statistically significant different to the control. The LR_{50} was estimated to be 1191 mL product/ha.

The reproduction rate was statistically significant at product rates of 120, 213, 379 and 675 compared to the control. No values of the reproduction rate of the highest product rate (1200 mL product/ha) were reported. The ER_{50} was estimated to be > 675mL product/ha, the highest dose rate where reproduction was tested.

I. MATERIAL AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); Specification No.: 102000027828-01, Batch ID: 2013-002135, sample description: TOX10113-00, content of a.s.: 6.49 % w/w (65.61 g/L) bixafen, 6.35 % w/w (64.21 g/L) fluopyram and 12.7 % w/w (128.5 g/L) prothioconazole; density: 1.011 g/mL.

Test design: Protonymphs of the predatory mite, *Typhlodromus pyri* (less than 24 h old) were exposed to dried spray deposits of 120, 213, 379, 675 and 1200 mL product/ha (diluted in 200 L deionised water/ha) on treated bean leaves (*Phaseolus vulgaris*). For each treatment and control 10 replicates with 10 individuals were used. Deionised water was used as a control treatment and Dimethoate (Perfekthion: 40.0 mL product/ha diluted in 200 L deionised water/ha) as a reference treatment.

The number of living, dead and escaped mites was counted twice in the first week (on day 3 and at day 7) after test initiation. Dead mites were removed, escaped mites were considered as dead. The reproduction performance of the surviving mites in the control and all product rates was then evaluated by counting the number of eggs laid and number of live and dead juvenile stages per female, which were removed afterwards, on 3 assessment days from day 7 on with a maximum interval of 3 days up to day 14 (inclusive). Eggs laid until day 7 inclusive were removed from the test arena and were not counted. No reproduction assessment was performed for the reference item.

Test conditions The climatic test conditions during the study were 24 - 26 °C temperature and 67 - 74 % relative humidity with a photoperiod of 16 hours light and a light intensity range of 420 - 940 lux.

Statistics: The LR_{50} of the mortality was calculated by applying the Weibull Analysis. Mortality data were analysed for significance using the Fisher's Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior analysis. Reproduction data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ($\alpha = 0.05$) and the Levene's test ($\alpha = 0.05$). Because reproduction data were normally distributed and homogenous the Dunnett's t-test (multiple comparison, one-sided, $\alpha = 0.05$) was used. The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, © ToxRat Solutions GmbH.

Dates of work: January 20, 2014 - February 03, 2014

II. Results and Discussion

In this extended laboratory test the effects on the survival and reproduction of the predatory mite *Typhlodromus pyri* by exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) applied on treated bean leaves were determined at 120, 213, 379, 675 and 1200 mL product/ha.

The corrected mortality was 4.5 % at 120 mL product/ha, 9.0 % at 213 mL product/ha, 6.7 % at 379 mL product/ha, 11.2 % at 675 mL product/ha and 59.6 % at 1200 mL product/ha, respectively, compared to the control where 11.0 % mortality was observed. The mortality of two highest product rates 675 and 1200 mL product/ha is statistically significant different to the control.

The LR_{50} for mortality effects is 1191 mL product/ha (95 % confidence limits could not be determined).

The reproductive capacity of *T. pyri* was tested at 120, 213, 379 and 675 mL product/ha. The effect on reproduction was 23.3 % at 120 mL product/ha, 26.3 % at 213 mL product/ha, 37.9 % at 379 mL product/ha and 40.4 % at 675 mL product/ha, respectively, compared to the control. The reproduction rate was statistically significant at product rates of 120, 213, 379 and 675 compared to the control.

The ER_{50} for reproduction effects was estimated to be > 675 mL product/ha, the highest dose rate where reproduction was tested.

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

Table 10.3.2.2- 3: Effects of dried spray residues on treated bean leaves on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) in an extended laboratory study

Test item:	BIX + FLU + PTZ EC 260 (65+65+130 g/L)
Test organism:	*Typhlodromus pyri*
Exposure on:	treated bean leaves

Treatment	Rate [mL product/ha] [1]	Mortality after 7 days [%]		Reproduction	
		Uncorrected [2]	Corrected [3]	Rate [eggs per female] [4]	Reduction relative to control [%] [5]
Control	-	11.0		8.8	-
Test item	120	15.0	4.5	6.7 *	23.3
	213	19.0	9.0	6.5 *	26.3
	379	17.0	6.7	5.4 *	37.9
	675	21.0 *	11.2	5.2 *	40.4
	1200	64.0 *	59.6	-	
Toxic ref.	40.0	100.0 *	100.0	-	-

LR_{50} = 1191 mL product/ha [6]
ER_{50} > 675 mL product/ha

1) Application rate in 200 L water/ha
2) Mortality: after 7 days of exposure to spray residues on leaf surfaces (Fisher's Exact Test, α = 0.05; * = significant)
3) Corrected mortality according to Abbott and improvements by Schneider-Orelli
4) Reproduction: mean number of eggs/female, (Dunnett's t-test, α = 0.05; * = significant)
5) Calculated on the exact raw data
6) LR_{50} was calculated with Weibull Analysis; 95 % confidence limits could not be determined due to mathematical reasons

Validity criteria:

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

Table 10.3.2.2- 4: Validity criteria

Validity criteria	Required	Obtained
Mortality/ escaping rate in the control group on day 7	≤ 20 %	11.0 %
Corrected mortality in the reference item group on day 7	≥ 50 %	100.0 %
Cumulative mean number of eggs per female in the control group (from day 7 to day 14)	≥ 4	8.8

III. CONCLUSION

The LR_{50} was estimated to be 1191 mL product/ha (95 % confidence limits could not be determined).

The ER_{50} was estimated to be > 675mL product/ha, the highest dose rate where reproduction was tested.

The figures obtained fulfilled the validity criteria of the laboratory method for exposure on leaves 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: ER_{50} > 675 mL product/ha

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Data Point:	KCP 10.3.2.2/03
Report Author:	[redacted]
Report Year:	2015
Report Title:	Toxicity to the predatory mite Typhlodromus pyri (Acari: Phytoseiidae) using an extended laboratory test with aged residues on apple bixafen + fluopyram + prothioconazole EC 260 (65 + 65 + 130 g/L)
Report No:	CW15/037
Document No:	M-535877-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable BLUMEL ET AL. (2000) modified
Deviations from current test guideline:	Current Guideline: Bluemel et al. (2000) Deviations: None; All validity criteria were met
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

In an extended laboratory test the effects on the survival and reproduction of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the predatory mite *Typhlodromus pyri* were investigated. One product rate of 2 x 1.5 L product/ha (interval of 14 days) was tested. Per test item rate 5 replicates of 20 individuals were exposed to aged residues BIX + FLU + PTZ EC 260 (65+65+130 g/L) on potted apple seedlings. On day 7 – 10 – 12 and 14 in the 1st and 3rd bioassay and day 7 – 8 – 11 and 14 in the 2nd bioassay the number of dead and living mites was counted, the dead mites were removed and the number of escaped mites was calculated. In all three bioassays the reproduction rate of surviving mites was evaluated over the period of 7 - 14 days after exposure by counting the total number of offspring (eggs and larvae) produced.

All validity criteria according to Blümel *et al.* (2000) were met.

The exposure of the test organisms to fresh residues (0 DAT) resulted in a mortality of 15.7 %. After 14 days (14 DAT) 7.2 % mortality occurred and 2.3 % corrected mortality was detected in the final bioassay started four weeks after the second application of the test item. In the first bioassay a reduction in reproductive success relative to the control of 71.6 % could be detected. The effect on reproduction decreased to 19.7 % reduction compared to the control after two weeks of aging. In the third bioassay no reduction was found anymore.

I. MATERIAL AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no.: 102000027828; batch no.: 2015-000475; sample description: TOX 10922-00; analysed content of active substance: 64.25 g/L bixafen, 64.82 g/L fluopyram, 129.2 g/L prothioconazole; density: 1.011 g/mL.

Test design: The product was applied two times with 1.5 L product/ha diluted in 400 L deionised water/ha on potted apple seedlings (*Malus sylvestris*). The application interval between was 14 days. The control was treated with deionised water in the same way as the test item. The toxic reference dimethoate was applied at 0.0476 L product/ha (20 g a.s./ha) diluted in 400 L deionised water/ha on the day of the second application on potted apple seedlings as well (for the first bioassay). For the further exposure dates it was applied directly on detached apple leaves (with 0.0476 L diluted in 200 L deionised water/ha; under laboratory conditions). It was included to indicate the relative susceptibility of the test organisms and the test system.

Aging of the spray deposits of the test item on the potted apple seedlings took place under semi-field conditions with UV permeable rain protection during the whole study. Three bioassays were performed, the first started on the day of the second application (0 DAT 2 = 0 days after treatment 2) and the last one four weeks later (28 DAT 2).

Predatory mites (*Typhlodromus pyri*) were exposed to these residues on the treated leaf surfaces (day 0). On day 1 and 4 the number of dead and living mites was counted, dead mites were removed and the number of escaped mites was calculated. On day 7, 10, 12 and 14 in the 1st and 3rd bioassay and day 7, 8, 11 and 14 in the 2nd bioassay the number of dead and living mites was counted, the dead mites were removed and the number of escaped mites was calculated. The number of females, males, eggs and juveniles was counted. Eggs and juveniles were removed. Mortality of 100 protonymphs was assessed up to 14 days after exposure in all bioassays.

In all three bioassays the reproduction rate of surviving mites was evaluated over the period of 7 - 14 days after exposure by counting the total number of offspring (eggs and larvae) produced.

Test conditions: The climatic conditions (temperature, relative humidity and light intensity) in the outdoor area were continuously recorded using a data logger. The temperature ranged from 9.5 - 42.0 °C and the relative humidity from 13 % - 99 % during the aging time of the apple seedlings. The laboratory phase for each exposure date was performed in a controlled environment room with temperature of 25 ± 2 °C and 60 - 90 % relative humidity.

Statistic: The mortality data were analysed for significance using the Fisher Exact test (one-sided with Bonferroni-Holm adjustment; α = 0.05). The reproduction data were tested for normal distribution using the Shapiro-Wilk test and for homogeneity of variance using the Levene test. As the reproduction data in the 1st bioassay were normally distributed but not homogenous the Welch test (α = 0.05) was used. As the reproduction data in the 2nd bioassay were not normally distributed the Wilcoxon test (one-sided with Bonferroni-Holm adjustment; α = 0.05) was used. As the reproduction data in the 3rd bioassay were normally distributed and homogenous one-way ANOVA and the Williams test (one-sided; α = 0.05) were used. The computer program SAS (Version 9.2, 2002-2008) was used to perform the statistical analyses.

Dates of work: June 25, 2015 – August 20, 2015

II. RESULTS AND DISCUSSION

In this extended laboratory test the effects of product residues (aged under semi-field conditions, with rain protection during the whole study) on the survival of the predatory mite *Typhlodromus pyri* were determined after two applications of 1.5 L product/ha with an application interval of 14 days onto apple seedlings (*Malus sylvestris*).

In the 1st bioassay started at the second application day of the test item, a corrected mortality of 15.7 % occurred which was statistically significant compared to the control. In the 2nd bioassay started 14 days later, a corrected mortality of 7.2 % was found. Only 2.3 % corrected mortality were detected in the 3rd bioassay started four weeks after the second application of the test item.

In the 1st bioassay a reduction in reproductive success relative to the control of 71.6 % could be detected, which was statistically significant compared to the control. The effect on reproduction decreased to 19.7 % reduction compared to the control after two weeks of aging. In the third bioassay no reduction was found anymore.

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

Table 10.3.2.2- 5: Effects of dried spray deposits on potted apple seedlings on the predatory mite *Typhlodromus pyri* (Acari: Phytoseiidae) in an extended laboratory study

Test item:	BIX + FLU + PTZ EC 260 (65+65+130 g/L)
Application:	2 x 1.5 L product/ha (interval of 14 days)
Test organism:	*Typhlodromus pyri*
Exposure on:	potted apple seedlings

Treatment	Mortality [%]			Reproduction		
	Uncorrected	Corrected	p-Value	Rate (eggs per female)	Reduction relative to control [%]	p-Value
0 DAT2 (0 weeks) 1)						
Control	17.0	-	-	4.2	-	-
Test item	30.0	15.7	0.022 *2)	1.2	71.6	0.004 *3)
Toxic ref.	97.0	96.4	-	-	-	-
14 DAT2 (2 weeks) 1)						
Control	17.0	-	-	5.1	-	-
Test item	23.0	7.2	0.188 2)	4.1	19.7	0.148 4)
Toxic ref.	100.0	100	-	-	-	-
28 DAT2 (4 weeks) 1)						
Control	14.0	-	-	4.6	-	-
Test item	16.0	2.3	0.422 2)	5.3	- 16.6	0.111 5)
Toxic ref.	99.0	98.8	-	-	-	-

* significant
1) Days after second treatment
2) Fisher´s Exact test (one-sided, α = 0.05), p-values adjusted according to Bonferroni-Holm
3) Welch test, α = 0.05
4) Wilcoxon test, (one-sided), p-values adjusted according to Bonferroni-Holm
5) one-way ANOVA, Williams test (one-sided, α = 0.05)

Validity criteria:

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

Table 10.3.2.2- 6: Validity criteria

Validity criteria	Required	Obtained
Mortality/ escaping rate in the control group on day 7	≤ 20 %	≤ 17 %
Corrected mortality in the reference item group on day 7	≥ 50 %	96.4 %
Cumulative mean number of eggs per female in the control group (from day 7 to day 14)	≥ 4	≥ 4.2

III. Conclusion

Two weeks after the application of 2 x 1.5 L product/ha with an application interval of 14 days, the effects on mortality and reproduction were less than 50 %.

The figures obtained fulfilled the validity criteria of the laboratory method for exposure on 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.

Two weeks after the application of 2 x 1.5 L product/ha with an application interval of 14 days, the effects on mortality and reproduction were less than 50 %.

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Data Point:	KCP 10.3.2.2/04
Report Author:	[redacted]
Report Year:	2013
Report Title:	Effects of bixafen + fluopyram + prothioconazole EC 260 (65 + 65 + 130 g/L) on the lacewing Chrysoperla carnea, extended laboratory study - Dose response test
Report No:	83873047
Document No:	M-476030-01-1
Guideline(s) followed in study:	Vogt et al. 2000; this guideline was modified for exposure of Chrysoperla carnea on natural substrate.
Deviations from current test guideline:	Current Guideline: Vogt et al. (2000); this guideline was modified for exposure of Chrysoperla carnea on natural substrate. Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In an extended laboratory test the effects on the survival and reproduction of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the lacewing *Chrysoperla carnea* were investigated. Five test item rates from 255 to 2550 mL product/ha were tested. Per test item rate 40 replicates of 1 individual were exposed to BIX + FLU + PTZ EC 260 (65+65+130 g/L) on treated bean leaves. The number of living and dead larvae and number of pupae developed was determined at least 3 times a week after test start and number of adults emerged was checked regularly. The number of eggs was counted after 24 hours egg-laying periods (checks) and 2 checks were done within one week. The number of larvae was determined after hatching of all larvae and the hatching rate was calculated.

All validity criteria according Vogt *et al.* (2000) were met.

Statistically significant different effects on mortality were observed at two highest product rates of 1434 (32.5 %) and 2550 (47.5 %) mL product/ha compared to the control. The LR_{50} was estimated to be > 2550 mL product/ha.

The reproduction was not statistically significant different compared to the control at all product rates.

I. Material and Methods

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L), specification no.: 102000027828-01; Batch ID: 2013-002935, sample description: TOX10413-001, content of a.s.: 6.49 % w/w (65.61 g/L) bixafen, 6.35 % w/w (64.21 g/L) fluopyram and 12.7 % w/w (128.5 g/L) prothioconazole; density: 1.011 g/mL.

Test design: Under extended laboratory conditions 2 - 3 day old larvae of the lacewing *Chrysoperla carnea* were exposed to dried spray deposits of 255, 453, 806, 1434 and 2550 mL product/ha (diluted in 200 L deionised water/ha) on treated bean leaves (*Phaseolus vulgaris*) (40 replicates, each containing 1 larva per treatment group). Deionised water was used as a control treatment and dimethoate (Perfekthion: 140 mL product/ha diluted in 200 L deionised water/ha) as a reference treatment. Exposure time (12 - 20 days; reference item only 5 days) lasted until pupae were transferred to the reproduction units for development of adults.

During the assessments the larvae were fed with UV-sterilized eggs of *Sitotroga cerealella*.

Mortality checks were carried out regularly until eclosion of adult lacewings (up to 23 days after test start). In addition, for the control and the test item treatment groups where the corrected mortality was < 50 %, the reproduction performance, *i.e.* egg deposition and larval hatching rate, was determined (2

checks/week, 24 hours period each check). The number of living and dead larvae and number of pupae developed was determined at least 3 times a week after test start and number of adults emerged was checked regularly.

The number of eggs was counted after 24 hours egg-laying periods (checks) and 2 checks were done within one week. The number of larvae was determined after hatching of all larvae and the hatching rate was calculated. No reproduction testing was performed with the reference item.

Test conditions: The climatic test conditions during the study were 23 - 26 °C temperature and 70 - 82 % relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 1020 - 1340 lux.

Statistics: Mortality data were analysed for significance using the Fisher's Exact Test, which is a distribution-free test and does not require testing for normality or homogeneity prior analysis.
The software used to perform the statistical analysis was ToxRat Professional, Version 2.10.05, ® ToxRat Solutions GmbH.

Dates of work: September 25, 2013 - November 01, 2013

II. RESULTS AND DISCUSSION

In this extended laboratory test the effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) residues on the survival of the green lacewing *Chrysoperla carnea* were determined at the rates of 255, 453, 806, 1434 and 2550 mL product/ha applied on treated bean leaves (*Phaseolus vulgaris*).

The pre-imaginal uncorrected mortality in the two highest product rates of 1434 and 2550 mL product/ha was statistically significant with 32.5 and 47.5 %, respectively.

The reproductive capacity of *C. carnea* was tested at all test item rates. There were no adverse effects of the test item on the reproductive performance.

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

Table 10.3.2.2- 7: Effects of dried spray residues on treated bean leaves on the green lacewing *Chrysoperla carnea* in an extended laboratory study

Test item	BIX + FLU + PTZ EC 260 (65+65+130 g/L)
Test organism	*Chrysoperla carnea*
Exposure on	treated bean leaves

		Preimaginal mortality [%]		Reproduction	
Treatment	**Rate [mL product/ha]** [1]	**Uncorrected** [2]	**Corrected** [3]	**Eggs per female and day**	**Fertility [hatching rate in %]**
Control	0	10.0	-	28.0	89.4
Test item	255	17.5	8.3	31.4	91.6
	453	7.5	-2.8	30.8	88.4
	806	15.0	5.6	37.8	89.2
	1434	32.5 *	25.0	37.8	89.2
	2550	47.5 *	41.7	17.9	86.5
Toxic ref.	140	100.0 *	100.0	-	-
LR_{50} > 2550 mL product/ha					

1) Application rate in 200 L deionised water/ha
2) Pre-imaginal mortality after exposure to spray residues on leaf surfaces (Fisher's Exact Test, α = 0.05, * = significant)
3) Corrected pre-imaginal mortality according to Abbott and improvements by Schneider-Orelli; negative values indicate better survivorship compared to control

Validity criteria:

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

Table 10.3.2.2- 8: Validity criteria

Validity criteria	Required	Obtained
Mortality in water control	≤ 20 %	10 %
Corrected mortality reference item	≥ 50 %	100 %
Mean number of eggs per female and day in water control	≥ 15	28
Mean hatching rate of the eggs (fertility) in water control	≥ 70 %	89.4 %

III. CONCLUSION

The LR_{50} was estimated to be > 2550 mL product/ha.

The figures obtained fulfil the validity criteria of the laboratory method for the exposure on glass according to Vogt *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} > 2550 mL product/ha

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Data Point:	KCP 10.3.2.2/05
Report Author:	[redacted]
Report Year:	2013
Report Title:	Effects of bixafen + fluopyram + prothioconazole EC 260 (65 + 65 + 130 g/L) on the ladybird beetle Coccinella septempunctata, extended laboratory study - Dose response test
Report No:	83874012
Document No:	M-476172-01-1
Guideline(s) followed in study:	Schmuck et al. 2000; this guideline was modified for exposure of C. septempunctata on natural substrate
Deviations from current test guideline:	Current Guideline: Schmuck et al. (2000) Deviations: None. All validity criteria were met.
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

In this extended laboratory study the effects of BIX+FLU+PTZ EC 260 (65+65+130 g/L) residues to larvae of the ladybird beetle *Coccinella septempunctata* were determined at 255, 453, 806, 1434 and 2550 mL product/ha. The application was done onto bean leaves *(Phaseolus vulgaris)* with 40 replicates, each containing 1 larva per treatment group. Mortality of the adults was assessed daily except at weekends. The number of living and dead larvae and pupae and number of adults hatched were counted daily except weekends.

Number of eggs was counted daily except at weekends, within the subsequent two weeks of oviposition. The number of larvae hatched was counted daily.

All validity criteria according to Schmuck *et al*. (2000) were met.

At the second highest product rate (1434 mL product/ha), a statistically significant difference in effect on mortality was observed compared to the control. The LR_{50} was estimated to be > 2550 mL product/ha.

No statistically significant differences in hatching rate was occurred at all product rates compared to the control.

I. Material and Methods

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no: 02000027828; Batch ID: 2013-002135; Sample Description: TOX10113-00; content of a.s.: 6.49 % w/w (65.61 g/L) bixafen, 6.35 % w/w (64.21 g/L) fluopyram and 12.7 % w/w (128.5 g/L) prothioconazole; density: 1.011 g/mL.

Test design: Under extended laboratory conditions 4 - 5 day old larvae of the ladybird beetle *Coccinella septempunctata* were exposed to dried spray deposits of 255, 453, 806, 1434 and 2550 mL product/ha

(diluted in 200 L deionised water/ha) on treated bean leaves (40 replicates, each containing 1 larva per treatment group). Deionised water was used as a control treatment and dimethoate (Perfekthion: 50 mL product/ha diluted in 200 L deionised water/ha) as a reference treatment. Exposure time lasted until the hatching of the adults.

During the assessments the larvae were fed with live aphids (*Acyrthosiphon pisum, Megoura viciae*) each day until larvae had entered the pupal stage.

The adults were fed with broad bean plants (*Vicia faba*) infested with aphids, *Acyrthosiphon pisum, Megoura viciae*. The infested plants were replaced once a week by fresh ones. If necessary, additional aphids were added. Fine ground pollen and honey were given *ad libitum*.

The number of living and dead larvae and pupae and number of adults hatched were counted daily except weekends. Mortality of the adults was assessed daily except at weekends, and the sex of the dead beetles was determined.
Number of eggs was counted daily except at weekends, within the subsequent two weeks of oviposition. The average number of eggs laid per female beetle per day was determined by dividing the total number of eggs laid within each group by the average number of viable females in that group. The number of larvae hatched was counted daily. Reproduction was performed where the corrected mortality was ≤ 50 %. No reproduction test was performed with the reference item.

Test conditions: The climatic test conditions during the study were 23 - 26 °C temperature and 70 - 80 % relative humidity. The light / dark cycle was 16:8 h with a light intensity range of 1000 - 2410 lux during the study.

Statistics: Mortality data were analysed for significance using the Fisher's Exact Test, 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 2.10.05, ® ToxRat Solutions GmbH.

Dates of work: September 04, 2013 - October 17, 2013

II. RESULTS AND DISCUSSION

In this extended laboratory study the effects of BIX+FLU+PTZ EC 260 (65+65+130 g/L) residues to larvae of the ladybird beetle *Coccinella septempunctata* were determined at 255, 453, 806, 1434 and 2550 mL product/ha. The application was done onto bean leaves *(Phaseolus vulgaris).*

The pre-imaginal mortality was 7.5 % in the control, 5.0 % at 255 and 453 mL product/ha, 0.0 % at 806 mL product/ha, 40.0 % at 1434 mL product/ha, which was statistically significant compared to the control, and 20.0 % at 2550 mL product/ha, respectively. This resulted in corrected mortalities of - 2.7 % at 255 and 453 mL product/ha, - 8.1 % at 806 mL product/ha, 35.1 % at 1434 mL product/ha and 13.5 % at 2550 mL product/ha, respectively. The LR_{50} was estimated to be > 2550 mL product/ha in 200 L water/ha.

The reproductive capacity of *C. septempunctata* was tested at all test item rates. Reproduction was > 2 fertile eggs per viable female per day at all tested test item rates, so the reproductive output is within the historical data base for control beetles and therefore this parameter is considered as not impacted by the treatment (Schmuck *et al.*, 2000) up to and including 2550 mL product/ha. No statistically significant differences in hatching rate was occurred at all product rates compared to the control.

Table 10.3.2.2- 9: Effects of dried spray residues on treated bean leaves on the ladybird beetle *Coccinella septempunctata* in an extended laboratory study

Test item		BIX + FLU + PTZ EC 260 (65+65+130 g/L)				
Test organism		*Coccinella septempunctata*				
Exposure on		treated bean leaves				
		Preimaginal mortality [%]		**Reproduction**		
Treatment	**Rate [mL product/ha]** 1)	**Uncorrected** 2)	**Corrected** 3)	**Eggs per female per day**	**Fertile eggs per female and day** 4)	**Hatching rate [%]**
Control	0	7.5	-	16.5	13.4	82.7
Test item	255	5.0	- 2.7	14.1	12.3	86.9
	453	5.0	- 2.7	9.0	7.9	85.5
	806	0.0	- 8.1	15.1	13.2	87.1
	1434	40.0 *	35.1	15.2	12.9	84.8
	2550	20.0	13.5	12.2	10.5	86.4
Toxic ref.	50	100.0 *	100.0	-	-	-
LR_{50} > 2550 mL product/ha						

S.D.: Standard deviation
1) Application rate in 200 L water/ha
2) Pre-imaginal mortality after exposure to spray residues on leaf surfaces (Fisher's Exact Test, α = 0.05, * = significant)
3) Corrected pre-imaginal mortality according to Abbott and improvements by Schneider-Orelli; negative values indicate better survivorship compared to control
4) Reproduction: mean number of fertile eggs/female/day

Validity criteria:

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

Table 10.3.2.2- 10: Validity criteria

Validity criteria	Required	Obtained
Preimaginal mortality in water control	≤ 30 %	7.5 %
Preimaginal mortality reference item	≥ 40 %	100 %
Mean number of fertile eggs per female and day in water control	≥ 2	13.4

III. CONCLUSION

The LR_{50} was estimated to be > 2550 mL product/ha.
The reproductive performance is not considered to be impacted by the test item.
The figures obtained fulfil the validity criteria of the laboratory method for exposure on glass plates according to Schmuck *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} > 2550 mL product/ha

CP 10.3.2.3 Semi-field studies with non-target arthropods

In view of the results presented above, no semi-field studies were deemed necessary.

CP 10.3.2.4 Field studies with non-target arthropods

In view of the results presented above, no field studies were deemed necessary.

CP 10.3.2.5 Other routes of exposure for non-target arthropods

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

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev. 2 final, 2002).

Predicted environmental concentrations used in risk assessment

For details of PEC_{soil} calculations refer to MCP Summary Section 9, Point 9.1.3.

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

Table 10.4- 1: Maximum PECsoil values for fluopyram, its metabolites and BIX+FLU+PTZ EC 260 in barley (for details see MCP Section 9, Point 9.1.3)

Compound	Barley		
	$PEC_{soil, initial}$ [mg/kg]	$PEC_{soil, plateau, 5\ cm}$ [mg/kg]	$PEC_{soil, acc}$*
1 × 39 g a.s./ha			
Fluopyram	0.010	0.009	0.019
Fluopyram-7-hydroxy	<0.001	<0.001	<0.001
Trifluoroacetic acid (TFA)	<0.001	<0.001	<0.001
1 × 78 g a.s./ha			
Fluopyram	0.021	0.018	0.039
Fluopyram-7-hydroxy	0.001	<0.001	0.001
Trifluoroacetic acid (TFA)	<0.001	<0.001	<0.001
1 × 0.6 L prod./ha			
BIX+FLU+PTZ EC 260	0.162 [1)]		
1 × 1.2 L prod./ha			
BIX+FLU+PTZ EC 260	0.324 [2)]		

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

1) The PEC_{soil} value for the product BIX+FLU+PTZ EC 260 is calculated based on the initial rate of the product (0.6 L/ha) in a single application, the portion reaching soil (BBCH 30-61, worst case interception of 80 % for spring cereals), the standard soil density (1.5 g/cm^3), the standard soil depth (5 cm) and the density of the formulation (1.011 g/mL).

2) The PEC_{soil} value for the product BIX+FLU+PTZ EC 260 is calculated based on the initial rate of the product (1.2 L/ha) in a single application, the portion reaching soil (BBCH 30-61, worst case interception of 80 % for spring cereals), the standard soil density (1.5 g/cm^3), the standard soil depth (5 cm) and the density of the formulation (1.011 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 BIX+FLU+PTZ EC 260, Fluopyram and its metabolites

Test item	Test species, test design	Ecotoxicological endpoint	Reference
BIX+FLU+PTZ EC 260	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 178 mg prod./kg dws $NOEC_{corr}$ = 89 mg prod./kg dws [A] EC_{10} = 136 mg prod./kg dws $EC_{10,corr}$ = 68 mg prod./kg dws [A]	[redacted] (2013) M-476477-01-1 KCP 10.4.1.1/01
FLU SC 500	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 134 mg a.s./kg dws [B] $NOEC_{corr}$ = 67 mg a.s./kg dws [A, B] EC_{10} = 117.5 mg a.s./kg dws [A] $EC_{10,corr}$ = 59 mg a.s./kg dws [A, B]	[redacted] (2020) M-680776-01-1 KCA 8.4.1/02 KCP 10.4.1.1/02 [C]
Fluopyram-7-hydroxy	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 18 mg p.m./kg dws $NOEC_{corr}$ = 9 mg p.m./kg dws [A] EC_{10} = calculation not possible	[redacted] (2021) M-762139-01-1 KCA 8.4.1/03
Trifluoroacetic acid (TFA)	*Eisenia fetida* reproduction 56 d, mixed	NOEC = 320 mg p.m./kg dws [D]	[redacted] (2005) M-251328-01-1 KCA 8.4.1/04

dws = dry weight soil, prod. = product

[A] Endpoint corrected by a factor of 2 due to lipophilic substance (log P_{OW} > 2)

[B] Endpoint calculated on the basis of analysed fluopyram content in the formulation (42.4 % w/w; as given in study report)

[C] Full details on this study are described in the corresponding MCP for the formulation FLU SC 500.

[D] NOEC of 320 mg/kg dws is based on effects on the body weight in the concentration 1000 mg/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 BIX+FLU+PTZ EC 260

Compound	Species, study type	Endpoint [mg prod./kg]	PEC_{soil} [mg prod./kg]	TER_{LT}	Trigger
Barley, 1 × 0.6 L prod./ha					
BIX + FLU +PTZ EC 260	Earthworm, reproduction	NOEC = 89	0.162	549	5
Barley, 1 × 1.2 L prod./ha					
BIX + FLU +PTZ EC 260	Earthworm, reproduction	NOEC = 89	0.324	275	5

Table 10.4.1- 3: TER calculation for earthworms for fluopyram and its metabolites

Compound	Species, study type	Endpoint [mg/kg]	PEC_{soil} [mg/kg]	TER_{LT}	Trigger
Barley, 1 × 39 g a.s./ha					
Fluopyram	Earthworm, reproduction	NOEC = 67	0.019	3526	5
Fluopyram-7-hydroxy	Earthworm, reproduction	NOEC = 9	<0.001	>45000 [A]	5
Trifluoroacetic acid (TFA)	Earthworm, reproduction	NOEC = 320	<0.001	>320000	5
Barley, 1 × 78 g a.s./ha					
Fluopyram	Earthworm, reproduction	NOEC = 67	0.039	1718	5
Fluopyram-7-hydroxy	Earthworm, reproduction	NOEC = 9	0.001	45000 [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 BIX + FLU +PTZ EC 260 in barley.

CP 10.4.1.1 Earthworms sub-lethal effects

Data Point:	KCP 10.4.1.1/01
Report Author:	[redacted]
Report Year:	2013
Report Title:	Bixafen + fluopyram + prothioconazole EC 260 (65+65+130) G: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Report No:	13 10 48 183 S
Document No:	M-476477-01-1
Guideline(s) followed in study:	OECD 222 (2004), ISO 11268-2 (1998)
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 BIX + FLU + PTZ EC 260 (65+65+130 g/L) on survival and reproduction of adult earthworms *Eisenia fetida* was tested during an exposure of 4 weeks (first part) in artificial soil by comparing control and treatment. After this period, the adult earthworms were removed from the test vessels and the cocoons and juvenile earthworms remained in the test vessels for additional 4 weeks (second part). The total duration of the study was 8 weeks. Five test item rates from 100 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 BIX + FLU + PTZ EC 260 (65+65+130 g/L) mixed into artificial soil.
After a period of 4 weeks the survivors were counted, and their fresh weight was measured. From these data mortality and biomass effects were determined. After an additional four weeks exposure of the cocoons and juvenile earthworms the reproduction was determined by counting the number of off-spring hatched from the cocoons per test vessel.

The study fulfilled all validity criteria of OECD 222 guideline.

The endpoints were: $NOEC_{mortality}$ ≥1000 mg prod./kg dry weight artificial soil, $LOEC_{mortality}$ >1000 mg prod./kg dry weight artificial soil, $NOEC_{growth}$ = 562 mg prod./kg dry weight artificial soil, $LOEC_{growth}$ = 1000 mg prod./kg dry weight artificial soil, $NOEC_{reproduction}$ = 178 mg prod./kg dry weight artificial soil, $LOEC_{reproduction}$ = 316 mg prod./kg dry weight artificial soil.
The EC_{10} and EC_{20} related to reproduction are determined to be 136 and 195 mg prod./kg dry weight artificial soil, respectively.

I. MATERIALS AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L), specification no.: 102000027828 - 01; Batch No.: 2013-002135; TOX10113-00, analytical findings: 6.49 % w/w (65.61 g/L) bixafen; 6.35 % w/w (64.21 g/L) fluopyram, 12.7 % w/w (128.5 g/L) prothioconazole, density (20 °C): 1.011 g/mL.
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): 68.5 % industrial quartz sand, 10% Sphagnum peat (air dried and finely ground), 20 % Kaolinite clay.
After four weeks, the adult worms were removed from the test vessels. The number of surviving worms (adult mortality) and their biomass change were determined, behaviour (including feeding activity) and pathological symptoms were recorded. The adult worms were discarded after counting and weighing.
At the end of the test after 8 weeks, the number of surviving juveniles per test vessel was determined and emerging earthworms were removed and counted.
Test conditions: The climatic conditions were in the temperature range 19.6 - 22.0 °C with a photoperiod of 16 hours light and a light intensity of 570 lux.
Statistics: The statistical analysis was performed with the software ToxRat Professional 2.10.06. The EC_{10} and EC_{20} (number of juveniles) were estimated by Probit analysis using the maximum likelihood method. Confidence limits (95 %) of the EC values were computed by normal approximation. Fisher`s Exact Binomial Test with Bonferroni Correction and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

Dates of work: September 13, 2013 - November 08, 2013

II. RESULTS AND DISCUSSION

Table 10.4.1.1- 1: Effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on *Eisenia fetida*

Parameter	Treatment [mg prod./kg dry weight artificial soil]					
	Control	**100**	**178**	**316**	**562**	**1000**
Mortality of adult earthworms [%] after 28 days	1.3	0.0	2.5	0.0	2.5	2.5
Significance (Mortality)	-	-	-	-	-	-
Mean change of body fresh weight of the adults from day 0 to day 28 [%] *	57.3	56.7	57.7	55.9	56.0	40.1
Standard deviation	33.1	17.0	12.1	21.5	38.7	16.3
Significance (body fresh weight)	-	-	-	-	-	+
Mean number of off-spring per test vessel after 56 days *	119.0	120.8	99.8	63.3*	48.3*	10.5*
Standard deviation	22.8	21.8	15.5	19.2	9.9	6.2
Coefficient of variance (%)	19.1	18.1	15.6	30.3	20.5	59.5
% of control	100	101.5	83.8	53.2	40.5	8.8
Significance (reproduction)	-	-	-	+	+	+
	Adult Mortality			**Growth**	**Reproduction**	
NOEC [mg prod./kg dry weight soil]	**≥ 1000**			**562**	**178**	
LOEC [mg prod./kg dry weight soil]	**> 1000**			**1000**	**316**	
EC_{10} [mg prod./kg dry weight artificial soil] ** (95% confidence limits)					136 (73 – 252)	
EC_{20} [mg prod./kg dry weight artificial soil] ** (95% confidence limits)					195 (124 – 308)	

* Statistically significantly different compared to control (Williams-t-test, α = 0.05, one-sided smaller)

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

Mortality:

No statistically significant mortality compared to the control was observed at any test item concentration (Fisher's Exact Binomial Test with Bonferroni Correction, α = 0.05, one-sided greater). 1.3 % mortality occurred in the control group. With the exception of 2.5 % mortality at concentrations of 178, 562 and 1000 mg prod./kg dry weight soil no mortality was observed in any other test item treatment group. 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.

No effects on behaviour (including feeding activity) of the worms were observed during the test.

Effects on growth:

The test item caused no statistically significant change in biomass compared to the control group up to the highest test item rate (1000 mg prod/kg soil d.w.). At a concentration of 1000 mg prod./kg soil d.w. the biomass change of 40.1 % was statistically significant (Williams-t-test, α = 0.05, one-sided smaller) compared to the control group. Therefore, the endpoints related to growth were: NOEC: 562 mg prod./kg dry weight artificial soil and LOEC: 1000 mg prod./kg dry weight artificial soil.

Effects on reproduction:
Statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on number of juveniles compared to the control group were recorded at the three highest test item rates (316, 562 and 1000 mg prod./kg d.w.). Therefore, the endpoints related to reproduction were: NOEC: 178 mg prod./kg dry weight artificial soil and LOEC: 316 mg prod./kg dry weight artificial soil. The EC_{10} (C.I.) and EC_{20} (C.I.) values were calculated to be 136 (73 - 252) mg prod./kg dry weight soil and 195 (124 - 308) mg prod./kg dry weight soil, 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 %	1.3 %
Number of juveniles (earthworms per control vessel)	≥ 30	≥ 110
Coefficient of variance of reproduction in the control	≤ 30 %	19.1 %

Reference test:
The reference item Nutdazim 50 FLOW (Carbendazim, SC 500) was tested in a separate study at concentrations of 5 and 10 mg product/kg soil dry weight.
In the most recent study with Nutdazim 50 FLOW (BioChem project No. R 13 10 48 005 S, dated November 22, 2013), the number of juveniles was reduced by 39 and 100 % at concentrations of 5 and 10 mg product/kg soil dry weight (mean number of juveniles = 77 and 0) after 8 weeks of test duration when compared to control (mean number of juveniles = 127).

III. Conclusion

All validity criteria were met. The endpoints were:

$NOEC_{mortality}$: > 1000 mg prod./kg d.w. artificial soil
$LOEC_{mortality}$: > 1000 mg prod./kg d.w. artificial soil

$NOEC_{growth}$: = 562 mg prod./kg d.w. artificial soil
$LOEC_{growth}$: = 1000 mg prod./kg d.w. artificial soil

$NOEC_{reproduction}$: = 178 mg prod./kg d.w. artificial soil
$LOEC_{reproduction}$: = 316 mg prod./kg d.w. artificial soil
$EC_{10reproduction}$: = 136 mg prod./kg d.w. artificial soil
$EC_{20reproduction}$: = 195 mg prod./kg d.w. artificial soil

According to EFSA (2015) the level of protection for the EC_{10} is classified as “medium”. The normalised width of confidence interval (NW) rating for the EC_{10} is “poor”.

Assessment and conclusion by applicant:

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

The endpoint is: $NOEC_{reproduction}$ = 178 mg prod./kg dws

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 BIX+FLU+PTZ EC 260 and fluopyram metabolites

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
Springtails, reproduction			
BIX+FLU+PTZ EC 260	*Folsomia candida* reproduction 28 d, mixed	NOEC = 100 mg prod./kg dws $NOEC_{corr}$ = 50 mg prod./kg dws [A] EC_{10} = 149 mg prod./kg dws $EC_{10,corr}$ = 74.5 mg prod./kg dws [A]	[redacted] (2014) M-481544-01-1 KCP 10.4.1.2/01
FLU SC 500	*Folsomia candida* reproduction 28 d, mixed	NOEC = 75.5 mg a.s./kg dws [B] $NOEC_{corr}$ = 37.8 mg a.s./kg dws [A, B] EC_{10} = 102 mg a.s./kg dws [B] $EC_{10,corr}$ = 51 mg a.s./kg dws [A, B]	[redacted] (2019) M-675002-01-1 KCA 8.4.2.1/01 KCP 10.4.2.1/03 [C]
Fluopyram-7-hydroxy	*Folsomia candida* reproduction 28 d, mixed	NOEC = 562 mg p m./kg dws $NOEC_{corr}$ = 281 mg p m./kg dws [A] EC_{10} = 611 mg p m./kg dws	[redacted] (2020) M-755397-01-1 KCA 8.4.2.1/03
Trifluoroacetic acid (TFA)	*Folsomia candida* reproduction 28 d, mixed	NOEC ≥ 100 mg p m./kg dws (Na-TFA) ≥ 84 mg p.m./kg dws (TFA) [D] EC_{10} calculation not possible	[redacted] (2012) M-436127-01-1 KCA 8.4.2.1/05
Soil mites, reproduction			
BIX+FLU+PTZ EC 260	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC = 178 mg prod./kg dws $NOEC_{corr}$ = 89 mg prod./kg dws [A] EC_{10} = 281 mg prod./kg dws $EC_{10,corr}$ = 140.5 mg prod./kg dws [A]	[redacted] (2017) M-473037-02-1 KCP 10.4.1.2/02
FLU SC 500	*Hypoaspis aculeifer* reproduction 14 d, mixed	NOEC ≥ 424 mg a.s./kg dws [B] $NOEC_{corr}$ ≥ 212 mg a.s./kg dws [A, B] EC_{10} calculation not possible	[redacted] (2020) M-678468-01-1 KCA 8.4.2.1/02 KCP 10.4.2.1/04 [C]

Test substance	Test species, test design	Ecotoxicological Endpoint	Reference
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) [D] 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 Full details on this study are described in the corresponding MCP for the formulation FLU SC 500.

D As the study was conducted with sodium trifluoroacetate which is the sodium salt of trifluoroacetic acid, the endpoint was converted to trifluoroacetic acid with factor 0.84.

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

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 BIX+FLU+PTZ EC 260

Compound	Species, study type	Endpoint [mg prod./kg]	PEC_{soil} [mg prod./kg]	TER_{LT}	Trigger
Barley, 1 × 0.6 L prod./ha					
BIX+FLU+PTZ EC 260	*Folsomia candida*	NOEC = 50	0.162	309	5
BIX+FLU+PTZ EC 260	*Hypoaspis aculeifer*	NOEC = 89	0.162	549	5
Barley, 1 × 1.2 L prod./ha					
BIX+FLU+PTZ EC 260	*Folsomia candida*	NOEC = 50	0.324	154	5
BIX+FLU+PTZ EC 260	*Hypoaspis aculeifer*	NOEC = 89	0.324	275	5

Table 10.4.2- 3: TER calculation for other non-target soil meso- and macrofauna for fluopyram and its metabolites

Compound	Species, study type	Endpoint [mg/kg]	PEC_{soil} [mg/kg]	TER_{LT}	Trigger
Barely, 1 × 39 g a.s./ha					
Fluopyram [A]	*Folsomia candida*	NOEC = 37.8	0.019	1989	5
Fluopyram [A]	*Hypoaspis aculeifer*	NOEC ≥ 212	0.019	≥ 11158	5
Fluopyram-7-hydroxy	*Folsomia candida*	NOEC = 281	<0.001	140500 [B]	5
Fluopyram-7-hydroxy	*Hypoaspis aculeifer*	NOEC ≥ 500	<0.001	≥ 250000 [B]	5
Trifluoroacetic acid (TFA)	*Folsomia candida*	NOEC ≥ 84	<0.001	≥ 84000	5
Trifluoroacetic acid (TFA)	*Hypoaspis aculeifer*	NOEC = 84	<0.001	≥ 84000	5
Barley, 1 × 78 g a.s./ha					
Fluopyram [A]	*Folsomia candida*	NOEC = 37.8	0.039	969	5
Fluopyram [A]	*Hypoaspis aculeifer*	NOEC ≥ 212	0.039	≥ 5436	5
Fluopyram-7-hydroxy	*Folsomia candida*	NOEC = 281	0.001	140500 [B]	5
Fluopyram-7-hydroxy	*Hypoaspis aculeifer*	NOEC ≥ 500	0.001	≥ 250000 [B]	5
Trifluoroacetic acid (TFA)	*Folsomia candida*	NOEC ≥ 84	<0.001	≥ 84000	5
Trifluoroacetic acid (TFA)	*Hypoaspis aculeifer*	NOEC ≥ 84	<0.001	≥ 84000	5

[A] endpoint derived from study performed with FLU SC 500

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

All TER values clearly exceed the trigger value of 5 indicating that no unacceptable adverse effects on soil macro-organisms are to be expected from the intended use of BIX + FLU +PTZ EC 260 in barley.

CP 10.4.2.1 Species level testing

Data Point:	KCP 10.4.2.1/01
Report Author:	[redacted]
Report Year:	2014
Report Title:	Bixafen + fluopyram + prothioconazole EC 260 (65+65+130) G: Influence on the reproduction of the collembolan species Folsomia candida tested in artificial soil
Report No:	FRM-Coll-166/14
Document No:	M-481544-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable OECD 232 (2009)
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) Deviations: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

In a laboratory study the effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on survival and reproduction of the collembolan species *Folsomia candida* was tested during an exposure of 28 days in artificial soil by comparing control and treatment. In the 1st test run five test item rates from 10 to 100 mg product/kg dry weight soil were tested. Since the 1st test run on the test item did not provide a final result, a 2nd test run was performed studying higher test concentrations with four test item rates from 178 to 1000 mg prod./kg dry weight soil. Per test item rate 4 replicates and for the control group 8 replicates with 10 collembolans each were exposed to BIX + FLU + PTZ EC 260 (65+65+130 g/L) mixed into artificial soil. Mortality and reproduction of the collembolans was assessed after 28 days.

The study fulfilled all validity criteria of OECD 232 guideline.

Concerning the number of juveniles statistical analysis revealed no significant difference between control and any treatment group (10 - 100 mg prod./kg dry weight artificial soil) in the 1st run. In the 2nd test run statistical analysis revealed statistically significant difference between control and all treatment groups (178 - 1000 mg prod./kg dry weight artificial soil).

Therefore, the No-Observed-Effect-Concentration (NOEC) for reproduction is 100 mg prod./kg artificial dry weight soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 178 mg prod./kg artificial dry weight soil.

I. MATERIALS AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no.: 102000027828-01; Batch No.: 2013-002135; TOX10113-00; analytical findings: 6.49 % w/w bixafen equivalent to 65.61 g/L; 6.35 % w/w fluopyram equivalent to 64.21 g/L; 12.7 % w/w prothioconazole equivalent to 128.5 g/L; density: 1.191 g/mL (20 °C).

Test design: Since the 1st test run on the test item did not provide a final result, a 2nd test run was performed studying higher test concentrations. In the 1st test run 10 collembolans (9 - 12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated), 10, 18, 32, 56, and 100 mg prod./kg artificial dry weight soil. In the 2nd test run

10 collembolans (9 - 12 days old) per replicate (8 replicates for the control group and 4 replicates for each treatment group) were exposed to control (water treated) and 178, 316, 562 and 1000 mg prod./kg artificial dry weight 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.
Test 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 Welch-t test, one-sided-smaller, α = 0.05, for the 1st run and William's-t test, one-sided-smaller, α = 0.05, for the 2nd run was applied to reproduction data. The ECx values were calculated with Probit analysis.

Dates of work: October 21, 2013 – February 20, 2014

II. Results and Discussion

Table 10.4.2.1- 1: Effects on mortality and reproduction of *Folsomia candida* after treatment with BIX + FLU + PTZ EC 260 (65+65+130 g/L)

Test concentration [mg prod./dry weight artificial soil]	Adult mortality [%]	Mean number of juveniles per test vessel ± SD	Reproduction [% of control]	Significance (*)
1st run				
Control	6.3	1526.9 ± 76.1		
10	10.0	1481.7 ± 60.5	97.0	-
18	13.0	1437.5 ± 138.2	94.1	-
32	10.0	1553.8 ± 24.3	101.8	-
56	15.0	1500.3 ± 55.4	98.3	-
100	5.0	1490.0 ± 73.6	97.6	-
2nd run				
Control	7.5	1504.0 ± 186.8		
178	5.0	1214.8 ± 267.1	80.8	+
316	32.5	515.3 ± 107.6	34.3	+
562	92.5	32.8 ± 38.0	2.2	+
1000	100.0	0.0 ± 0.0	0.0	+

Endpoints	Reproduction
NOEC [mg prod./kg dry weight artificial soil]	100
LOEC [mg prod./kg dry weight artificial soil]	178
EC_{10} [mg prod./kg dry weight artificial soil] [1] (95 % confidence limits)	149 (128 - 167)
EC_{20} [mg prod./kg dry weight artificial soil] [1] (95 % confidence limits)	181 (162 - 197)

Calculations were done with unrounded values.
SD: Standard deviation
[1] ECx = Probit analysis
* (Welch-t test one-sided-smaller, α = 0.05, + = significant, - = not significant) 1st run
* (William's-t test one-sided-smaller, α = 0.05, + = significant, - = not significant) 2nd run

Mortality:
In the control group 6.3 % (1st run) and 7.5 % (2nd run) of the adult *Folsomia candida* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Welch-t test, one-sided smaller, α = 0.05) revealed no significant difference between control and any treatment group in the 1st run. In the 2nd test run William's-t test, one-sided smaller, α = 0.05 revealed statistically significant difference between control and all treatment groups.
Therefore the NOEC for reproduction is 100 mg prod./kg artificial dry weight soil. The LOEC for reproduction is 178 mg prod./kg artificial dry weight soil.

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	
		1st run	2nd run
Mean adult mortality in control	≤ 20 %	6.3 %	7.5 %
Mean number of juveniles per replicate (with 10 collembolans introduced)	≥ 100	1526.9	1504.0
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	5.0 %	12.4 %

All validity criteria of the OECD 232 guideline were fulfilled.

Reference test:
The most recent non-GLP-test (FRM-Coll-Ref-24/14) with the reference item Boric acid was performed at test concentrations 44 – 67 – 100 – 150 and 225 mg Boric acid/kg artificial dry weight soil.
Boric acid showed an EC_{50} of 90 mg test item/kg artificial dry weight soil (95 % confidence limits from 68 mg to 119 mg Boric acid/kg artificial dry weight soil) for reproduction according Probit analysis using maximum likelihood regression.
The result is in the recommended range of the guideline (about 100 mg Boric acid/kg artificial dry weight soil).
The $NOEC_{reproduction}$ was calculated to be < 44 mg Boric acid/kg artificial dry weight soil and accordingly the $LOEC_{reproduction}$ is 44 mg Boric acid/kg artificial dry weight soil according Williams multiple t-test procedure, α = 0.05, one-sided smaller. This shows that the test organisms are sufficiently sensitive.

III. Conclusion

All validity criteria were met. The endpoints were:

$NOEC_{reproduction}$: 100 mg prod./kg artificial dry weight soil
$LOEC_{reproduction}$: 178 mg prod./kg artificial dry weight soil
$EC_{10\ reproduction}$: 149 mg prod./kg artificial dry weight soil
$EC_{20\ reproduction}$: 181 mg prod./kg artificial dry weight 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 = 100 mg prod./kg dws

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Data Point:	KCP 10.4.2.1/02
Report Author:	[redacted]
Report Year:	2017
Report Title:	Amendment no. 1: Bixafen + fluopyram + prothioconazole EC 260 (65+65+130) G: Influence on mortality and reproduction of the soil mite species Hypoaspis aculeifer tested in artificial soil
Report No:	E 428 4570-0
Document No:	M-473037-02-1
Guideline(s) followed in study:	EU Directive 91/414/EEC; Regulation (EC) No. 1107/2009; US EPA OCSPP: not applicable; OECD 226 from October 03, 2008: OECD guideline for the Testing of Chemicals - Predatory mite (Hypoaspis (Geolaelaps) aculeifer) reproduction test in soil
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 BIX + FLU + PTZ EC 260 (65+65+130 g/L) on survival and reproduction of the soil mite species *Hypoaspis aculeifer* was tested during an exposure of 14 days in artificial soil by comparing control and treatment. Five test item rates from 100 to 1000 mg product/kg dry weight soil were tested. Per test item rate 4 replicates and for the control group 8 replicates with 10 soil mites each were exposed to BIX + FLU + PTZ EC 260 (65+65+130 g/L) mixed into artificial soil. Mortality of the soil mites was assessed after 14 days.

The study fulfilled all validity criteria of OECD 226 guideline.

No statistically significant differences compared to the control occurred.

The reproduction rate of the soil mites was assessed after 14 days. Concerning the number of juveniles statistical analysis (Williams t-test, one-sided smaller, α = 0.05) revealed significant differences between control and the three highest treatment groups. Therefore the No-Observed-Effect-Concentration (NOEC) for reproduction is 178 mg prod./kg artificial dry weight soil. The Lowest-Observed-Effect-Concentration (LOEC) for reproduction is 316 mg prod./kg artificial dry weight soil.

I. MATERIALS AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no.: 102000027828-01; Batch No.: 2013-002135; TOX10113-00; analytical findings: 6.49 % w/w bixafen equivalent to 65.61 g/L; 6.35 % w/w fluopyram equivalent to 64.21 g/L; 12.7 % w/w prothioconazole equivalent to 128.5 g/L; density: 1.011 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 3 days after start of egg laying). Concentrations of 100, 178, 316, 562 and 1000 mg prod./kg dry weight artificial soil were mixed into the artificial soil. During the test, the *Hypoaspis aculeifer* were fed with cheese mites bred on brewer's 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, 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.

Test 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 to 800 lux.

Statistics: Williams t-test (one-sided smaller, α = 0.05) was applied to reproduction data. The ECx values were calculated with Weibull analysis.

Dates of work: October 21, 2013 – November 12, 2013

II. RESULTS AND DISCUSSION

Table 10.4.2.1- 3: Effects on mortality and reproduction of *Hypoaspis aculeifer* after treatment with BIX + FLU + PTZ EC 260 (65+65+130 g/L)

Test concentration [mg prod./dry weight artificial soil]	Adult mortality [%]	Mean number of juveniles per test vessel ± SD	Reproduction [% of control]	Significance (*)
Control	0.0	345.9 ± 18.8	-	-
100	2.5	327.8 ± 17.3	94.8	-
178	0.0	321.5 ± 15.6	93.0	-
316	7.5	282.0 ± 38.6	81.5	+
562	0.0	272.0 ± 7.9	78.6	+
1000	7.5	123.3 ± 25.5	35.6	+

Endpoints	Reproduction
NOEC [mg prod./kg dry weight artificial soil]	178
LOEC [mg prod./kg dry weight artificial soil]	316
EC_{10} [mg prod./kg dry weight artificial soil] 1) (95% confidence limits)	281 (26 – 448)
EC_{20} [mg prod./kg dry weight artificial soil] 1) (95 % confidence limits)	434 (105 – 598)

SD: Standard deviation
1) ECx = Weibull analysis
(*) Williams t-test, one-sided smaller, α = 0.05, "-": non-significant; "+": significant

Mortality:
In the control group 0 % of the adult *Hypoaspis aculeifer* died which is below the allowed maximum of ≤ 20 % mortality.

Reproduction:
Concerning the number of juveniles statistical analysis (Williams t-test, one-sided smaller, $\alpha = 0.05$) revealed significant differences between control and the three highest treatment groups.
Therefore the NOEC for reproduction is 178 mg prod./kg dry weight artificial soil. The LOEC for reproduction is 316 mg prod./kg dry weight artificial soil.

Validity criteria:
Validity criteria for the untreated control of the study according OECD 226 from July 29, 2016 were used.

All validity criteria of the OECD 226 guideline were fulfilled.

Table 10.4.2.1- 4: Validity criteria

Validity criteria acc. to OECD 226 (adopted 2016)	Required	Obtained
Mean adult mortality	≤ 20 %	0 %
Mean number of juveniles per replicate (with 10 mites introduced)	≥ 50	345.9
Coefficient of variation calculated for the number of juveniles per replicate	≤ 30 %	5.4 %

Reference test:
The most recent non-GLP-test (kra/HR-O-12/13) with the reference item dimethoate was performed at test concentrations 1.0, 1.8, 3.2, 5.6 and 10.0 mg dimethoate/kg dry weight artificial soil.

Dimethoate showed a LC_{50} of 4.32 mg a.s./kg (95 % confidence limits from 4.31 mg a. s./kg to 4.32 mg a. s./kg) for mortality of the adult mites according Probit analysis using maximum likelihood regression.

The reproduction of the soil mites was not significantly reduced in comparison to the control up to 3.2 mg a.s./kg dry weight artificial soil. Therefore the NOEC is calculated to be 3.2 mg a.s./kg and accordingly the LOEC is 5.6 mg a.s./kg. Since variances of the data were homogenous Williams-t test $\alpha = 0.05$, one-sided smaller was used. Dimethoate EC 400E G showed an EC_{50} of 5.67 mg a. s./kg (95 % confidence limits from 5.58 mg a. s./kg to 5.79 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:

$NOEC_{reproduction}$: 178 mg prod./kg dry weight artificial soil
$LOEC_{reproduction}$: 316 mg prod./kg dry weight artificial soil
$EC_{10\ reproduction}$: 281 mg prod./kg dry weight artificial soil
$EC_{20\ reproduction}$: 434 mg prod./kg dry weight artificial soil

According to EFSA (2015) the level of protection for the EC_{10} is classified as “medium”. The normalised width of confidence interval (NW) rating for the EC_{10} is “poor”.

Assessment and conclusion by applicant:

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

The endpoint is: NOEC = 178 mg prod./kg dws

CP 10.4.2.2 Higher tier testing

In view of the results presented in Section CP 10.4.2, no further testing is necessary.

CP 10.5 Effects on soil nitrogen transformation

Table 10.5- 1: Studies on nitrogen transformation with BIX+FLU+PTZ EC 260, Fluopyram and its metabolites

Test substance	Test species, test design	Ecotoxicological endpoint	Reference
N-transformation			
BIX+FLU+PTZ EC 260	Study duration 42 d	No unacceptable effects at an appl. rate of: 20.22 mg prod./kg dws	[redacted] (2015) M-472714-01-1 KCP 10.5/01
Fluopyram	Study duration 28 d	No unacceptable effects at an appl. rate of: **3.33 mg a.s./kg dws**	[redacted] (2020) M-781177-01-1 KCA 8.5/04
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 BIX+FLU+PTZ EC 260 for soil micro-organisms

Compound	Species	Endpoint [mg prod./kg]	$PEC_{soil, max}$ [mg prod./kg]	Refinement required
Barley, 1 × 0.6 L prod./ha				
BIX+FLU+PTZ EC 260	Soil micro-organisms	20.22	0.162	No
Barley, 1 × 0.12 L prod./ha				
BIX+FLU+PTZ EC 260	Soil micro-organisms	20.22	0.324	No

Table 10.5- 3: Risk Assessment for Fluopyram and its metabolites for soil micro-organisms

Compound	Species	Endpoint [mg/kg]	$PEC_{soil, max}$ [mg/kg]	Refinement required
Barley, 1 × 39 g a.s./ha				
Fluopyram	Soil micro-organisms	3.33	0.019	No
Fluopyram-7-hydroxy	Soil micro-organisms	10	<0.001	No [A]
Trifluoroacetic acid (TFA)	Soil micro-organisms	1.344	<0.001	No
Barley, 1 × 78 g a.s./ha				
Fluopyram	Soil micro-organisms	3.33	0.039	No
Fluopyram-7-hydroxy	Soil micro-organisms	10	0.001	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:	2013
Report Title:	Bixafen + fluopyram + prothioconazole EC 260 (65+65+130) G: Effects on the activity of soil microflora - (Nitrogen transformation test
Report No:	13 10 48 115 N
Document No:	M-472714-01-1
Guideline(s) followed in study:	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 BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the activity of soil microflora with regard to nitrogen transformation was tested during an exposure of 42 days in a loamy sand soil by comparing control and treatment. Two test item rates of 2.02 and 20.22 mg product/kg dry weight soil (equivalent to 1.5 and 15 L test item/ha) were tested. Per treatment there were 3 replicates. The soil was enriched by 0.5 % lucerne meal and a water content of 43 - 44 % of the water holding capacity was maintained during the test.

The study fulfilled all validity criteria of OECD 216 guideline.

Nitrogen transformation was determined after 3 hours, 7, 14, 28 days and 42 days. No adverse effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on nitrogen transformation in soil could be observed at 2.02 mg prod./kg dry weight soil 28 days after application (time interval 14 - 28 days after application). The test item caused a temporary inhibition and a temporary stimulation of the daily nitrate rate at the tested concentration of 20.22 mg prod./kg soil dry weight at time interval 7 - 14 and time interval 14 - 28 days after application, respectively.

I. MATERIALS AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L), Specification No.: 102000027828-01, Batch ID: 2013-002135, Sample description: TOX10113-00, analytical findings: 6.49 % w/w, 65.61 g bixafen/L, 6.35 % w/w, 64.21 g fluopyram/L, 12.7 % w/w, 128.5 g prothioconazole/L, density: 1.011 g/mL.

Test design: A loamy sand soil (DIN 4220) with pH 6.5, 1.47 % C_{org} and with the water holding capacity of 15.62 - 16.17 g/100 g dry soil was exposed for 42 days to 2.02 and 20.22 mg prod./kg soil dry weight. Application rates were equivalent to 1.5 and 15 L prod./ha. For calculation of the test concentrations (mg/kg dry weight soil) 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 nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). 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 2.16 mg/100 g dry weight soil.

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. NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an autoanalyzer at different sampling intervals (0, 7, 14, 28 and 42 days after treatment).
Soil samples (10 g soil d.w. per replicate) were taken at intervals of 3 hours, 7, 14, 28 and 42 days after application and the NH_4-N-, NO_3-N- and NO_2-N-contents were determined.
Test conditions: The test vessels were kept in darkness in a climatic room and the temperature ranged between 19.3 -20.9 °C during the test. The water content of the soil ranged between 42.55 - 44.04 % of WHC. The pH value of the soil ranged between 6.1 and 6.3.

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).

Dates of work: September 16, 2013 – October 28, 2013

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 2.2 % 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 BIX+FLU+PTZ EC 260 (65+65+130 g/L)

Time interval [days]	Control			2.02 mg prod./kg dry weight soil equivalent to 1.5 L prod./ha				20.22 mg prod./kg dry weight soil equivalent to 15 L prod./ha			
	Nitrate-N[1]			Nitrate-N[1]			% difference to control	Nitrate-N[1]			% difference to control
0 - 7	3.37	±	0.17	3.20	±	0.19	-5.1 n s	3.97	±	0.49	+17.8 n s
7 - 14	1.76	±	0.16	1.88	±	0.47	+6.8 n s	0.91	±	0.49	-48.2 *s
14 - 28	0.88	±	0.08	0.96	±	0.24	+9.8 n s	1.17	±	0.14	+34.0 *s
28 - 42	0.77		0.03	-[2]		-[2]	-[2]	0.71	±	0.06	-8.0 n s

The calculations were performed with unrounded values
n.s. No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)
*s statistically significantly different to control (Student-t-test for homogeneous variances, 2-sided, p ≤ 0.05)
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
2) Since in this treatment group the deviation from the control was below 25% on day 28, no further evaluations were performed.

No adverse effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on nitrogen transformation in soil could be observed at 2.02 mg prod./kg dry weight soil 28 days after application (time interval 14 - 28 days after application).
The test item caused a temporary inhibition and a temporary stimulation of the daily nitrate rate at the tested concentration of 20.22 mg prod./kg dry weight soil at time interval 7 - 14 and time interval 14 - 28 days after application, respectively.
However, no adverse effects on nitrogen transformation in soil could be observed at tested concentration of 20.22 mg prod./kg dry weight soil at the end of the test, 42 days after application (time interval 28 - 42).
Differences from the control of + 9.8 % (test concentration 2.02 mg prod./kg dry weight soil) and - 8.0 % (test concentration 20.22 mg/kg dry weight soil) were measured 28-days (time interval 14 - 28 days) and 42 days (time interval 28 - 42 days) after application, respectively.

Reference test:
In a separate study the reference item Dinoterb caused a stimulation of nitrogen transformation of +33.7 % and +42.6 % at 16.00 mg and 27.00 mg Dinoterb per kg dry weight soil, respectively, determined 28 days after application.

III. CONCLUSION

BIX + FLU + PTZ EC 260 (65+65+130 g/L) caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (measured as NO_3-N production) at the end of the 42-day incubation period. The study was performed in a field soil at concentrations up to 20.22 mg prod./kg dry weight soil, which are equivalent to application rates up to 15 L prod./ha.

Assessment and conclusion by applicant:

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

The endpoint is: 20.22 mg prod./kg dws

CP 10.6 Effects on terrestrial non-target higher plants

For the formulation BIX + FLU + PTZ EC 260 two single dose studies (testing 11 and 10 species) on Terrestrial Plant Vegetative Vigour and two single dose studies (testing 11 and 10 species) and one dose response study (testing 2 species) on Terrestrial Plant Seedling Emergence were conducted to determine potential effects on terrestrial non-target higher plants. In none of the studies listed below adverse effects > 50% were observed in any species tested. The only exceptions are *Glycine max* and *Allium cepa* in the seedling emergence limit test (M-688446-01-1) at 1.5 L prod./ha. For *Allium cepa* a dry weight reduction of 66.7 % and for *Glycine max a* reduction of shoot height of 67.4 % was measured. A follow-up dose-response study was conducted for both species (M-690855-01-1) showing an ER_{50} of > 1.5 L prod./ha for *Allium cepa* and for *Glycine max*. Thus, the relevant ER_{50} for the risk assessment of all species is set as > 1.5 L prod./ha.

Table 10.6- 1: Effect values relevant for the risk assessment for non-target terrestrial plants for BIX+FLU+PTZ EC 260

Test organism	Study type	Endpoint	References
Beta vulgaris [d] *Brassica napus* [d] *Cucumis sativus* [d] *Fagopyrum esculentum* [d] *Glycine max* [d] *Helianthus annuus* [d] *Solanum lycopersicum* [d] *Allium cepa* [m] *Avena sativa* [m] *Lolium multiflorum* [m] *Zea mays* [m]	Vegetative vigour; Tier 1, single dose, 20 days	ER_{50} > 1.5 prod./ha (all species)	[redacted] (2013) M-476383-01-1 KCP 10.6.2/04
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} > 1.5 prod./ha (all species)	[redacted] (2020) M-696912-01-1 KCP 10.6.2/05
Beta vulgaris [d] *Brassica napus* [d] *Cucumis sativus* [d] *Fagopyrum esculentum* [d] *Glycine max* [d] *Helianthus annuus* [d] *Solanum lycopersicum* [d] *Allium cepa* [m] *Avena sativa* [m] *Lolium multiflorum* [m] *Zea mays* [m]	Seedling emergence; Tier 1, single dose, 21 days	ER_{50} > 1.5 prod./ha (all species)	[redacted] (2014) M-478325-01-1 KCP 10.6.2/01
Beta vulgaris [d] *Brassica napus* [d] *Cucumis sativus* [d] *Fagopyrum esculentum* [d] *Glycine max* [d] *Helianthus annuus* [d] *Allium cepa* [m] *Avena sativa* [m] *Lolium perenne* [m] *Zea mays* [m]	Seedling emergence; Tier 1, single dose, 21 days	ER_{50} > 1.5 prod./ha (all species except *Glycine max* and *Allium cepa*) ER_{50} < 1.5 L prod./ha (67.4 % reduction of shoot height for *Glycine max*; 66.7 % reduction of shoot dry weight for *Allium cepa*)	[redacted] (2020) M-688446-01-1 KCP 10.6.2/02
Glycine max [d] *Allium cepa* [m]	Seedling emergence; Tier 2, dose response, 21 days	ER_{50} > 1.5 prod./ha	[redacted] (2020) M-690835-01-1 KCP 10.6.2/03

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 1.5 L prod./ha does not result in effects > 50 % according to the "Guidance Document on Terrestrial Ecotoxicology" (SANCO/10329/2002 rev. 2 final, 2002), no risk for non-target terrestrial plants is expected. The limit test rate is higher than the highest field application rate and is thus considered as indicator for an acceptable risk.

A detailed deterministic risk assessment is in addition presented below. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the highest use rate also covers the risk for non-target terrestrial plants from the use rate with the lower application rate.

Deterministic risk assessment

Table 10.6-2: Deterministic assessment of the risk for non-target plants due to the use of BIX+FLU+PTZ EC 260 in barley

Intended use	Barley, 1× 1.2 L prod./ha, BBCH 30-61			
product	BIX+FLU+PTZ EC 260			
Application rate (L prod./ha)	1 × 1.2			
Test species	**ER_{50} (L prod./ha)**	**Drift rate (%)**	**$PER_{off\text{-}field}$ (L prod./ha)**	**TER criterion: TER ≥ 5***
All species -seedling emergence	>1.5	2.77	0.0332	>45.13
All species -vegetative vigour	>1.5	2.77	0.0332	>45.13

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 BIX + FLU + PTZ EC 260 will not produce unacceptable effects on terrestrial non-target plants growing near treated fields. No mitigation measures are necessary for the intended use rates.

CP 10.6.1 Summary of screening data

Studies were not necessary since guideline GLP studies for terrestrial non-target plants are available (see Point 10.6.2 in this MCP Summary).

CP 10.6.2 Testing on non-target plants

Data Point:	KCP 10.6.2/01
Report Author:	[redacted]
Report Year:	2014
Report Title:	Effect of bixafen+fluopyram+prothioconazole EC 260 (65+65+130 g/L) - On the seedling emergence and seedling growth of terrestrial plants (Short code of test item: BIX+FLU+PTZ EC 260)
Report No:	AS317
Document No:	M-478325-01-1
Guideline(s) followed in study:	OECD Guideline for the Testing of Chemicals Guideline 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test (adopted July 2006)
Deviations from current test guideline:	Current Guideline: OECD 208 (2006) Deviations: Temporary deviation from climate condition (humidity). Light intensity was not reported. Deviation from recommended plant density. All validity criteria were met. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	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 BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the seedling emergence and growth of eleven species of non-target terrestrial plants, following a pre-emergence application of the product to the soil surface. A total of 11 species, 7 dicotyledonous and 4 monocotyledonous species from 9 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* var. *altissima* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Fagopyrum esculentum* (buckwheat) *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Solanum lycopersicum* (tomato), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium multiflorum* (ryegrass), *Zea mays* (corn). Planting density included 6 seeds per pot, with 5 replicate pots, respectively, for a total of 30 seeds per treatment level. The sown seeds of the plant species were treated with a single application rate of 1.5 L product/ha and a water control. The application was done at a volume rate of 200 L/ha. Assessments for seedling emergence and phytotoxicity were done 7, 14 and 21 (± 1 day) after 50 % of the seedlings in the control had emerged (species dependent). At day 21 after 50 % emergence of the control seedlings, survival of emerged plants, shoot dry weight and plant development (BBCH) were determined.

The study fulfils all validity criteria of OECD 208 guideline.

Only minor phytotoxicity was observed with single species. No adverse effects on emergence, post-emergence mortality and shoot dry weight occurred. Therefore the ER_{50} (based on emergence, survival and shoot dry weight) was determined to be > 1.5 L prod./ha.

I. MATERIAL AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no.: 102000027828-01; batch ID.: 2013-002135; Sample description: TOX 10113-00; active substance (analysed content): bixafen: 6.49 % w/w (65.61 g/L), fluopyram: 6.35 % w/w (64.21 g/L), prothioconazole: 12.7 % w/w (128.5 g/L); density: 1.011 g/mL).

Test design: A total of eleven species, 7 dicotyledonous and 4 monocotyledonous species from 9 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* var. *altissima* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Fagopyrum esculentum* (buckwheat) *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Solanum lycopersicum* (tomato), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium multiflorum* (ryegrass), *Zea mays* (corn). The seeds were sown one day prior to application of the product to the soil surface in 13 cm plastic pots (filled with approx. 875 g fresh soil). The used soil was a very silty sand.

Planting density included 6 seeds per pot, with 5 replicate pots, respectively, for a total of 30 seeds per treatment level. The sown seeds of the plant species were treated with a single application rate of 1.5 L product/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 control pots of each species were observed for the number of seedlings emerged until 50 % of the seedlings had emerged (= day 0). Assessments for seedling emergence and phytotoxicity were done 7, 14 and 21 (± 1 day) after 50 % of the seedlings in the control had emerged (species dependent). At day 21 after 50 % emergence of the control seedlings, survival of emerged plants, shoot dry weight and plant development (BBCH) were determined.

Fagopyrum esculentum had to be repeated according to study plan amendment no. 1 (insufficient germination in the control the first test run).

The phytotoxicity was rated in % affected plant material per replicate compared to the control.

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

Test conditions: Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. Light intensity was not reported. The temperature was regulated to maintain 18 to 24 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was regulated to maintain 55 to 75 % in the first test run and 38 to 61 % in the repeated test with *Fagopyrum esculentum*.

Statistics: Statistical analysis was made for the mean values, standard deviations, analysis of variance (ANOVA) followed by Student-t Test (α = 5 %) or Mann-Whitney U-test. All calculations were done using Microsoft® Excel software Version 2010 SP2 and ToxRat Standard 2.10.05.

Dates of work: September 09, 2013 – December 19, 2013

II. RESULTS AND DISCUSSION

Validity criteria:

The validity criteria of OECD 208 were fulfilled.

The seedling emergence of the control plants was ≥70 % The mean survival of emerged control seedlings was ≥ 90 % 21 days after at least 50 % emergence in the control. There was no visual phytotoxicity in controls and 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.

Analytical results:

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

The analysis of bixafen content in the aqueous solution revealed measured concentrations of 98.0 - 101.7 % of nominal.

Biological findings:

There were no statistically significant effects on seedling emergence and shoot dry weight. Inhibitions in shoot dry weight were determined for all plant species except of Sugar beet, Soybean, Tomato, Rye grass and Oats. No effects on plant survival were found and only minor phytotoxicity was observed with single species. Also, no effects on plant development (BBCH) were found.

Visual phytotoxicity was observed in *Brassica napus* at test termination. The observed symptom was growth reduction and the average phytotoxicity score was 1 %. In *Glycine max* the observed phytotoxicity was 4 %. The observed symptoms were necrosis and growth reductions. In *Helianthus annuus* symptoms of phytotoxicity were observed in one replicate of the test item rate of 1.5 L product/ha at test termination. The observed symptom was growth reduction. The average phytotoxicity score was 6 %. No phytotoxicity was observed in the other plants.

The effects on mortality, emergence, phytotoxicity, plant growth stage and dry weight are summarized for each of the plant species in the following tables for the final assessment (on day 21 after application).

Table 10.6.2- 1: Summary of seedling emergence and shoot dry weight following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	Seedling emergence			Shoot dry weight		
	Control	1.5 L prod./ha		Control	1.5 L prod./ha	
	Mean [number of seedlings/ replicates]	Mean [number of seedlings/ replicates]	% Inhibition [A]	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Brassica napus	5.8	5.2	10	2.782	2.746	1
Cumis sativus	6.0	6.0	0	4.331	4.195	3
Beta vulgaris	6.0	6.0	0	2.354	2.366	- 1
Glycine max	4.8	4.8	0	3.624	3.621	0
Helianthus annuus	5.8	5.4	7	3.703	3.183	14
Solanum lycopersicum	5.8	5.8	0	1.649	1.762	- 7
Fagopyrum esculentum	5.4	5.0	7	3.210	2.856	11
Allium cepa	5.8	5.6	3	0.133	0.113	15
Lolium multiflorum	5.6	5.8	- 4	1.470	1.621	- 10
Avena sativa	6.0	5.8	3	2.488	2.520	- 1
Zea mays	6.0	5.8	3	5.134	5.087	1

[A] Negative figures indicate that there was an increase in biomass (dry weight) when compared to the untreated control.

Table 10.6.2- 2: Summary of phytotoxicity and growth stages (BBCH) following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	Phytotoxicity [%] [A]		BBCH growth stages	
	Control	1.5 L prod./ha	Control	1.5 L prod./ha
Brassica napus	0	1	14-15	14 - 15
Cumis sativus	0	0	12-13	12 - 13
Beta vulgaris	0	0	13 - 14	13 - 14
Glycine max	0	1	13 - 14/51	13 - 14/51
Helianthus annuus	0	6	14 - 16	14 - 16
Solanum lycopersicum	0	0	14	14
Fagopyrum esculentum	0	0	14 - 16/21 -23/59-63	14 - 16/21 - 23/59 - 63
Allium cepa	0	0	11 - 12	11 - 12
Lolium multiflorum	0	0	15 - 17/22 - 23	15 - 17/22 - 23
Avena sativa	0	0	15/21	15/21
Zea mays	0	0	15 - 16	15 - 16

[A] Phytotoxicity: 0: no phytotoxicity or effect

III. CONCLUSION

In a seedling emergence and growth study, BIX + FLU + PTZ EC 260 (65+65+130 g/L) was tested under greenhouse conditions for effects on the seedling emergence, survival, growth and shoot dry weight of 11 non-target terrestrial plant species, following a pre-emergence application of the product to the soil surface.

No adverse effects on emergence, post-emergence mortality and shoot dry weight occurred. Therefore the ER_{50} (based on emergence, survival and shoot dry weight) was determined to be > 1.5 L prod./ha.

Assessment and conclusion by applicant:
The study and its data are considered as acceptable and reliable for use in risk assessment.
The endpoint is: ER_{50} > 1.5 L prod./ha

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Data Point:	KCP 10.6.2/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram + bixafen + prothioconazole EC 260 (65+65+130 g/L): Effects on the seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions (Tier I)
Report No:	S19-22929
Document No:	M-688446-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) no. 1107/2009 US EPA OCSPP 850.4100 (2012) OECD 208 (2006)
Deviations from current test guideline:	Current Guideline: OECD 208 (2006) Deviations: Temporary deviation from climate condition (light). All validity criteria were met. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this specific study was to evaluate the potential effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the seedling emergence and growth of ten species of non-target terrestrial plants, following a pre-emergence application of the product to the soil surface. A total of ten species, 6 dicotyledonous and 4 monocotyledonous species from 8 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn). Planting density included 2 or 4 seeds per pot, with 10 or 5 replicate pots, respectively, for a total of 20 seeds per treatment level. The sown seeds of the plant species were treated with a single application rate of 1.5 L product/ha and a water control. The application was done at a volume rate of 200 L/ha. Plants were assessed for seedling emergence, post-emergence mortality and visual injuries 7, 14 and 21 days after at least 50% emergence of the seedlings in the control group. Additionally, the effects on the BBCH growth stage, shoot height and shoot dry weight were determined for day 21 (21 DA50E).

The study fulfils all validity criteria of OECD 208 guideline.

Visual phytotoxicity occurred only in *Helianthus annuus* (deformations) and *Allium cepa* (necrosis) with a mean effect value below 10 %, respectively, compared to the control group.

Adverse effects on shoot height above the 50 % effect level occurred for *Glycine max*. Adverse effects on shoot dry weight above the 50 % effect level occurred for *Allium cepa*. Except for *Glycine max* and *Allium cepa* the ER_{50} for all species (based on survival, growth stage development and shoot dry weight) can be set to be > 1.5 L product/ha.

I. Material and methods

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no.: 102000027828; supplier batch no.: EM4L025147; Sample description: TOX 21235-00; active substance (analysed content): bixafen: 6.44 % w/w (65.10 g/L), fluopyram: 6.56 % w/w (66.33 g/L), prothioconazole: 12.5 % w/w (126.7 g/L); density: 1.011 g/mL.

Test design: A total of ten species, 6 dicotyledonous and 4 monocotyledonous species from 8 plant families were tested in this seedling emergence and growth test: *Beta vulgaris* (sugar beet), *Brassica napus* (oilseed rape winter), *Cucumis sativus* (cucumber), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium perenne* (ryegrass), *Zea mays* (corn). The plant species used in this study are representative of a wide range of plant families and were chosen because they are readily cultivated test organisms and widely used in research. The seeds were sown on the day of application of the product to the soil surface in 15 cm plastic pots (filled with approx. 1.5 kg soil/pot). The used soil was a loamy sand.

Planting density included 2 or 4 seeds per pot, with 10 or 5 replicate pots, respectively, for a total of 20 seeds per treatment level. The sown seeds of the plant species were treated with a single application rate of 1.5 L product/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. 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 individually for each species on this day (= day 0) and 7, 14 and 21 days post emergence of 50 % of the control seedlings. Plants were assessed for seedling emergence, post-emergence mortality and visual injuries 7, 14 and 21 days after at least 50% emergence of the seedlings in the control group. Additionally, the effects on the BBCH growth stage, shoot height and shoot dry weight were determined for day 21 (21 DA50E). A gradual rating was assigned to describe the extent of the visual phytotoxicity in comparison to the control, taking into account necrosis, deformation and change in colour (e.g. chlorosis, bleaching, reddening). The ratings referred to the whole plants within a replicate and range from 10 to 90 %.

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

Statistics: The data of seedling emergence and post-emergence morality 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 or shoot dry weight or if no post-emergence mortality occurred, no statistical evaluation was conducted. Statistical analysis was performed using the program ToxRat Professional Version 3.3.0.

Test conditions: Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. Light intensity was in the range of 315 - 430 µmol/m²/s. The temperature was regulated to maintain 16 to 31 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was regulated to maintain 48 to 79 %.

Dates of work: January 27, 2020 – February 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 85% and 100%). The control seedlings of each species did not exhibit visible visual injuries (e.g. change in colour, necrosis and deformations) and control plants exhibited normal variation in growth and morphology for that particular species. The mean survival of emerged control seedlings was ≥ 90 % (actually 100 %) 21 days after at least 50% emergence in the control. The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

Analytical results:

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

The analysed concentration of fluopyram in the product solution corresponded to 118 % of the nominal concentration.

Biological findings:

Visual phytotoxicity observed at the final assessment (on day 21 after application) in this seedling emergence and growth study included chlorosis, necrosis, deformation and stunting of the plants. Visual phytotoxicity occurred only in *Helianthus annuus* (deformations) and *Allium cepa* (necrosis) with a mean effect value below 10 %, respectively, compared to the control group.

No statistically significant effects on the parameter seedling emergence were observed for any of the plant species tested. The highest inhibition occurred for *Glycine max* with 26.3 % compared to the control group.
No statistically significant effects on the parameter post-emergence mortality were observed for any of the plant species tested. The highest mortality occurred for *Cucumis sativus* with 5.6 %.
No differences in the growth stages between the test item and the control group of all ten plant species tested were observed.
Statistically significant differences in shoot height compared to the control group were observed for *Brassica napus* (11.0 %), *Cucumis sativus* (31.1 %), *Fagopyrum esculentum* (19.5 %), *Glycine max* (67.4%), *Helianthus annuus* (19.0 %), *Lolium perenne* (21.8 %) and *Zea mays* (11.4 %).
Statistically significant differences in shoot dry weight compared to the control group were observed for *Allium cepa* (66.7 %).

The effects on mortality, emergence, phytotoxicity, plant growth stage as well as shoot height and dry weight are summarized for each of the plant species in the following tables for the final assessment (on day 21 after application).

Table 10.6.2- 3: Summary of growth stages (BBCH) and phytotoxicity following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	BBCH growth stage [Min - Max]		Phytotoxicity: mean % / Symptoms A	
	Control	Test item [1.5 L product/ha]	Control	Test item [1.5 L product/ha]
Beta vulgaris	12 - 12	12 - 12	0	0 / -
Brassica napus	12 - 12	12 - 12	0	0 / -
Cucumis sativus	12 - 12	12 - 12	0	0 / -
Fagopyrum esculentum	55 - 55	55 - 55	0	0 / -
Glycine max	14 - 14	14 - 14	0	0 / -
Helianthus annuus	16 - 16	16 - 16	0	4 / DE
Allium cepa	11 - 11	11 - 11	0	8 / NE
Avena sativa	13 - 13	13 - 13	0	0 / -
Lolium perenne	14 - 14	14 - 14	0	0 / -
Zea mays	14 - 14	14 - 14	0	0 / -

* Statistically significantly different compared to the control (Student's t-test; one-sided smaller, α = 0.05)
A Visual symptoms: None (-), CC = change in colour, DE = deformation

Table 10.6.2- 4: Summary of shoot height and shoot dry weight and corresponding percent inhibitions following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	Shoot height			Shoot dry weight		
	Control	1.5 L prod./ha		Control	1.5 L prod./ha	
	Mean [cm]	Mean [cm]	% inhibition A	Mean dry weight [g]	Mean dry weight [g]	% Inhibition A
Beta vulgaris	5.0	5.4	8.0	0.038	0.061	- 60.5
Brassica napus	10.9	9.7	11.0 *	0.100	0.130	- 30.0
Cucumis sativus	7.4	5.1	31.1 *	0.154	0.194	- 26.0
Fagopyrum esculentum	23.6	19.0	19.5 *	0.133	0.119	10.5
Glycine max	60.1	19.6	67.4 *	0.925	0.924	0.1
Helianthus annuus	8.4	6.8	19.0 *	0.330	0.379	- 14.8
Allium cepa	8.8	8.0	9.1	0.009	0.003	66.7 *
Avena sativa	37.0	37.6	- 1.6	0.169	0.179	- 5.9
Lolium perenne	28.4	22.2	21.8 *	0.060	0.049	18.3
Zea mays	59.6	52.8	11.4 *	0.571	0.779	- 36.4

* Statistically significantly different compared to the control (Student's t-test; one-sided smaller, α = 0.05)
A Negative values indicate that there was an increase compared to the control

Table 10.6.2- 5: Summary of emergence and cumulative mortality following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	Seedling emergence			Cumulative mortality	
	Control	1.5 L prod./ha		Control	1.5 L prod./ha
	% emerged	% emerged	% Inhibition	% mortality	% mortality
Beta vulgaris	95	95	0	0	0
Brassica napus	90	100	- 11.1	0	0
Cucumis sativus	95	90	5.3	0	5.6
Fagopyrum esculentum	100	95	5.0	0	0
Glycine max	95	70	26.3	0	0
Helianthus annuus	100	100	0	0	0
Allium cepa	95	100	- 5.3	0	0
Avena sativa	100	80	20.0	0	0
Lolium perenne	85	75	11.8	0	0
Zea mays	95	90	5.3	0	0

III. Conclusion

In a seedling emergence and growth study, BIX + FLU + PTZ EC 260 (65+65+130 g/L) was tested under greenhouse conditions for effects on the seedling emergence, survival, growth and shoot dry weight of ten non-target terrestrial plant species, following a pre-emergence application of the product to the soil surface. Adverse effects on shoot height above the 50 % effect level occurred for *Glycine max*. Adverse effects on shoot dry weight above the 50 % effect level occurred for *Allium cepa*. Except for *Glycine max* and *Allium cepa* the ER_{50} for all species (based on survival, growth stage development and shoot dry weight) can be set to be > 1.5 L product/ha.

Assessment and conclusion by applicant:

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

The endpoint is: ER_{50} > 1.5 L prod./ha

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Data Point:	KCP 10.6.2/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram + bixafen + prothioconazole EC 260 (65+65+130 g/L): Effects on the seedling emergence and seedling growth of two non-target terrestrial plant species under greenhouse conditions (Tier 2)
Report No:	S20-03149
Document No:	M-690835-01-1
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: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this specific study was to evaluate the potential effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the seedling emergence and growth of ten species of non-target terrestrial plants, following a pre-emergence application of the product to the soil surface. A total of two species, 1 dicotyledonous and 1 monocotyledonous species from 2 plant families were tested in this seedling emergence and growth test: *Glycine max* (soybean) and *Allium cepa* (onion). 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 five application rates of 0.094, 0.188, 0.375, 0.75 and 1.5 L product/ha and a water control. The application was done at a volume rate of 200 L/ha. Plants were assessed for seedling emergence, post-emergence mortality and visual injuries 7, 14 and 21 days after at least 50% emergence of the seedlings in the control group. Additionally, the effects on the BBCH growth stage, shoot height and shoot dry weight were determined for day 21 (21 DA50E).

The study fulfils all validity criteria of OECD 208 guideline.

For *Glycine max* a LOER of 0.375 L prod./ha and a NOER of 0.188 L prod./ha based on shoot height were determined. For *Allium cepa* a LOER could not be calculated and the NOER is considered to be the highest rate tested, 1.5 L prod./ha. The ER_{25} and ER_{50} could not be calculated for the two plant species tested due to a lack of inhibition equal to or above 25% and is reported as > 1.5 L prod./ha, respectively.

I. MATERIAL AND METHODS

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no.: 102000027828; supplier batch no.: EM4L025147; Sample description: TOX 21235-00; active substance (analysed content): bixafen: 6.44 % w/w (65.10 g/L), fluopyram: 6.56 % w/w (66.33 g/L), prothioconazole: 12.5 % w/w (126.7 g/L); density: 1.011 g/mL.

Test design: A total of two species, 1 dicotyledonous and 1 monocotyledonous species from 2 plant families were tested in this seedling emergence and growth test: *Glycine max* (soybean) and *Allium cepa* (onion). The plant species had shown relevant effects in a preceding limit test and were therefore investigated in this rate-response study. The seeds were sown on the day of application of the product to the soil surface in 15 cm plastic pots (filled with approx. 1.5 kg soil/pot). The used soil was a loamy sand.

Planting density included 2 or 4 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 five application rate of 0.094, 0.188, 0.375, 0.75 and 1.5 L product/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. 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 individually for each species on this day (= day 0) and 7, 14 and 21 days post emergence of 50 % of the control seedlings. Plants were assessed for seedling emergence, post-emergence mortality and visual injuries 7, 14 and 21 days after at least 50% emergence of the seedlings in the control group. Additionally, the effects on the BBCH growth stage, shoot height and shoot dry weight were determined for day 21 (21 DA50E). A gradual rating was assigned to describe the extent of the visual phytotoxicity in comparison to the control, considering necrosis, deformation and change in colour (e.g. chlorosis, bleaching, reddening). The ratings referred to the whole plants within a replicate and range from 10 to 90 %.

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

Test conditions: Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. The temperature was regulated to maintain 19 to 28 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was regulated to maintain 50 to 83 %.

Statistics: The data of seedling emergence were tested with Fisher`s exact test. As no post-emergence 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 Dunnett`s t-test in case that both requirements were fulfilled and the trend analysis by contrast was not significant. The Multiple Welch`s t-test with Bonferroni-Holm adjustment was conducted in case the data were normal distributed but not homogenous. If the data were not normal distributed but homogenous and 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. 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: May 20, 2020 – June 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 75% for *Glycine max* and 95% for *Allium cepa*). The control seedlings of each species did not exhibit visible visual injuries (e.g. change in colour, necrosis and deformations) and control plants exhibited normal variation in growth and morphology for that particular species. The mean survival of emerged control seedlings was ≥ 90% (actually 100%) 21 days after at least 50% emergence in the control. The environmental conditions for each particular species were identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source.

Analytical results:

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

The analysed concentration of fluopyram in the product solution corresponded to 115 % of the nominal concentration.

Biological findings:

No statistically significant effects on the parameter seedling emergence were observed for the two plant species tested. No post-emergence 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. No visual injuries occurred in the two plant species tested.
Statistically significant reductions in shoot height compared to the control group were observed for *Glycine max*. A LOER of 0.375 L product/ha and a NOER of 0.188 L product/ha were determined for this species. For *Allium cepa* a LOER could not be calculated and the NOER is considered to be the highest rate tested, 1.5 L product/ha. A reliable ER_{25} and ER_{50} could not be calculated for both plant species tested due to a lack of inhibition equal to or above 25% and is reported as > 1.5 L product/ha, respectively.
No statistically significant differences in shoot dry weight compared to the control group were observed for both plant species tested. Thus, for both species a LOER could not be calculated and the NOER is considered to be the highest rate tested, 1.5 L product/ha. The ER_{25} and ER_{50} could not be calculated for the two plant species tested due to a lack of inhibition equal to or above 25% and is reported as > 1.5 L product/ha, respectively.

Table 10.6.2- 6: Summary of endpoints for seedling emergence following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Glycine max	-	1.5	> 1.5	> 1.5
Monocotyledonous species				
Allium cepa	-	1.5	> 1.5	> 1.5

Table 10.6.2- 7: Summary of endpoints for post-emergence mortality following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Glycine max	-	1.5	> 1.5	> 1.5
Monocotyledonous species				
Allium cepa	-	1.5	> 1.5	> 1.5

Table 10.6.2- 8: Summary of endpoints for shoot height following exposure to BIX + FLU + PTZ EC 260 at the final assessment on day 21

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Glycine max	0.375 [A]	0.188	> 1.5	> 1.5
Monocotyledonous species				
Allium cepa	-	1.5	> 1.5	> 1.5

[A] Multiple Welch`s t-test with Bonferroni-Holm adjustment; one-sided smaller, $\alpha = 0.05$

Table 10.6.2- 9: Summary of endpoints for shoot dry weight following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Species	LOER	NOER	ER_{25} (95 % confidence limits)	ER_{50} (95 % confidence limits)
Dicotyledonous species				
Glycine max	-	1.5	> 1.5	> 1.5
Monocotyledonous species				
Allium cepa	-	1.5	> 1.5	> 1.5

III. CONCLUSION

In a seedling emergence and growth study, BIX + FLU + PTZ EC 260 (65+65+130 g/L) was tested under greenhouse conditions for effects on the seedling emergence, survival, growth and shoot dry weight of two non-target terrestrial plant species, following a pre-emergence application of the product to the soil surface.

For *Glycine max* a LOER of 0.375 L prod./ha and a NOER of 0.188 L prod./ha based on shoot height were determined. For *Allium cepa* a LOER could not be calculated and the NOER is considered to be the highest rate tested, 1.5 L prod./ha. The ER_{25} and ER_{50} could not be calculated for the two plant species tested due to a lack of inhibition equal to or above 25%/50% and is reported as > 1.5 L prod./ha, respectively.

Assessment and conclusion by applicant:

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

The endpoint is: ER_{50} > 1.5 L prod./ha

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Data Point:	KCP 10.6.2/04
Report Author:	[redacted]
Report Year:	2013
Report Title:	Bixafen+Fluopyram+Prothioconazole EC 260 (65+65+130 g/L) - On vegetative vigour of terrestrial plants
Report No:	AS318
Document No:	M-476483-01-1
Guideline(s) followed in study:	OECD-Guideline for Testing of Chemicals, Guideline 227: Terrestrial Plant Test: Vegetative Vigour Test (adopted July 2006)
Deviations from current test guideline:	Current Guideline: OECD 227 (2006) Deviations: Deviation from recommended plant density. Light intensity was not reported. All validity criteria were met but the germination rate of the seeds used in this study was not reported. However, as routine germination tests were carried out on the seeds to ensure their viability, the germination rate can be considered to be in the acceptable range. The deviations listed above had no influence on the reliability of the study and endpoints.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	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 BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the vegetative vigour of eleven non-target terrestrial plant species, following a post-emergence application of the product onto the foliage of plants at the 2 - 4 leaf stage. A total of 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), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Solanum lycopersicum* (tomato), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium multiflorum* (ryegrass), *Zea mays* (corn). Planting density included 5 plants per pot with 6 replicate pots, respectively, for a total of 30 plants per treatment level. The plant species were treated with a single application rate of 1.5 L product/ha and a water control. The application was done at a volume rate of 200 L/ha. Assessments for phytotoxicity were done 7, 14 and 20 days after application (DAA) for all plants. 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.

With the exception of *Avena sativa*, for each of the tested plant species symptoms of phytotoxicity could be observed. For none of the species tested, effects on survival, phytotoxicity or shoot dry weight reaching or exceeding the 50 % threshold for further testing were found. Therefore the ER_{50} (based on survival and drey weight) was determined to be >1.5 L prod./ha.

I. Material and Methods

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no.: 102000027828-01; supplier batch ID.: 2013-002135; Sample description: TOX 10113-00; active substance (analysed content): bixafen: 6.49 % w/w (65.10 g/L), fluopyram: 6.35 % w/w (64.21 g/L), prothioconazole: 12.7 % w/w (128.5 g/L); density: 1.011 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), *Fagopyrum esculentum* (buckwheat), *Glycine max* (soybean), *Helianthus annuus* (sunflower), *Solanum lycopersicum* (tomato), *Allium cepa* (onion), *Avena sativa* (oat), *Lolium multiflorum* (ryegrass), *Zea mays* (corn). The plants were grown in a greenhouse in commercial non porous 13 cm plastic pots (filled with approx. 875 g fresh soil). The used soil was a very silty sand.

Planting density included 5 plants per pot with 6 replicate pots, respectively, for a total of 30 plants per treatment level. The plant species were treated at the 2 – 4 leaf stage with a single application rate of 1.5 L product/ha and a water control. The test solutions were applied onto the plants at a volume rate of 200 L/ha. Control pots were sprayed with 200 L/ha of deionized water.

After application, the pots with plants were transferred back to the greenhouse.

Assessments for phytotoxicity were done 7, 14 and 20 days after application (DAA) for all plants. Final assessments were made for plant survival, visual phytotoxicity, plant growth stage and shoot dry weight. The phytotoxicity was rated in % affected plant material per replicate compared to the control.

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

Test 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 18 to 23 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was 59 to 79 %.

Statistics:Statistical analysis was made for the mean values, standard deviations, analysis of variance (ANOVA) followed by Student-t Test ($\alpha = 5\%$). All calculations were done using Microsoft® Excel software Version 2010 SP2 and ToxRat Standard 2.10.05, SpiritSoftware, Alsdorf, Germany. All calculations for statistical assessments (analysis of variance) were done using the maximum number of digits available for each software program.

Dates of work: September 09, 2013 – October 30, 2013

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:

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

The analysis of bixafen content in the initial product stock solution revealed measured concentrations of 101.7 % of nominal.

Biological findings:

Mortality was not observed during the test. With the exception of Oats, for each of the tested plant species symptoms of phytotoxicity could be observed. Statistically significant inhibitions of shoot dry weight were observed for Oilseed rape, Cucumber, Sugar beet, Tomato and Buckwheat, however, all inhibitions were much lower than the 50 % trigger for further testing. No clear effects on plant development (BBCH) were found.

The effects on phytotoxicity, plant growth stage and shoot dry weight are summarized for each of the plant species in the following tables for the final assessment (on day 20 after application).

Table 10.6.2- 10: Summary of shoot dry weight following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	Shoot dry weight		
	Control	1.5 L prod./ha	
	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Brassica napus	4.841	4.108*	14
Cumis sativus	6.480	5.067*	22
Beta vulgaris	2.747	2.357*	14
Glycine max	10.740	10.154	5
Helianthus annuus	4.503	4.038	10
Solanum lycopersicum	6.854	5.476*	20
Fagopyrum esculentum	6.583	4.975*	24
Allium cepa	1.013	0.964	5
Lolium multiflorum	5.278	5.178	2
Avena sativa	5.345	5.634	-7
Zea mays	6.804	6.186	9

[A] Negative figures indicate that there was an increase in biomass (dry weight) when compared to the untreated control.

* Statistically significant (Student-t test, one-sided smaller; $p \leq 0.05$).

Table 10.6.2- 11: Summary of phytotoxicity and growth stages (BBCH) following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	Phytotoxicity [%] [A]		BBCH growth stages	
	Control	1.5 L prod./ha	Control	1.5 L prod./ha
Brassica napus	0	20	15 - 16	15 - 16
Cumis sativus	0	30	16/53-54	15/52 - 53
Beta vulgaris	0	28	14	14
Glycine max	0	30	16 - 17/26 - 27	16 - 17/26 - 27
Helianthus annuus	0	20	16	16
Solanum lycopersicum	0	33	16	16
Fagopyrum esculentum	0	33	16 - 17/65	16 - 17/65
Allium cepa	0	8	13 - 15	13 - 14
Lolium multiflorum	0	10	15 - 17/22 – 24	15 - 17/22 - 24
Avena sativa	0	0	16/34	16/34
Zea mays	0	18	15 - 16	15

[A] Phytotoxicity: 0: no phytotoxicity or effect

III. Conclusion

As a result of this vegetative vigour and growth study, in which the effect of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on eleven non-target terrestrial plant species was tested under greenhouse conditions, for none of the species tested, effects on survival, phytotoxicity or shoot dry weight reaching or exceeding the 50 % threshold for further testing were found. Therefore the ER_{50} (based on survival and drey weight) was determined to be > 1.5 L prod./ha.

Assessment and conclusion by applicant:

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

The endpoint is: ER_{50} > 1.5 L prod./ha

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Data Point:	KCP 10.6.2/05
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopyram + bixafen + prothioconazole EC 260 (65+65+130 g/L): Effects on the vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions (Tier 1)
Report No:	S19-22930
Document No:	M-696912-01-1
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: None. All validity criteria were met.
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this study was to evaluate the potential effects of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on the vegetative vigour of ten non-target terrestrial plant species, following a post-emergence application of the product onto the foliage of plants at the 2 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 multiflorum* (ryegrass), *Zea mays* (corn). Planting density included 2 or 4 plants per pot with 10 or 5 replicate pots, respectively, for a total of 20 plants per treatment level. The plant species were treated with a single application rate of 1.5 L product/ha and a water control. The application was done at a volume rate of 200 L/ha. Plants were assessed for mortality and visual injuries on day 7, 14 and 21. Additionally, the BBCH growth stage and shoot height were determined for all treatment groups on day 21. The effects on plant shoot dry weight were determined for day 21.

The study fulfils all validity criteria of OECD 227 guideline.

For none of the species tested, effects on survival, phytotoxicity, shoot height and shoot dry weight reaching or exceeding the 50 % threshold for further testing were found. Therefore the ER_{50} (based on survival, shoot height and dry weight) was determined to be > 1.5 L prod./ha.

I. Material and Methods

Test item: BIX + FLU + PTZ EC 260 (65+65+130 g/L); specification no.: 102000027828; supplier batch ID: 2013-002135; Sample description: TOX 21235-00; active substance (analysed content): bixafen: 6.44 % w/w (65.10 g/L), fluopyram: 6.56 % w/w (66.33 g/L), prothioconazole: 12.5 % w/w (126.7 g/L); density: 1.011 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 multiflorum*

(ryegrass), *Zea mays* (corn). The plants were grown in a greenhouse in commercial non porous 15 cm plastic pots (filled with approx. 1.5 kg/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 rate of 1.5 L product/ha and a water control. The test solutions were applied onto the plants 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.

Plants were assessed for mortality and visual injuries on day 7, 14 and 21. Additionally, the BBCH growth stage and shoot height were determined for all treatment groups on day 21. The effects on plant shoot dry weight were determined for day 21.

Analysis of the product solution and the control solution were conducted by LC – MS/MS.

Test conditions: Following application, the pots with plants were maintained under greenhouse conditions and natural daylight was supplemented by artificial lighting. The temperature was 17 to 29 °C during the light cycle (16 h) and during the dark cycle (8 h). The relative humidity was 50 to 77 %.

Statistics: As no mortality occurred, no statistical evaluation was performed for this endpoint.
The data of shoot height and shoot dry weight were tested for normal distribution and homoscedasticity using Shapiro-Wilk`s Test and Levene-Test, respectively. For all species tested both requirements were fulfilled, therefore Student t-test was conducted. The significance level was set to $\alpha = 0.05$ for all tests. In case of an increase in the test item group compared to the control group for shoot height or shoot dry weight, no statistical evaluation was conducted.
Statistical analysis was performed using the program ToxRat Professional Version 3.3.0.

II. RESULTS AND DISCUSSION

Validity criteria:

The validity criteria of OECD 227 were fulfilled.

The seedling emergence was ≥ 70 % for all species included in this test. The control seedlings of each species did not exhibit visible visual injuries (e.g. change in colour, necrosis and deformations) and control plants exhibited normal variation in growth and morphology for that particular species. The mean survival of emerged control seedlings was ≥ 90 % for all species included in this test (actually 100 %). 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 analysis of fluopyram content in the initial product stock solution revealed measured concentrations of 103 % of nominal.

Biological findings:

Mortality was not observed during the test. Visual injuries occurred in all dicotyledonous species tested as well as in *Zea mays* with a highest mean effect of 30 % for *Cucumis sativus, Fagopyrum esculentum* and *Glycine max*, respectively.

Differences in the BBCH growth stage with BBCH 54 in the control group compared to BBCH 45 in the test item group occurred for *Avena sativa*. In all other species tested no differences in growth stage occurred between the control group and the test item group.

Statistically significant differences in shoot height compared to the control group were observed for *Beta vulgaris* (6.3 %), *Cucumis sativus* (16.7 %), *Fagopyrum esculentum* (7.4 %), *Glycine max* (27.1 %), *Avena sativa* (9.0 %) and *Zea mays* (13.7 %).

Statistically significant differences in shoot dry weight compared to the control group were observed for *Beta vulgaris* (35.4 %), *Cucumis sativus* (9.6 %), *Fagopyrum esculentum* (7.1 %), *Glycine max* (22.9 %) and *Lolium perenne* (25.5 %).

The effects on shoot height, shoot dry weight, phytotoxicity and 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- 12: Summary of shoot height and shoot dry weight following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	Shoot height			Shoot dry weight		
	Control	1.5 L prod./ha		Control	1.5 L prod./ha	
	Mean [cm]	Mean [cm]	% Inhibition [A]	Mean dry weight [g]	Mean dry weight [g]	% Inhibition [A]
Beta vulgaris	14.2	13.3	6.3 *	0.995	0.643	35.4 *
Brassica napus	16.6	15.4	7.2	1.500	1.440	4.0
Cucumis sativus	37.1	30.9	16.7 *	2.272	2.055	9.6 *
F. esculentum	88.8	88.2	7.4 *	1.435	1.333	7.1 *
Glycine max	107.6	78.4	27.1 *	2.347	1.810	22.9 *
Helianthus annuus	13.5	14.2	-5.2	1.675	1.917	-14.4
Allium cepa	30.4	29.6	2.6	0.397	0.295	25.7
Avena sativa	44.6	40.6	9.0 *	0.393	0.369	6.1
Lolium perenne	46.1	44.6	3.3	0.627	0.467	25.5 *
Zea mays	65.8	56.8	13.7 *	1.495	1.543	-3.2

* Statistically significantly different compared to the control (Student's t-test; one-sided smaller, α = 0.05)
[A] Negative values indicate that there was an increase compared to the control

Table 10.6.2- 13: Summary of phytotoxicity and growth stages (BBCH) following exposure to BIX + FLU + PTZ EC 260 (65+65+130 g/L) at the final assessment on day 21

Plant species	Phytotoxicity [%] [A]		BBCH growth stages	
	Control	1.5 L prod./ha	Control	1.5 L prod./ha
Beta vulgaris	0 / --	20 / CC, NE	16 - 16	16 - 16
Brassica napus	0 / --	8 / CC, NE, DE	15 - 15	15 - 15
Cucumis sativus	0 / --	30 / CC, NE	65 - 65	65 - 65
F. esculentum	0 / --	30 / CC, NE	65 - 65	65 - 65
Glycine max	0 / --	30 / CC, NE	52 - 52	52 - 52
Helianthus annuus	0 / --	10 / CC, NE	18 - 18	18 - 18
Allium cepa	0 / --	0 / --	14 - 14	14 - 14
Avena sativa	0 / --	0 / --	54 - 54	45 - 45
Lolium perenne	0 / --	0 / --	23 - 23	23 - 23
Zea mays	0 / --	10 / CC	15 - 15	15 - 15

[A] Phytotoxicity: Visual symptoms: -- = None, CC = Change in colour, NE = Necrosis, DE = Deformation

III. CONCLUSION

As a result of this vegetative vigour and growth study, in which the effect of BIX + FLU + PTZ EC 260 (65+65+130 g/L) on ten non-target terrestrial plant species was tested under greenhouse conditions, for none of the species tested, effects on survival, phytotoxicity, shoot height and shoot dry weight reaching or exceeding the 50 % threshold for further testing were found. Therefore the ER_{50} (based on survival, shoot height and dry weight) was determined to be >1.5 L prod./ha.

Assessment and conclusion by applicant:

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

The endpoint is: ER_{50} > 1.5 L prod./ha

CP 10.6.3 Extended laboratory studies on non-target plants

In view of the results presented under Point CP 10.6.2 above, no further studies are deemed necessary.

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

In view of the results presented under Point CP 10.6.2 above, no further studies are deemed necessary.

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

In view of the study results presented above no studies on other terrestrial organisms are considered necessary. However, further investigation has been conducted on fungicidal activity with no adverse effects observed; for details see MCA 8.7.

CP 10.8 Monitoring data

No monitoring data has been collected by the applicant nor have they been reported in any of the public literature references as evaluated in Document MCA, Section 9. No monitoring of non-target organism is deemed to be necessary.