Document Title

Summary of the ecotoxicological studies Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L)

Data Requirement(s)

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

Document MCP

Section 10: Ecotoxicological studies

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

Date

2020-08-07

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M-690102-01-2

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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

[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

Fluopicolide was included in Annex I to Council Directive 91/414/EEC in 2010 (Commission Directive 2010/15/EU, Entry into Force on June 1, 2010). The expiration of approval of fluopicolide is May 31, 2023 (Commission Implementing Regulation (EU) 2017/1527). The Supplementary Dossier contains only data which were not submitted at the time of the Annex I inclusion of fluopicolide 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 AG.

The formulation Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L), abbreviation FLC + FXA FS 350, is a flowable concentrate for seed treatment formulation (FS) containing 200 g/L of fluopicolide. This formulation is registered in Europe under the trade name Scenic Gold®. Scenic Gold® was not a representative formulation of Bayer AG for the Annex I inclusion of fluopicolide under Council Directive 91/414/EEC.

Fluopicolide (AE C638206) is a fungicidal active substance developed by Bayer. It is the only active substance in Europe representing a class of chemistry (pyridinylmethyl-benzamides) with a unique mode of action via delocalization of a spectrin-like protein in the Oomycetes fungi.

Fluopicolide is active against a wide range of Oomycete fungi low dose rates against a wide range of Oomycete (Phycomycetes) diseases including downy mildews (Pseudoperonospora, Peronospora, Bremia), late blight (Phytophthora). It is also effective against downy mildews and some Pythium species causing damping off at emergence time.

Fluopicolide is redistributed via the xylem and effective disease control can be achieved from foliar and seed applications. Fluopicolide is used in mixture in a range of foliar formulations in potatoes, horticultural crops and industrial crops such as oilseed.

Fluopicolide has a long track record of safe use in a large number of targeted crops within industrial crops.

Fluopicolide can be formulated with other active ingredients in different types of formulations to optimise and complete its activity.

The development of resistances of Oomycetes against existing, well-established fungicide groups represent a threat for European farmers by increasing the complexity of their plant protection programs leading to severe economic impacts. With fluopicolide, farmers in EU-27 have access to a modern tool for their integrated crop protection programs, contributing to effective and sustainable management of resistance development and preserving high level of protection against Oomycete diseases.

By reducing the Oomycete damages, applications of fluopicolide + fluoxastrobin FS 350 on target crops contribute to the achievement of optimum emergence insuring yield and quality, thus securing sufficient supply of high-quality oilseed for European consumer destinations and markets abroad, for the processing industry.

Use pattern considered in this risk assessment

Table 10- 1: Intended application pattern

Crop	Formulation	Application				Application rate per treatment		
	Conc. of a.s.	Method	Timing	Number	Interval between applications	g a.s./100 kg seeds min - max	kg seeds/ha min - max	g a.s./ha min - max
Rape, winter	FLC: 200 g/L FXA: 150 g/L	Seed treatment	BBCH 00	1	n.a.	FLC: 200 FXA: 150	2.5 - 6	FLC: 5 - 12 FXA: 3.75 - 9

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:

Definition of the residue for risk assessment

Soil: Fluopicolide, M-01 (AE C653711), M-02 (AE C678188), M-03 (AE 0608000)

Surface water: Fluopicolide, M-01 (AE C653711), M-02 (AE C657188), M-03 (AE 0608000)

Sediment: Fluopicolide

In June 2019 EFSA issued a Technical Report Outcome of the pesticides Peer Review Meeting on general recurring issues in ecotoxicology, doi:10.2903/sp.efsa.2019.EN-1673

As part of this document guidance and a template were provided to complete the questionnaire for the use of residue data extracted from vol. 3 B.7.to support the ecotoxicological assessment of pesticides.

Data Point:	KCP Section 10/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Fluopicolide - Residue information supporting the ecotoxicological assessment of pesticides
Report No:	VC/19/038B
Document No:	M-687286-01-1
Guideline(s) followed in study:	--
Deviations from current test 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]

The following metabolism studies are available for fluopicolide:

Report reference	Author, Year	Crop Category	Crop	Application	Fluopicolide label
M-241268-02-1	[redacted] 2004	Fruit crop (F)	Grapes	Foliar	[U-^{14}C-phenyl]- and [2,6-^{14}C-pyridyl]-Fluopicolide
M-241267-03-1	[redacted] 2004	Root and tuber crop (R)	Potato	Foliar	[U-^{14}C-phenyl]- and [2,6-^{14}C-pyridyl]-Fluopicolide

[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.

M-241269-02-1	[redacted] 2004	Leafy crop (L)	Lettuce	Foliar	[U-^{14}C-phenyl]- and [2,6-^{14}C-pyridyl]-Fluopicolide
		Leafy crop (L)	Lettuce	Soil drench	[U-14C-phenyl]-Fluopicolide
M-358357-01-1	[redacted] 2009	Pulses and oilseed (P/O)	Oilseed rape	Seed treatment	[U-^{14}C-phenyl]- and [2,6-^{14}C-pyridyl]-Fluopicolide

Metabolism studies have been conducted in three crop groups with foliar applications, namely fruit (F), root (R) and leafy (L), and since the metabolism is similar in all three crop groups thus all crops are covered. Additional studies are available covering the drench and seed treatment uses. All of the foliar applied metabolism studies have been previously reviewed at the EU level, the following conclusion was made for these studies:

Lettuce, grapes and potatoes (foliar application)
When fluopicolide was applied as a foliar treatment, the quantity of the metabolites formed was extremely low. When fluopicolide reached the soil during application, it was degraded and there was an increased quantity of the metabolites M-01 (AE C653711) and M-02 (AE C657188) taken up into the plant parts. This information is sufficient to cover the proposed representative uses on foliar treated potatoes, lettuce and cucumbers.

Lettuce (soil drench)
Following soil drench application with [U-14C-phenyl]-fluopicolide the majority of the residue consisted of fluopicolide, with significant amounts of M-01 (AE C653711) and minor amounts of M-06 (AE C643890). No other single metabolite comprised more than 1% of the total residue in any matrix.

Oilseed rape (seed treatment)
The only prominent metabolite observed was M-01 (BAM, AE C653711), when fluopicolide is applied as a seed treatment to oilseed rape seed. All other metabolites were detected in low amounts (≤ 4.1% of TRR and ≤ 0.002 mg/kg). The only observed metabolic reaction is the cleavage of fluopicolide to form M-01. This information is sufficient to cover the proposed representative use on seed treated oilseed rape.

For the lettuce (soil drench) and oilseed rape (seed treatment) metabolism, while these studies have been reviewed by EU Member States, they are only representative of their own respective commodity groupings (‘leafy vegetable’ and ‘pulses / oilseeds’), as there is only one study available for each of these application types, so it would not be possible to infer whether the metabolic pathway would be similar for other crop groups based on this information alone.

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]?

In the metabolism in primary crop studies and metabolism in rotational crop study conducted with [phenyl-U-^{14}C]-fluopicolide or [2,6-pyridyl-^{14}C]-fluopicolide, a number of plant metabolites were detected in matrices considered as relevant for leafy substrates above the criteria of > 10% of the total radioactive residue (TRR) or present at concentrations > 0.05 mg/kg thus triggering the need to be considered for ecotoxicological assessments.

The matrices available from radiolabelled studies were lettuce, potato foliage (as a substitute for palatable leaves), radish tops, wheat forage and oilseed rape forage.

For lettuce and potato, foliage investigations with both radiolabelled test items were conducted in metabolism in primary crop studies after foliar application and for lettuce further investigations were conducted after soil application with [phenyl-U-^{14}C]-fluopicolide. Additionally, for lettuce, radish tops and wheat forage, seed was sown 29 days, 133 days and 1 year after treating soil with [phenyl-U-^{14}C]-fluopicolide or [2,6-pyridyl-^{14}C]-fluopicolide in the metabolism in rotational crop study.

Finally, for oilseed rape foliage (BBCH 17-19) investigations were conducted in a metabolism in primary crop study with both [phenyl-U-^{14}C]-fluopicolide and [2,6-pyridyl-^{14}C]-fluopicolide after seed treatment. The dose rate in this study was 10 times (nominal 120 g/kg seed) the normal field application rate to aid investigation into the metabolism of fluopicolide in oilseed rape.

The metabolites M-01 (AE C653711), M-02 (AE C657188), M-04 (AE C657378), M-05 (AE 1344122), M-06 (AE C643890) and M-09 (AE B102859) met the criteria of > 10% TRR or > 0.05 mg/kg. It should be noted the maximum overall concentration of each metabolite either as %TRR or as mg/kg did not typically come from the same matrix sample.

Metabolite	Overall Maximum Concentration		
	%TRR	mg/kg (as metabolite)	Comment
M-01	87.5	2.170	Maximum values from different matrices
M-02	43.0	1.087	Maximum values from same matrix
M-04	59.3	0.870	Maximum values from different matrices
M-05	41.0	0.108	Maximum values from different matrices
M-06	2.8	0.068	Maximum values from different matrices
M-09	10.5	0.052	Maximum values from different matrices

[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).

Residue data from supervised residue trials and rotational residue trials are available for each of the metabolites listed above for the metabolites M-01 and M-02 in oilseed rape (green material) and for M-01, M-02, M-04, M-05, M-06 and M-09 in lettuce. The purpose of supervised residue trials is to determine the magnitude of the residues under realistic field conditions and data from these trials should be considered in the ecotoxicological assessments in preference to data from radiolabelled studies. In addition, a number of conjugated metabolites were detected and identified in samples of wheat forage sown 29 days after treating soil with [phenyl-U-^{14}C]-fluopicolide or [2,6-pyridyl-^{14}C]-fluopicolide in an addendum to the metabolism in rotational crop study. These metabolites are reported as %TRR only (and in some cases the %TRR is for a radio-peak subsequently separated into different metabolites). In all cases amounts do not exceed 10% TRR. The metabolite concentration has been calculated from the reported data and exceeds 0.05 mg/kg for M-18, M-23, M-25, M-26, M-27, M-28 and M-32.

Metabolite	Overall Maximum Concentration			
	%TRR	mg/kg (as conjugate)	mg/kg (as free metabolite)	Comment
M-18 (P11)	1.6	0.086	0.071	Animal metabolite observed in hen, cow & rat. Sulfate conjugate of M-06 or its isomer
M-23 (P2a,b)	2.2 [A]	0.129	0.058	2 malonyl glucoside conjugates of M-04 and its isomer
M-25 (P4a)	6.3 [A]	0.394	-	Major rat metabolite in bile. Conjugate which contains both phenyl and pyridyl rings
M-26 (P4b)	3.4 [A]	0.284	-	Conjugate which contains both phenyl and pyridyl rings
M-27 (P4c)	0.7 [A]	0.052	-	Conjugate which contains both phenyl and pyridyl rings
M-28 (P5)	3.5	0.293	0.180	Malonyl glucoside conjugate of M-06
M-32 (P10)	2.4	0.155	-	Conjugate which contains both phenyl and pyridyl rings

[A] Individual %TRR for M-23, M-25, M-26 and M-27 (called P2a,b, P4a, P4b and P4c in the report) have been recalculated from reported data.

M-18 and M-25 are known animal metabolites, and M-25 is the main metabolite observed in rat bile (ca. 50% dose).

M-23 is a malonyl glucoside conjugate of M-04 (AE C657378) and its isomer, while M-18 and M-28 are sulfate and malonyl glucoside conjugates of M-06 (AE C643890). If ingested by a small mammal it is presumed malonyl glucoside plant conjugates will be cleaved to their aglycons via glucoside conjugates. When considered in combination with the levels of the free unconjugated metabolite the overall maximum concentrations are as follows.

Metabolite	Overall Maximum Concentration	
	%TRR	mg/kg
M-04	59.3 (59.3)	0.928 (0.870)
M-06	5.6 (2.8)	0.251 (0.068)

Values for unconjugated metabolite are in parentheses

Metabolites seen in the confined rotational crop study are presented within Appendix 1 of this document.

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]

Translocation of radioactive residues from the soil was observed (for all crops, at all plant back intervals) in the confined rotational crop study ([redacted], 2003; M-240707-03-1). The relevant information is summarised within the following table. In general, the highest residues were found at the shortest interval, in this case 29 days after soil application.

Total radioactive residues (mg/kg fluopicolide equivalents) in crops (mean values):

Phenyl Label			
Crop	**Total Radioactive Residue (mg/kg fluopicolide equivalents)**		
	29 Day	**133 Day**	**365 Day**
Lettuce	1.01	0.10	0.53
Radish Tops	6.40	0.23	1.75
Radish Roots	0.13	0.02	0.03
Immature Wheat	4.95	0.22	0.86
Wheat Grain	0.16	0.02	0.05
Wheat Straw	13.56	0.84	2.37
Pyridyl Label			
Crop	**Total Radioactive Residue (mg/kg fluopicolide equivalents)**		
	29 Day	133 Day	365 Day
Lettuce	0.27	0.03	0.05
Radish Tops	1.96	0.23	0.40
Radish Roots	0.09	0.02	0.02
Immature Wheat	4.29	0.16	0.24
Wheat Grain	2.60	0.10	0.18
Wheat Straw	7.05	0.35	1.01

The total radioactivity in soil was found to decline steadily over the course of the study. Total radioactive residues in plant matrices declined with longer soil ageing. The mean residues in 29-day (Raw Agricultural Commodities) RACs ranged from 0.09 ppm (radish root) to 13.56 ppm (wheat straw), but residues declined greatly in the 133-day and 365-day ageing periods. The 133-day crop

[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.

residues ranged from 0.02 ppm (radish root) to 0.84 (wheat straw). The 365-day crop residues were observed to increase slightly, ranging from 0.02 ppm (radish root) to 2.37 ppm (wheat straw). This was considered to be a result of seasonal variation. The 133-day plots were planted in October and developed through the winter when formation of soil metabolites from the degradation of parent would be slowest. In contrast, the 365-day plots were planted in March when the plant uptake would be less pronounced, due to the increased degradation.

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?

In the confined rotational crop studies [redacted] 2003; M-240707-03-1), [^{14}C] phenyl and pyridinyl ring labelled fluopicolide was applied to soil at a rate of 0.4 kg a.s./ha. Lettuce, wheat and radish were planted after 29, 133 and 365 days of ageing. The highest total radioactive residue (TRR) levels were observed at a plant back interval (PBI) of 29 days, in wheat straw (up to 13.6 mg eq./kg), radish tops (up to 6.71 mg eq./kg), wheat grain (up to 2.6 mg eq./kg) and lettuce (up to 1.01 mg eq./kg). Although total radioactivity tends to decline over time in the succeeding crops, significant levels were also found at the PBI of 365 days (up to 2 mg eq./kg in radish tops, 1.0 mg eq./kg in wheat straw and 0.62 mg eq./kg in lettuce).

Based on this information, residues tend to accumulate within the leafy (aerial) potions of the crops and cereal grains (for the early PBIs), but lower levels tend accumulate within the roots (based on the data for radishes).

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?

The seed treatment use for winter oilseed rape (product – Scenic Gold®) is included among the representative uses sought for the fluopicolide renewal:

The residues field trials did not cover the magnitude of the residues for early post emergence (BBCH <10). The studies included an initial assessment of the residue adhered to the surface of the treated seed. The first assessment of the residues for the plant was made at BBCH 19 (green material) and for the seed and rest of the plant at commercial harvest (BBCH 89). The residues BBCH 19 and 89 were <LOQ (0.01 mg/kg) for metabolites M-01 and M-02. For the fluopicolide residues, two of the

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

trials showed levels of 0.02 mg/kg at BBCH 19, with the remaining trials showing levels <LOQ (0.01 mg/kg). No residues above the LOQ were found in the seed / rest of the plant at BBCH 89 for fluopicolide.

Study references

Test commodity	Report reference	Author, Year	Dossier reference
Oilseed rape	M-390353-01-1	[redacted] 2010a	M-CA 6.3.5
Oilseed rape	M-396237-02-1	[redacted] 2010a	M-CA 6.3.5
Oilseed rape	M-390357-01-1	[redacted] 2010b	M-CA 6.3.5

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]

Of the representative uses, only lettuce has any residue decline trials which contain meaningful information for the ecotoxicology risk assessment. While some decline trials are available for potatoes, the vast majority of the residue levels at all pre-harvest intervals (PHIs) were <LOQ (<0.01 mg/kg), with only a few of the results being at or slightly above the LOQ. Decline trials were not conducted in the seed treated oilseed rape and (indoor) cucumber residue trial studies.

The supervised residue trials for lettuce are summarised and referenced within Appendix 2 of this document. In the trials, fluopicolide residue levels were typically found to gradually decline in lettuce heads over a 14-day period.

For metabolite M-01, many of the trials showed <LOQ (0.01 m/kg) residue levels. In some cases, residues were observed and some of the trials showed decline from day 0, to day 7 and finally to day 14. In other cases, an upturn in the residue content was observed from day 7 to day 14, which may be explained by an uptake of M-01 from the soil.

Residue levels of metabolite M-02 (which does not form part of the risk assessment residue definition for consumers) were practically <LOQ (<0.01 mg/kg) in all of the trials, with two exceptions (0.012 and 0.015 mg/kg).

[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).

The residues field trials were conducted according to the guidance in place at the time when they were conducted. All of the trials were conducted at rates and timings comparable to the requested GAPs for the fluopicolide 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.

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

Residues trials have been conducted to support the representative uses on potatoes, lettuces, cucumbers (indoor only) and oilseed rape (seed treatment only). According to the EU 'Guidelines on comparability, extrapolation, group tolerances and data requirements for setting MRLs' (SANCO 7525/VI/95Rev. 10.3), it is possible to extrapolate the data generated on the representative commodities to support other similar crops (those specifically identified within the EU guidance document). However, for the purposes of the renewal, no additional uses for extrapolated commodities have been sought.

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]

A fish metabolism study has not been undertaken for fluopicolide. According to the current EU guidance (SANCO/11187/2013 rev. 3) the metabolism in fish is only required where the partition coefficient (Log P_{OW}) is ≥ 3. Based on the partition coefficients available in the physical-chemical properties data package for fluopicolide, neither the parent (fluopicolide) nor the primary metabolite (BAM) are considered to be fat soluble. However, a fish bioaccumulation study is available ([redacted] 2003; M-241273-01-1). The bioaccumulation of the fluopicolide residues in fish was determined using a continuous flow-through set-up over 45 days (which included a 24-day uptake period and a 21-day depuration period). The

[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.

study was previously assessed during the original EU inclusion for fluopicolide (DAR, 2006; RMS = UK). A brief summary of the study is described within the following paragraph:
The study showed that [2,6-14C-pyridinyl]-fluopicolide accumulates rapidly in fish tissues (bluegill sunfish), principally in the non-edible portions, regardless of the exposure concentration. The steady-state bioconcentration factors (BCFs) for the low treatment (0.8 µg/L) were 48x, 117x and 197x in edible, whole fish, and non-edible, respectively. For the high treatment (8.0 µg/L) were 40x, 104x, and 175x in edible, whole fish and non-edible, respectively. Fluopicolide cleared rapidly from fish tissues regardless of the exposure concentration. The depuration appeared to be biphasic with the "fast" phase as the major component. Based on a one-compartment model with whole fish, the calculated bioconcentration concentration factor's (BCFs) were 121x and 102x for the low and high treatment, respectively. The time to reach 90% of the steady-state was about 2 days for both treatments. The depuration half-life was much shorter at about 0.5 day for both treatments.

The major residue in all fish tissues was unchanged parent fluopicolide:

Treatment	Tissue type	Residue in analysed extracts		Fluopicolide residues		% Identified	Largest single unidentified component	
		mg/kg	%	mg/kg	%		mg/kg	%
Low (0.8 µg/L)	Edible	0.039	87.6	0.039	87.6	100	-	-
	Non-edible	0.158	91.4	0.128	73.8	73.8	0.013	7.8
High (8.0 µg/L)	Edible	0.271	85.5	0.271	85.5	100	-	-
	Non-edible	1.228	91.8	0.908	67.9	67.9	0.169	12.7

While the bioaccumulation study noted that fluopicolide rapidly accumulates within the tissues of fish, the study also shows that the fluopicolide residue levels rapidly clear from the fish tissues during the depuration phase. No metabolites of fluopicolide were identified and only fluopicolide was present within the edible portions of the analysed fish.
As low BCFs were obtained during the study for the low and high treatment rates, it can be concluded there is no indication of any significant accumulation of residues within fish tissues.

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?

The following tables summarised the residue levels found within animal tissues / products from the metabolism studies:

Fluopicolide poultry metabolism study (dose level of 10 mg/kg in the diet):

Tissue	Residue level (ppb)	Extracted (ppb)	Total ^{14}C-residue identified/characterised (ppb)						
			FLC	M-06	M-01	Metabolite 1	Unknown	Polar	Non-extracted
Egg white	43	42	1	n.d.	n.d.	22	n.d.	n.d.	1
Egg yolk	154	126	17	n.d.	n.d.	n.d.	20	69	35
Liver	976	762	n.d.	53	361	n.d.	212	n.d.	214
Skin	69	47	n.d.	10	n.d.	7	n.d.	23	22
Fat	61	46	4	n.d.	n.d.	23	n.d.	12	15
Muscle	39	22	n.d.	n.d.	n.d.	n.d.	n.d.	22	17

n.d. = not detected

M-01 (BAM) poultry metabolism study (dose level of 2 mg/kg in the diet):
Limited metabolism of M-01 (BAM) in the hen was observed with M-01 (BAM) excreted essentially unchanged following 14 days repeated oral administration. There was indication of accumulation of M-01 (BAM) in eggs and in the high proportion of the cumulative dose detected in edible tissues at sacrifice. Only minor metabolites of BAM were detected in the excreta, mainly hydroxylation products.

[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.

Metabolite	Liver		Omental Fat		Egg Yolk[a]		Egg White[a]		Muscle	
	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg	% TRR	mg eq./kg
Chromatographed radioactivity	98.2	10.34	98.3	1.90	98.9	5.20	93.3	2.59	96.5	3.34
Identified metabolites										
M-01 (BAM)	96.4	10.16	96.2	1.86	97.9	5.15	93.3	2.59	96.0	3.32

a) Pool of egg yolks and whites Day 7 – Day 14

Fluopicolide ruminant metabolism study (dose level of 10 mg/kg in the diet):

Tissue	Residue level (ppb)	% Extracted	% Total ^{14}C-residue identified/characterised					
			FLC	M-06	M-07	M-01	Polar§	Non-extracted
Urine		NA	-	39	8.5	-	47	NA
Faeces		21.6	14.0	1.7	0.92	-	1.7	78.4
Milk	19	85.9	36.9	-	-	3.9*	37.8	14.1
Fat	41	84.9	78.4	-	-	-	-	16.8
Muscle	24	28.2	5.1	-	-	-	13.2	74.2
Liver	644	89.9	0.9	1.6	1.2	-	79.7	10.9
Kidney	302	92.4	0.7	6.8	3.3	-	77.5	7.6

NA = Not Applicable
* The presence of this metabolite could not be confirmed in a second system or by HPLC/MS.
§ In most cases there were a number of areas of radioactivity in the polar region, each of which could contain more than one metabolite.

M-01 (BAM) ruminant metabolism study:
There was indication of accumulation of M-01 (BAM) in milk and in the high proportion of the cumulative dose detected in edible tissues at sacrifice. More extensive metabolism was found in the liver and kidneys.

Conventional extraction:

Sample	Skimmed Milk Day 2-4 Pool		Muscle Pool		Fat Pool		Liver		Kidney	
TRR [mg/kg]	0.104		0.690		0.238		13.977		6.249	
	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg	% of TRR	mg/kg
M-01 (BAM)	82.1	0.085	69.6	0.481	92.4	0.220	16.3	2.278	9.4	0.586
L1, Glutathione conjugate	---	---	---	---	---	---	14.3	2.007	---	---
L2, USHD/9 relation	---	---	---	---	---	---	23.3	3.263	---	---
L3/K4, USHD/6	---	---	---	---	---	---	35.8	5.015	22.8	1.423
L5/K13, FSHD/8	---	---	---	---	---	---	1.3	0.188		
L6							0.8	0.118		

K1/K2 USHD/3									19.2	1.198
K3									11.1	0.693
K7 USHD/10b									9.9	0.615
K13									9.7	0.607
Total identified	**82.1**	**0.085**	**69.6**	**0.481**	**92.4**	**0.220**	**91.9**	**12.869**	**82.1**	**5.122**

References

Test animal (test compound)	Report reference	Author, Year	Dossier reference
Poultry (FLC)	M-233361-02-1	[redacted] 2003	M-CA 6.2.2
Poultry (FLC)	M-233977-03-1	[redacted] 2009	M-CA 6.2.2
Cow (FLC)	M-233391-02-1	[redacted] 2003	M-CA 6.2.3
Cow (FLC)	M-218626-02-1	[redacted] 2008	M-CA 6.2.3
Poultry (M-01)	Not available	[redacted] 2020	Preliminary results provided – report not yet finalised
Goat (M-01)	Not available	[redacted] 2020	Preliminary results provided – report not yet finalised

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CP 10.1 Effects on birds and other terrestrial vertebrates

CP 10.1.1 Effects on birds

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".

Table 10.1.1- 1: Endpoints used in risk assessment

Test substance	Risk assessment	Test species	Endpoint		Reference
Fluopicolide	Acute	Mallard duck	LD_{50}	> 2250 mg a.s./kg bw	[redacted] 2001; M-240576-01-1 KCA 8.1.1.1/01
			LD_{50}	= 4248 mg a.s./kg bw [a)]	Extrapolated acc. to EFSA GD 2009
		Bobwhite quail	LD_{50}	> 2250 mg a.s./kg bw	[redacted] 2001; M-240577-01-1 KCA 8.1.1.1/02
			LD_{50}	= 4248 mg a.s./kg bw [a)]	Extrapolated acc. to EFSA GD 2009
		Zebra finch	LD_{50}	= 1105 mg a.s./kg bw	[redacted] 2015; M-544294-01-1 KCA 8.1.1.1/03
		Bird	**LD_{50}**	**= 2711 mg a.s./kg bw** [b)]	Geometric mean acc. To EFSA GD 2009
	Short-term	Bobwhite quail	LC_{50} LDD_{50}	> 5620 ppm > 1744 mg a.s./kg bw/day	[redacted] 2002; M-240713-01-1 KCA 8.1.1.2/01
		Mallard duck	LC_{50} LDD_{50}	> 5620 ppm > 2343 mg a.s./kg bw/day	[redacted] 2002; M-240714-01-1 KCA 8.1.1.2/02
	Long-term	Bobwhite quail	NOAEC NOAEL EC_{10}	≥ 1000 ppm ≥ 88.9 mg a.s./kg bw/day = 46.7 (29.7 – 89.7) mg a.s./kg bw/d	[redacted] 2003; M-225403-01-2 KCA 8.1.1.3/01 EC_{10} calculation [redacted] 2019; M-660212-01-1 KCA 8.1.1.3/03

Test substance	Risk assessment	Test species	Endpoint	Reference
		Mallard duck	NOEC ≥ 1000 ppm NOEL ≥ 140.8 mg a.s./kg bw/day EC_{10} **= 32.2 (31.1 – 33.4) mg a.s./kg bw/d**	[redacted] 2003; M-225404-01-2 KCA 8.1.1.3/02 EC_{10} calculation [redacted] 2019; M-663971-01-1 KCA 8.1.1.3/04
M-01 (2,6-dichloro-benzamide)	Short-term	Bobwhite quail	LC_{50} = 3867 ppm LDD_{50} = 1171 mg a.s./kg bw/day	[redacted] 2003; M-225551-01-2 KCA 8.1.1.2/03
Fluoxastrobin	Acute	Bobwhite quail	LD_{50} > 2000 mg a.s./kg bw	EFSA Scientific Report 102 (2007)
			LD_{50} = 3776 mg a.s./kg bw [a)]	Extrapolated acc. to EFSA GD 2009
	Long-term	Mallard duck	NOEL = 51 mg/kg bw/day	EFSA Scientific Report 102 (2007)
Fluopicolide + Fluoxastrobin	Acute	Bird	**$LD_{50\ MIX}$** **= 3084 mg total a.s./kg bw**	Table 10.1.1-6

Endpoints in **bold** considered relevant for risk assessment

a) The study endpoint was extrapolated according to EFSA GD 2009. The extrapolation factor of 1.888 was derived from EFSA GD 2009, section 2.1.2, table 1 for studies in which 10 animals were dosed and no mortality occurred.

b) In accordance with EFSA GD 2009, the geometric mean LD_{50} of the three species mallard duck (LD_{50} = 4248 mg a.s./kg bw), bobwhite quail (LD_{50} = 4248 mg a.s./kg bw) and zebra finch (LD_{50} = 1105 mg a.s./kg bw) was used.

Metabolites of fluopicolide

The metabolites of fluopicolide do not pose higher risk to birds than the parent compound. This is also confirmed by the EFSA Scientific report 299 (2009), wherein it is stated, that the risk to birds from plant metabolites of fluopicolide is considered to be low. Furthermore, a study conducted in 2019 (M-683112-01-1 see this document MCP 10.1.1.2/03) shows residue levels of the most relevant metabolites M-01 (AE C653711) and M-02 (AE C657188) to reach maximum concentration of only 0.019 mg p.m./kg and <0.01 mg p.m./kg, respectively. Therefore, a potential risk from metabolites should be covered by the risk assessment of the parent compound fluopicolide (see below). As a further line of evidence for M-01 and M-02, a worst case risk assessment for herbivorous bird exposure to plant metabolites can be based on the maximum RUDs determined by [redacted] 2020 (M-686445-01-1, MCP Infinito 10.1.1.2/01) in foliage sampled during the course of rotational crop studies. Here, the toxicity endpoint is set at one tenth of the reproductive risk assessment endpoint for the parent (see 'Refined risk assessment for birds feeding on rape shoots' further below).

Table 10.1.1- 2: Calculation of the maximum amount of active substances on one dressed seed

Crop	Product loading [L prod./dt seeds]	Content of a.s. within the product [g a.s./L prod.]	Nominal loading/ application rate (NAR) [mg a.s./kg seeds]	Max. amount of a.s. on one dressed seed [a)] [µg a.s./seed]
Winter rape	1.0	FLC: 200 FXA: 150	FLC: 2000 FXA: 1500	FLC: 14.0 FXA: 10.5

a) Assuming a weight of thousand seeds of 4 – 7 g according to GAP. For the calculations 7 g was used as a worst case.

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

Scenario	Generic focal species	Calculation of residues	
		Acute assessment	Reproductive assessment
Birds feeding on seeds (small seeds)	Small granivorous bird	NAR × 0.3	NAR × 0.3 × ftwa
Birds feeding on seedlings	Small omnivorous bird	NAR/5 × 0.5	NAR/5 × 0.5 × ftwa

NAR= Nominal loading/application rate

ACUTE DIETARY RISK ASSESSMENT

Birds feeding on seeds

Table 10.1.1- 4: First-tier acute risk assessment for birds feeding on seeds (fluopicolide)

Crop	Generic focal species	NAR [mg a.s./kg seeds]	FIR/bw	NAR × FIR/bw	LD_{50} [mg a.s./kg bw]	TER_a	Trigger
Winter rape	Small granivorous bird	2000	0.3	600	2711	**4.52**	10

The TER_A value for fluopicolide calculated in the acute risk assessment for birds feeding on seeds is below the acceptability trigger of 10. Therefore, further refinement steps are provided further below.

Birds feeding on seedlings

Table 10.1.1- 5: First-tier acute risk assessment for birds feeding on seedlings (fluopicolide)

Crop	Generic focal species	NAR [mg a.s./kg seeds]	FIR/bw	NAR/5 × FIR/bw	LD_{50} [mg a.s./kg bw]	TER_a	Trigger
Winter rape	Small omnivorous bird	2000	0.5	200	2711	13.56	10

The TER_A value for fluopicolide calculated in the acute risk assessment for birds feeding on seedlings is above the acceptability trigger of 10. Therefore, no further refinement steps are necessary.

Please note: For the active substance fluoxastrobin the scenario of birds feeding on seedlings does not apply as the uptake of fluoxastrobin into the plant is relatively low and the substance in general can be regarded to be non-systemic. Therefore, the use of the $LD_{50\ MIX}$ is not considered for the seedling eater risk assessment in the combined toxicity risk assessment below.

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 performed.

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 the FLC+FXA FS 350 in this AIR-evaluation, but it may be conducted post-AIR according to the respective zonal guidances.

For the assessment of acute effects (mortality), a surrogate LD_{50mix} 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_{50mix} 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 FLC + FXA FS 350 addressed in this dossier is 200 g fluopicolide/L prod. and 150 g fluoxastrobin/L prod., making up a total of 350 g a.s./L product.

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

Table 10.1.1- 6: Avian LD_{50} (mix) for fluopicolide and fluoxastrobin when combined as FLC+FXA FS 350 (step 1 in Appendix B of EFSA GD 2009)

	Fluopicolide	Fluoxastrobin
Content of a.s. in product [g a.s./L prod.]	200	150
Fraction in the a.s. mixture	0.5714	0.4286
LD_{50} of a.s. [mg a.s./kg bw]	2711	3776
Fraction / LD_{50}	0.0002108	0.0001135
Sum	0.0003243	
1/sum = predicted LD_{50} (mix) [mg total a.s./kg bw]	3084	

Fluopicolide contributes to 65 % to mixture toxicity, while fluoxastrobin has 35 % impact on the mixture toxicity (see table below). Consequently, the risk assessment cannot be performed only for the most toxic active substance alone and further considerations according to Steps 2–4 are necessary.

Table 10.1.1- 7: Avian "tox per fraction" for FLC+FXA FS 350 (step 1 in Appendix B of EFSA GD 2009)

	Fluopicolide	Fluoxastrobin	"mix"
Content of a.s. in product [g a.s./L prod.]	200	150	350
Fraction in the a.s. mixture	0.5714	0.4286	1
LD_{50} of a.s. [mg a.s./kg bw]	2711	3776	3084
Tox per fraction	4744	8810	13555
Contribution to predicted toxicity	65 %	35 %	100 %

EFSA GD 2009 recommends as next step (2a and 2b in Appendix B) to check the predicted toxicity against measured toxicity from LD_{50} studies conducted with the formulation. However, no study with the formulation was submitted for birds. Therefore, the calculated surrogate endpoint is used for the risk assessment.

Table 10.1.1- 8: First-tier acute risk assessment for birds feeding on seeds (product)

Crop	Generic focal species	NAR [mg total a.s./kg seeds]	FIR/bw	NAR × FIR/bw	$LD_{50\,MIX}$ [mg total a.s./kg bw]	TER_a	Trigger
Winter rape	Small granivorous bird	3500	0.3	1050	3084	**2.9**	10

The TER_A value, calculated for a surrogate endpoint, does not exceed the trigger value of 10. Therefore, further refinement is provided further below.

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

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/day (EFSA GD 2009, Appendix K).

An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER less than direct dietary consumption.

LONG-TERM REPRODUCTIVE ASSESMENT

Birds feeding on seeds

Table 10.1.1- 9: First-tier reproductive risk assessment for birds feeding on seeds (fluopicolide)

Crop	Generic focal species	NAR [mg a.s./ kg seeds]	FIR/bw	f_{twa}	NAR × FIR/bw × f_{twa}	EC_{10} [mg a.s./ kg bw/day]	TER_{lt}	Trigger
Winter rape	Small granivorous bird	2000	0.3	0.79 [a)]	474	32.2	**0.07**	5

a) Worst case value based on a germination time of 7 days and a default DT_{50} of 10 days

The TER_{lt} value for fluopicolide calculated in the reproductive risk assessment for birds feeding on seeds is below the acceptability trigger of 5. Therefore, further refinement steps are provided further below.

Birds feeding on seedlings

Table 10.1.1- 10: First-tier reproductive risk assessment for birds feeding on seedlings (fluopicolide)

Crop	Generic focal species	NAR [mg a.s./ kg seeds]	FIR/bw	f_{twa}	NAR/5 × FIR/bw × f_{twa}	EC_{10} [mg a.s./ kg bw/day]	TER_{lt}	Trigger
Winter rape	Small omnivorous bird	2000	0.5	0.53	106	32.2	**0.30**	5

The TER_{lt} value for fluopicolide calculated in the reproductive risk assessment for birds feeding on seedlings is below the acceptability trigger of 5. Therefore, further refinement steps are provided further below.

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

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/day (EFSA (2009), Appendix K).

An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER less than direct dietary consumption.

RISK ASSESSMENT OF SECONDARY POISONING

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

The log Pow value of fluopicolide is 2.9 and thus below the trigger value of 3. The active substance has a negligible potential to bioaccumulate in animal tissues. No formal risk assessment from secondary poisoning is therefore required.

REFINED RISK ASSESSMENT

Refined risk assessment for seed eating birds - weight of evidence approach

The EFSA Guidance Document (5.2, Risk assessment for treated seeds) states following: *"Tier 1 assumes that granivorous birds and mammals feed entirely on readily available, freshly treated seeds. The failure rate of pesticides used as seed treatments to meet the EU triggers for acute and reproductive risks under such a scenario is likely to be high. […] The outcome of a refined assessment would, in most cases take the form of a weight-of-evidence approach, rather than a quantitative assessment (e.g. TER)"*

Note that the current higher tier risk assessment follows a weight of evidence approach as indicated above. This means that all available data (quantitative as well as qualitative) were gathered together to provide a more realistic assessment of any potential risk.

Focal species, attractiveness of freshly drilled winter OSR fields and PT consideration

Different studies were performed to generate a list of real focal species on bare soil. For winter OSR the following small seed eaters, which are the birds potentially most at risk, were regularly found ([redacted] 2001, M-031392-01-1; [redacted] 2006, M-279936-01-1; [redacted] 2018, M-629338-01-1, LoA M-631447-01-1): **Linnet**, **Skylark**, **Chaffinch**, and **Yellowhammer**. For these species, PT values were determined in the study by [redacted] (2006, M-279936-01-1) as well as in the study by [redacted] (2018, M-629338-01-1) (see tables below). In the study by [redacted] (2006, M-279936-01-1) stomach and faeces samples were additionally investigated. **For Yellowhammers and Chaffinches, no OSR seeds were found**. However, OSR seeds were detected in one linnet's stomach. The author suggested that this may have originated from spilled seeds (i.e. untreated harvest remains).

Table 10.1.1- 11: PD values for real focal species as found in the study by [redacted] (2006, M-279936-01-1)

DIET of species in oilseed rape fields				
	Food items	Yellowhammer (n = 10)	Chaffinch (n = 12)	Linnet (n = 5)
Numerical portion of food items [%] after the analysis of faeces (25) and samples of stomach flushing (1) and stomach contents (1) gathered in or near by oilseed rape fields	*Brassica napus* (OSR) seeds	0	0	(19.6)*
	Brassica seeds - unspecified	2.6	7.7	19.6
	cereal seeds	5.3	3.1	0
	wheat seeds	56.6	12.3	0
	other seeds	6.6	21.5	37.0
	other plant material	0	7.7	23.9
	Coleoptera	3.9	20.0	0
	Dermaptera	6.6	13.8	0
	Diptera	11.8	3.1	0
	Hymenoptera	2.6	7.7	0
	other animals	2.6	3.1	0
	Unknown	1.3	0	0

* Oil seed rape found in the stomach of a dead bird (road kill) near the farm, they may originate from spilled seeds (untreated harvest remains) on the premises of the agrarian cooperative.

According to the study by [redacted] (2018, M-629338-01-1) the **Woodpigeon** can additionally be identified as being a potentially relevant species due to its high frequency of occurrence. Although considerably larger than the other focal species indicated above, and therefore not further investigated in a radiotracking study, the Woodpigeon will also be considered in the refined risk assessment, further below.

Table 10.1.1- 12: PT values for real focal species as found in the study by [redacted] (2006, M-279936-01-1) see MCP 10.1.1.2/11

Species	PT - 90th percentile [%]	PT - max [%]	Tracking sessions (individuals' total/consumers)
Yellowhammer	17.24	17.45	10 (10/3)
Chaffinch	27.32	27.32	9 (9/5)
Linnet	--	--	3 (3/0)

Table 10.1.1- 13: PT values for real focal species as found in the study by [redacted] (2018, M-629338-01-1) see MCP 10.1.1.2/07

Species	PT - 90th percentile [%] Consumer approach	PT - max [%]	Tracking sessions (individuals' total/consumers)
Yellowhammer	0.3	0.3	10 (10/1)
Chaffinch	--	--	12 (12/0)
Skylark	23	24.4	23 (20/6)

In general, there is a concern over the use of the 'consumer approach' for a PT calculation, i.e. taking only those individuals into account which have been tracked on the field at least once. This approach might make sense if individuals have been trapped (and equipped with a transmitter) some distance away from the investigated fields. In recent studies, however, care is taken to trap focal species in the direct vicinity/at the direct border of the fields under investigation, so that tracked individuals **do easily have access to these fields**. Furthermore, whenever choosing an area for a study conduct researches take care that not only one appropriate field is accessible for a focal bird species but that it has access to several fields of the same crop and crop stage (i.e. in the current case freshly drilled winter OSR fields). Under a landscape perspective this is considered a conservative approach.

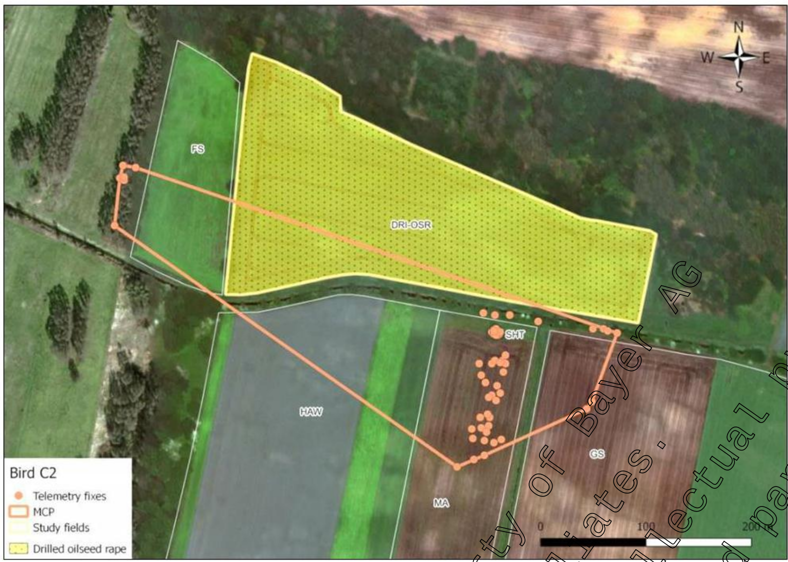

Figure 10.1.1- 1: A representative example for a radiotracked Chaffinch in the study by [redacted] (2018, M-629338-01-1) which is not considered in a PT calculation as it did not enter the field (not a defined consumer). The figure clearly shows the accessibility of the field. However, it was obviously not attractive to the bird.

In the study by [redacted] (2018, M-629338-01-1) only 1 out of 10 Yellowhammers, 0 out of 12 Chaffinches and 6 out of 20 Skylarks entered the freshly drilled OSR fields. Irrespective of how a PT is calculated and/or what the calculated value looks like, this numbers clearly show the low attractiveness of freshly drilled winter OSR fields in general. The study by [redacted] (2006, M-279936-01-1) showed similar results as only 3 out of 10 Yellowhammers and 5 out of 9 Chaffinches entered the fields. The number of radio-tracked linnets in this study was too low to give much indication, however none of the 3 tracked Linnets visited freshly drilled winter OSR fields. Because the linnet was the only species for which OSR seeds were found to be ingested (see above, [redacted] 2006, M-279936-01-1) a new study was conducted ([redacted] 2020, M-684638-01-1) to provide more evidence on a potential risk to linnets from treated OSR seeds. In advance of this study the conducting researchers carefully mapped the broader area and took care that not only several fields were available on which farmers intended to drill OSR but also fields were abundant where OSR was grown the season before. On the latter fields remains of the previous year's harvest were available. Note that harvested OSR fields in the vicinity of freshly drilled fields is considered representative.

The purposes of the study of [redacted] (2020, M-684638-01-1), were;

i) to assess the proportion of diet (PT) that linnets (*Carduelis cannabina*) obtain in winter oilseed rape fields, both freshly drilled and harvested, during the pre-emergence period; and

ii) to provide observational data of the occurrence of linnets in freshly drilled OSR fields in comparison to harvested OSR fields, in Germany.

Four trapping locations were used to trap and radio-tag linnets. Moreover, 20 harvested fields and 20 freshly drilled fields were used as reference for the agricultural status during the telemetry sessions conducted.

Of the study fields used for scan sampling, 10 were harvested and 10 were freshly drilled in pre-emergence period (from BBCH 00 to 07). They were selected by their surrounding habitat structure in order to match requirements of different species of birds, especially for the linnets. Continuous radio tracking sessions on 21 linnets allowed a representative assessment of potential foraging times in order to calculate PT values. Thirteen of the radio-tracked linnets (n=21) had freshly drilled oilseed rape fields in their home range and none of them entered the pre-emergence OSR fields while radio-tracked (PT = 0). In contrast, 20 of 21 linnets entered a harvested oilseed rape field during the radio tracking period (95.2% of all birds). PT values for consumer individuals in harvested fields ranged between 0.58 and 0.08 (90%ile = 0.52). This results clearly show that linnets are not attracted to freshly drilled OSR seeds.

Despite the general low attractiveness, refined TER_{lt} values are depicted below using 90%tile PTs for the focal species Yellowhammer, Chaffinch and Skylark (17.24, 27.32, and 23%, respectively). For this, individual FIR/bw were calculated, assuming that focal species would eat 100% seeds whenever they were found active on the field. Again, this is a highly conservative approach, as activity on a field does not necessarily mean food intake of treated seeds (note that in the faeces of potentially exposed Yellowhammer and Chaffinch no OSR seed remains were found).

Table 10.1.1.2- 14: Higher tier reproductive risk assessment for birds feeding on seeds (fluopicolide)

Generic focal species	Body weight (g)	NAR [mg a.s./ kg seeds]	FIR/bw	f_{twa}	PT [%]	NAR × FIR/bw × f_{twa} × PT	EC_{10} [mg a.s./ kg bw/day]	TER_{lt}	Trigger
Yellowhammer	26.5	2000	0.24	0.79 a)	17.24	65.4	32.2	**0.49**	5
Chaffinch	20.9	2000	0.26	0.79 a)	27.32	112.2	32.2	**0.29**	5
Skylark	37.2	2000	0.21	0.79 a)	23	76.3	32.2	**0.42**	5
Linnet	15.3	2000	0.28	0.79 a)	0	0	32.2	- b)	5

a) Worst case value based on a germination time of 7 days and a default DT_{50} of 10 days
b) TER non-calculable because PT = 0 and consequently DDD = 0. Risk is negligible.

The refined TER_{lt} values for fluopicolide calculated in the reproductive risk assessment for birds feeding on seeds are below the acceptability trigger of 5 for Yellowhammer, Chaffinch and Skylark. Therefore, further refinement steps are necessary.

Drilling and reproduction period of relevant focal bird species

A potential reproductive issue can be assumed if the exposure of a plant protection product falls into or at least overlaps with the reproduction period of the relevant focal bird species. Relevant bird species have been elaborated above, based on several field studies. These were the Linnet, Skylark, Chaffinch, Yellowhammer and the Woodpigeon.

A report is available from [redacted] (2017, M-616722-01-1; LoA M-620031-01-1) in which reproductive information of different bird species were compiled. The knowledge of the duration of bird breeding seasons will help to consider relevance of any potential exposure in the assessments. For this, volunteer collected data from pulli ringing across Europe was used to model the breeding season period across Europe for 14 species common to farmland habitats in relation to differences in habitat, elevation, climate, longitude and latitude. In order to achieve a conservative estimate of breeding season timing rather than an estimate of the mean value across the species, a quantile regression was used. The reliability and predictive ability of these models was explored across the fourteen species.

Predictions were generated from the quantile regression models for first egg date (FED), last egg date (LED) and fledging date (FLG) for each species. The table below displays the predictions for the whole region using the 0.10 quantile for FED and the 0.90 quantile for LED and FLG. Country specific predictions were also generated.

	Whole region FED (0.10 quantile)	Whole region LED (0.90 quantile)	Whole Region FLG (0.90 quantile)
Chaffinch	01-Apr (28-Mar to 05-Apr)	28-Jun (18-Jun to 07-Jul)	16-Jul (08-Jul to 26-Jul)
Linnet	11-Apr (06-Apr to 16-Apr)	28-Jul (20-Jul to 06-Aug)	02-Sep (25-Aug to 10-Sep)
Skylark	09-Apr (04-Apr to 13-Apr)	06-Jul (02-Jul to 12-Jul)	03-Aug (30-Jul to 08-Aug)
Woodpigeon	26-Feb (15-Feb to 11-Mar)	28-Aug (20-Aug to 10-Sep)	15-Oct (07-Oct to 27-Oct)
Yellowhammer	05-Apr (29-Mar to 13-Apr)	19-Jul (12-Jul to 28-Jul)	19-Aug (12-Aug to 27-Aug)

If considering that the drilling peak of winter OSR is mid to end of August (for more precise drilling dates see below) a potential **reproductive risk can be excluded for the Chaffinch, Skylark and Yellowhammer**, as their reproductive period is clearly over when drilling starts. Woodpigeons might breed over a longer period of time. However, due to their large body size a considerable larger amount of seeds would have to be ingested to exceed the regulatory acceptable dose for potential reproductive effects (RAD_{LT} = NOAEL/5; for further explanation of RAD calculation see below). A **Woodpigeon** would need to ingest **more than 225 seeds every day over a considerably long time period**. Considering its omnivorous behaviour this seems to be unrealistic.

In contrast to Chaffinch, Skylark and Yellowhammer, Linnets theoretically might have a small overlap between drilling and reproduction, so that additional and more in-depth evaluations were done for Germany (M-680746-01-1), Czech Republic (M-680747-01-1) and UK (M-680745-01-1). The graph below is depicted for the UK as here the available database was the most comprehensive.

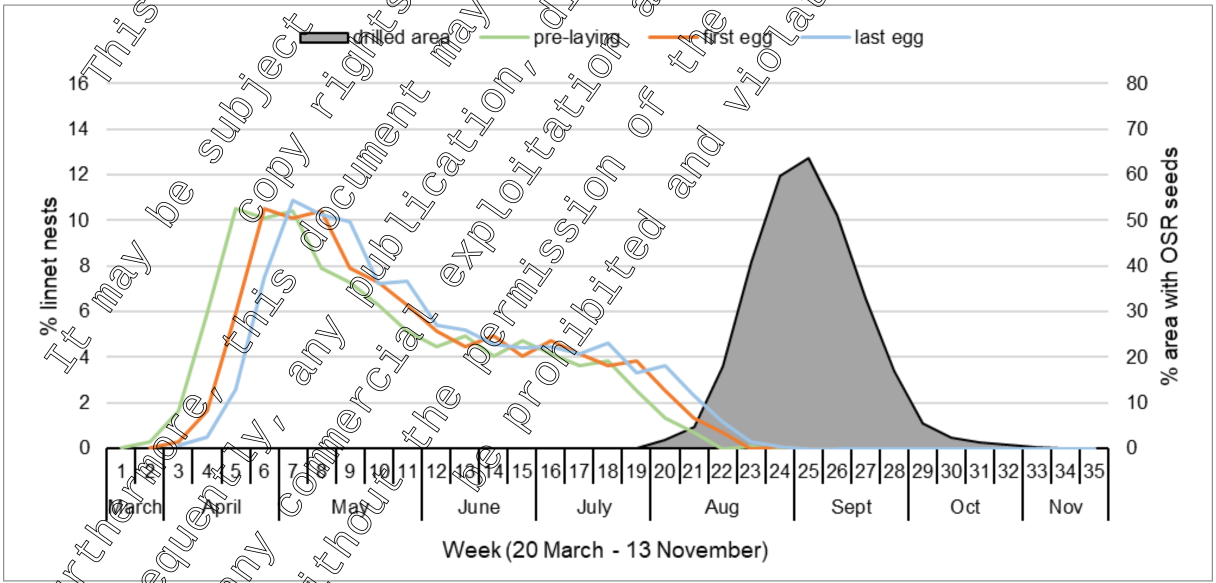

Figure 10.1.1- 2: Percentage of Linnet nests and percentage of area with OSR seeds for the weeks from 20 March until 13 November for UK, as an example. Data are presented by week from 20 March until 19 November. Percentages of Linnet nests are given for nests in the pre-laying phase, nests with the first egg laid and nests with complete clutch.

The evaluation aimed to assess the relevance of a reproductive risk assessment for Linnets potentially exposed to treated winter oilseed rape (OSR) seeds by comparing data on the timing of reproduction in Linnets to regionally specific drilling data. Overall, both periods overlap in August when the breeding season of Linnets' ceases. However, this results in a very small proportion of potentially affected nests ranging between a) 0.08% for the pre-laying period and 0.74% for the egg laying period in Germany; b) 0% for the pre-laying period and 0.07% for the egg laying period in the Czech Republic; c) 0.10% for the pre-laying period and 0.57% for the egg laying period in the UK.

Also note that the EFSA GD (2009) proposes a phase-specific approach for reproductive risk assessment (Appendix J). In this approach different test endpoints are presented in order to assess different breeding phases within a birds' reproductive period. Considering that an overlap between breeding and reproduction might occur during very late stages also test endpoints should be considered for late phases. According to the EFSA GD this would be phase 4 and phase 5, namely 'juvenile growth and survival until fledging' and 'Post-fledging survival', respectively. For these two phases test endpoints are defined as 1/10 of LD50, NOAEL for proportion of 14-day-old juveniles per number of hatchlings per hen, and NOAEL for 14-day-old juvenile weights per hen. Note that the LD50 is considered high (2711 mg/kg bw) and that the two other endpoints did not show any significant effects up to the highest concentration of 1000 ppm. Overall, a negative effect on reproductive performance is therefore not expected. Furthermore, radiotracking shows PT = 0 and thus negligible risk of exposure of linnets.

Exposure of OSR seeds and calculation of regulatory acceptable doses (RAD_A and RAD_{LT})

Modern drilling techniques minimize the number of seeds remaining on the surface. In this way the food availability is generally low for birds on freshly drilled fields, resulting in low abundances of birds. In reality only a small portion of the bird population is exposed to a low number of treated seeds remaining on the soil surface. This was demonstrated in a field study in France ([redacted] 2010, M-362200-01-1) in which exposure of rape seeds and bird abundances were reported.

Although the differences in the use of equipment and seed types were high, the exposure of seeds on the surface of the fields was quite similar and generally very low: In midfield areas the mean number of rape seeds per m² amounted to 0.7 (SD 1.1); at the end row areas it amounted to 1.6 seeds per m² (SD 1.5).

The low number of exposed rape seeds after precision drilling was the key finding of the study. Therefore, the drilled field was not attractive for granivorous birds as was demonstrated by the relative low abundance of birds on the fields. Furthermore, the relative low number of individuals and species occurring on the drilled fields were also considered a typical seasonal pattern. Rape seed drilling is performed predominantly during a time (August) when most of the birds are moulting. In that period, they avoid open landscapes and prefer sheltered areas. At that time of the year the autumn migration of passerine birds had not yet started, and the attractiveness of the fields is low due to a reduced food availability.

A similar result was shown in a field study in Great Britain ([redacted] 2001, M-031392-01-1): The number of findings of rape seeds amounted to 1.09 (midfield) and 2.69 (end row) seeds/m². The abundance of small seed eating birds on freshly drilled rape fields was very low. No increased feeding activity after drilling was found. The author concluded: "Because of the low amount of exposed oilseed rape seeds under good agricultural practice, freshly drilled oilseed rape fields in autumn are not profitable feeding habitats for small seed eating birds. Thus, the potential risk from oilseed rape, treated with pesticides as dressings, for wild birds appears negligible".

As a further piece of evidence demonstrating the acceptability of the formulation, an assessment of the area which would carry the number of exposed rape seeds to exceed the regulatory acceptable dose ($RAD_A = LD_{50}/TER_A$; $RAD_{LT} = NOEL/TER_{LT}$) was done.

The total a.s. on one rape seed is 0.0245 mg, based on a TGW of 7 g. Expressed in terms of total a.s. the LD_{50} for birds of the formulation was calculated as 3084 mg/kg bw. Applying a TER_A of 10 reveals a regulatory acceptable dose for acute risk assessment of 308.4 mg total a.s./kg bw. For a small granivorous bird (e.g. Linnet, the smallest of the five focal species for winter rape) weighing 15.3 g, this dose is 4.72 mg total a.s./animal. Accordingly, a small granivorous bird could ingest 193 seeds without exceeding the RAD_A of 4.72 mg total a.s./animal. These 193 seeds would be dispersed over an area of 72 to 120 m² in the end row area or ca. 177 to 275 m² in the midfield. Considering the Woodpigeon, being the largest of the focal species with 490 g bw, the RAD_A values would amount to 151.1 mg total a.s./animal. Accordingly, a Woodpigeon could ingest 6,167 seeds without exceeding the RAD_A of 151.1 mg total a.s./animal. These 6,167 seeds would be dispersed over an area of 2,293 to 3,855 m² in the end row area or ca. 5,659 to 8,811 m² in the midfield. Due to the negligible overlap between breeding and drilling, a long-term RAD calculation is not considered necessary for the focal species Yellowhammer, Chaffinch, Skylark and Linnet.

As there might be some overlap between breeding and drilling for the Woodpigeon, however, a RAD_{LT} is calculated below.

Regarding the reproduction toxicity, EFSA GD (2009) Appendix B, step 3 states: "*As regards the risk to reproduction from exposure to more than one active substance, it is currently not recommended to consider the use of predicted toxicity values as surrogates in the risk assessment. […] If a given formulation contains several active substances all known to cause similar effects via a similar biochemical mechanism (e.g. aromatase inhibition) and if this type of effects is actually driving the risk assessment, it is thus recommended to perform an assessment for combined effects on a case-by-case basis.*" In the avian reproduction studies the most sensitive effect produced by fluopicolide was a reduction in hatchling body weight. For fluoxastrobin, the NOEL was driven by effects on female body weights, number of eggs laid, and number of eggs set. These effects indicate that the two active substances are very unlikely to act via a similar biochemical mechanism when it comes to the effects driving the risk assessment. For this reason, a combined risk assessment concerning reproductive effects is not required by the EFSA Guidance Document.

The a.s. loading on one oilseed rape seed is 0.0140 mg fluopicolide, based on a TGW of 7 g. For fluopicolide the EC_{10} is 32.2 mg a.s./kg bw. Applying a TER_{LT} of 5 reveals a regulatory acceptable dose of fluopicolide for the long-term/reproductive risk assessment of 6.44 mg a.s./kg bw. For a Woodpigeon weighing 490 g, this is equivalent to 3.16 mg fluopicolide per animal. Accordingly, a Woodpigeon would need to ingest more than 225 seeds per day over a considerably long period to exceed the RAD_{LT} for fluopicolide (3.16 mg per animal). These 225 seeds would be dispersed over an area of 84 to 141 m² in the end row area or ca. 207 to 322 m² in midfield.

Conclusion

The considerably low seed availability, their low attractiveness as well as the negligible overlap of drilled OSR seeds with the reproduction period of relevant focal species render a risk to birds acceptable.

Accordingly, a risk to birds from oilseed rape seeds treated with FLC + FXA FS 350 is considered to be low.

Refined risk assessment for birds feeding on rape shoots

A study was conducted by [redacted] (2020, M-683112-01-1) to quantify the amount of residues of fluopicolide and its metabolites in winter oilseed rape seedlings (wOSR) after drilling of dressed seeds with Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L) under field conditions at a rate of nominal 10 mL prod/kg seed. The first sampling, corresponding to BBCH 10, was 10 to 13 days after drilling. Maximum residue concentrations of fluopicolide were 0.46 to 0.93 mg/kg in seedlings and 0.97 to 1.30 mg/kg in roots. Therefore, a residue concentration of 1.30 mg fluopicolide/kg is used as a worst case for the refined risk assessment for birds feeding on rape shoots.

In the above-mentioned residue study first samplings were taken at BBCH stage 10. Results are supported by a study from [redacted] (2009, M-358357-01-1, KCA 6.2.1/04) in which measured residue concentrations of fluopicolide in rapeseed plants were taken at a later stage, namely at BBCH stage 17–19 after seed treatment with the FS 540 formulation (fluopicolide + fluoxastrobin + clothianidin). In 10× overdose experiments, (NAR = 20 g fluopicolide/kg seeds), the measured loading rate in shoots was 0.1 mg a.s./kg.

Table 10.1.1- 15: First-tier reproductive risk assessment for birds feeding on seedlings (fluopicolide and metabolites M-01 and M-02)

Compound	Generic focal species	Residues on seedling [mg a.s./kg]	FIR/bw	f_{twa}	Residues × FIR/bw × f_{twa}	NOEL [mg a.s./ kg bw/day]	TER_{lt}	Trigger
Fluopicolide	Small omnivorous bird	1.3	0.5	0.53	0.345	32.2	93	5
M-01 (metabolite)		0.021 [a)]	0.5	0.53	0.006	3.22 [c)]	537	5
M-02 (metabolite)		0.006 [b)]	0.5	0.53	0.002	3.22 [c)]	1610	5

a) RUDmax of 1.714 mg/kg as noted in M-686445-01-1, MCP Infinito 10.1.1.2/01 and re-calculated to the current application rate of 12 g a.s./ha

b) RUDmax of 0.498 mg/kg as noted in M-686445-01-1, MCP Infinito 10.1.1.2/01 and re-calculated to the current application rate of 12 g a.s./ha

c) The toxicity endpoint is set at one tenth of the reproductive risk assessment endpoint for the parent.

This refinement step demonstrates that the risk for birds feeding on emerged rape seedlings is acceptable.

CP 10.1.1.1 Acute oral toxicity

For animal welfare reasons, no acute oral toxicity study with the formulation was performed.

CP 10.1.1.2 Higher tier data on birds

Data Point:	KCP 10.1.1.2/01
Report Author:	[redacted]
Report Year:	2001
Report Title:	Generic avian field study on freshly drilled oilseed rape fields in Great Britain
Report No:	[redacted]/FS03
Document No:	M-031392-01-1
Guideline(s) followed in study:	Pesticides and Wildlife - Field Testings. Recommendations of an international workshop on terrestrial field testing of pesticides, 12-15 Sept. 1988 Selwyn Collage, Cambridge UK
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

An avian field monitoring was conducted in Great Britain at five study fields with freshly drilled oilseed rape and their surroundings. The observation technique used was the scan sampling. Attention was paid to the exposure of OSR seeds and remaining seeds of the previous crop on the soil surface after drilling. The study showed that freshly drilled OSR fields are not attractive feeding habitats for seed eating bird species: 91% of all small seed eating birds preferred hedges and other boundary habitats, only 9% of all small seed eating birds were observed on field habitats, predominately on ploughed fields. The mean number of exposed oilseed rape and remaining winter wheat seeds at the soil surface immediately after drilling were 1.09 grains/m^2 in the midfields and 2.69 grains/m^2 in the end rows.

I. MATERIAL AND METHODS:

The field monitoring was conducted in Great Britain at five study fields and their surroundings from 2000-08-18 (first bird observation) until 2000-09-19 (last bird census). The fields were located in East Anglia at Suffolk and Essex and were cultivated by commercial farmers. The test used commercial oilseed rape seed (osr) regardless of the seed treatment. Attention was paid to the exposure of OSR seeds and remaining seeds of the previous crop on the soil surface after drilling (transect counts on five main study fields and 6 additional sites). Also, of importance was bird activity on the fields (behaviour observations to estimate bird activity before and after drilling with focus on species, number of individuals and behaviour, especially feeding, on the five main study sites). The observation technique used was the scan sampling with observation intervals of 5 minutes. Observations were performed in the early morning and in the evening before sunset with a duration of 3 hrs per session. On the day after drilling observations were made throughout the whole daylight period. Additionally, also bird activity in the surroundings of the study fields were recorded by transect counts (habitat mapping and bird census to assess the general bird abundance and habitat preference).

II. Results and Discussion:

Findings:

Test substance	Commercial oil seed rape seed (generic study)
Test object	Natural bird community on five study fields
Number of exposed oilseed rape and remaining winter wheat seeds at the soil surface immediately after drilling [grains/m^2] (mean of 11 fields)	Oil seed rape — Midfield: 1.09 — Endrow: 2.69
Calculated seed mass of oil seed rape of 4 fields) as potential food source for birds	Oil seed rape: 0.01 [g/m^2]
Habitat preference of small seedeating birds	• 91% of all small seed eating birds preferred hedges and other boundary habitats, covering only approx. 5 % of the area • only 9% of all small seed eating birds were observed on field habitats, predominately on ploughed fields
Results from behaviour observations	• very low abundance of small seedeating birds on freshly drilled OSR fields • no increased feeding activity on freshly drilled OSR fields

Transect counts showed that on average 53% of birds were recorded on field habitats that covered 95% of the area. Up to 90% of small seed eating birds were recorded in boundary habitats like hedges and there were relatively very few recorded on drilled fields where large birds were most prevalent (see table below).

Counts and densities of bird guilds in different habitats from transect counts

Guild	No. counted across all landscape	No. counted in all fields	Density of birds in all fields (ind/km transect)	No. counted in drilled fields (incl oilseed rape)	Density of birds in drilled fields (ind/km transect)
small seedeater	1095	93	0.67	10	0.39
large seedeater	1893	1123	8.06	318	12.52
small non-seedeater	348	9	0.06	0	0
large non-seedeater	1956	1567	11.25	413	16.26
Total	5292	2792		741	-

The table below shows scan counts of small and large granivores in fields before and after drilling of oilseed rape. In general, bird densities did not increase in response to drilling. In 2 cases where this did occur this was explained by factors other than the presence of newly drilled seed. On field 1 several hundred woodpigeons and 3 yellowhammers were observed foraging on residual wheat seed. On field 5 woodpigeons were present on the field in large numbers after spreading manure. There were either no or very low densities of small granivores foraging on oilseed rape field after drilling and no observation of them eating oilseed rape seed.

Counts of small and large granivores in drilled oilseed rape fields before and after drilling

Field	Small granivore (<50g)		Large granivore (>50g)	
	Before drilling (max count per 5 min scan/ha)	After drilling (max count per 5 min scan/ha)	Before drilling (max count no's/ha)	After drilling (max count no's/ha)
Field 1	0.16	0.24	0.88	2.73
Field 2	1.87	0.51	2.24	1.31
Field 3	0.37	0.00	1.67	0.89
Field 3a	0.18	0.00	1.77	2.43
Field 4	0.02	0.00	1.32	4.88
Field 5	0.0	0.02	4.43	5.49

Drilling efficiency

The drilling efficiency was evaluated in the midfield and end-rows in the 22 transects with in total 220 counted plots. The details of the drilling procedure are given in the table below:

Field No	Seeding rate [kg seeds/ha]	Equipment used	Number of seeds per m^2	
			Midfield	End-rows
1	8.77	Roger Module – R MR305 4m 32	1.2	4.2
2	6.24	Vaderstad Rapid 400 P	0.2	4.0
3	8.16	Accord pneumatic tandem DL	2.6	2.6
4	4.34, 6.55 and 5.38 (depending on the variety)	Bettinson TC4 disc coulter drill	1.6	1.6
5	5.4	Bettinson TC4 disc coulter drill	0.2	0.4
6	No data, drilling efficiency on these fields was performed in addition to target fields (1-5)		1.0	5.6
7			0.8	2.0
8			0.8	3.0
9			0.8	1.8
10			1.0	1.8
11			1.8	2.6
		Mean	1.09	2.69

The maximum number of seeds was 20 seeds/m2 found in the end-row are of one plot in field No 1.

III. CONCLUSIONS:

Freshly drilled OSR fields are not attractive feeding habitats for seed eating bird species. Small seed eating bird species in August/September prefer hedges and trees as habitats. Although large seed eating bird species use drilled fields as feeding habitat they were more attracted by remaining seeds from the previous crop on the fields. Remaining winter wheat seeds are, compared with OSR seeds, of much higher importance concerning seed number and especially seed mass on the soil surface. Because of the low amount of exposed OSR seeds under good agricultural practice, freshly drilled OSR fields in autumn are not profitable feeding habitats for small seed eating birds. Thus, the potential risk from oilseed rape, treated with pesticides as seed dressings, for wild birds appears negligible.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. The abundance of small seed eating birds on freshly drilled oilseed rape fields was very low. The availability of treated oilseed rape seeds, especially in terms of seed mass, was very low compared to cereal grain leftover from the previous crop.

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Data Point:	KCP 10.1.1.2/02
Report Author:	[redacted]
Report Year:	2020
Report Title:	Occurrence and PT of linnets in new-drilled, preemergence winter oilseed rape in comparison to harvested oilseed rape fields in Germany (2019)
Report No:	P19034
Document No:	M-684638-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009, Directive 2003-01 (Canada/PMRA) US EPA OCSPP Not Applicable No official test guideline available at present type of study. The study was conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009)
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was i) to assess the proportion of diet (PT) that linnets (*Carduelis cannabina*) obtain in winter oilseed rape fields, both freshly drilled and harvested, during the pre-emergence period; and ii) to provide observational data of the occurrence of linnets in freshly drilled in comparison to harvested fields, in Germany, central zone. Four trapping locations were used to trap and radio-tag linnets. Moreover, 20 harvested fields and 20 freshly drilled fields were used as reference for the agricultural status during the telemetry sessions conducted. Additionally, 10 harvested fields and 10 freshly drilled fields in pre-emergence period were selected for scan sampling. This study demonstrated that harvested oilseed rape fields are attractive as feeding habitat for linnets but not pre-emergence oilseed rape fields. During the tracking sessions, none of the 21 linnets entered freshly drilled oilseed rape fields, but 20 out of 21 entered harvested fields. The linnet was the species with highest values of abundance, frequency of occurrence and dominance, in harvested oilseed rape fields during the scan sampling. Considering data from all sessions, their mean PT was 0.35 (90%ile = 0.52). Since none of the tracked linnets foraged in freshly drilled oilseed rape fields, their PT was 0 for all approaches.

I. MATERIAL AND METHODS:

The study was conducted in the administrative district of Lüchow-Dannenberg, located in the north-east of Germany (province of Lower Saxony). In the chosen area, representative for the cultivation of oilseed rape, harvested oilseed rape fields are present during the drilling period of oilseed rape. Four trapping locations were used to trap and radio-tag linnets. Moreover, 20 harvested fields and 20 freshly drilled fields were used as reference for the agricultural status during the telemetry sessions conducted. The study fields used for scan sampling, 10 harvested and 10 freshly drilled in pre-emergence period (from BBCH 00 to 07), were selected by their surrounding habitat structure in order to match requirements of different species of birds, especially for the linnets.

General information			
Study design	Generic		
Study area	North-East Germany		
Study period	August- September 2019		
Crop	Oilseed rape		
Test item	None		
Focal bird species	Linnet (*Carduelis cannabina*)		
Main methods	Trapping, radio-tracking, scan sampling, monitoring of crop availability, habitat mapping, seed availability		
Field information			
	Freshly drilled	**Harvested**	**TOTAL**
No. of monitored oilseed rape fields (selected fields approach)	20	20	40
No. of scan sampling fields[1]	10	10	20
Total scan area [ha]	35.52	32.86	68.38
Mean scan area [ha]	3.55	3.29	3.42
Drilling dates[2]	19 August - 01 September 2019		
Focal species information			
No. trapped birds	156		
No. tagged birds	33		
No. successful tracked birds	21		

[1] Scan sampling fields were used partly as crop monitoring fields
[2] Of fields included in the analysis

The field phase of the study was conducted from the beginning of August to the middle of September 2019. The schedule of the study was based on the status of the study fields selected as reference for the crop development of the pre-emergence oilseed rape fields. The radio tracking sessions of the birds started after the drilling of 3 of the study fields selected for crop monitoring (i.e. the fields selected as reference fields in order to illustrate the progress of drilling and therefore the availability of freshly drilled oilseed rape fields in the study area). Radio tagged linnets were tracked continuously over their activity period (max. 15:20h) to determine their location and behaviour.

In total, 21 complete radio-tracking sessions were successfully performed on 21 individuals. The proportion of time spent active and potentially foraging in pre-emergence and harvested oilseed rape fields (PT estimate) and the habitat preferences were analysed. Additionally, the radio tracking results enabled the calculation of the size and shape of the individuals' home range (during each session), using the minimum convex polygon method (MCP). The Jacobs' preference index (D), which indicates if an individual bird prefers or avoids pre-emergence or harvested oilseed rape fields as feeding habitat, was calculated for each tracking session.

The attractiveness of freshly drilled and harvested oilseed rape for birds was monitored in 10 freshly drilled fields between drilling and before emergence (from BBCH 00 to 07) and in 10 harvested oilseed rape fields by measuring the general abundance and behaviour of all bird species. The species, number of individuals and behaviour of every bird present on the surface of a defined scan area of at least 1 ha (average 3.42 ha) were recorded using the scan-sampling technique. This technique is commonly used to quantify bird presence as well as bird activities by steady visually scanning the study field with the aid of a scope and/or binoculars as optical devices. One session (approx. 4 hours in the morning or evening, to cover the times of maximum bird activity) was carried out per field. Mean abundance, dominance and frequency of occurrence (FO_{scan}, FO_{field}) were the main parameters used to describe the bird community in freshly drilled and harvested oilseed rape fields and to compare the occurrence of linnets in freshly drilled and harvested fields by the scan sampling approach.

The availability of freshly drilled fields in the study area was monitored by regular checks of 20 study fields for their drilling time and development of the crop (i.e. BBCH growth stages). The agricultural status of harvested oilseed rape fields in the study area was monitored by regular checks of 20 study fields (Selected fields approach). Moreover, 52 harvested fields were assessed before and after radio tracking activities started and 58 freshly drilled fields or fields for which drilling was planned were assessed after radio tracking activities were finished in order to complete the status of the crop development in the area (Comprehensive approach). Also seed availability in pre-emergence and in harvested fields was assessed during crop monitoring, scan sampling and home range-habitat mapping.

II. Results and Discussion:

Continuous radio tracking sessions on 21 linnets allowed a representative assessment of potential foraging times in order to calculate PT values. Thirteen of the radio-tracked linnets (n=21) had freshly drilled oilseed rape fields in their home range and none of them entered the pre-emergence oilseed rape fields while radio-tracked. In contrast, 20 of 21 linnets entered a harvested oilseed rape field during the radio tracking period (95.2% of all birds). PT values for consumer individuals in harvested fields ranged between 0.58 and 0.08. Linnets had large home ranges; the mean home range size (n = 21 individuals) was 176.47 ha.

The proportion of oilseed rape fields in relation to the agricultural area in the home ranges of all individuals was 2.28% for freshly drilled fields and 7.77% for harvested fields. The Jacobs' index for habitat preferences showed a mean value of 0.68 for harvested fields and -1 in freshly drilled fields; hence, harvested fields are a preferred foraging habitat for linnets.

Summarised radio-tracking results in freshly drilled oilseed rape fields				
PT approach	**Parameter**	**Number of birds**	**PT in OSR fields**	**Jacobs' index [D][1]**
Freshly drilled OSR fields				
Consumer[2]	Mean	0	-	-
	Median		-	-
	90%ile		-	-
Home range[3]	Mean	13	0	-1
	Median		0	-1
	90%ile		0	-1
All indviduals[4]	Mean	21	0	-
	Median		0	-
	90%ile		0	-

[1] calculated for birds with freshly drilled oilseed rape fields in their home ranges
[2] Considering only those individuals that actually used freshly drilled oilseed rape for foraging during their tracking session
[3] Considering those individuals that had freshly drilled oilseed rape fields in their home range during tracking session
[4] Considering all tracked individuals

Summarised radio-tracking results in harvested oilseed rape fields				
PT approach	**Parameter**	**Number of birds**	**PT in OSR fields**	**Jacobs' index [D][1]**
Harvested OSR fields				
Consumer[2]	Mean	20	0.37	-
	Median		0.38	-
	90%ile		0.52	-
Home range[3]	Mean	21	0.35	0.68
	Median		0.38	0.79
	90%ile		0.52	0.90
All indviduals[4]	Mean	21	0.35	-
	Median		0.38	-
	90%ile		0.52	-

[1] calculated for birds with harvested oilseed rape fields in their home ranges
[2] Considering only those individuals that actually used harvested oilseed rape for foraging during their tracking session
[3] Considering those individuals that had harvested oilseed rape fields in their home range during tracking session
[4] Considering all tracked individuals

The scan sampling approach offered 28,075 individual bird observations belonging to 40 different bird species during 997 scans performed in 20 oilseed rape fields (10 freshly drilled before emergence and 10 harvested). In each field one scan sampling session comprising approx. 50 scans was conducted.

In freshly drilled fields, 574 bird observations of 18 different species were observed; in harvested OSR fields, 27501 sightings of 33 species were recorded.

No linnet was observed in freshly drilled fields during the study, while 11,602 sightings were recorded in harvested oilseed rape fields.

The mean abundance of birds was 0.30 ind./scan/ha in freshly drilled oilseed rape fields, and 17.06 ind./scan/ha in harvested fields.

In harvested oilseed rape fields, the linnet was the most abundant species with 7.13 ind./scan/ha. In freshly drilled fields woodpigeon (0.12 ind./scan/ha), feral pigeon (0.08 ind./scan/ha) and carrion crow (0.05 ind./scan/ha) were the most abundant species.

Of the individuals observed, on average 81.4% and 83.7% showed foraging behaviour in freshly drilled and harvested oilseed rape fields, respectively.

Almost all linnets (99.99%) recorded in harvested oilseed rape fields showed foraging behaviour.

Bird monitoring - Scan sampling			
	Freshly drilled OSR	**Harvested OSR**	**TOTAL**
No. of scans	500	497	997
No. of linnet observations	0	11602	11602
No. of bird observations	574	27501	28075
No. of bird species	18	33	40
Mean abundance [ind./scan/ha]	0.30	17.06	-
Mean foraging birds [%]	81.4	83.7	-

The spatial and temporal distribution of the avian community, as indicated by the frequency of occurrence (FO_{field} and FO_{scan}) in the harvested oilseed rape fields, showed the linnet and yellow wagtail as the most relevant species; they were present in 80% of the 10 scan sampling study fields and observed in ≈ 50% of the scans. In freshly drilled fields, the most frequent species were the carrion crow and woodpigeon (FO_{field}=60%; FO_{scan}>10%).

Using the frequency of occurrence by field ($FO_{field} \geq 20\%$) as the major criterion, together with body mass, diet guild, frequency of occurrence per scan (FO_{scan}) and dominance as ranking parameters, 7 and 17 species were listed as the most relevant species for freshly drilled and harvested oilseed rape fields, respectively. The main species present in freshly drilled fields were white wagtail, yellow wagtail, yellowhammer, jay, carrion crow, woodpigeon and feral pigeon; the main species present in harvested oilseed rape fields were yellow wagtail, white wagtail, black redstart, northern wheatear, stonechat, great tit, robin, linnet, greenfinch, chaffinch, goldfinch, serin, tree sparrow, common starling, jay, carrion crow and woodpigeon.

The linnet was the species with highest values of abundance, frequency of occurrence and dominance, in harvested oilseed rape fields.

Ranking of most relevant species in harvested oilseed rape fields						
Species	**Mean abundance [ind./scan/ha]**	**FO_{field} [%]**	**FO_{scan} [%]**	**Dominance [%]**	**$FO_{foraging}$ [%]**	**Foraging stratum**
Insectivorous (small)						
Yellow wagtail[1]	1.44	80.0	48.89	8.46	47.89	ground
White wagtail[1]	1.01	60.0	29.98	5.90	29.78	ground
Black redstart	0.06	20.0	11.07	0.37	10.66	ground
Northern wheatear	0.01	20.0	4.23	0.08	3.02	ground
Stonechat	< 0.01	20.0	1.01	0.02	1.01	ground
Great tit	< 0.01	20.0	0.40	0.01	0.40	ground
Robin	< 0.01	20.0	0.40	0.01	0.40	ground
Granivorous (small)						
Linnet[1]	7.13	80.0	49.50	41.81	49.50	ground
Greenfinch[1]	0.94	60.0	19.11	5.50	19.11	ground
Chaffinch[1]	0.16	50.0	9.46	0.94	9.46	ground
Goldfinch	0.07	30.0	6.04	0.43	6.04	ground
Serin	0.01	30.0	2.41	0.08	2.41	ground
Omnivorous (small)						
Tree sparrow[1]	0.07	30.0	4.63	0.42	4.63	ground
Omnivorous (medium)						
Common starling[1]	5.83	50.0	18.71	34.18	18.71	ground
Jay	< 0.01	30.0	1.81	0.04	1.81	ground
Omnivorous (large)						
Carrion crow	0.01	30.0	1.41	0.06	1.21	ground
Herbivorous/Granivorous (medium)						
Woodpigeon	0.08	50.0	10.66	0.44	10.66	ground

[1] species with flocking behaviour
Species with FOfield ≥20% and ranked according to diet guild, FOfield > FOscan > dominance
Data from one scan sampling session. Information on diet guild, size and predominant foraging strata during season from Cramp et al. (1998), Dunning (2008), Buxton et al. (1998) and Dietzen et al. (2014.)

Ranking of most relevant species in freshly drilled oilseed rape fields						
Species	**Mean abundance [ind./scan/ha]**	**FO_{field} [%]**	**FO_{scan} [%]**	**Dominance [%]**	**$FO_{foraging}$ [%]**	**Foraging stratum**
Insectivorous (small)						
White wagtail	< 0.01	30.0	0.60	1.09	0.40	ground
Yellow wagtail	< 0.01	30.0	0.60	0.53	0.40	ground
Granivorous (small)						
Yellowhammer	0.03	30.0	6.40	8.47	6.40	ground
Omnivorous (medium)						
Jay	< 0.01	30.0	1.20	1.08	1.00	ground
Omnivorous (large)						
Carrion crow	0.05	60.0	10.60	16.26	10.40	ground
Herbivorous/Granivorous (medium)						
Woodpigeon	0.12	60.0	10.80	39.91	10.80	ground
Feral pigeon[1]	0.08	30.0	4.40	25.27	4.40	ground

[1] species with flocking behaviour
Species with FOfield ≥20%, and ranked according to diet guild, FOfield > FOscan > dominance
Data from one scan sampling session. Information on diet guild, size and predominant foraging strata during season from Cramp et al. (1998), Dunning (2008), Buxton et al. (1998) and Dietzen et al. (2014.)

The crop development monitoring showed that freshly drilled fields were continuously available for the linnets. The monitoring of the cultivation status of harvested fields showed that tillage practices were eventually practiced in all the monitored fields and that the number of fields with low vegetation cover was higher at the beginning of the study period.

In crop monitoring fields, the availability of seeds expressed as the mean number of seeds per ha was much lower in freshly drilled OSR fields than in harvested OSR fields (mean = 30900 (n = 19) seeds/ha and mean = 396314 seeds/ha (n = 20), respectively).

III. CONCLUSIONS:

This generic study provides bird observations under realistic agricultural conditions in freshly drilled and in harvested oilseed rape fields in Germany. The range of locations, habitat structures, and timing covers a typical spectrum of potential scenarios, making the results of the study representative for oilseed rape fields in the central zone.

This study demonstrated that harvested oilseed rape fields are attractive as feeding habitat for linnets but not pre-emergence oilseed rape fields.

- In total, 21 tracking sessions (continuous recording from dawn to dusk) were conducted between 9th August and 10th September 2019 on 21 linnets. The birds were trapped inside harvested oilseed rape fields. During the tracking sessions, none of the 21 linnets entered freshly drilled oilseed rape fields, but 20 out of 21 entered harvested fields.
- For 'consumers' in harvested fields, the mean PT was 0.37 (90%ile = 0.52).
  For individuals with harvested oilseed rape fields in their home range during their tracking session ('home range approach') the mean PT was 0.35 (90%ile = 0.52). Considering data from all sessions ('all individuals approach') the mean PT was 0.35 (90%ile = 0.52).
  Since none of the tracked linnets foraged in freshly drilled oilseed rape fields, their PT was 0 for all approaches.

- During the scan sampling, no linnet was observed in the freshly drilled oilseed rape study fields, in comparison with the 11602 sightings recorded in the harvested oilseed rape study fields. Overall, the diversity and abundance of birds in freshly drilled fields was very low in comparison with harvested oilseed rape fields.
- Foraging was the most prevalent behaviour in both type of fields, as shown by the percentage of foraging observations and frequency of foraging behaviour across all observed birds.
- The linnet (together with other nine species) showed strong flocking behaviour in the harvested oilseed rape fields.
- Seven and seventeen species in freshly drilled and harvested fields, respectively, are considered as most relevant species. The white wagtail (insectivore), yellowhammer (granivorous), jay and carrion crow (as omnivore guild) and woodpigeon (herbivore/granivore) were the most frequent bird species in freshly drilled oilseed rape fields during the survey period. In harvested fields yellow wagtail, linnet, tree sparrow, carrion crow and woodpigeon were most frequently observed. These species are representative for all body size classes and different diet guild profiles.
- The linnet was the species with highest values of abundance, frequency of occurrence and dominance, in harvested oilseed rape fields during the scan sampling.
- The preference of harvested OSR fields over freshly drilled OSR by linnets can most probably be explained because volunteer seeds in harvested OSR fields showed a much higher abundance on the soil surface than OSR seeds in freshly drilled fields.

<u>Assessment and conclusion by applicant:</u> This study is considered reliable and can be used for risk assessment. It shows that no linnets were observed or radiotracked on freshly drilled oilseed rape fields, although they were abundant in the landscape and often found on freshly harvested oilseed rape fields. With a PT value of 0, the risk of winter oilseed rape seeds treated with Scenic Gold® for small granivorous birds is negligible.

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Data Point:	KCP 10.1.1.2/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Measured residues in winter oilseed rape seedlings emerging from fluopicolide + fluoxastrobin FS 350 treated seed in the central zone, 2019 (GLP)
Report No:	P19036
Document No:	M-683112-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009, Directive 2003-01 (Canada/PMRA) US EPA OCSPP Not Applicable No official test guideline available at present type of study. The study was conducted under consideration of the EFSA Guidance Document on Risk Assessment for Birds & Mammals (EFSA 2009)
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of the study was to quantify the amount of residues of fluopicolide and its metabolites in winter oilseed rape seedlings (wOSR) after drilling of dressed seeds with Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L) under field conditions. Four residue trials were conducted on farmers' fields at different locations distributed over Germany. At each site, one control plot and one treatment plot with a size of 75 m2 each were drilled with untreated and treated seeds, respectively. The test item was applied to wOSR with nominal 10 mL/kg seeds, corresponding to 200 g fluopicolide/100 kg seeds. The actual loading of fluopicolide on wOSR seeds was 180.14 g/100kg seeds. The target drilling rate was 6 kg/ha. The first sampling, corresponding to BBCH 10, was 10 to 13 days after drilling. The results on one of the 4 sites were considered unreliable and excluded from further calculations. Of the other three sites, maximum residue concentrations of fluopicolide were 0.46 to 0.93 mg/kg in seedlings (mean: 0.70 mg/kg) and 0.97 to 1.30 mg/kg in roots (mean 0.78 mg/kg).

I. MATERIAL AND METHODS

Study Sites: Four residue trials were conducted on farmers' fields at different locations distributed over Germany, France and the Netherlands: (RR) Reusrath, NRW, western Germany; (ZP) Zuelpich, NRW, western Germany; (DA) Douai, Nord, northern France; (SW) Swalmen, Limburg, Netherlands. Sites were well spread out with distance between sites ranging between 49 km and 265 km.

Trial design: At each site, one control plot (C1) and one treatment plot (P2) with a size of 75 m2 each were drilled with untreated and treated seeds, respectively.

Test item and application: Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L) was applied to winter oilseed rape (wOSR) with nominal 10 mL/kg seeds, corresponding to 200 g fluopicolide/100 kg seeds. The actual loading of fluopicolide on wOSR seeds was 180.14 g/100kg seeds. The target drilling rate was 6 kg/ha.

Drilling dates were 16 Aug 2019 at sites RR and ZP, 26 Aug at site DA and 17 Sep at site SW.
Sampling: Sampling started soon after emergence at BBCH 10. The first sampling was defined as DAE 0 (DAE= Day After Emergence). Seedlings emerging from drilled treated seeds (P2) were collected at

DAE 0, 1, 2, 3, 5, 7, 10, 14 and 21. Untreated seeds (C1) were collected at DAE 0, 14, 21. Samples were taken randomly in a 'W'-shaped design.

Whole seedlings were pulled out by hand and loose root soil was removed by gently shaking the seedling. The plants were then cut with scissors to separate the 'above soil-surface' part of each seedling (green leaf material, coded SL) from the 'below soil-surface' part (stem and roots, coded R). Whole seedlings were counted. Every seedling sample contained at least 40 seedlings of a targeted minimum 'above soil surface' biomass of 5 g wet weight and 'below soil surface' biomass of minimum 2 g wet weight.

Seeds were sampled before drilling directly from the seed package to verify seed loading. Separated seedling and roots samples were stored and shipped deep-frozen until residue analysis.

Residue analysis: All samples were analysed for their content of fluopicolide (analytical method 01209/M001) and its 2 metabolites M-01 (AE C653711) and M-02 (AE C657188) via HPLC-MS/MS. Residues are reported in terms of mg active substance/kg (mg a.s./kg) or mg metabolite/kg (mg met/kg). The Limit of Quantification (LOQ) value was 0.01 mg/kg for parent and metabolites.

Calculations: Mean concentrations were calculated with MS Office Excel 2010.

II. RESULTS AND DISCUSSION

Concentrations of fluopicolide and its metabolites M-01 (AE C653711) and M-02 (AE C657188) in control seedlings and roots were all < LOQ.

The first sampling (DAE 0), corresponding to BBCH 10, was 10 to 13 days after drilling. Maximum residue concentrations of fluopicolide in 3 of the 4 sites were 0.46 to 0.93 mg/kg in seedlings (mean: 0.70 mg/kg), and 0.97 to 1.30 mg/kg in roots (mean: 0.78 mg/kg).
At site SW a maximum concentration of 4.10 mg/kg was measured. Roots were not cleaned before freezing of samples in the field. Remaining soil particles on root material might have been the cause for the unrealistically high concentrations in SW samples. The leaf material of the same seedlings was not soiled and resulted in residue concentrations comparable to the other sites. The residue values of the SW site were considered unreliable and excluded from calculation of mean concentrations.

Mean measured concentrations of fluopicolide in seedlings

DAE	Fluopicolide [mg/kg] in seedlings Site RR	Site ZP	Site DA	*Site SW*	mean**
0	0.46	0.93	0.71	*1.1*	0.70
1	0.32	0.35	0.91	*0.40*	0.53
2	0.22	0.34	0.82	*0.49*	0.46
3	0.26	0.14	0.32	*0.49*	0.24
5	0.071	0.11	0.35	*0.25*	0.18
7	0.014	0.084	0.14	*0.14*	0.08
10	0.010	0.036	0.071	*0.14*	0.04
14	0.025	0.025	0.031	*0.14*	0.03
21	0.070	0.016	0.013	*0.037*	0.03
Conc. seeds	1239	1315	1344	330*	1299
Transfer rate	0.00037	0.00071	0.00068	-	0.00054
Time between drilling and 1st sampling [days]	12	13	10	13	-

values < 0.01 were set to 0.01 for calculations
DAE = Day After Emergence (DAE 0 = start of sampling at BBCH 10)
Transfer rate = maximum concentration after DAE 0 / concentration on seeds
* Analytical concentration on seeds at SW was almost 4 times lower as compared to the other sites
**Mean concentration on seeds, seedlings and transfer rate were calculated excluding SW.

Mean measured concentrations of fluopicolide in roots

DAE	Fluopicolide [mg/kg] in roots Site RR	Site ZP	Site DA	*Site SW*	Mean**
0	0.83	0.97	0.53	*4.1*	0.78
1	0.75	0.63	0.75	*2.0*	0.71
2	0.50	0.63	0.83	*3.7*	0.65
3	1.0	0.51	1.2	*2.4*	0.90
5	0.37	0.49	0.73	*1.9*	0.53
7	0.61	0.51	1.1	*1.8*	0.74
10	0.51	0.38	1.3	*1.5*	0.73
14	0.68	0.30	0.96	*1.9*	0.65
21	0.27	0.23	1.2	*0.93*	0.57
Conc. seeds	1239	1315	1344	330*	1299
Transfer rate	0.00081	0.00074	0.00097	-	0.00070
Time between drilling and 1st sampling [days]	12	13	10	13	-

values < 0.01 were set to 0.01 for calculations
DAE = Day After Emergence (DAE 0 = start of sampling at BBCH 10)
Transfer rate = maximum concentration after DAE 0 / concentration on seeds
* Analytical concentration on seeds at SW was almost 4 times lower as compared to the other sites
**Mean concentrations on seeds, roots and transfer rate were calculated excluding SW

Full details and acceptable validation data to support this method (01209/M001) are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Residues of the two metabolites M-01 (AE C653711) and M-02 (AE C657188) in seedling and root samples were all < LOQ, except for sites RR and ZP. Residues of the metabolite M-01 (AE C653711) were detected on DAE 0, 1, 2 and 3 (maximum concentration of 0.019 mg met/kg) at site RR and on DAE 0 and 1 (maximum concentration of 0.013 mg met/kg) at site ZP in seedling samples.

Rainfall until DAE 0 differed between study sites with 16.0 mm at RR, 14.8 mm at ZP, 9.2 mm at DA and 36.0 mm at SW.

III. Conclusion

The study provides field residue data for fluopicolide in winter oilseed rape seedlings emerging from treated seeds at a rate of nominal 10 mL prod/kg seed. Whole seedlings with roots were separated into seedlings (above soil part of the plant) and roots (below soil part of the plant). This terminology was used because the root is very small as compared to stem and leaf part and at lower BBCH stages 10-11, the stem is partly below the soil surface.

The first sampling (DAE 0), corresponding to BBCH 10, was 10 to 13 days after drilling. Maximum residue concentrations of fluopicolide in 3 of the 4 sites were 0.46 to 0.93 mg/kg in seedlings (mean: 0.70 mg/kg), and 0.97 to 1.30 mg/kg in roots (mean: 0.78 mg/kg). The residue values of the 4th site were considered unreliable and excluded from calculation of mean concentrations.

Assessment and conclusion by applicant: This study is considered reliable and can be used for risk assessment. Mean peak concentrations of fluopicolide were 0.70 mg/kg in seedlings and 0.78 mg/kg in roots.

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Data Point:	KCP 10.1.1.2/04
Report Author:	[redacted]
Report Year:	2020
Report Title:	Linnet breeding period compared to drilling season of winter oilseed rape in UK
Report No:	R1960071_P
Document No:	M-680745-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	none
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

This report aims to assess the relevance of a reproductive risk assessment for linnets potentially exposed to treated winter oilseed rape (OSR) seeds in the UK by comparing data on the timing of reproduction in linnets to UK drilling data. Overall, both periods very slightly overlap in August when the breeding season of linnet's ceases. This results in a very small proportion of potentially affected nests (ranging between 0.10% for the pre-laying period and 0.57% for the egg laying period). A negative effect on reproductive performance is not expected.

I. Material and Methods

OSR drilling data and ringing data of linnet nestlings were obtained for the UK. For an evaluation of the temporal overlap, the percentage of linnet nests was multiplied with the percentage of the sown area for each week of overlap and summed up. This was done for the nest stages "pre-laying", "first egg laid" and "last egg laid" for all UK regions combined and additionally for the "last egg laid" stage for all UK regions separately. These nest stages were selected because the correspond with the phases of the avian reproduction studies where the parental birds are exposed over the pre-egglaying and until the end of the egg-laying phase, but where there is no treatment during incubation or the nestling phase.

II. Results and Discussion

There are only 43 days of overlap between the beginning of drilling and the end of egg laying at a time when relatively few linnets still breed and only a few fields are drilled. This results in only 0.57% (indicators for overall spatial variation between the regions: 5th percentile 0.09%, 95th percentile 1.28%) of linnet nests being potentially exposed to treated seeds until the end of the egg-laying period. For the different UK regions, this ranged between 0.04% in South West England and 1.4% for North East England, Yorkshire and Humberside.
These results suggest that only a negligible proportion of linnets is potentially affected by OSR seed treatments during their reproduction. Furthermore, the percentage of ingested treated OSR seeds is most likely also very low. Finally, studies on other passerines suggest that successful reproduction (i.e. surviving offspring in one year and reproduction of this offspring in the subsequent breeding season) is generally very low for late breeding linnets.

III. Conclusion

To conclude, there is only little overlap between winter OSR drilling and the breeding season of linnets. This suggests that only a very small and negligible proportion of linnets is potentially exposed by OSR seed treatments during their reproductive period. Therefore, a negative effect on reproductive performance is not expected.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. There is very little overlap between drilling of winter oilseed rape and the breeding season of linnets.

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Data Point:	KCP 10.1.1.2/05
Report Author:	[redacted]
Report Year:	2020
Report Title:	Linnet breeding period compared to drilling season of winter oilseed rape in Germany
Report No:	R1960071_2
Document No:	M-680746-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	none
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

This report aims to assess the relevance of a reproductive risk assessment for linnets potentially exposed to treated winter oilseed rape (OSR) seeds in Germany by comparing data on the timing of reproduction in linnets to German drilling data. Overall, both periods overlap in August when the breeding season of linnet's ceases. This results in a very small proportion of potentially affected nests (ranging between 0.08% for the pre-laying period and 0.74% for the egg laying period). A negative effect on reproductive performance is not expected.

I. Material and Methods

OSR drilling data and ringing data of linnet nestlings were obtained for Germany. For an evaluation of the temporal overlap, the percentage of linnet nests was multiplied with the percentage of the sown area for each week of overlap and summed up. This was done for the nest stages "pre-laying", "first egg laid" and "last egg laid" for all regions combined. These nest stages were selected because the correspond with the phases of the avian reproduction studies where the parental birds are exposed over the pre-egglaying and until the end of the egg-laying phase, but where there is no treatment during incubation or the nestling phase.

II. Results and Discussion

There are only 34 days of overlap between the beginning of drilling and the end of egg laying, at a time when relatively few linnets still breed and only a few fields are drilled. This results in only 0.74% of linnet nests being potentially exposed to treated seeds until the end of the egg-laying period.
These results suggest that only a negligible proportion of linnets is potentially affected by OSR seed treatments during their reproduction. Furthermore, the percentage of ingested treated OSR seeds is most likely also very low. Finally, studies on other passerines suggest that successful reproduction (i.e. surviving offspring in one year and reproduction of this offspring in the subsequent breeding season) is generally very low for late breeding linnets.

III. Conclusion

To conclude, there is only little overlap between winter OSR drilling and the breeding season of linnets. This suggests that only a very small and negligible proportion of linnets is potentially exposed by OSR seed treatments during their reproductive period. Therefore, a negative effect on reproductive performance is not expected.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. There is very little overlap between drilling of winter oilseed rape and the breeding season of linnets

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Data Point:	KCP 10.1.1.2/06
Report Author:	[redacted]
Report Year:	2020
Report Title:	Linnet breeding period compared to drilling season of winter oilseed rape in the Czech Republic
Report No:	R1960071_3
Document No:	M-680747-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	none
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

This report aims to assess the relevance of a reproductive risk assessment for linnets potentially exposed to treated winter oilseed rape (OSR) seeds in the Czech Republic by comparing data on the timing of reproduction in linnets to Czech drilling data. Overall, both periods overlap in the first week of August when the breeding season of linnets' ceases. This results in a very small proportion of potentially affected nests (ranging between 0% for the pre-laying period and 0.07% for the egg laying period). A negative effect on reproductive performance is not expected.

I. Material and Methods

OSR drilling data and ringing data of linnet nestlings were obtained for the Czech Republic. For an evaluation of the temporal overlap, the percentage of linnet nests was multiplied with the percentage of the sown area for each week of overlap and summed up. This was done for the nest stages "pre-laying", "first egg laid" and "last egg laid" for all regions combined. These nest stages were selected because the correspond with the phases of the avian reproduction studies where the parental birds are exposed over the pre-egg-laying and until the end of the egg-laying phase, but where there is no treatment during incubation or the nestling phase.

II. Results and Discussion

There is only about one week of overlap between the beginning of drilling and the end of egg laying at a time when relatively few linnets still breed and only a few fields are drilled. This results in only 0.07% of linnet nests being potentially exposed to treated seeds until the end of the egg-laying period.
These results suggest that only a negligible proportion of linnets is potentially affected by OSR seed treatments during their reproduction. Furthermore, the percentage of ingested treated OSR seeds is most

likely also very low. Finally, studies on other passerines suggest that successful reproduction (i.e. surviving offspring in one year and reproduction of this offspring in the subsequent breeding season) is generally very low for late breeding linnets.

III. Conclusion

To conclude, there is only little overlap between winter OSR drilling and the breeding season of linnets. This suggests that only a very small and negligible proportion of linnets is potentially exposed by OSR seed treatments during their reproductive period. Therefore, a negative effect on reproductive performance is not expected.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. There is very little overlap between drilling of winter oilseed rape and the breeding season of linnets.

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Data Point:	KCP 10.1.1.2/07
Report Author:	[redacted]
Report Year:	2018
Report Title:	Occurrence and PT of bird species in new-drilled, pre-emergence winter oilseed rape in Germany (2017)
Report No:	P16049
Document No:	M-629338-01-1
Guideline(s) followed in study:	EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) Regulation (EC) No 1107/2009 No official test guideline available. The Study Plan was prepared under consideration of recommendations in the current guidance document (EFSA 2009) on risk assessment for birds and mammals.
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCP 10.1.1.2/08
Report Author:	[redacted]
Report Year:	2018
Report Title:	Letter of access for generic behavioural ecology data - Study report: Occurrence and PT of bird species in new-drilled, pre-emergence winter oilseed rate in Germany (2017) (Syngenta Report no. TK0319846)
Report No:	M-631447-01-1
Document No:	M-631447-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

A generic study was conducted to a) assess the proportion of diet (PT) that the focal species skylark (*Alauda arvensis*), chaffinch (*Fringilla coelebs*), linnet (*Carduelis cannabina*) and yellowhammer (*Emberiza citrinella*) obtain in freshly-drilled oilseed rape fields during the pre-emergence period in summer; and b) provide bird observations under practical conditions and determine bird ‘focal species’ in oilseed rape fields. For this purpose, radiotracking and scan sampling was conducted in the area of Lüchow-Dannenberg (north-east of Germany). In total, 27 oilseed rape study fields were used for the study (7 trapping fields, 11 scan fields and 9 fields used for both purposes). The radiotracking demonstrated that pre-emergence oilseed rape fields are not attractive as feeding habitat for skylarks, chaffinches and yellowhammers. Full day radiotracking of linnets was not conducted for logistical reasons, but no linnets were observed on freshly drilled oilseed rape fields during visual observations. Furthermore, the scan sampling conducted during this study also showed that for bird species in general, pre-emergence oilseed rape fields are not used extensively as foraging habitat.

I. Material and Methods:

The purposes of the study were: a) to assess the proportion of diet (PT) that the focal species skylark (*Alauda arvensis*), chaffinch (*Fringilla coelebs*), linnet (*Carduelis cannabina*) and yellowhammer (*Emberiza citrinella*) obtain in freshly-drilled oilseed rape fields during the pre-emergence period in summer; and b) to provide bird observations under practical conditions and determine bird ‘focal species’ in oilseed rape fields.

The study was conducted on commercially managed oilseed rape (OSR) fields in the area of Lüchow-Dannenberg, in the north-east of Germany (province of Lower Saxony). The chosen study area is representative for oilseed rape cultivation.

The field phase of the study was conducted from the beginning of August to the middle of September 2017. The schedule of the study was developed based on the status of the 27 study fields selected as reference for the crop development of the pre-emergence oilseed rape fields. These study fields were associated with the trapping locations for capturing and tagging of the focal species and/or were the study fields selected for the scan sampling approach (see below).

The radio tracking sessions of the focal bird species were conducted during the pre-emergence period of the trapping study fields. The sessions started after the drilling of these fields and finished when the oilseed rape emerged in these fields (26th August – 13th September). Radio-tagged individuals of the focal species were tracked continuously over their activity period (max. 15hr15min) to determine their location and behaviour.

In total, 45 complete radio-tracking sessions were successfully performed on 42 individuals: 12 sessions for chaffinch, 23 for skylark (with three repeated sessions) and 10 for yellowhammer. Radio-tracking sessions on the linnets had to be withdrawn from the schedule due to unforeseen circumstances related to weather and agricultural practice (see 3.7.3 of the report). The proportion of time spent active and potentially foraging in pre-emergence oilseed rape fields (PT estimate) and the habitat preferences were analysed. Additionally, the radio tracking results enabled the calculation of the size and shape of the individuals' home range (during each session), using the minimum convex polygon method (MCP). The Jacobs' preference index (D), which indicates if an individual bird prefers or avoids pre-emergent oilseed rape fields as feeding habitat, was calculated for each tracking session.

The attractiveness of pre-emergence oilseed rape for birds was monitored in 20 fields between drilling and before emergence (from BBCH 00 to 08) by measuring the general abundance and behaviour of all bird species.

The species, abundance and behaviour of every bird present on the surface of the study field or a defined scan plot of at least 1.40 ha (average 3.5 ha) were recorded using the scan-sampling technique. This technique is commonly used to quantify bird presence as well as bird activities by steadily visually scanning the study field with the aid of a telescope and/or binoculars as optical devices. One session of approximately 4 hours in the morning or evening to cover the times of maximum bird activity was carried out per field. Mean abundance, dominance and frequency of occurrence (FO_{scan}, FO_{field}) were the main parameters used to describe the bird community in pre-emergence oilseed rape fields.

The availability of pre-emergent oilseed rape fields in the study area was monitored in 60 oilseed rape fields; by regular checks of the 27 study fields for their status, drilling time and development of the crop and by recording drilling information or BBCH status on 33 more oilseed rape fields of the study area.

Summary of general information

General information			
Study design	Generic		
Study period	August - September 2017		
Crop	Oilseed rape		
Test item	None		
Focal species	Chaffinch (*Fringilla coelebs*), Skylark (*Alauda arvensis*), Yellowhammer (*Emberiza citrinella*), Linnet* (*Carduelis cannabina*)		
Field information			
No. OSR study fields	27		
No. known OSR fields in area	60		
Drilling dates	23 August - 11 September 2017		
Radio tracking			
Species	Chaffinch	Skylark	Yellowhammer
No. of radio tracked birds	12	20	10
Radio tracking sessions	12	23	10
Bird monitoring - Scan sampling			
No. study fields	20		
Total scan area [ha]	69.8		
Mean scan area [ha]	3.5		

No. of scans	995
No. of bird observations	1050
No. of bird species	27

* Linnet was withdrawn from radio-tracking session schedule

II. Results and Discussion:

Continuous radio tracking sessions on 12 chaffinches, 20 skylarks and 10 yellowhammers for the pre-emergence period allowed a representative assessment of potential foraging times in order to calculate PT values. Seven skylarks and one yellowhammer entered the drilled oilseed rape fields during 45 sessions. Additionally, 3 chaffinch and 3 yellowhammer individuals flew over this habitat during the tracking session i.e. crop was in their daily home range. Moreover, some individuals (3 chaffinches, 9 skylarks and 4 yellowhammers) were trapped inside or close-by (≤10m) a drilled or soon-to-be drilled OSR field, i.e. these fields were in their daily home range at the trapping time. All these individuals were therefore considered potential consumers (50%, 75% and 80% of all individuals of chaffinch, skylark and yellowhammer respectively).

Mean PT values for consumer individuals were low but highly dependent on the species. For chaffinches (no consumer) and for yellowhammers (only one consumer), PT values are zero or nearly zero. For the skylarks, mean value was 0.15 and the 90%ile was 0.23 over all sessions. Skylarks had in general reduced home ranges; the mean home range size (n = 23 sessions) was 2.97 ha, while chaffinches and yellowhammers (respective mean = 17.59 and 24.95 ha; n = 12 and 10 individuals) used more extended areas. Jacobs' index for habitat preferences showed a mean value of -0.26 for skylarks. Overall, freshly drilled OSR fields were not very commonly used as feeding habitat by the focal bird species.

In total, 1050 bird observations of 27 different bird species were made during 995 scans performed throughout one session for each of 20 pre-emergence oilseed rape fields.
The mean abundance of birds was 0.30 ind./ha. Of the individuals observed, on average 80.7% showed foraging behaviour.

The most abundant and also dominant species (only species that foraged in the fields and did not show flocking behaviour) were the white wagtail (0.037 ind./ha, 12.49% of sightings), the carrion crow (0.021 ind./ha, 6.94% of sightings) and the skylark (0.015 ind./ha, 4.94% of sightings).

The temporal distribution of the avian community (only species that foraged in the fields), as indicated by the frequency of occurrence (FO_{scan}) in the pre-emergence fields, showed the skylark and white wagtail as the most relevant species. Using the frequency of occurrence by field (FO_{field} > 20%) as the major criterion, together with body mass, diet guild, frequency of occurrence per scan (FO_{scan}) and dominance, 4 species were listed as candidates for focal species for pre-emergence oilseed rape fields across all guilds: the yellow wagtail (small insectivore), yellowhammer (small omnivore), common starling (medium omnivore) and woodpigeon (medium herbivore/granivore). When considering potential focal species that might consume drilled oilseed rape seeds (i.e. small granivores and omnivores), the relevant candidates are the yellowhammer, skylark and chaffinch, as the only three small omnivores which might potentially feed on oilseed rape seeds observed on new-drilled OSR fields during scan sampling. No pure granivore species were seen in scan sampling of oilseed rape fields.

PT values for new-drilled, pre-emergence OSR fields (BBCH 00-09)

Yellowhammer			Chaffinch			Skylark		
Bird ID	**Status**	**PT**	**Bird ID**	**Status**	**PT**	**Bird ID**	**Status**	**PT**
Y3	pot.consumer	0.000	C2	pot.consumer	0.000	S1	pot.consumer	0.000
Y4	pot.consumer	0.000	C3	-	0.000	S3	pot.consumer	0.000
Y5	pot.consumer	0.000	C4	-	0.000	S4	pot.consumer	0.000
Y7	pot.consumer	0.000	C5	-	0.000	S5	consumer	0.153
Y8	pot.consumer	0.000	C6	pot.consumer	0.000	S6	pot.consumer	0.000
Y9	pot.consumer	0.000	C7	-	0.000	S10	consumer	0.203
Y10	pot.consumer	0.000	C8	-	0.000	S10*		
Y11	consumer	0.003	C10	-	0.000	S11	pot.consumer	0.000
Y12	-	0.000	C13	pot.consumer	0.000	S13	pot.consumer	0.000
Y13	-	0.000	C14	pot.consumer	0.000	S14	pot.consumer	0.000
			C15	pot.consumer	0.000	S15	consumer	0.050
			C16	pot.consumer	0.000	S17	-	0.000
						S18	consumer	0.217
						S19	pot.consumer	0.000
						S20	-	0.000
						S21	-	0.000
						S22	consumer	0.244
						S22*		
						S23	consumer	0.004
						S23*		
						S24	pot.consumer	0.000
						S25	-	0.000
						S27	-	0.000

* Repeat session of a consumer individual (mean of two sessions presented)

Summary of radio-tracking results

Radio tracking results				
PT approach	Parameter	Number of sessions/birds	PT in drilled OSR fields	Jacobs' index [D]
Chaffinch				
Consumer	Mean	0	-	-
	Median		-	-
	90%ile		-	-
Potential consumer	Mean	6	0.00	-
	Median		0.00	-
	90%ile		0.00	-
All birds	Mean	12	0.00	-
	Median		0.00	-
	90%ile		0.00	-
Skylark				
Consumer	Mean	7/6	0.15	-0.26
	Median		0.18	-0.45
	90%ile		0.23	0.42
Potential consumer	Mean	18/15	0.06	-
	Median		0.00	-

Radio tracking results				
PT approach	Parameter	Number of sessions/birds	PT in drilled OSR fields	Jacobs' index [D]
	90%ile		0.21	-
All birds	Mean	23/20	0.04	-
	Median		0.00	-
	90%ile		0.20	-
Yellowhammer				
Consumer	Mean	1	0.003	-
	Median		0.003	-
	90%ile		0.003	-
Potential consumer	Mean	8	<0.001	-
	Median		0.000	-
	90%ile		0.001	-
All birds	Mean	10	<0.001	-
	Median		<0.001	-
	90%ile		<0.001	-

III. CONCLUSIONS:

This generic study provides bird observations under realistic agricultural conditions in pre-emergence oilseed rape fields in Germany.

This study demonstrated that pre-emergence oilseed rape fields are not attractive as feeding habitat for skylarks, chaffinches and yellowhammers.

– In total 45 tracking sessions (continuous recording from dawn to dusk) were conducted between 26th August and 13th September 2017 on 20 skylarks (23 sessions), 12 chaffinches (12 sessions) and 10 yellowhammers (10 sessions). The birds were trapped inside or close to oilseed rape fields that were in the pre-emergence stage during radio tracking, in order to measure use of these fields as feeding habitat.
– During the tracking sessions, 6 out of 20 skylark individuals, none of the 12 chaffinches and one of the 10 yellowhammers entered pre-emergence oilseed rape fields.
– For 'consumers', the mean PT was 0.15 for the skylark (90%ile = 0.23) and 0.003 for the yellowhammer (90th percentile = 0.003).
  For individuals with oilseed rape fields in their home range during their tracking session or during trapping ('potential consumer approach') the mean PT was 0.06 for the skylarks (90%ile = 0.21) and <0.001 for the yellowhammer (90%ile = 0.001).
  Considering data from all sessions ('all birds approach') the mean PT was 0.04 for the skylarks (90th percentile = 0.20) and <0.001 for the yellowhammer (90th percentile <0.001). Since none of the tracked chaffinches foraged in pre-emergence oilseed rape fields, their PT was 0 for all approaches.

The scan sampling conducted during this study also showed that for bird species in general, pre-emergence oilseed rape fields are not used extensively as foraging habitat.

– During 995 single scans performed across one sampling session for each of 20 pre-emergence oilseed rape fields, a total of 1050 bird observations of 27 different bird species were recorded.
– Foraging was the most prevalent behaviour across all observed birds.

- Four species proposed as focal species across all guilds were the yellow wagtail (small insectivore), yellowhammer (small omnivore), common starling (medium omnivore) and woodpigeon (medium herbivore/granivore).
- When considering potential focal species that might consume drilled oilseed rape seeds (i.e. small granivores and omnivores), the relevant candidates are the yellowhammer, skylark and chaffinch, as the only three small omnivores observed which might potentially feed on oilseed rape seeds on new-drilled OSR fields during scan sampling. No purely granivore species were seen in scan sampling of oilseed rape fields.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. Freshly drilled OSR fields were not very commonly used as feeding habitat by the focal bird species skylark, chaffinch and yellowhammer. Linnets were never observed on freshly drilled fields.

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Data Point:	KCP 10.1.1.2/09
Report Author:	[redacted]
Report Year:	2018
Report Title:	Modelling breeding season period of farmland birds at a European scale
Report No:	TK0231883
Document No:	M-616722-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted Approval renewal of Prothioconazole
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data Point:	KCP 10.1.1.2/10
Report Author:	[redacted]
Report Year:	2018
Report Title:	Letter of access for generic behavioural ecology data. Study report: Modelling breeding season perio of farmland birds at a European Scale (Syngenta Report no. TK0231883). Crop Grouping: Multi-crop and landscape
Report No:	M-620031-01-1
Document No:	M-620031-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

Volunteer collected data from pulli ringing across Europe was used to model the breeding season period across Europe for 14 species common to farmland habitats in relation to differences in habitat, elevation, climate, longitude and latitude. Breeding season start and end dates varied between species but there were a few consistent patterns. In general species bred earlier in more southerly regions, where spring and summer temperatures were warmer. For the suite of species considered here and for the geographical area considered, the key period when the bulk of the population of most species are breeding in farmland habitat spans late March to late August/early September.

I. Material and Methods:

Volunteer collected data from pulli ringing across Europe was used to model the breeding season period across Europe for 14 species common to farmland habitats in relation to differences in habitat, elevation, climate, longitude and latitude. In order to achieve a conservative estimate of breeding season timing rather than an estimate of the mean value across the species, a quantile regression was used. The reliability and predictive ability of these models was explored across the fourteen species.

II. Results and Discussion:

Predictions were generated from the quantile regression models for first egg date (FED), last egg date (LED) and fledging date (FLG) for each species. The table below displays the predictions for the whole region using the 0.10 quantile for FED and the 0.90 quantile for LED and FLG. Country specific predictions were also generated and are in the main report below. Predictive accuracy, as measured by cross validation and the prediction confidence intervals, varied between species and within a species spatially, due to variations in data availability; predictions for species which had more data are more accurate. Extreme quantiles tended to be less accurate; using the 0.10 and 0.90 quantiles is recommended to achieve a balance between a conservative estimate and good model accuracy.

	Whole region FED (0.10 quantile)	**Whole region LED (0.90 quantile)**	**Whole Region FLG (0.90 quantile)**
Blackbird	15-Mar (13-Mar to 17-Mar)	25-Jun (22-Jun to 29- Jun)	23-Jul (20-Jul to 27-Jul)
Blackcap	18-Apr (15-Apr to 20-Apr)	07-Jul (30-Jun to 15-Jul)	29-Jul (22-Jul to 09-Aug)
Blue Tit	27-Mar (26-Mar to 28-Mar)	17-May (16-May to 18- May)	15-Jun (14-Jun to 16-Jun)
Crow sp.	25-Mar (20-Mar to 29-Mar)	17-May (12-May to 24- May)	05-Jul (30-Jun to 13-Jul)

	Whole region FED (0.10 quantile)	Whole region LED (0.90 quantile)	Whole Region FLG (0.90 quantile)
Chaffinch	01-Apr (28-Mar to 05-Apr)	28-Jun (18-Jun to 07-Jul)	16-Jul (08-Jul to 26-Jul)
Great Tit	29-Mar (29-Mar to 29-Mar)	01-Jun (30-May to 03- Jun)	06-Jul (04-Jul to 08-Jul)
Linnet	11-Apr (06-Apr to 16-Apr)	28-Jul (20-Jul to 06-Aug)	02-Sep (25-Aug to 11-Sep)
Skylark	09-Apr (04-Apr to 13-Apr)	06-Jul (02-Jul to 12-Jul)	03-Aug (30-Jul to 08-Aug)
Starling	01-Apr (30-Mar to 02-Apr)	05-Jun (02-Jun to 11- Jun)	08-Jul (04-Jul to 13-Jul)
Song Thrush	22-Mar (19-Mar to 24-Mar)	01-Jul (25-Jun to 08-Jul)	27-Jul (21-Jul to 03-Aug)
Woodlark	09-Feb (27-Jan to 24-Feb)	01-Jul (13-Jun to 01-Aug)	20-Jul (07-Jul to 14-Aug)
Woodpigeon	26-Feb (15-Feb to 11-Mar)	28-Aug (20-Aug to 10-Sep)	15-Oct (07-Oct to 27-Oct)
Yellowhammer	05-Apr (29-Mar to 13-Apr)	19-Jul (12-Jul to 28-Jul)	19-Aug (12-Aug to 27-Aug)
Yellow Wagtail	01-May (24-Apr to 09-May)	03-Aug (03-Jul to 07- Sep)	31-Aug (31-Jul to 18-Oct)

III. CONCLUSIONS:

Breeding season start and end dates varied between species but there were a few consistent patterns. In general species bred earlier in more southerly regions, where spring and summer temperatures were warmer. For the suite of species considered here and for the geographical area considered, the key period when the bulk of the population of most species are breeding in farmland habitat spans late March to late August/early September.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. For most species there is little overlap between the egg-laying season and the drilling of winter oil-see rape.

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Data Point:	KCP 10.1.1.2/12
Report Author:	[redacted]
Report Year:	2010
Report Title:	Field monitoring of birds and mammals after the drilling of rape seeds, treated with Methiocarb FS 500 (1.0 - 1,5 kg/ 100 kg seed) in France 2009
Report No:	[redacted]/FS 052
Document No:	M-362200-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted Previously submitted for Methiocarb
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data on methiocarb is not relevant for the risk assessment, information on the number of seeds on the soil surface and species observed is relevant for the revised risk assessment. Only data relevant for the refined risk assessment is summarised.

Executive Summary

A study was conducted in the French Department Haute-Marne, Champagne-Ardennes to evaluate possible side-effects of seeds treated with Methiocarb FS 500 on natural bird and mammal communities after drilling. The exposure of rape seeds on the soil surface was determined on the drilling day (day 0) or day +1. On every field two bird observation sessions of 2 hours were performed between day 0 and day +3. Activity signs of mammals were also recorded. In midfield areas the mean number of rape seeds per m² amounted to 0.7 (SD 1.1); in end row areas it amounted to 1.6 seeds per m² (SD 1.5). In general, the abundance of birds was low. the most frequently observed species were Carrion Crow, Jay, Mistle Thrush, Buzzard, Chaffinch, and Yellowhammer. Only few mammalian activities were recorded.

I. Material and Methods:

The study aimed to evaluate possible side-effects of seeds treated with Methiocarb FS 500 (1.0 – 1.5 kg a.s./100 kg seed) on natural bird and mammal communities after drilling.

The monitoring was performed in August 2009 on 10 fields in the French Department Haute-Marne, Champagne-Ardennes, which is a major region for rape growing in France.

From each used batch of treated seeds, a sample was collected, which was analysed for the loading with methiocarb.

The exposure of rape seeds on the soil surface was determined on the drilling day (day 0) or day +1. On each field, 80 squares (50 cm x 50 cm) along eight transect lines of 50 m (4 in midfield area, 4 in endrow areas, per transect 10 squares) were randomly chosen, on which the number of remaining rape seeds was counted. It was as well intended to report any spillages found during the exposure assessment.

After the application, on each field 3 carcass searches for dead or impacted birds and mammals were performed (always one search on day +1, and 2 further searches between day +2 and +6). During the carcass search, a team of 2 – 4 people paced the test area. In total 66.3 ha were covered.

The commitment and search efficiency of the carcass search team was checked twice, by distributing dummies. The recovery rate was 83 % and 92 %, indicating an appropriate search efficiency by the carcass search teams.

Carcasses were collected when they were suitable for residue analysis. The place of finding, the circumstances of the finding and the conditions of the carcasses including signs of intoxication were recorded. The carcasses were submitted to residue analysis for methiocarb. On the application day, no carcass search was carried out in order not to chase the birds away.

On every field two bird observation sessions of 2 hours were performed between day 0 and day +3. During the bird observation all birds seen on the field were recorded. Based on the results, the frequency of occurrence was calculated for each species of concern. Activity signs of mammals were also recorded.

II. Results and Discussion:

Test item	Rape seeds, treated with Methiocarb FS 500 (1.0 – 1.5 kg/100 kg seed)
Test object	Bird and mammal populations

Content of methiocarb on rape seeds

Chemical analysis of samples from each used batch confirmed that the content of active substance on the treated seeds was in the required range.

Application and exposure:

The rape drilling was always performed as precise drilling. On the 10 fields 6 different machines were used (Amazone, Horsch, Kverneland, Nodet, Sulky Unidrill, Väderstad). The diversity of different seed types and batches was high as well.

Although the differences in the use of equipment and seed types were high, the exposure of seeds on the surface of the fields was always similar and in general low:

In midfield areas the mean number of rape seeds per m² amounted to 0.7 (SD 1.1); in end-row areas it amounted to 1.6 seeds per m² (SD 1.5): A spillage of ca. 300 seeds each was detected on field 7 and on field 14.

Bird observation

In total, 26 bird species were detected on the fields and their surroundings, of which 25 visited regularly or occasionally the drilled rape fields.

The frequency of occurrence of the different birds is expressed in percentage related to all fields (n=10; frequency of occurrence – fields) and related to all censuses (n=20; frequency of occurrence – survey).

Based on the frequency of occurrence – field, the most frequently observed species were carrion crow, jay, mistle thrush, buzzard (each 40 %), chaffinch, and yellow hammer (each 30 %). Related to the number of surveys, the most frequent bird species were the same with little differences in the ranking list: jay 30 %; carrion crow, buzzard, mistle thrush and yellow hammer: each 25 %; chaffinch: 20 %.

Generally, all species with a high frequency of occurrence may be candidates for focal species. For this special monitoring small granivorous birds, potentially feeding on rape seeds, are of most concern. Those are chaffinches and yellow hammers.

In general, the abundance of birds was low.

All observed birds behaved normally and were above any suspicion of being impacted by methiocarb.

Mammal observation

During bird observation sessions no activities of mammals were detected. On the fields 4, 5 and 7 fox droppings were detected. On field 7 mole hills, holes of rodents and marks of boars were found. During the bird observation on field 10 a mouse of the Genus *Apodemus* (probably a wood mouse) and a fox were seen running around on the field.

Carcass searches

In total, 29:50 hrs or 102:20 man hrs (hh:mm) were spent on carcass searches.

The only carcasses found were two small wood mice (*Apodemus sylvaticus*). They were detected in the middle of field 11 on day +6, when most of the rape was emerged. The distance between the two carcasses was less than 10 cm. No further carcass was found.

Some single feathers of different species, e.g. wood pigeon, rook, jay, grey heron, were detected.

Analysis of the carcasses

The biometric and pathological analysis of the two wood mouse carcasses verified that both mice were juveniles.

The intestines and stomachs of both mice were submitted to residue analysis. The analysis showed that residues of methiocarb in/on the mouse carcass samples were always below the LOD (0.025 mg/kg). The analysis supports the conclusion of the biometric and pathological examination that the juveniles did not die on intoxication.

III. CONCLUSIONS:

The monitoring program aimed to describe and identify possible effects on birds and mammals after the drilling of rape seeds, treated with methiocarb. Carcass searches and bird observation did not reveal any suspicion of intoxication or mortality of birds or mammals by methiocarb.

A key finding is the low exposure of rape seeds after precise drilling. Therefore, the drilled field is not attractive for granivorous birds as it can be demonstrated by the relative low abundance of birds on the fields. Furthermore, the relative low number of individuals and species occurring on the drilled fields is considered a typical seasonal pattern. The rape drilling is performed during a time (August) when most of the birds are moulting. In that period, they avoid open landscapes and prefer sheltered areas. At that time of the year the autumn migration of passerine birds had not yet started and the attractiveness of the fields is low due to reduced food availability.

The low exposure combined with the low abundance of birds is in line with the absence of noticeable effects after drilling of the seeds treated with methiocarb.

Only few mammalian activities were recorded. Negative impacts on mammals were not visible. The carcasses of two juvenile wood mice were found, which were analysed on residues of methiocarb, but with negative results. The mice were most likely abandoned by their mother and unable to survive.

Drilling efficiency:

The number of seeds present at the soil surface after the precise drilling is presented in the table below:

Field No	Seeding rate [kg seeds/ha]	Equipment used	Number of seeds per m²	
			Midfield	End-rows
1	2.2	Horsch Sprinter 4ST	0.1	0.4
2	2.2	Nodet, mortiser upstream	0.3	1.8
4	1.8	Amazone D9-40	0.1	0.5
5	1.8	Amazone D9-40	0.6	1.1
7	2.2	Kverneland NGS 401	0	0.9
10	2.0	Horsch-CO6	0	0.8
11	2.0	Horsch-CO6	0.3	0.2
12	2.0	Sulky Unidrill	3.7	3.9
13	2.0	Sulky Unidrill	1.2	4.7
14	1.8	Väderstad	0.2	1.4
		Mean	**0.7**	**1.6**
		SD	1.1	1.5

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. Exposure of birds to treated oilseed rape seeds on freshly drilled fields is low.

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Data Point:	KCP 10.1.1.2/11
Report Author:	[redacted]
Report Year:	2006
Report Title:	Generic field monitoring of birds in freshly drilled oilseed rape fields in summer in Germany
Report No:	[redacted]/FS 037
Document No:	M-279936-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

A generic study was performed in the western part of Saxony-Anhalt, Germany to evaluate the extent to which birds utilize freshly drilled treated oilseed rape (*Brassica napus*) seeds as a food source. Based on information gained from the literature, three main focal species were selected: the Yellowhammer, the Linnet and the Chaffinch. In order to assess the exposure of these species to treated oilseed rape seeds, the portion of time they spent 'potentially foraging' in freshly drilled fields was acquired by radio tracking. Furthermore, census counts were carried out along four different transects, representing all main agrarian habitat types within the study area. Additionally, in six defined subareas of oilseed rape fields a scan sampling procedure was conducted. Information on food items selected by the focal species, samples of faeces and stomach contents were analysed quantitatively for their composition. The study showed that fields of freshly drilled oilseed rape were only used as a minor feeding habitat by Yellowhammers, Chaffinches and Linnets. Furthermore, no proof for freshly drilled oilseed rape seeds as part of their diet was found.

I. Material and Methods:

This generic study was performed to evaluate the extent to which birds utilize freshly drilled treated oilseed rape (*Brassica napus*) seeds as a food source.

Test locality:
The study was conducted in the farming area of the agrarian co-operative Warnstedt, which is located in the district of Quedlinburg in the nature preservation region 'Nördliches Harzvorland' (federal state of Sachsen-Anhalt, Germany). This region is a typical area for oilseed rape (OSR) cultivation in Europe and known to hold an essential population of the three preselected focal species Yellowhammer (*Emberiza citrinella*), Linnet (*Carduelis cannabina*) and Chaffinch (*Fringilla coelebs*).

Methods:
Based on information gained from the literature three main focal species were selected: the

Yellowhammer, the Linnet and the Chaffinch. These species were expected to feed on treated freshly drilled oilseed rape, because of distribution, habitat selection and food preferences. In order to assess the exposure of these species to treated oilseed rape seeds, the portion of time they spent 'potentially foraging' in freshly drilled fields was acquired by radio tracking. Ten Yellowhammers, nine Chaffinches and three Linnets were trapped and tagged with radio transmitters in freshly drilled oilseed rape fields and adjacent habitats. Radio tracking was carried out during late summer (tracking period 2005-08-16 to 2005-09-11). The individual birds were continuously radio-tracked for one daylight period.

In order to assess the general relevance of freshly drilled oilseed rape fields as feeding locations for birds compared to other habitats, census counts were carried out along four different transects, representing all main agrarian habitat types within the study area. These transects were walked six times each to attain a full overview of the abundant bird life.

Additionally, in six defined subareas of oilseed rape fields – including a small adjacent section outside the field - a scan sampling procedure was conducted. Here, any bird activity was monitored from dawn until dusk. Scan sampling was carried out once before drilling, and twice after drilling to record any changes in bird activity possibly caused by the availability of treated oilseed rape seeds. Information on food items selected by the focal species, samples of faeces and stomach contents were analysed quantitatively for their composition: discernible whole seeds or parts of OSR seeds, remains of plants and arthropods according to taxonomic orders and other identifiable items.

II. Results and Discussion:

PORTION OF TIME potentially foraging (PT) in oilseed rape fields by radio tracked species				
potential foraging time radio tracked birds spent in freshly drilled oilseed rape fields	Species	mean [%]	90^{th} %ile [%]	tracking sessions (individuals)
	Yellowhammer	4.14	17.24	10 (10)
	Chaffinch	6.60	27.32	9 (9)
	Linnet	0	0	3 (3)

HABITAT PREFERENCE of species according to radio tracking		
preference of oilseed rape as a feeding habitat (Jacobs Index [D], Range: -1 to +1; MCP [100%])	Yellowhammer	-0.91
	Chaffinch	-0.60
	Linnet	-1.00

DIET of species in oilseed rape fields				
Numerical portion of food items [%] after the analysis of faeces (25) and samples of stomach flushing (1) and stomach contents (1) gathered in or near by oilseed rape fields	food items	Yellowhammer (n = 10)	Chaffinch (n = 12)	Linnet (n = 5)
	Brassica napus (OSR) seeds	0	0	(19.6)*
	Brassica seeds - unspecified	2.6	7.7	19.6
	cereal seeds	5.3	3.1	0
	wheat seeds	56.6	12.3	0
	other seeds	6.6	21.5	37.0
	other plant material	0	7.7	23.9
	Coleoptera	3.9	20.0	0
	Dermaptera	6.6	13.8	0
	Diptera	11.8	3.1	0
	Hymenoptera	2.6	7.7	0
	other animals	2.6	3.1	0
	Unknown	1.3	0	0

BIRD ABUNDANCE in oilseed rape fields according to transect counts (based on population)			
abundance of focal species and three other most abundant species after four transect counts covering 116.24 ha	Species	no. of ind.	[ind./transect count and ha]
	Yellowhammer	7	0.169
	House Sparrow	7	0.169
	Blue Tit	1	0.024
	Tree Sparrow	1	0.024
	Chaffinch	-	-
	Linnet	-	-
BIRD FREQUENCY OF OCCURRENCE per scan in oilseed rape fields according to scan sampling			
frequency of occurrence per scan (mean of the results for each session; n = 12) of focal species and five other prevalent species on six fields	Species	[%]	
	Chaffinch	7.77	
	Blackbird	5.67	
	Wood Pigeon	4.92	
	Yellowhammer	4.27	
	Buzzard	3.81	
	Mistle Thrush	3.60	
	Song Thrush	3.14	
	Linnet	0.14	

[1] sum of behaviour categories 'foraging', 'active, maybe foraging' and 'unknown'
* Oil seed rape found in the stomach of a dead bird (road kill) near the farm, they may originate from spilled seeds (untreated harvest remains) on the premises of the agrarian cooperative

III. CONCLUSIONS:

Radio tracking Yellowhammers, Chaffinches and Linnets in an agrarian landscape with a high number of freshly drilled oilseed rape fields in the western part of Saxony- Anhalt showed that this field type was used only as a minor feeding habitat. Furthermore, no proof for freshly drilled oilseed rape seeds as part of their diet was found.

Ten individually radio tracked Yellowhammers (n = 10 sessions) did not prefer freshly drilled oilseed rape field as feeding habitats. Only three individuals did use freshly drilled oilseed rape fields as a feeding habitat at all. Oilseed rape fields were on average avoided, i.e. selected to a lower portion as to be derived from the available portion in the birds' home ranges. Additionally, no clear evidence for the ingestion of oilseed rape seeds was found, according to stomach and faeces samples. At most, not specified *Brassica* spec. seeds (2.6 % of all food items) provide an indication that Yellowhammers may occasionally feed on rape seeds.

Radio tracking of nine individual Chaffinches (n = 9 sessions) in the same area showed that freshly drilled oilseed rape fields did not present an exclusive feeding habitat, but they were visited on average more often than in Yellowhammers. More than half of the tracked Chaffinches (five individuals) fed during the tracking sessions on freshly drilled oilseed rape fields, whilst four individuals were not observed using oilseed rape fields as feeding habitat at all. Calculation of abundance and mean frequency of occurrence according to scan sampling showed them to be most abundant and frequent in freshly drilled oilseed rape fields. However, in stomach and faeces samples no Brassica seeds were found, which were identified as *Brassica napus* (OSR) seeds for sure. Some unspecified Brassica spec. seeds (7.7 % of all food items) indicate that Chaffinches may feed on oilseed rape.

Radio tracking of three individual Linnets (n = 3 sessions) showed that the birds mainly fed on stubble fields and in the habitat 'tree/bush/hedge'. None of the tracked Linnets used freshly drilled oilseed rape fields as feeding habitat. However, sample size for radio-tracked Linnets is low, because of difficulties in trapping them. Therefore, results have to be discussed with caution.

Although, bird census data confirmed that Linnets clearly prefer stubble fields and non-crop habitats to freshly drilled oilseed rape fields and results from the scan sampling approach confirmed this finding. No oilseed rape seeds were detected in faeces samples. A number of Brassica spec. seeds (19.6 % of all food items) give a clue that Linnets may feed on freshly drilled oilseed rape seeds. OSR seeds found in the stomach of a dead bird (stomach sample) may originate from spilled seeds (untreated harvest remains) on the premises of the agrarian cooperative.

For risk assessment purposes a value for portion of time spent foraging in freshly drilled oilseed rape fields (PT) for Yellowhammer, Chaffinch and Linnet, can be derived from the study results: Yellowhammers spent on average 4.14 % of their potentially foraging time in freshly drilled oilseed rape fields (90th percentile = 17.24%), Chaffinches on average 6.60 % (90th percentile = 27.32 %) and Linnets spent 0% of their 'potentially foraging' time in oilseed rape fields (90th percentile = 0 %).

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

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".

Table 10.1.2- 1: Endpoints used in risk assessment

Test substance	Risk assessment	Species	Endpoint		Reference
Fluopicolide	Acute	Rat	**LD_{50}**	**> 5000 mg a.s./kg bw**	[redacted] 2000; M-197224-01-1 KCA 5.2.1/01
	Long-term	Rat	**$BMDL_{10}$ for 21-d bw in rodents**	**= 119 mg a.s./kg bw/day**	[redacted] 2019; M-667414-01-1 KCP 10.1.2.2/07
			$BMDL_{10}$ for 21-d bw gain in rodents	= 116 mg a.s./kg bw/day	[redacted] 2019; M-667312-01-1 KCP 10.1.2.2/08
		Rabbit	**NOAEL**	**= 20 mg a.s./kg bw/day**	[redacted] 2004; M-202513-02-1 KCA 5.6.2/04
M01 (AE C653711, BAM)	Acute	Rat	LD_{50}	=1470 mg/kg bw	[redacted] 1967; M-228905-01-1 KCA 5.8.1/02
	Long-term	Rat	**NOAEL**	**= 7.5 mg/kg bw/day**	[redacted] 1993; M-301025-01-1 KCA 5.8.1/49

Test substance	Risk assessment	Species	Endpoint		Reference
Fluoxastrobin	Acute	Mouse	LD_{50}	> 2000 mg a.s./kg bw	EFSA Scientific Report 102 (2007)
	Long-term	Rat	NOEL	= 742–764 mg a.s./kg bw/day	EFSA Scientific Report 102 (2007)
Fluopicolide + Fluoxastrobin	Acute	Rat	$LD_{50\ MIX}$	**> 3041 mg total a.s./kg bw**	Table 10.1.2-6

Endpoints in **bold** considered relevant for risk assessment

Higher Tier endpoint

Based on the different feeding behaviour of rabbits and rodents (mice) and the different observed effects in the toxicity studies, further described in M-683114-01-1, it is considered justified to employ distinct risk assessments for rabbit scenarios (herbivores) with the rabbit endpoint, and for granivore scenarios of seed eating mice with the corresponding rodent endpoint.

The treatment of rodents (rats, mice) with fluopicolide mainly results in moderate effects on body weight changes, in the rat typically associated with initially reduced food consumption which is overcome by week 3. The duration of the environmental exposure scenario of mice to treated seeds in a landscape with freshly drilled oil seed rape fields can also be conservatively estimated not to exceed 3 weeks. Therefore, 3-week body weight effects in rodents were considered as appropriate point of departure for the risk assessment on seed eating mice in freshly drilled oil seed rape fields.

For the use in the Toxicity Exposure Ratio (TER) calculation, 3-week body weight effects were derived with a benchmark dose (BMD) calculation. For this purpose, body weight data for the first 3 weeks were excerpted from all dietary studies with fluopicolide in rodents (28-d, 90-d, chronic, reproduction) which include a comparable exposure setting in the initial 21 days.

BMDs were calculated with the tools recommended by EFSA (2017), and the reliability of the fit was assessed based on the criterion of normalized width (EFSA 2015). BMD values were calculated both for body weight differences and for body weight gain differences. Because 10 % effect on body weight over a few weeks is considered a more severe finding than 10 % percent effect on body weight gain, and the lowest reliable $BMDL_{10}$ values were very similar, preference in a refined risk assessment should be given to the BMD for body weight effects.

As a point of departure for the refined risk assessment, the $BMDL_{10}$ is proposed, i.e. the left confidence limit of the BMD for 10% effect on body weight. **The lowest reliable $BMDL_{10}$ was 119 mg/kg bw/d.**

This endpoint of 119 mg/kg bw/d was used as a refinement step for the seed eating mammal scenario. The seedling eater scenario was conducted with the rabbit endpoint of 20 mg/kg bw/d.

Metabolites of fluopicolide

The metabolites of fluopicolide do not pose higher risk to birds than the parent compound. This is also confirmed by the EFSA Scientific report 299 (2009), wherein it is stated that the risk to birds from plant metabolites of fluopicolide is considered to be low. Furthermore, a study conducted in 2019 (M-683112-01-1, see this document MCP 10.1.1.2/03) shows residue levels of the most relevant metabolites M-01 (AE C653711) and M-02 (AE C657188) to reach maximum concentration of only 0.019 mg p.m./kg and <0.01 mg p.m./kg, respectively. Therefore, a potential risk from metabolites is covered by the risk assessment of the parent compound fluopicolide (see below). As a further line of evidence for M-01 and

M-02, a worst case risk assessment for herbivorous mammals exposure to plant metabolites can be based on the maximum RUDs determined by [redacted] 2020 (M-686445-01-1, MCP Infinito 10.1.1.2/01) in foliage sampled during the course of rotational crop studies (see 'Refined risk assessment for mammals feeding on rape shoots' further below).

Table 10.1.2- 2: Calculation of the maximum amount of active substances on one dressed seed

Crop	Product loading [L prod./dt seeds]	Content of a.s. within the product [g a.s./L prod.]	Nominal loading/ application rate (NAR) [mg a.s./kg seeds]	Max. amount of a.s. on one dressed seed[a)] [µg a.s./seed]
Winter rape	1.0	FLC: 200 FXA: 150	FLC: 2000 FXA: 1500	FLC: 14.0 FXA: 10.5

b) Assuming a weight of thousand seeds of 4 – 7 g according to GAP. For the calculations 7 g was used as a worst case.

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

Scenario	Generic focal species	Calculation of residues	
		Acute assessment	Reproductive assessment
Mammals feeding on seeds (Small seeds)	Small omnivorous mammal	NAR × 0.24	NAR × 0.24 × ftwa
Mammals feeding on seedlings	Small omnivorous mammal	NAR/5 × 0.24	NAR/5 × 0.24 × ftwa

NAR = Nominal loading/application rate

ACUTE DIETARY RISK ASSESSMENT

Mammals feeding on seeds

Table 10.1.2- 4: First-tier acute risk assessment for mammals feeding on seeds (fluopicolide)

Crop	Generic focal species	NAR [mg a.s./kg seeds]	FIR/bw	NAR × FIR/bw	LD_{50} [mg a.s./kg bw]	TER_a	Trigger
Winter rape	Small omnivorous mammal	2000	0.24	480	5000	10.42	10

The TER_A value for fluopicolide calculated in the acute risk assessment for mammals feeding on seeds is above the acceptability trigger of 10. Therefore, no further refinement steps are necessary.

Mammals feeding on seedlings

Table 10.1.2- 5: First-tier acute risk assessment for mammals feeding on seedlings (fluopicolide)

Crop	Generic focal species	NAR [mg a.s./kg seeds]	FIR/bw	NAR/5 × FIR/bw	LD_{50} [mg a.s./kg bw]	TER_a	Trigger
Winter rape	Small omnivorous mammal	2000	0.24	96	5000	52.08	10

The TER_A value for fluopicolide calculated in the acute risk assessment for mammals feeding on seeds is above the acceptability trigger of 10. Therefore, no further refinement steps are necessary.

Please note: For the active substance fluoxastrobin the scenario of mammals feeding on seedlings does not apply as the uptake of fluoxastrobin into the plant is relatively low and the substance in general can be regarded to be non-systemic. Therefore, the use of the $LD_{50\,MIX}$ is not considered for the seedling eater risk assessment in the combined toxicity risk assessment below.

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.

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 the FLC+FXA FS 350 in this AIR-evaluation, but it may be conducted post-AIR according to the respective zonal guidance.

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

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

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 FLC + FXA FS 350 addressed in this dossier is 200 g fluopicolide/L prod. and 150 g fluoxastrobin/L prod., making up a total of 350 g a.s./L product.

Table 10.1.2-6 shows the calculation of the predicted LD_{50} (mix) of fluopicolide and fluoxastrobin when mixed in these proportions (step 1 in Appendix B of EFSA GD 2009).

Table 10.1.2- 6: Mammalian LD_{50} (mix) for fluopicolide and fluoxastrobin when combined as FLC+FXA FS 350 (step 1 in Appendix B of EFSA GD 2009)

	Fluopicolide	Fluoxastrobin
Content of a.s. in product [g a.s./L prod.]	200	150
Fraction in the a.s. mixture	0.5714	0.4286
LD_{50} of a.s. [mg a.s./kg bw]	> 5000	> 2000
Fraction / LD_{50}	0.0001143	0.0002143
Sum	0.0003286	
1/sum = predicted LD_{50} (mix) [mg total a.s./kg bw]	> 3043	

Fluopicolide contributes to 35 % to mixture toxicity, while fluoxastrobin has 65 % impact on the mixture toxicity (see table below). Consequently, the risk assessment cannot be performed only for the most toxic active substance alone and acute risk assessments are also done with the $LD_{50\,MIX}$.

Table 10.1.2- 7: Mammalian "tox per fraction" for FLC+FXA FS 350 (step 1 in EFSA GD 2009, Appendix B)

	Fluopicolide	Fluoxastrobin	"mix"
Content of a.s. in product [g a.s./L prod.]	200	150	350
Fraction in the a.s. mixture	0.5714	0.4286	1
LD_{50} of a.s. [mg a.s./kg bw]	> 5000	> 2000	3043
Tox per fraction	8750.437522	4666.355576	13416.7931
Contribution to predicted toxicity	35 %	65 %	

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

Left side of the equation: $\sum_i \frac{X(a.s._i)}{LD_{50}(a.s._{i.})} = \frac{0.5714}{\frac{5000\text{ mg a.s}}{\text{kg bw}}} + \frac{0.4286}{\frac{2000\text{ mg a.s}}{\text{kg bw}}} = 0.0003$

Right side of the equation: $\frac{1}{LD_{50}(mix)} = \frac{1}{\frac{602\text{ mg total a.s.}}{\text{kg bw}}} = 0.0017$

0.0003 < 0.0017

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. However, note that both derived endpoints, the calculated as well as the measured, are based on a 'greater than' value due to low toxicity of both substances and consequently no effect was observed at the highest test level. Therefore, it is considered justified to use the larger of the two endpoints, namely > 3043 mg/kg bw/d for further calculations.

Table 10.1.2- 8: First-tier acute risk assessment for mammals feeding on seeds (product)

Crop	Generic focal species	NAR [mg total a.s./kg seeds]	FIR/bw	NAR × FIR/bw	$LD_{50\ MIX}$ [mg total a.s./kg bw]	TER_a	Trigger
Winter rape	Small omnivorous mammal	3500	0.24	840	> 3043	**> 3.62**	10

The TER_A value, calculated for a surrogate endpoint, does not exceed the trigger value of 10. However, there were no mortalities or other significant effects at the top dose levels of the acute oral LD_{50}-studies conducted with the individual active substances or their combination. Therefore, this TER of 3.62 is actually calculated with a NOLEL rather than with a 50% mortality endpoint, adding an additional margin of safety to ensure that wild mammal mortality is not expected. Furthermore, further refinement provided under CP 10.1.2.2 confirms that dietary exposure of wood mice to residues on treated oilseed rape seeds is very low under field conditions. Thus, it can be concluded that the risk of visible mortalities in wild mammals after use of fluopicolide on treated oilseed rape seeds is low.

Acute risk assessment for mammals drinking contaminated water from puddles

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/day (EFSA GD 2009, Appendix K).

An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER less than direct dietary consumption.

LONG-TERM REPRODUCTIVE ASSESSMENT

Mammals feeding on seeds

Table 10.1.2- 9: First-tier reproductive risk assessment for mammals feeding on seeds (fluopicolide)

Crop	Generic focal species	NAR [mg a.s./ kg seeds]	FIR/bw	f_{twa}	NAR × FIR/bw × f_{twa}	NOEL [mg a.s./ kg bw/day]	TER_{lt}	Trigger
Winter rape	Small omnivorous mammal	2000	0.24	0.79 [a)]	379	20	**0.05**	5

a) Worst case value based on a germination time of 7 days and a default DT_{50} of 10 days

The TER_{lt} values for fluopicolide calculated in the reproductive risk assessment for mammals feeding on seeds are below the acceptability trigger of 5. Therefore, further refinement steps are provided further below.

Mammals feeding on seedlings

Table 10.1.2- 10: First-tier reproductive risk assessment for mammals feeding on seedlings (fluopicolide)

Crop	Generic focal species	NAR [mg a.s./ kg seeds]	FIR/bw	f_{twa}	NAR/5 × FIR/bw × f_{twa}	NOEL [mg a.s./ kg bw/day]	TER_{lt}	Trigger
Winter rape	Small omnivorous mammal	2000	0.24	0.53	51	20	**0.39**	5

The TER_{lt} value for fluopicolide calculated in the reproductive risk assessment for mammals feeding on seedlings is below the acceptability trigger of 5. Therefore, further refinement steps are provided further below.

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

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/day (EFSA GD 2009, Appendix K).

An assessment of the risk potentially posed by consumption of contaminated drinking water after the use of a pesticide as seed treatment is not required since this route seems unlikely to be a critical one or to lead to TER less than direct dietary consumption.

RISK ASSESSMENT OF SECONDARY POISONING

According to the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009), substances with a log $P_{OW} \geq 3$ have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains.

The log Pow value of fluopicolide is 2.9 and thus below the trigger value of 3. The active substance has a negligible potential to bioaccumulate in animal tissues. No formal risk assessment from secondary poisoning is therefore required.

REFINED RISK ASSESSMENT

Use of freshly drilled OSR fields

A study was conducted by [redacted] (2020; M-680740-01-1) to determine Portion of Time (PT) values of wood mice (*Apodemus sylvaticus*) in freshly drilled oilseed rape fields.

For PT analysis, 23 radio tracking sessions of 14 individuals were used. The tracking sessions were conducted from 15 August 2019 until 02 September 2019 and covered the time span between several days pre-drilling and emergence of the oilseed rape seeds.
PT estimates were calculated for "potential consumers" and for "confirmed consumers". Since all radio tracked wood mice were captured either in the oilseed rape field or in the directly adjacent off-crop habitat, they all had access to the study fields and could therefore be determined as "potential consumers" for the PT calculations. This corresponds to the term "consumers" according to EFSA (2009). Therefore, all successfully radio tracked individuals with all sessions were considered to calculate a PT estimate regardless of the use of the oilseed rape fields during radio tracking. Additionally, PT was estimated only from radio tracking sessions of wood mice recorded (by trapping, by single telemetry fixes, or during radio tracking) at least once within in an oilseed rape field (i.e. "confirmed consumers").
During pre-drilling radio tracking, four valid radio tracking sessions of four individuals were conducted, resulting in a mean PT value of 0.05 (90th percentile 0.11). PT values ranged from 0.00 to 0.13. Of these, two sessions were "confirmed consumer" sessions, resulting in a mean PT for the "confirmed consumers" of 0.10 (90th percentile 0.12).
During post-drilling radio tracking, 19 valid radio tracking sessions of 14 individuals were conducted, resulting in a mean PT for all sessions (i.e. "potential consumers") of 0.04 (90th percentile 0.12). Post-drilling PT values ranged from 0.00 to 0.33. In total, 10 individuals in 11 radio tracking sessions were confirmed consumers, resulting in a mean PT for the "confirmed consumers" of 0.08 (90th percentile 0.3). Comparing pre- and post-drilling, no correlation between potential foraging time in the oilseed rape field and exposed seeds on the soil surface, which might have triggered the attractiveness, was apparent.

Table 10.1.2- 11: PT values for radio tracking sessions conducted post-drilling (between drilling and emergence of oilseed rape plants)

	PT target crop potential consumers (N=19)	PT target crop confirmed consumers (N=11)
Mean	0.04	0.08
90th percentile	0.12	0.30

Table 10.1.2- 12: Refined risk assessment for fluopicolide for the seed eating mammal scenario based on the revised endpoint, a more realistic diet composition and a conservative assumption on field use (PT) by wood mice

Generic focal species	Food item	NAR [mg a.s./ kg seeds]	FIR/bw	f_{twa}	PT [%]	NAR × FIR/bw × f_{twa} × PT	$BMDL_{10}$ [mg a.s./ kg bw/day]	TER_{lt}	Trigger
Small omnivorous mammal wood mouse	Seeds	2000	0.12 a)	0.79 b)	0.30 c)	56.9	119	**2.09**	5
					0.12 d)	22.8		5.23	

a) Calculated with diet 50 % ground-dwelling invertebrates + 50 % weed seeds
b) calculated with DT_{50} = 10 d, averaging interval = 7 d
c) 90th percentile confirmed consumer PT
d) 90th percentile consumer PT *sensu* EFSA GD (2009): trapped on or immediately adjacent to the field

The TER_{lt} value for fluopicolide is below the trigger of 5 with the worst-case PT for confirmed consumers but exceeds the trigger of 5 with the 90th percentile PT for potential consumers (which is the appropriate category according to the EFSA GD 2009 since all animal were trapped on or in the immediate vicinity of the fields).

Further refinement steps are provided below in form of a weight of evidence approach.

Weight of evidence approach

EFSA GD 2009 (5.2, Risk assessment for treated seeds): "*Tier 1 assumes that granivorous birds and mammals feed entirely on readily available, freshly treated seeds. The failure rate of pesticides used as seed treatments to meet the EU triggers for acute and reproductive risks under such a scenario is likely to be high.* […] *The outcome of refined assessment would, in most cases take the form of a weight-of-evidence approach, rather than a quantitative assessment (e.g. TER)*". This judgment is clearly supported by the tier 1 risk assessment presented above and the TER values for granivorous mammals. From this starting point it seems unlikely that by refining exposure estimates a TER above the respective trigger value of 10 or 5 can be achieved for the use presented. For this reason, the following further TER calculations are not carried out anymore for treated seeds. As proposed by the EFSA GD, a weight-of-evidence approach is presented to demonstrate the safety of this product for wild mammals.

The argumentation is based on five main findings:

1. low exposure of treated seeds on the surface of the field
2. low abundance of small granivorous mammals after drilling
3. low preference for rape seeds as food source for small granivorous mammals
4. de-husking of rape seeds by mice
5. high reproductive rate in focal species (high "plasticity" of population)

Finding 1: As discussed in chapter 4 (avian risk assessment), the exposure of treated seeds on the surface of the field is very low after sowing: Modern drilling techniques minimize the number of seeds remaining on the surface. In this way the food availability is generally low for mammals on freshly drilled fields, making them an unattractive habitat. Low seed density on the soil surface after drilling was demonstrated in a field study in France ([redacted] 2010, M-362200-01-1) in 10 fields.

Although the differences in the use of equipment and seed types were high, the exposure of seeds on the surface of the fields was always similar and in general very low: In midfield areas the mean number of rape seeds per m² amounted to 0.7 (SD 1.1); at the end row areas it amounted to 1.6 seeds per m² (SD 1.5).

The same results were shown in a field study in Great Britain: ([redacted] 2001, M-031392-01-1, CP 10.1.1.2/01) on 4 fields. The number of exposed rape seeds amounted to 1.09 (midfield) and 2.69 (end row) seeds/ m².

In order to put the exposure into perspective with the risk to small granivorous mammals, the area is calculated on which the number of seeds are dispersed and a small granivorous mammal has to collect to reach the regulatory acceptable dose ($RAD_A = LD_{50}/TER_A$; $RAD_{LT} = NOEL/TER_{LT}$).

The total a.s. on rape seeds is 0.0245 mg, based on a TGW of 7 g. Expressed in terms of total a.s. the LD_{50} for the formulation was determined as 3043 mg total a.s./kg bw (Table 10.1.2- 7). Applying a TER of 10 reveals a regulatory acceptable dose for acute risk assessment of 304.3 mg total a.s./kg bw. For a wood mouse weighing 21.7 g, this dose is 66.03 mg total a.s./animal.

Accordingly, a wood mouse could ingest 2,695 seeds without exceeding the regulatory acceptable dose of 66.03 mg total a.s./animal. These 2,695 seeds would be dispersed over an area of 1,002 to 1,685 m² in the end row area or 2,473 to 3,850 m² in midfield.

The fluopicolide loading on one oilseed rape is 0.0140 mg, based on a TGW of 7 g. Applying a TER_{LT} of 5 to the NOEL of 119 mg/kg bw/d reveals a RAD_{LT} of 23.8 mg fluopicolide/kg bw. For a wood mouse weighing 21.7 g, this dose is 0.52 mg fluopicolide/animal. Applying a dehusking factor of 0.6 (see finding 4 below), a wood mouse could ingest 61 seeds without exceeding the RAD_{LT} of 0.52 mg fluopicolide/animal. These 61 seeds would be dispersed over an area of 23 to 38 m² in the end row area or 56 to 88 m² in midfield.

Given the absence of cover on a freshly drilled rape field and the few numbers of seeds per unit area, it can be considered unlikely that a mouse would regularly graze on such a relatively large area. In addition, since the mouse would have to quantitatively eat all the exposed seeds, it would have to search a new plot of that size each day, which would make it a total area of ca. 1,185 m² in midfield or ca. 480 m² in the end row area over a period of 21 days. Accordingly, it would be reasonable to assume that the risk of falling to a predator would very likely be much higher than exceeding the regulatory dose from ingesting treated rape seeds.

Finding 2: Seedbed preparation with ploughing leaves a bare field with little food for granivores and herbivores, largely destroyed burrows and devoid of vegetative cover. The local (in-field) population is greatly diminished or almost extinct. Over the growing season a new population eventually develops in the field started from individuals immigrating from the habitats surrounding the field. This immigration will take some time and the establishment of an in-field population is slowed down by the lack of vegetative cover and the absence of intact burrows. Therefore, there will not be a relevant number of individuals in the field after sowing and at emergence of the rape seeds. Accordingly, exposure on a population level is very low to negligible.
When the seeds emerge and grow to a height that provides appropriate cover for the mice, exposure concentrations will be very low in the seedlings because of growth dilution and degradation of the active substances.

Low abundance of wood mice was demonstrated in a field study in Germany on freshly drilled oil-seed rape fields ([redacted] 2006, M-281405-01-1). Although the agricultural practice on these fields followed a minimum tillage philosophy and consequently had a lot of remaining weed and cereals seeds on the surface, the abundance of wood mice was very low on the fields compared to the surroundings (in the field: 1.89 catches/100 trap nights, in the surrounding: 21.68 catches/100 trap nights).

Finding 3: Feeding studies were performed with house mice and wood mice, in which treated and untreated rape seeds were offered:

Mice (*Mus domesticus*) received untreated rape seeds and rape seeds treated with clothianidin, fluopicolide and fluoxastrobin ([redacted] 2009, M-357355-01-1). When exclusively fed with untreated rape seeds (day -3 and -2), the mice consumed significantly lower amounts (mean of 0.3 to 0.7 g) which did not cover the daily energy demand. In order to avoid effects of emaciation mingled with potential toxic effects, the mice were granted a recovery day, when they received standard food consisting of oat flakes and rape seeds. On that day (-1) the average food consumption was 3 to 4 times higher than on the previous days. The oat flakes were completely consumed, only rape seeds were left over.

On the exposure day the consumption of rape seeds in the control and the treatment group was similar (control: 0.5 g /mouse; treatment group: 0.4 g/mouse), and in the range of the amount consumed during the acclimation period on the rape (only days -3 and -2).

Remarkably similar findings were observed with wood mice (*Apodemus sylvaticus*), to which untreated rape and rape treated with methiocarb were offered ([redacted] 2007, M-295311-01-1). Again, the reduced consumption of untreated rape seeds leads to a body weight loss. On the recovery day the mice ate 10 times more food than on the previous days, where only rape seeds were offered.

The results proved that rape seeds are not sufficient and not appropriate as exclusive food for mice. Rape seed husks observed after exposure confirmed qualitatively that the mice were de-husking the rape seeds before consumption.

A clear avoidance regarding drilled oilseed rape seeds was also shown in a feeding trial during a study conducted by [redacted] (1994; M-682041-01-1), in which it was revealed that wood mice indeed fed on young oilseed rape seeds, but avoided old seeds (which are used for drilling). The wood mice very much preferred eating mature cereal seeds and invertebrates than mature oilseed rape seeds. These findings are highly relevant for the risk assessment scenario for treated winter oilseed rape seeds. Winter oilseed rape fields are typically established on previous cereal fields harvested a few weeks before oilseed drilling. On these fields, wood mice would encounter both mature oilseed rape seeds (treated) and mature cereal seed (untreated harvest leftover), in modest quantities in case of proper seed bed preparation and even more on low-tillage fields. The results of the cafeteria experiments suggest that wood mice are much more likely to forage on the mature cereal seeds remaining from the previous crop than on the mature oilseed rape seeds from the new drilling. Thus, the estimation of the portion of diet with radiotracking data for foraging time on freshly drilled winter oilseed rape fields likely overestimates the portion of oilseed rape seeds, because cereal seeds are likely to be taken up in similar if not higher quantities, as suggested by the much higher preference index. Invertebrates (mealworms) were also much preferred over oilseed rape seeds in the cafeteria experiments, again suggesting that the small portion of time wood mice were found spending on freshly drilled oilseed rape fields is not much releated to foraging on treated seeds.

Finding 4: Wood mice are able to dehusk seeds (see also finding 3). DEFRA, UK (2010; M-406213-01-1) presented data on de-husking of different seeds by wood-mice and proposed a default value of 0.6 for rape seeds. Thus, the area a mouse would have to forage, as demonstrated under finding 1, would increase accordingly, increasing further the risk of predation and reducing the risk from intoxication.

Finding 5: Arable fields are a very unstable environment from the perspective of animals living in these fields. Several times per year the fields undergo dramatic changes. After seedbed preparation, drilling adds another disturbance, yet not so dramatic. Emergence of the crop changes the environment again providing increasingly more shelter for small animals living on the ground or in the vegetation. Harvest again changes the environment, leaving plenty of food for granivores but reducing significantly the shelter.

Animals inhabiting such changing (unstable) environments, if they are unable to leave the area, suffer a high mortality rate due to these drastic changes. To be successful as a species (or population), this high mortality rate has to be countered by a high reproductive rate that allows a quick growth of the population when conditions are favourable. This high reproductive rate usually is achieved by rapid maturation, an early age at first reproduction, and a relatively large number of off-spring at a time. Wood mice (as the real focal species) comprise all of these traits. Therefore, changes in population density, irrespective of their origin, are unlikely to endanger a Wood mouse population at a regional or even local level.

These five factors, low exposure of treated seeds on the field, low abundance of real focal species at the time of drilling, low preference for rape seeds as a food item, dehusking of the seeds, and high potential to increase population density quickly and immensely when circumstances become favourable, all indicate that a risk to wood mouse populations living in areas of oil-seed rape cultivation, from the use of FLC + FXA FS 350 as a seed treatment in this crop can be excluded.

Refined risk assessment for mammals feeding on rape shoots

The refinement for mammals feeding on seedlings is based on measured residues of fluopicolide in oilseed rape seedlings. Please refer to CP 10.1.1 of this document for detailed explanations.

Table 10.1.2- 13: First-tier reproductive risk assessment for mammals feeding on seedlings (fluopicolide and metabolites)

Compound	Generic focal species	Residues on seedling [mg a.s./kg]	FIR/bw	f_{twa}	Residues × FIR/bw × f_{twa}	NOEL [mg a.s./kg bw/day]	TER_{lt}	Trigger
Fluopicolide	Small omnivorous mammal	1.30	0.24	0.53	0.1654	20	121	5
M-01 (metabolite)		0.021 a)	0.24	0.53	0.003	7.5	2500	5
M-02 (metabolite)		0.006 b)	0.24	0.53	0.0008	2.0 c)	2500	5

a) RUD_{max} of 1.714 mg/kg as noted in M-686445-01-1, MCP Infinito 10.1.1.2/01 and re-calculate to the current application rate of 12 g a.s./ha
b) RUD_{max} of 0.498 mg/kg as noted in M-686445-01-1, MCP Infinito 10.1.1.2/01 and re-calculate to the current application rate of 12 g a.s./ha
c) The toxicity endpoint is set at one tenth of the reproductive risk assessment endpoint for the parent.

This refinement step demonstrates that the risk for mammals feeding on emerged rape seedlings is acceptable.

CP 10.1.2.1 Acute oral toxicity to mammals

The result from the acute study with the formulated product FLC + FXA FS 350 confirms the predicted toxicity of > 3043 mg total a.s./kg bw, calculated in Table 10.1.2- 7 above.

Table 10.1.2.1- 1: Mammalian toxicity data of the formulated product FLC + FXA FS 350

Test substance	Risk assessment	Species	Endpoint		Reference
FLC + FXA FS 350	Acute	Rat	LD_{50}	> 2000 mg prod./kg bw (≙ 602 mg total a.s./kg bw) a)	[redacted] 2015; M-531437-01-1 KCP 7.1.1/01

a) Based on a total a.s. content of 30.1% w/w (FLC 17.0% w/w and FXA 13.1% w/w according to certificate of analysis)

CP 10.1.2.2 Higher tier data on mammals

Data Point:	KCP 10.1.2.2/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Scenic Gold - Fluopicolide, 200 g/L - Fluoxastrobin, 150 g/L - Refined seed-eater mammal endpoint for fluopicolide
Report No:	M-683114-01-1
Document No:	M-683114-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

Scenic Gold® is proposed by Bayer AG as a fungicidal seed treatment for winter oil seed rape (OSR) in the EU.

The reproductive risk to mammals is driven by the chronic toxicity of the active substance fluopicolide, resulting in a Tier 1 TER_{LT} below the required trigger of 5. This TER is calculated with the lowest overall endpoint of potential relevance for wild mammals, i.e. the NOAEL of 20 mg/kg bw/d from the rabbit developmental toxicity study.

This paper presents a re-consideration of the higher tier risk assessment refinement, primarily the proposal to use a rodent endpoint for the scenario of seed eating rodents (mice) in fields drilled with treated oilseed rape.

This proposal is justified by the fact that rabbits do not eat seeds, but rodents do. A comparison of the toxicity profile of fluopicolide in the developmental toxicity studies in rats and rabbits is provided in detail, since these studies are conducted with a similar design and thus allow a comparison of like with like.

In the developmental toxicity study with fluopicolide in rabbits, severe and overt toxicity including mortality and abortion were observed at dose levels of 60 mg/kg bw/d and above. In the developmental studies with fluopicolide in the rat, no mortalities, abortions or other clinical signs of severe toxicity were observed up to the top test level of 700 mg/kg bw/d (i.e., a factor of 10 less sensitive).

Based on the different feeding behaviour of rabbits and rodents (mice) and the different observed effects in the toxicity studies it is considered justified to employ distinct risk assessments for rabbit scenarios (herbivores) with the rabbit endpoint, and for granivore scenarios of seed eating mice with the rodent endpoint.

The treatment of rodents (rats, mice) with fluopicolide mainly results in moderate effects on body weight changes, in the rat typically associated with initially reduced food consumption which is overcome by week 3. The duration of the environmental exposure scenario of mice to treated seeds in a landscape with freshly drilled oil seed rape fields can also be conservatively estimated not to exceed 3 weeks.

Therefore, 3-week body weight effects in rodents were considered as appropriate point of departure for the risk assessment on seed eating mice in freshly drilled oil seed rape fields.

For the use in the Toxicity Exposure Ratio (TER) calculation, 3-week body weight effects were derived with a benchmark dose (BMD) calculation. For this purpose, body weight data for the first 3 weeks were excerpted from all dietary studies with fluopicolide in rodents (28-d, 90-d, chronic, reproduction) which include a comparable exposure setting in the initial 21 days.

BMDs were calculated with the tools recommended by EFSA (2017), and the reliability of the fit was assessed based on the criterion of normalized width (EFSA 2015). As a point of departure for the refined risk assessment, the $BMDL_{10}$ is proposed, i.e. the left confidence limit of the BMD for 10% effect. BMD values were calculated both for body weight differences and for body weight gain differences. Because 10 % effect on body weight over a few weeks is considered a more severe finding than 10 % percent effect on body weight gain, and the lowest reliable $BMDL_{10}$ values were very similar, preference in a refined risk assessment should be given to the BMD for body weight effects. **The lowest reliable $BMDL_{10}$ for body weight was 119 mg/kg bw/d**.

I. Material and Methods:

A review of the regulatory guidance (EFSA 2009) was conducted, supported by public literature, to confirm that the relevant focal species for scenarios on consumption of treated seeds are granivorous mice (represented by the Wood mouse, Apodemus sylvaticus), rather than rabbits which are suitable focal species for scenarios on consumption of foliage germinated from treated seeds, but not for the treated seeds themselves.

Since the current wild mammal reproductive risk risk assessment endpoint for fluopicolide was taken from a rabbit developmental toxicity study, the toxicological profile of fluopicolide in rabbits was compared with its profile in rodents, in order to check which rodent endpoint would be more appropriate for the seed eater risk assessment.

II. Results and Discussion:

Rabbits do not eat OSR seeds, rodents do.

Rabbits (and hares) are strictly herbivorous ([redacted] 1990; M-074076-01-1; KCP 10.1.2.2/10) and usually feed on a variety of green plants not too far from their burrows. They are known to forage in fields with young cereals ([redacted] 1989; M-064651-01-1; KCP 10.1.2.2/11) and OSR ([redacted]., 1990; M-619312-01-1; KCP 10.1.2.2/09). Rabbits are not considered to eat OSR seeds after drilling, but to eat seedlings grown from treated seeds (EFSA; 2009). Available rabbit toxicity studies can be used to determine the relevant endpoint for the TER assessment for seedling eaters but available rodent (rat, mice) toxicity studies should be used to determine the relevant endpoint for the TER assessment for seed-eaters such as the woodmouse.

Rabbits are more sensitive to fluopicolde than rodents

Several mammalian toxicity data are available, in rats and rabbits as well as mice. Developmental toxicity data are available for rats and rabbits; reproductive toxicity data are only available for rats (Table 10.1.2.2-1). The susceptibility of rabbits compared to rats for toxicity due to fluopicolide exposure can be best compared based on the developmental toxicity studies in each species, which have been conducted with a largely comparable design.

It is evident that rabbits are substantially more susceptible to fluopicolide than rodents (rats) when comparing the oral (gavage) developmental toxicity studies in these two species. The severe toxicity/mortality observed in the rabbit study (NOAEL 20 mg/kg bw/d) was not reflected in rats or mice. Fluopicolide administration did not affect mortality in rodents at any dose and the clinical signs and gross necropsy finding were also not apparent in rodents.

Marked reductions in food consumption and bodyweight gain that preceded the deaths in the rabbit study (and also affected surviving animals), were less marked in the rat and occurred at much higher doses than in the rabbit.

Table 10.1.2.2- 1: Comparison of fluopicolide in development toxicity studies with rabbit and rodent (rat)

Study	Doses	Main effects
Rabbit developmental toxicity Dose range finding study Oral, gavage 4/dose [redacted] 2000a M-211192-01-1	25, 50, 100, 250, 500 & 1000 mg/kg bw/d	1000, 500, 250 and 100 mg/kg bw/d All animals died (or were sacrificed) 50 mg/kg bw/d ↓ Bodyweight gain (BWG) and food consumption (FC) 25 mg/kg bw/d ↓ FC
Rabbit developmental toxicity Definitive study Oral, gavage 23/group [redacted] 2001a M-202513-02-1	0, 5, 20 & 60 mg/kg bw/d	60 mg/kg bw/d 18/23 deaths ↓ BWG (-86%) ↓ FC 20 mg/kg bw/d No adverse effects
Rat developmental toxicity Dose range-finding study Oral, gavage 4/dose [redacted] 2000b M-198488-01-1	500 & 1000 mg/kg bw/d	There were no treatment-related deaths at either dose 1000 mg/kg bw/d Clinical signs: pultaceous/loose faeces ↓ BWG ↑ FC 500 mg/kg bw/d ↓ FC
Rat developmental toxicity Definitive study Oral, gavage 23/dose [redacted] 2001b M-202155-02-1	0, 5, 60 and 700 mg/kg bw/d	No deaths or clinical signs of toxicity at any dose 700 mg/kg bw/d ↓ BWG (8%) 60 & 5 mg/kg bw/d No effects

The dietary toxicity studies in rats and mice (28 days, 90 days or chronic) and the reproductive study in rats did not indicate any overt systemic toxicity as no effects on reproduction or survival were linked to fluopicolide exposure (Table 10.1.2.2-2, Table 10.1.2.2-3). These data strongly support the conclusion that the rabbit is more sensitive than the rat.

Table 10.1.2.2- 2: Toxicity of fluopicolide in dietary studies with rodents

Study	**Doses**	**Relevant adverse effects (relative to controls unless otherwise stated)**
Rats		
28-day rat [redacted] 2000 M-199377-01-1	0, 20, 200, 2,000 & 20,000 ppm Equivalent to: 0, 1.78, 17.8, 179 & 1770 mg/kg bw/d (combined sexes)	No treatment-related deaths or clinical signs of toxicity at any dose <u>20,000 ppm</u> ↓BWG (days 1-29) in M (32%) & F (37%) ↓ BW (day 29) in M (14%) & F (11%) ↓ FC (week 1) in M (41%) & F (28%) <u>2,000, 200 & 20 ppm</u> No effects on body weight or food consumption
90-day rat [redacted] 2000a M-197622-01-1	0, 100, 1400 & 20000 ppm Equivalent to: 0, 7.9, 114, 1671 mg/kg bw/d (combined sexes)	No treatment-related deaths at any dose <u>20000 ppm</u> Clinical signs: hair loss in M & F, body-soiling & loss of coat condition in M and urogenital staining in F ↓ Final BW in M (30%) & F (18%) ↓ BWG (days 1-92) in M (41%) & F (29%) ↓ Food consumption in M (17%) & F (15%) <u>1400 & 100 ppm</u> No clinical signs of toxicity or effects on body weight or food consumption (NOAEL based on organ weight increases and haematology/clinical chemistry findings)
2-year rat (104-week carcinogenicity phase) [redacted] 2003 M-225616-01-1	0, 50, 200, 750 & 2500 ppm Equivalent to: Males: 0, 2.1, 8.4, 31.5 & 109.4 mg/kg bw/d Females: 0, 2.8, 10.8, 41 & 142.2 mg/kg bw/d	No effect on mortality <u>2500 ppm</u> ↓ BW in M (max 8.7% in week 104) & F (max 12.3% in week 104) ↓ BWG in M (max 32.7% during week 0-1) & F (max 42.3% during week 1-2) ↓ FC during week 1 in M (19.5%) and F (7%) <u>750 ppm</u> ↓ BWG during weeks 0-1 in F (39%) <u>200 & 50 ppm</u> No effects on body weight or food consumption
2-year rat (52-week chronic phase) [redacted] 2003 M-225616-01-1	0, 50, 200, 750 & 2300 ppm Equivalent to: Males: 0, 2.5, 9.8, 37, 125.5 mg/kg bw/d Females: 0, 3.3, 12.9, 48.7, 163.6 mg/kg bw/d	No effect on mortality ↓ BW in F (max 16.8% in week 52) ↓ BWG in M (max 38.6% during week 0-1) & F (max 53.6% during week 0-1) ↓ FC during week 1 in M (13%) & F (7%) ↓ FC (weeks 0-52) in F (8%) <u>750 ppm</u> ↓ BW in F (max 9.5% in week 52) ↓ BWG during weeks 0-1 in M (12.3%) & F (39.3%) <u>200 & 50 ppm</u> No effects on body weight and food consumption
Mice		
28-day mouse [redacted] 2000b M-197342-01-1	0, 6, 64, 640 & 6400 ppm Equivalent to: 0, 1.07, 11.6, 115 & 1111 mg/kg bw/w (combined sexes)	No treatment-related deaths or clinical signs of toxicity & no effects on bodyweight or food consumption at any dose (NOAEL based on ↑ liver weights and hypertrophy at high dose)

Study	Doses	Relevant adverse effects (relative to controls unless otherwise stated)
90-day mouse [redacted] 2006 M-205579-02-1	0, 50, 200, 800 & 3200 ppm Equivalent to: Males: 0, 10.4, 37.8, 161 & 770 mg/kg bw/d Females: 0, 12.6, 52.8, 207 & 965 mg/kg bw/d	No treatment-related deaths or clinical signs of toxicity at any dose 3200 ppm ↓ BWG days 1-90 at in M (7%) & F (14%) ↓ BW (day 8) in M (10%) & F (7%) 800, 200 & 50 ppm No body weight effects (NOAEL based on liver effects)
90-day mouse [redacted] 2000c M-197623-01-1	0, 32, 320, 3200 & 6400 ppm Equivalent to: Males: 0, 4.7, 46, 461 & 944 mg/kg bw/d Females: 0, 6.2, 60, 629 & 1239 mg/kg bw/d	No treatment-related deaths or clinical signs of toxicity and food consumption was not affected at any dose. 6400 ppm ↓ BWG (days 1-92) in M (20%) & F (32%) 3200 ppm ↓ BWG (days 1-92) in F (22%) 320 & 32 ppm No treatment-related effects on body weight
18-month mouse [redacted] 2003 M-225595-01-1	0, 50, 400 & 3200 ppm Equivalent to: Males: 0, 7.9, 64.5 & 551 mg/kg bw/w Females: 0, 11.5, 91.9 & 772.3 mg/kg bw/d	No effect on mortality 3200 ppm ↓ BW in M (max 22% in week 52) & F (max 20% in week 52) Body weight losses during weeks 1-2 ↓ FC in M (max 11% during weeks 29-51) & F (max 14% during weeks 1-12)

Table 10.1.2.2- 3: Toxicity of fluopicolide in the rat reproduction study ([redacted] 2003, M-215068-01-1)

Phase of study	Test substance, purity, doses	Relevant adverse effects (relative to controls unless otherwise stated)
Pre-mating & mating	0, 50 ,200, 750 & 2500 ppm Equivalent to: Males: 0, 4, 17, 65 & 197 mg/kg bw/w Females: 0, 4, 18, 67 & 204 mg/kg bw/w	2500 ppm ↓BWG in M (-25% on days 0-7, -27% on days 0-14 & -22% on days 0-49) & F (-40% on days 0-7 & -20% on days 0-14) ↓ FC on days 0-14 in M (-10%) & F (-9%) 750 ppm ↓BWG in F (-28% on days 0-7) 200 & 50 ppm No relevant effects
Gestation	0, 50, 200, 750 & 2500 ppm Equivalent to: 0, 4.7, 18.7, 65.7 & 216	2500 ppm ↓BWG on GD 0-6 (-18%) & GD 0-13 (-19%*) ↓FC on GD 3-5 (-7%**), days 6-9 (-13%**), GD 10-12 (-7%**) & GD 17-19 (-18%*) 750 ppm ↓BWG on GD 0-6 (-18%) ↓FC on GD 3-5 (-7%*), GD (6-9 (-10%**) & GD 10-12 (-7%**) 200 & 50 ppm No relevant effects
Lactation	0, 50 ,200, 750 & 2500 ppm Equivalent to: 0, 8, 32.3, 119.7 & 408.3 mg/kg bw/d	2500 ppm ↓FC on LD 7-13 (-38%) 750, 200 & 50 ppm No relevant effects

Phase of study	Test substance, purity, doses	Relevant adverse effects (relative to controls unless otherwise stated)
F1 generation	Males: 0, 6, 24, 93 & 316 mg/kg bw/d Females: 0, 6, 25, 95 & 313 mg/kg bw/w	F1 Pups 2500 ppm ↓ BW on day 14 (-20.1%*) & day 21 (-12.1%*) ↓BWG on days 1-21 (-14.8%*) 750, 200 & 50 ppm No relevant effects *F1 adults* 2500 ppm ↓BWG in M on week 1-3 (-11.3%*) ↓ FC in M (-10-13%) & F (-7-9%) 750, 200 & 50 ppm No relevant effects
Pre-mating & mating	0, 100, 500 & 2000 ppm Equivalent to: Males: 0, 5.2, 25.5, & 103.4 mg/kg bw/d Females: 0, 6.4, 32.9 & 127.3 mg/kg bw/d	2000 ppm ↓ BW in M (-6.4%* on week 8) ↓BWG in M (-9.7%* on week 0-8 & -8.9%* on week 0-12) & F (-11.1%* on week 0 - 4, -13.4%** on week 0 - 8 & -13.7%** on week 0 - 10) 500 & 100 ppm No relevant effects
Gestation	0, 100, 500 & 2000 ppm Equivalent to: 0, 7.4, 38, & 150.8 mg/kg bw/d	2000 ppm ↓ BW on GD 13 (-7.5%**) ↓ BWG on GD 0-6 (-16.7%**) & GD 0-13 (-16.1%**) ↓ FC on GD 0-5 (-8.3%**) & GD 6-12 (-7.4%**) 500 & 100 ppm No relevant effects
Lactation	0, 100, 500 & 2000 ppm Equivalent to: 0, 13.5, 134 & 281.4 mg/kg bw/d	2500 ppm ↓ BW on LD 1 (-8.1%**), LD 4 (-5.9%*), LD 7 (-6%*) & LD 14 (-8.2%**) ↓ FC on LD 7-13 (-13.3%**) 500 & 100 ppm No relevant effects
F1 generation	Fluopicolide Purity: 95.9% 0, 100, 500 & 2000 ppm Equivalent to: Males: 0, 10.7, 53 & 220 mg/kg bw/d Females: 0, 11, 55 & 230 mg/kg bw/d (pre-mating), 0, 8, 39 & 156 mg/kg bw/d (gestation), 0, 16, 75 & 320 mg/kg bw/w (lactation)	*F1 pups* 2000 ppm ↓ BW in M on days 14, 21, 25 & 28 (-7.9%**, -8.2%**, -8.7%** & -8.5%**) & F on days 14, 21, 25 & 28 (-7.8%** -7.6%**, -7.7%** & -7.1% ↓ BWG on days 1-28 in M (-9.6%**) & F (-7.9%**) 500 & 100 ppm No adverse effects *F1 parents* 2000 ppm Pre-mating: ↓ BW in M on days 0, 7 & 21 (-11%**, -10%* & -6%*) & F at all measurements (max -9%** on day 70) ↓BWG in F (-8%* on days 0-70) ↓FC in M (-7%) & F (-6%) Gestation: ↓ BW at all measurements (max -11%** GD 6 and 13) ↓ BWG on GD 0-6 (-15%*), GD 0-13 (-14%**) & GD 0-20 (-7%**) ↓ FC (up to -16% on GD 13-19) Lactation: ↓ BW at all measurements (max -12%** on LD 4, 7 & 14) ↓ FC (-10% LD 4-6) 500 & 100 ppm No relevant effects

Phase of study	Test substance, purity, doses	Relevant adverse effects (relative to controls unless otherwise stated)
F2 pups	Fluopicolide Purity: 95.9% 0, 100, 500 & 2000 ppm	2500 ppm ↓ BW on days 14, 21, 25 & 28 in M (-9%**, -13%**, -13%** & -12%**) & F (-9%**, -12%**, -11%** & -10%**) ↓ BWG on days 1-28 in M (-14%) & F (-11%**) 500 & 100 ppm No relevant effects

In the rat dietary studies, reductions in bodyweight gain were often most marked at the start of the study and were secondary to initial reductions in food consumption. This was not the case following gavage administration in rats in the developmental study, suggesting that the initial reductions in bodyweight gain and food consumption in the dietary studies with rodents were related to the palatability of the test substance in the diet and not a specific toxic effect of fluopicolide.

It is proposed that the length of the exposure period in the toxicity assessment should match the exposure period in the field, in order to obtain realistic risk estimates ([redacted]. 2019; M-669217-01-1, KCP 10.1.2.2/12). Thus, in risk assessments on uses for short environmental exposure like treated OSR seeds, a 21-day toxicity endpoint can be considered as realistic worst case for exposure of granivorous rodents.

The calculated BMDs for bodyweight effects over a period of 3 weeks (21 days) are considered to appropriately encompass both the initial avoidance phase and the subsequent recovery of the food consumption. The alternative approach (including an explicit avoidance factor in the risk assessment to account for the initial food avoidance) is nowadays rarely accepted in regulatory risk assessments.

Therefore, it is considered appropriate to apply the most relevant toxicity data per exposure scenario:

Table 10.1.2.2- 4: Focal species and toxicity species for exposure assessment of fluopicolide OSR seed treatment

Scenario	Focal species	Toxicity species
Treated seeds	Woodmouse	Rodent (rat)
Seedlings grown from treated seeds	Rabbit	Rabbit

III. CONCLUSIONS:

The results of the present study demonstrate that the toxicity of fluopicolide in rodents is much lower than in rabbits. Rabbits are herbivores and relevant for oilseed rape leaf consumption scenarios, and rodents (wood mice) are relevant for assessing consumption of treated oilseed rape seeds.

Therefore, it is suggested to use the NOAEL from the rabbit developmental toxicity study only for the seedling eater scenario, and a rodent endpoint for the seed eater scenario.

Given the rather mild toxicity of flupicolide seen in rodents, effects of clear relevance are seen not even at comparatively high dosages. Typically, fluopicolide treatment induces initially reduced food consumption and correspondingly body weight effects in rodents.

Since the exposure window for treated oilseed rape seed availability in the field is short (TWA concentrations are calculated for a 7-day period), an exposure window of 3 weeks in the toxicological studies with rodents is considered appropriately conservative for deriving the endpoint for bodyweight effects.

Evaluation of all dietary studies with fluopicolide in rodents demonstrated that benchmark dose calculation is possible in several cases, in oder to identify the lowest reliable endpoint for body weight effects over 3 weeks of treatment. These calculations are reported by [redacted] for **body weight**

(2019 a; M-667414-01-1; KCP 10.1.2.2/07) and by for **body weight gain** ([redacted] 2019b, M-667312-01-1; KCP 10.1.2.2/08).

Because 10 % effect on body weight over a few weeks is considered a more severe finding than 10 % percent effect on body weight gain, and the lowest reliable $BMDL_{10}$ values were very similar, preference in a refined risk assessment should be given to the BMD for body weight effects. **The lowest reliable $BMDL_{10}$ for body weight was 119 mg/kg bw/d**.

Assessment and conclusion by applicant:
This statement is considered reliable and can be used for risk assessment. The lowest reliable $BMDL_{10}$ for body weight of 119 mg/kg bw/d for fluopicolide can be used in refined risk assessment for granivorous mammals risk assessment in winter oilseed rape risk assessments.

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Data Point:	KCP 10.1.2.2/02
Report Author:	[redacted]
Report Year:	1994
Report Title:	The ecology of wood mice(Apodemus sylvaticus) in fields of oilseed rape(Brassica napus oleifera)
Report No:	M-682041-01-1
Document No:	M-682041-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The ecology of wood mice (*Apodemus sylvaticus*) found in fields of oilseed rape (*Brassica napus oleifera*) in the UK was studied by live trapping with mark and recapture, radio-tracking, examination of stomach contents and feeding trials. Population densities of wood mice in oilseed rape fields, were lower than those of woodland mice, but comparable to those of wood mice on arable land found in other studies. Although there were no statistically significant differences between seasons, the mice tended to make less use of oilseed rape fields during the early stage of the crop but increased their use of oilseed rape fields as the oilseed rape plants grew older. The diet of wood mice caught in oilseed rape fields was similar to those of wood mice in agricultural fields in other studies, in that seed formed the major part of their diet while vegetative parts of plants and animal food were taken seasonally as supplementary foods. Oilseed rape was eaten by wood mice in a noticeable amount only in April, when flowering buds and flowers were always eaten, and in June, when young seeds of oilseed rape were frequently eaten. Only young seeds of oilseed rape, not old ones, were found to be as preferred by wood mice. However, wood mice were shown to eat very little mature oil seed rape seeds, based on samples from field catches and based on cafeteria experiments. Wood mice showed a clear preference for feeding on mature cereal

seeds or invertebrates over feeding mature oilseed rape seeds. Thus, wood mice on freshly drilled winter oilseed rape fields are likely to preferentially feed on harvest leftover grain or invertebrates, rather than on treated oilseed rape seeds.

I. Material and Methods:

The ecology of wood mice (*Apodemus sylvaticus*) found in fields of oilseed rape (*Brassica napus oleifera*) around Newburgh, Aberdeenshire was studied from October 1990 to December 1992. The actual drilling phase of oilseed rape is not covered by the field observations, but the general findings on food preferences can be considered as relevant and informative also for the exposure assessment of treated oilseed rape seeds. The animals' population dynamics were studied by live trapping with mark and recapture, their home range sizes and habitat utilisation were determined by radio-tracking, their diets were analysed by microscopic examination of stomach contents. Additionally, cafeteria experiments were conducted to assess the preferences of wood mice for the different food types available in the fields or their surroundings

II. Results and Discussion:

Population densities of wood mice in oilseed rape fields, at 1.6 to 12.5 per ha, were generally lower than those of woodland mice, but were comparable to those of wood mice on arable land found in other studies. The seasonal fluctuations in wood mouse densities in oilseed rape fields differed slightly from those described for woodland mice, with peak densities found in spring or summer. Both agricultural practices and the type of adjacent habitats affected the mean densities and seasonal fluctuations in densities of wood mice in oilseed rape fields.

The mean home range size of radio-tracked wood mice in this study (0.34-0.88 ha) was larger than those reported for woodland mice, but smaller than those reported for wood mice on sand dunes.

Although there were no statistically significant differences between seasons, the mice tended to make less use of oilseed rape fields during the early stage of the crop but increased their use of oilseed rape fields as the oilseed rape plants grew older.

The diet of wood mice caught in oilseed rape fields was similar to those of wood mice in agricultural fields in other studies, in that seed formed the major part of their diet, while vegetative parts of plants and animal food were taken seasonally as supplementary foods.

Based on stomach analysis in field catches, oilseed rape was eaten by wood mice in a noticeable amount only in April, when flowering buds and flowers were always eaten, and in June, when young seeds of oilseed rape were frequently eaten. In June, oilseed rape seeds were found in the diet analysis with the highest frequency of 100%, and the greatest percentage volume of 46%. The seeds were still eaten in later stages of the crop but in very small amounts.

High preference for immature seeds of oilseed rape, but much lower preferences for older oilseed rape seeds was also seen in the cafeteria experiments: immature seeds of oilseed rape were readily taken, even slightly more than immature wheat and barley seeds (not statistically significant), but mature oilseed rape seeds are clearly less preferred than mature cereal seeds (difference highly significant).

Results from cafeteria experiments (combined presentation of Tables 5.3 and 5.5 in the report)

Food item	**Mean standardized preference indices for wood mice**	
Stage	June (immature)	July (mature)
Wheat seed	0.737	0.927
Barley seed	0.766	0.755
Oilseed rape seed	0.892	0.336 **

The scenario with the mature seeds is highly relevant for the risk assessment scenario for treated winter oilseed rape seeds: winter oilseed rape fields are typically established on previous cereal fields harvested a few weeks before oilseed drilling. On these fields, wood mice would encounter both mature oilseed rape seeds (treated) and mature cereal seed (untreated harvest leftover), both in modest quantities in case of proper seed bed preparation or more in low-tillage fields. The results of the cafeteria experiments suggest that wood mice are much more likely to forage on the mature ceral seeds remaining from the previous crop than on the mature oilseed rape from the new drilling. Thus, the estimation of the portion of diet with radiotracking data for foraging time on freshly drilled winter oilseed rape fields likely overestimates the portion of oil seed rape seeds, because cereal seeds are likely to be taken up in similar if not higher quantities, as suggested by the much higher preference index.

Invertebrates (mealworms) were also much preferred over oilseed rape seeds in the cafeteria experiments (Table 5.4 in the report), again suggesting that the small portion of time wood mice were found on freshly drilled oilseed rape fields is not much related to foraging on treated seeds.

Food item	**Mean standardized preference indices for wood mice**
Mealworms	1.000
Oilseed rape seed	0.342

III. CONCLUSIONS:

The results of the present study suggest that oilseed rape crop is not the main food source but is a potential source of supplementary food for wood mice that live or forage in oilseed rape fields. Wood mice eat only small amounts of the vegetative parts of oilseed rape throughout the growing season of the crop, except in April when flowering buds and flowers are eaten in larger amounts, presumably because of the scarcity of other foods and the abundance of flowering buds and flowers of oilseed rape at that time. In addition, wood mice have also been found to eat immature seeds in early summer (June).

However, wood mice were shown to eat very little mature oil seed rape seeds (July), based on samples from field catches and based on cafeteria experiments. Wood mice showed a clear preference for feeding on mature cereal seeds or invertebrates over feeding mature oilseed rape seeds. Thus, wood mice on freshly drilled winter oilseed rape fields are likely to preferentially feed on harvest leftover grain or invertebrates, rather than on treated oilseed rape seeds.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. Wood mice clearly preferred immature oilseed rape seeds, mature cereal seeds and invertebrate prey over mature oilseed rape seeds.

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Data Point:	KCP 10.1.2.2/03
Report Author:	[redacted]
Report Year:	2020
Report Title:	Generic field study on PT of wood mice in freshly drilled oilseed rape fields in Central Europe
Report No:	EnSa-EBAC0091
Document No:	M-680740-01-1
Guideline(s) followed in study:	No official test guideline(s) available at present
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

This study aimed to determine Portion of Time (PT) values of wood mice (*Apodemus sylvaticus*) in freshly drilled oilseed rape fields. In total 19 post-drilling radio tracking sessions of 14 individuals were conducted. This resulted in reliable and appropriate data for wildlife risk assessments and showed that wood mice spend on average about 4% of their potential foraging time in freshly drilled oilseed rape fields (90th percentile 0.12).

I. Material and Methods

Study site:

The study site was located in the administrative districts Birkenfeld and Rhein-Hunsrück-Kreis in Rhineland-Palatinate (Germany). Five oilseed rape fields were selected as study fields based on Non-GLP pre-trapping that was conducted before the drilling of oilseed rape in order to select most suitable study fields regarding the presence of wood mice.

The study area was mapped for crop and habitat types (i.e. target crop: freshly drilled oilseed rape fields; other arable crops, natural off-crop habitats and anthropogenic off-crop habitats). Each change of habitat type within the mapped area during the Field Phase was recorded.

Seed counting:

Seed counting was performed to determine the availability of all seeds (oilseed rape seeds and other seeds) on the study fields as potential diet items. Seeds on the soil surface were counted on each study field twice, once before drilling and once after drilling (with ten counting frames deployed per counting, eight in the mainland and two in the headland, respectively).

Live trapping:

For live trapping of wood mice baited 'Ugglan' multiple capture live traps were used.

Live trapping was conducted on the selected study fields to identify suitable individuals for radio-tagging and subsequent radio tracking and followed a Capture-Mark-Recapture design, which allowed identification of individually marked animals upon recapture (via Passive Integrated Transponders).

Radio tracking:
During live trapping, suitable wood mouse individuals (≥20 g, if possible recaptured individuals on or close to the study fields) were equipped with radio tags that are designed as collars with a radio transmitter attached to a cable tie. Tags were fitted around the animal's neck. After emergence of oilseed rape plants on the study fields, trapping for radio tag removal was conducted.
Animals were not tracked on the day of tagging to exclude any bias during the initial adaptation process. During the tracking sessions, wood mice were tracked continuously, in order to record their location and any behavioural changes. Each location of the radio tracked individual was recorded on a map. The exact coordinates of the location of the radio tracked animals were calculated afterwards, from the information documented on the map.

II. RESULTS AND DISCUSSION

Seed counting
During pre-drilling seed counts, 92 seeds were found on the soil surface. The highest number of seeds was found on study field 3 with 59 seeds in total (mainly resulting from two frames with >10 seeds). The mean number per counting was 17.4 seeds in the mainland and 1.0 seeds in the headland, resulting in 8.7 seeds/m² in the mainland and 2.0 seeds/m² in the headland found during pre-drilling seed counts. From 18 seeds found post-drilling, eleven were identified as oilseed rape seeds and seven as harvest remains of the previous crop. The maximum number of oilseed rape seeds was four seeds, and the maximum number of post-harvest remaining seeds was six seeds (in total in ten frames, respectively). The mean number of post-harvest remaining during post-drilling seed counts was 1.4 seeds per counting (in the mainland, no harvest remains were found in the headland), resulting in 0.7 seed/m². The mean number of oilseed rape seeds per counting was 1.6 seeds in the mainland (M) and 0.6 seeds in the headland (H), resulting in 0.8 seeds/m² in the mainland (M) and 1.2 seeds/m² in the headland (H).

Summarized results from seed countings conducted pre- and post-drilling

	Pre-/post-drilling	No. of seeds (harvest remains)		No. of oilseed rape seeds		No. of seeds (harvest remains) per m²		No. of oil seed rape seeds per m²	
		H	M	H	M	H	M	H	M
Total	pre-drilling	5	87	-	-	-	-	-	-
	post-drilling	0	7	3	8	-	-	-	-
Mean	pre-drilling	1.0	17.4	-	-	2.0	8.7	-	-
	post-drilling	0	1.4	0.6	1.6	0	0.7	1.2	0.8

H = Headland (two frames per counting), M = Mainland (eight frames per counting)

Radio tracking:
For PT analysis, 23 radio tracking sessions of 14 individuals were used. The tracking sessions were conducted from 15 August 2019 until 02 September 2019 and covered the time span between several days pre-drilling and emergence of the oilseed rape seeds.
PT estimates were calculated for "potential consumers" and for "confirmed consumers". Since all radio tracked wood mice were captured either in the oilseed rape field or in the directly adjacent off-crop habitat, they all had access to the study fields and could therefore be determined as "potential consumers" for the PT calculations. This corresponds to the term "consumers" according to EFSA (2009). Therefore, all successfully radio tracked individuals with all sessions were considered to calculate a PT estimate regardless of the use of the oilseed rape fields during radio tracking. Additionally, PT was estimated only from radio tracking sessions of wood mice recorded (by trapping, by single telemetry fixes, or during radio tracking) at least once within in an oilseed rape field (i.e. "confirmed consumers").
During pre-drilling radio tracking, four valid radio tracking sessions of four individuals were conducted, resulting in a mean PT value of 0.05 (90th percentile 0.11). PT values ranged from 0.00 to 0.13. Of these,

two sessions were "confirmed consumer" sessions, resulting in a mean PT for the "confirmed consumers" of 0.10 (90th percentile 0.12).
During post-drilling radio tracking, 19 valid radio tracking sessions of 14 individuals were conducted, resulting in a mean PT for all sessions (i.e. "potential consumers") of 0.04 (90th percentile 0.12). Post-drilling PT values ranged from 0.00 to 0.33. In total, 10 individuals in 11 radio tracking sessions were confirmed consumers, resulting in a mean PT for the "confirmed consumers" of 0.08 (90th percentile 0.3). Comparing pre- and post-drilling, no correlation between potential foraging time in the oilseed rape field and exposed seeds on the soil surface, which might have triggered the attractiveness, was apparent.

PT values for radio tracking sessions conducted post-drilling (between drilling and emergence of oilseed rape plants)

	PT target crop potential consumers (N=19)	PT target crop confirmed consumers (N=11)
Mean	0.04	0.08
SEM	0.02	0.04
90th percentile	0.12	0.30

III. Conclusion

To conclude, this study showed that wood mice spent on average about 4% of their potential foraging time in freshly drilled oilseed rape fields. The PT data from 14 different individuals and 19 tracking sessions represent a robust data set for the use in wildlife risk assessments according to EFSA (2009).

Assessment and conclusion by applicant: This study is considered reliable and can be used for risk assessment. The 90th percentile PT for consumers *sensu* EFSA GD 2009 was 0.12.

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Data Point:	KCP 10.1.2.2/04
Report Author:	[redacted]
Report Year:	2009
Report Title:	Acceptance of rape seeds treated with clothianidin & fluopicolide & fluoxastrobin FS 400 + 80 + 60 (application rate: 25 mL/kg seed), observed with house mouse (Mus domesticus)
Report No:	[redacted]/ANN 152
Document No:	M-357355-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data on the effects of the formulation are not relevant to the risk assessment, data on the attractiveness of oil seed rape as a food item is relevant to the refined risk assessment.

Executive Summary

A feeding study was performed with house mice and wood mice, in which treated and untreated rape seeds were offered: Mice (*Mus domesticus*) received untreated rape seeds and rape seeds treated with clothianidin & fluopicolide & fluoxastrobin FS 400 + 80 + 60. When exclusively fed with untreated rape seeds (day -3 and -2), the mice consumed significantly lower amounts (mean of 0.3 to 0.7 g) which did not cover the daily energy demand. In order to avoid effects of emaciation mingled with potential toxic effects, the mice were granted a recovery day, when they received standard food consisting of oat flakes and rape seeds. On that day (-1) the average food consumption was 3 to 4 times higher than on the previous days. The oat flakes were completely consumed, only rape seeds were left over. On the exposure day the consumption of rape seeds in the control and the treatment group was similar (control: 0.5 g /mouse; treatment group: 0.4 g/mouse), and in the range of the amount consumed during the acclimation period on the rape (only days -3 and -2). The results proved that rape seeds are not sufficient and not appropriate as exclusive food for the mice.

I. Material and Methods:

Rape seeds treated with clothianidin & fluopicolide & fluoxastrobin FS 400 + 80 + 60 (application rate: 25 mL/kg seed), batch-ID: 2008-009793; TOX No.: 8457-00; Specification No.:102000021195; Treated rape seeds (TOX-No.: 08599-00; Specification No.:102000021195-01) were offered to 10 singly caged house mice for 24 hours, while 10 control house mice received exclusively untreated rape seeds.

During the first week of an acclimation period of 13 days, mice received oat flakes and standard pellets ad libitum. On day -8 oat flakes (5 g per mouse) was offered. The food consumption of oat flakes was determined on day -7. This procedure aimed to get information on the consumption of this attractive food item by the mice. From day -7 until day -4 all mice received standard diet ad libitum (50% oat flakes, 50% untreated rape seeds). On day -3 and on day -2 all mice received 5 g untreated rape seeds per mouse for 24 hours. The food from day -3 and day -2 remaining after 24 hours was reweighed. Since mice lose weight when offered only rape seeds, the day -1 was designed as recovery day, when the mice received 5 g of standard food.

On the exposure day (day 0), 5 g of treated rape seeds were offered to each of 10 house mice while 5 g untreated rape seeds were offered to each mouse of the control. During the 4 days post-exposure period the mice received standard food ad libitum.

The body weight was determined on day -7, day -1 and on day 5.
Observations on mortality, signs of intoxication and feeding activity were performed hourly during the exposure day over ca. 7 hours, and then once per day until test termination.

II. Results and Discussion:

Test item:	Clothianidin & Fluopicolide & Fluoxastrobin FS 400 + 80 + 60 (application rate: 25 mL/kg seed), TOX-No.: 8599-00
Test object:	House mouse (*Mus domesticus*)
Exposure:	Treated rape seeds
Results and observations:	No mortality, no intoxication Reduced food consumption No treatment related influence on body weight Dehusking of treated and untreated rape seeds

All mice behaved normally and did not show any signs of impairment, behavioural changes or intoxication.

Food consumption
On day -8 the mice consumed in average 3.6 (treatment group), resp. 3.7 g (control) of the oak flakes, a food item which is highly preferred. When exclusively fed with untreated rape seeds (day -3 and -2), the mice consumed significantly lower amounts (mean of 0.3 to 0.7 g) which did not cover the daily energy demand. This resulted in a body weight loss, measured on day -1. In order to avoid effects of emaciation mingled with potential toxic effects, the mice were granted a recovery day, when they received standard food consisting of oak flakes and rape seeds. On that day (-1) the average food consumption was 3 to 4 times higher than on the previous days. The oat flakes were completely consumed, only rape was left over.
On the exposure day the consumption of rape seeds in the control and the treatment group was similar (control: 0.5 g /mouse; treatment group: 0.4 g/mouse), and in the range of the amount consumed during the acclimation period on the rape-only days -3 and -2.
In the remainder of the feed, rape seed husks were observed. Differences in dehusking between control and treatment group were not visible.

Body weight development
The exclusive feeding with rape seeds during the acclimation period resulted in a significant body weight loss. Therefore, a recovery day with standard food was implemented on day -1. Since the mice consumed similarly small amounts of rape seeds on the exposure day, a similar body weight loss has to be assumed. A further body weight check was dispensed in order to reduce stress for the animals. During the post-exposure period, when the mice received standard food, body weight increased again.

III. CONCLUSIONS:

The results proved that rape seeds are not sufficient and not appropriate as exclusive food for the mice. Similar amounts of treated seeds were consumed as of the untreated ones. Rape seed husks observed after exposure confirmed qualitatively that the mice were dehusking the rape seeds before consumption. Consumption of the treated seeds did not cause any signs of intoxication in the mice.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. Oilseed rape seeds are not sufficient as food source for mice; seed dehusking was observed.

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Data Point:	KCP 10.1.2.2/05
Report Author:	[redacted]
Report Year:	2007
Report Title:	Acceptance of rape seeds treated with Methiocarb FS 500 G, observed with wood mice (Apodemus sylvaticus)
Report No:	[redacted]/ANN 141
Document No:	M-295311-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	none
Previous evaluation:	yes, evaluated and accepted in DAR (2005)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Data on methiocarb is not relevant for the risk assessment, data on the attractiveness of oil seed rape as a food item is relevant to the refined risk assessment.

Executive Summary

In this study, the food consumption of wood mice (*Apodemus sylvaticus*), feeding on rape seeds, treated with METHIOCARB FS500 G was investigated. Results were also obtained for untreated oilseed rape seeds. For this purpose, 10 wood mice were acclimated to rape seed with the following feeding regime: Day -8 to -5: standard food consisting of 50% oat flakes and 50 % untreated rape seeds; day -3 to -2: only untreated rape seed; day -1: standard food. When exclusively fed with untreated seeds (day -3 and -2), the mice consumed only small amounts of this food item, which did not cover the daily energy demand. This resulted in a body weight loss, measured on day -1. In order to avoid signs of emaciation mingled with potential toxic effect, the mice were granted a recovery day, when they received standard food, containing oat flakes and rape seeds. On that day (-1) the average food consumption was ten times higher than on the previous days. This proves that rape seeds are not sufficient and not appropriate as exclusive food for wood mice. Dehusking of the oilseed rape seeds was observed.

I. MATERIAL AND METHODS:

In this study, the food consumption of wood mice (*Apodemus sylvaticus*), feeding on rape seeds, treated with METHIOCARB FS500 G (Batch no. 2007-000877, TOX no. 07844-00) was investigated. 10 individually housed wood mice, caught in the arable land in Monheim (Germany) were acclimated to rape seed with the following feeding regime: Day -8 to -5 : standard food consisting of 50% oat flakes and 50 % untreated rape seeds; day -3 to -2: only untreated rape seed; day -1 : standard food (as recovery since the mice lost body weight on the previous days). Food was always offered for 24 hours in porcelain bowls.
On the exposure day the mice received 5 g treated rape seeds each for 24 hours. After the exposure day they were switched to standard diet until day +5.
The mice were observed on signs of intoxication five times on the exposure day and once per day during the post-exposure period.
Body weight was measured on day -8, -1 and at test termination.
Food consumption was measured for day -3 until day 0.

II. Results and Discussion:

Test substance:	Methiocarb FS500 G, Tox 07844-00, on rape seed, application rate 3L /100 kg, Tox no. 07951-00
Test object:	Wood mouse (*Apodemus sylvaticus*)
Exposure:	Treated rape seeds
Results and observations:	No mortality, no intoxication Reduced food consumption, No treatment related influence on body weight

When exclusively fed with untreated seeds (day -3 and -2), the mice consumed only small amounts of this food item, which did not cover the daily energy demand. This resulted in a body weight loss, measured on day -1. In order to avoid signs of emaciation mingled with potential toxic effect, the mice were granted a recovery day after the days on untreated seeds, when they received standard food, containing oat flakes and rape seeds. On that day (-1) the average food consumption was ten times higher than on the previous days.
The rape seeds were de-husked.
On the exposure day, when only treated seeds were offered, the mean food consumption was even lower than on day -3 and -2.

III. Conclusions:

The results proved that rape seeds are not sufficient and not appropriate as exclusive food for wood mice. De-husking occurred with both treated and untreated seeds.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. Woodmice lost weight when offered only (treated or untreated) oilseed rape seeds and consumed relatively low quantities. Dehusking was observed and found to prevent intoxication.

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Data Point:	KCP 10.1.2.2/06
Report Author:	[redacted]
Report Year:	2006
Report Title:	Generic field monitoring of mammals in freshly drilled oilseed rape fields in summer in Germany
Report No:	[redacted]/FS 036
Document No:	M-281405-01-1
Guideline(s) followed in study:	The test was specifically designed for this study
Deviations from current test guideline:	Not applicable
Previous evaluation:	yes, evaluated and accepted Study list relied upon, December 2011 (RMS: DE)
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

Small mammal species (particularly wood mice) were monitored within and around OSR fields in the region of Thale, Sachsen-Anhalt, Germany. The species and abundance of small mammals was investigated by live trapping. Furthermore, individual small mammals were radio tracked. The abundance of small mammals was additionally monitored by means of a thermal image camera. In order to receive information on the diet of small mammals, stomach contents were analysed. This focus of this summary is on granivorous mice, according to the refined risk assessment scenario where this study is used.

Wood mice were live-trapped on all selected study plots, though the surrounding habitats proved to be more attractive than the OSR fields, with much higher trapping efficiencies. Only 8.02% of the wood mice captures were made in traps set up in the field. Six samples of stomach contents could be obtained from wood mice on drilled oilseed rape fields. The main food items concerning their volume were seeds of Brassica spec. (mean 45.8%) and cereal grains (mean 36.7%). Furthermore, some animal matter could be found (mean 4.3%). Radio tracking of 15 wood mice showed that the freshly drilled OSR field habitat was only of minor importance For risk assessment purposes the portion of time spent potentially foraging in freshly drilled oilseed rape fields (PT) was calculated from the radio tracking data: wood mice spent in average 12.53 % (90th percentile 29.99%) of their potential foraging time in oilseed rape fields.

I. Material and Methods:

This generic study has been conducted in and around four different oilseed rape fields in the region of Thale, Sachsen-Anhalt, Germany. This region is a typical area for oilseed rape (OSR) cultivation in Europe. However, the cultivation procedures followed a minimum-soil cultivation philosophy. After harvest of the preceding crops, all plots were covered with significant amounts of harvest leftovers, i.e. rooted stem parts, leaves and ears, and leftover husks and grains (fallen out or still contained in ears). Since the fields were not ploughed afterwards, significant amounts of the leftover of the preceding crops remained on the soil surface until the end of the study period.

All in all, this lack of intense soil cultivation resulted in extraordinary favourable habitat conditions, and in order to counteract infestation of common voles, rodenticides were applied to hot spots inside some fields (but outside the trapping areas). This rodenticide application had no measurable impact on the rodent population in the trapping area but demonstrates the extraordinary favourable conditions for rodents in the fields where this study has been conducted.

Small mammal species (particularly wood mice) were monitored within and around OSR fields on four study plots. On each plot a grid of 64 life traps was installed with traps set up in the field as well as in the adjacent surrounding. The species and abundance of small mammals was investigated by live trapping (capture – mark - recapture method). Furthermore, individual wood mice were radio tracked continuously for the whole active period from dusk till dawn. The location, the type of habitat and the behaviour was recorded for each position. From the telemetry data the portion of time/potential foraging time in OSR fields, the habitat preference and individual home ranges were calculated. The abundance of small mammals was additionally monitored by means of a thermal image camera. In order to receive information on the diet of small mammals, stomach contents were analysed.

II. Results and Discussion:

Wood mice were live-trapped on all selected study plots, though the surrounding habitats proved to be more attractive than the OSR fields, with much higher trapping efficiencies. Only 8.02% of the wood mice captures were made in traps set up in the field.

Radio tracking of 15 wood mice showed that the freshly drilled OSR field habitat was only of minor importance. The highest proportion of time was spent in the habitat hedgerow/shrub (mean proportion 66.25%, N=7). The majority of individuals used a mixed habitat, characterized by structures like grassland, bushes and trees, during potential foraging time (mean proportion 65.10%, N=12). None of the tracked individuals used the oilseed rape field as nesting habitat. Based on the Minimum Convex Polygon (MCP) freshly drilled oilseed rape fields accounted in average for 27.24% (90th percentile 68.54%) in wood mice to the home ranges of radio tracked individuals.

Six samples of stomach contents could be obtained from wood mice on drilled oilseed rape fields. The main food items concerning their volume were seeds of *Brassica* spec. (mean 45.8%) and cereal grains (mean 36.7%). Furthermore, some animal matter could be found (mean 4.3%).

RELEVANT SPECIES in the OSR field habitat (based on live-trapping)				
Species	total trapping efficiency [captures/100 trapnights]		captures in the OSR field [%]	
	OSR field (based on 1536 trapnights)	Surrounding (based on 544 trapnights)		
Wood mouse (*Apodemus sylvaticus*)	1.89	21.69	8.02	
Common vole (*Microtus arvalis*)	11.52	43.20	21.05	
Yellow-necked mouse (*Apodemus flavicollis*)	1.56	34.74	4.30	
HABITAT USE of wood mice after radio tracking				
Proportion of habitat types to home range (MCP), based on 15 individuals tracked for one night (whole observed period) (mean of individuals, 90%ile)	OSR fields		27.24%	68.54
PORTION OF TIME (PT) in habitat of wood mice after radio tracking				
potential foraging time (surface activity only) spent per habitat; based on 15 individuals, (mean of individuals, 90%ile)	OSR fields		12.53%	29.99
PREFERENCE FOR OSR FIELDS in wood mice after radio tracking				
preference for OSR field habitat (mean Jacobs' index (D), Range: -1 to +1, MCP (100%), 90%ile); N=15			-0.56	-0.09

III. CONCLUSIONS:

For risk assessment purposes the portion of time spent potentially foraging in freshly drilled oilseed rape fields (PT) was calculated from the radio tracking data: wood mice spent in average 12.53 % (90th percentile 29.99%) of their potential foraging time in oilseed rape fields.

Assessment and conclusion by applicant: This study is considered reliable and can be used for risk assessment. The 90th percentile PT was 0.3 for consumers *sensu* EFSA GD 2009.

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Two calculations have been conducted to determine the benchmark dose (BMD) one based on body weight the other on body weight gain after three weeks of exposure.

Data Point:	KCP 10.1.2.2/07
Report Author:	[redacted]
Report Year:	2019
Report Title:	Fluopicolide: BMD calculations for body weight for mammal toxicity studies
Report No:	19036-BAY-1
Document No:	M-667414-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Not applicable
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 the present study the lower bound of the benchmark dose (BMD) confidence interval (BMDL) was calculated for the substance fluopicolide based on data from four mouse studies and four rat studies with dietary exposure. The analysed endpoint was body weight after three weeks (i.e. following exposure over approximately 21 days). The lowest reliable $BMDL_{10}$ was 119 mg a.s./kg bw/d. This value can be considered as reliable realistic worst-case short-term exposure rodent endpoint for fluopicolide , suitable for instance in OSR seed treatment risk assessments for granivorous rodents.

I. MATERIAL AND METHODS:

In the present study the lower bound of the benchmark dose (BMD) confidence interval (BMDL) was calculated for the substance fluopicolide. Calculations were conducted with the mean body weights after three weeks of exposure. The software PROASTweb (version 66.39) was used in accordance with EFSA (2017). Model averaging was performed when possible.
Data from four mouse studies and four rat studies with dietary exposure were available:

- A 28-day study with mice by [redacted] (2000a)
- A 90-day study with mice by [redacted] (2000c)
- A 90-day study with mice by [redacted] (2001)
- A chronic toxicity/carcinogenicity study with mice by [redacted] (2003)
- A 28-day study with rats by [redacted] (2000)
- A 90-day study with rats by [redacted] (2000b)
- A multi-generation study with rats by [redacted] (2003)
- A chronic toxicity/carcinogenicity study with rats by [redacted] (2003)

The analysed endpoint was body weight after three weeks (i.e. following exposure over approximately 21 days). It has been argued that the length of the exposure period in the toxicity assessment should match the exposure period in the field, in order to obtain realistic risk estimates (Wang et al. 2019). Thus, in risk assessments on uses for short environmental exposure like treated OSR seeds, a 21 day toxicity endpoint can be considered as realistic worst case for exposure of granivorous rodents.
The BMR of 10% corresponds with the EC10 required according to EU regulation 283/2013 and may therefore also be specified in the revision of the EFSA GD (2009).
The BMDL corresponds with the use of the lower limit of the 90%-confidence interval and is therefore a conservative estimate of the BMD.
The reliability of the BMD values can be assessed based on the normalized width NW, i.e. the ratio of the width of the 90%-confidence range over the BMD (EFSA 2015):

$$NW = (BMDU-BMDL) / BMD$$

A threshold of NW ≤ 1.0 was applied to identify reliable fits, corresponding to the EFSA (2015) categories “excellent” (NW < 0.2), “good” (NW < 0.5) and “fair” (NW < 1.0).
Fits with NW ≥ 1.0 (“poor” or “bad”) were not considered reliable enough to determine valid BMDL values.

II. Results and Discussion:

The results are presented in the table below. For some endpoints the BMDL10 could not be calculated, since no significant trend, i.e. no dose-response (no effect within the dose range), was observed.
The overall lowest BMDL10 of 72.2 mg a.s./kg bw/d (female body weight in the study by [redacted], 2000) should be rejected due to an unacceptable NW of 43.04).
The lowest reliable $BMDL_{10}$ (NW = 0.35, see chapter 3.5 of the full study report) was 119 mg a.s./kg bw/d (mean body weight of F1 female rats during gestation in the rat reproduction study by [redacted] (2003)).

Summary of BMD calculations for body weight after three weeks of exposure (the lowest reliable $BMDL_{10}$ is marked in bold):

Species	Study	Sex	BMDL10	NW	Reliability
Mouse	[redacted] (2000a)	Females	973	30.3	bad
	28-day study	Males	No dose-response	-	-
	[redacted] (2000c)	Females	No dose-response	-	-
	90-day study	Males	1100	1.56	poor
	[redacted] (2001)	Females	238	1.75	poor
	90-day study)	Males	708	1.29	poor
	[redacted] (2003) chronic toxicity/ carcinogenicity study	Females	966[1] 979[2]	0.13[1] 0.24[2]	excellent good
		Males	913[1] 884[2]	0.80[1] 0.93[2]	fair fair
Rat	[redacted] (2000)	Females	72.2	43.0	bad
	28-day study	Males	178	1.86	poor
	[redacted] (2000b)	Females	652	0.83	fair

Species	Study	Sex	BMDL10	NW	Reliability
	90-day study	Males	219	3.15	bad
	[redacted] (2003) multi-generation study	Females – prior to pairing (F0)	199	3.08	bad
		Females – during gestation (F0)	150	1.41	poor
		Females – during lactation (F0)	288	1.43	poor
		Females – pups (F1)	222	2.55	bad
		Females – prior to pairing (F1)	199	0.99	fair
		Females – during gestation (F1)	**119**	**0.35**	**good**
		Females – during lactation (F1)	203	0.47	good
		Females – pups (F2)	141	0.70	fair
		Males – prior to pairing (F0)	No dose-response	-	-
		Males – pups (F1)	235	1.62	poor
		Males – prior to pairing (F1)	No dose-response	-	-
		Males – pups (F2)	122	0.81	fair
	[redacted] (2003) chronic toxicity/ carcinogenicity study	Females	371[1] 274[2]	3.36[1] 8.16[2]	bad bad
		Males	275[1] 273[2]	0.97[1] 7.74[2]	fair bad

[1] refers to animals used for carcinogenicity assessment
[2] refers to animals used for chronic toxicity assessment.

III. Conclusions:

A $BMDL_{10}$ of 119 mg/kg bw/d for bodyweight effects of fluopicolide over 21 days of exposure can be considered as reliable realistic worst-case short-term exposure endpoint, suitable for instance in OSR seed treatment risk assessments for granivorous rodents.

Assessment and conclusion by applicant:

This study is considered reliable and can be used for risk assessment. A BMDL10 of 119 mg/kg bw/d for bodyweight effects of fluopicolide over 21 days of exposure can be used as short-term exposure endpoint in OSR seed treatment risk assessments for granivorous rodents.

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Data Point:	KCP 10.1.2.2/08
Report Author:	[redacted]
Report Year:	2019
Report Title:	Fluopicolide: BMD calculations for body weight gain for mammal toxicity studies
Report No:	19036-BAY-2
Document No:	M-667312-01-1
Guideline(s) followed in study:	None
Deviations from current test guideline:	Not applicable
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 the present study the lower bound of the benchmark dose (BMD) confidence interval (BMDL) was calculated for the substance fluopicolide based on data from four mouse studies and four rat studies with dietary exposure. The analysed endpoint was body weight gain after three weeks (i.e. following exposure over approximately 21 days). The lowest reliable $BMDL_{10}$ was 116 mg a.s./kg bw/d. This value can be considered as reliable realistic worst-case short-term exposure rodent endpoint for fluopicolide, suitable for instance in OSR seed treatment risk assessments for granivorous rodents.

I. Material and Methods

In the present study the lower bound of the benchmark dose (BMD) confidence interval (BMDL) was calculated for the substance fluopicolide. Calculations were conducted with the mean body weight changes after three weeks of exposure. The software PROASTweb (version 66.39) was used in accordance with EFSA (2017). Model averaging was performed when possible.
Data from four mouse studies and four rat studies with dietary exposure were available:

- A 28-day study with mice by [redacted] (2000a)
- A 90-day study with mice by [redacted] (2000c)
- A 90-day study with mice by [redacted] (2001)
- A chronic toxicity/carcinogenicity study with mice by [redacted] (2003)
- A 28-day study with rats by [redacted] (2000)
- A 90-day study with rats by [redacted] (2000b)
- A multi-generation study with rats by [redacted] (2003)
- A chronic toxicity/carcinogenicity study with rats by [redacted] (2003)

The analysed endpoint was body weight gain after three weeks (i.e. following exposure over approximately 21 days). It has been argued that the length of the exposure period in the toxicity assessment should match the exposure period in the field, in order to obtain realistic risk estimates (Wang et al. 2019). Thus, in risk assessments on uses for short environmental exposure like treated OSR seeds, a 21 day toxicity endpoint can be considered as realistic worst case for exposure of granivorous rodents.

The body weight gain in this evaluation is employed as the ratio of the terminal body weight (day 21) to the initial body weight (day 0) because this is the preferred expression for body weight gain in benchmark dose calculations (EFSA, 2017).
The BMR of 10% corresponds with the EC_{10} required according to EU regulation 283/2013 and may therefore also be specified in the revision of the EFSA GD (2009).
The BMDL corresponds with the use of the lower limit of the 90%-confidence interval, and is therefore a conservative estimate of the BMD.
The reliability of the BMD values can be assessed based on the normalized width NW, i.e. the ratio of the width of the 90%-confidence range over the BMD (EFSA 2015):
NW = (BMDU-BMDL) / BMD
A threshold of NW ≤ 1.0 was applied to identify reliable fits, corresponding to the EFSA (2015) categories “excellent” (NW < 0.2), “good” (NW < 0.5) and “fair” (NW ≤ 1.0).
Fits with NW ≥ 1.0 (“poor” or “bad”) were not considered reliable enough to determine valid BMDL values.

II. RESULTS AND DISCUSSION:

The results are presented in the table below. For some endpoints the $BMDL_{10}$ could not be calculated, since no significant trend, i.e. no dose-response (no effect within the dose range), was observed.
The overall lowest $BMDL_{10}$ was 116 mg a.s./kg bw/d (mean body weight gain of female rat pups (F2 generation) in the study by [redacted] 2003).

Summary of BMD calculations for body weight gain after three weeks of exposure (the lowest $BMDL_{10}$ is marked in bold):

Species	Study	Sex	$BMDL_{10}$	NW
Mouse	[redacted] (2000a) 28-day study	Females	1090	0.46
		Males	1190	-
	[redacted] (2000c) 90-day study	Females	No dose-response	-
		Males	196	6.30
	[redacted] (2001) 90-day study	Females	401	0.85
		Males	No dose-response	-
	[redacted] (2003) chronic toxicity/ carcinogenicity study	Females	1010[1] 926[2]	0.14[1] 0.29[2]
		Males	908[1] 972[2]	0.63[1] 0.88[2]
Rat	[redacted] (2000) 28-day Study	Females	139	3.65
		Males	228	1.80
	[redacted] (2000b) 90-day study	Females	862	0.62
		Males	203	1.60
	[redacted] (2003) multi-generation study	Females – prior to pairing (F0)	210	1.09
		Females – during gestation (F0)	No dose-response	-
		Females – during lactation (F0)	No adverse effect	-
		Females – pups (F1)	195	0.40
		Females – prior to pairing (F1)	No dose-response	-
		Females – during gestation (F1)	No dose-response	-
		Females – during lactation (F1)	No dose-response	-
		Females – pups (F2)	**116**	**0.83**

Species	Study	Sex	$BMDL_{10}$	NW
		Males – prior to pairing (F0)	223	12.02
		Males – pups (F1)	203	0.34
		Males – prior to pairing (F1)	No adverse effect	-
		Males – pups (F2)	119	0.82
	[redacted] (2003) chronic toxicity/ carcinogenicity study	Females	342[1] 316[2]	0.81[1] 7.85[2]
		Males	303[1] 293[2]	0.86[1] 61.13[2]

[1] refers to animals used for carcinogenicity assessment [2] refers to animals used for chronic toxicity assessment

III. CONCLUSIONS:

A $BMDL_{10}$ of 116 mg/kg bw/d for body weight gain effects of fluopicolide over 21 days can be considered as reliable realistic worst-case short-term exposure endpoint, suitable for instance in OSR seed treatment risk assessments for granivorous rodents.

<u>Assessment and conclusion by applicant:</u>
This study is considered reliable and can be used for risk assessment. A BMDL10 of 116 mg/kg bw/d for body weight gain effects of fluopicolide over 21 days can be used as short-term exposure endpoint, in OSR seed treatment risk assessments for granivorous rodents.

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Data Point:	KCP 10.1.2.2/09
Report Author:	Boag, B.; Macfarlane Smith, W.; Griffiths, D.
Report Year:	1990
Report Title:	Effects of grazing by wild rabbits (Oryctolagus cuniculus) on the growth and yield of oilseed and fodder rape (Brassica napus sub. sp. oleifera)
Report No:	M-619312-01-1
Document No:	M-619312-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

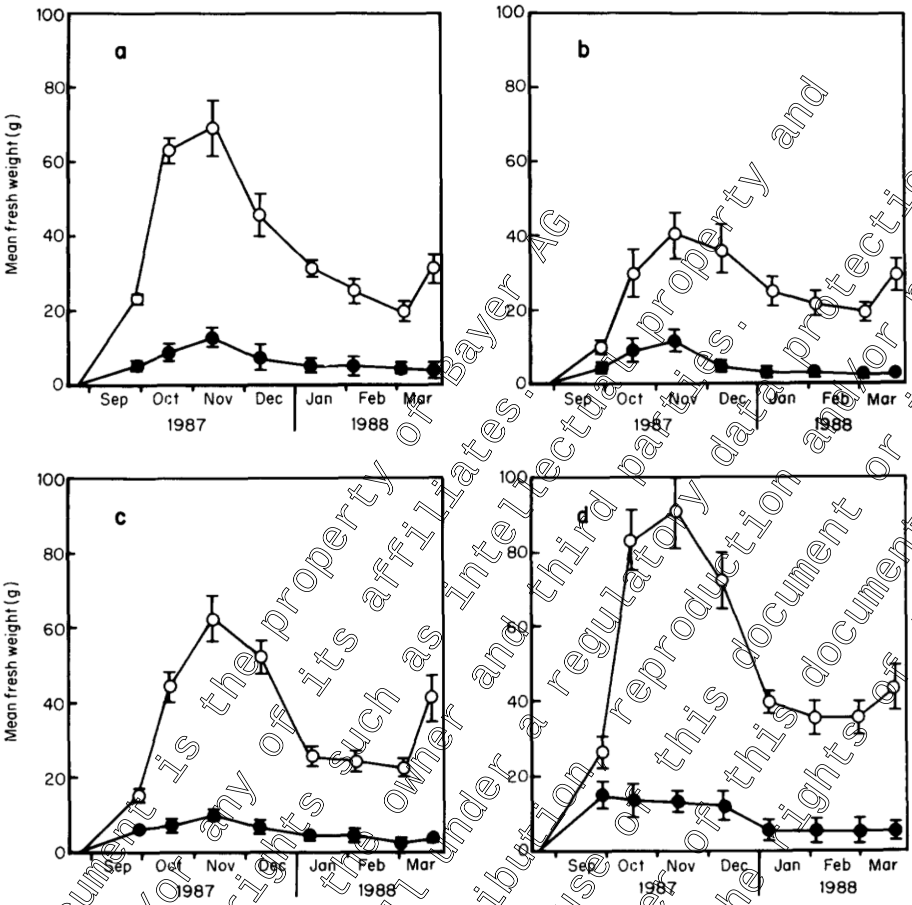
Executive Summary

The effects of rabbit (*Oryctolagus cuniculus*) grazing on the growth and yield of two oilseed rape (*Brassica napus* sub. sp. *oleifera*) cultivars (Bienvenu, a single-low cultivar and Ariana, a double-low cultivar) and two fodder rape cultivars (Hobson and Bonar, the latter low in progoitrin) were observed at an experimental site in eastern Scotland. No long-term significant differences were observed in the preference of the rabbits for any of the cultivars. Grazing by rabbits significantly reduced the yield of seed at harvest. When the crop was protected for part of the growing season, i.e. over winter or during the spring and summer, damage was reduced but yields were still significantly reduced. No harmful effects due to grazing the rape were observed on either the survival or reproductive capacity of the rabbits.

I. MATERIAL AND METHODS:

The experiment was at Littleton Farm, Inchture, Perthshire, Scotland, at approximately 150 m above sea level and on a loam soil. The experiment was situated approximately 12 m inside a stock-proof, but not rabbit-proof, 30 ha field growing the double-low oilseed rape cv. Ariana. The estimated five to seven adult rabbits which occupied a warren at the side of the field close to the experiment had, in addition to the rape, access to both a grass and a cereal stubble field throughout the winter. The two oilseed rape cultivars sown on 27 August 1987 were the double-low cv. Ariana and the single-low cv. Bienvenu while the two fodder rape cultivars were the conventional cv. Hobson and the low progoitrin cv. Bonar. The four rape cultivars were randomized in eight blocks. Initially, four of the blocks were protected from rabbits while the others were unprotected. Each block was 12.5 m long and 10 m wide. The blocks were immediately adjacent to one another. Within each block the four rape cultivars were grown in plots 2.5 m wide, each plot comprising five rows 50 cm apart. Ten randomly selected plants were collected from each plot each month from September until May and weighed. By March 1988, rabbit grazing damage to the plants in the unprotected blocks was so severe that two of the ungrazed blocks were opened to the rabbits to provide a differential measure of the effects of spring and summer grazing while two of the grazed blocks were fenced to determine a measure of the extent to which rape plants would make compensatory growth and recover.

II. Results and Discussion:



Effect of rabbit grazing on the mean fresh weight of two oilseed and two fodder rape cultivars, ○= rabbit-free plots; ● plots where rabbits had free access.

Effect of rabbit grazing on the fresh weight of two oilseed and two fodder rape varieties

Rabbit access		Mean weight of 10 plants, g (±SEM)			
Winter	**Summer**	**cv. Ariana**	**cv. Bienvenu**	**cv. Hobson**	**cv. Bonar**
Yes	Yes	14 (2.8)	12 (3.4)	3 (3.7)	12 (3.4)
Yes	No	51 (4.5)	47 (10.7)	52 (6.8)	51 (8.3)
No	Yes	89 (10.2)	87 (8.7)	87 (4.2)	104 (7.1)
No	No	153 (12.5)	204 (15.3)	218 (15.0)	183 (20.0)

SEM = Standard error of mean

The effect of rabbits on the growth of rape plants can be seen in the figure above. Although differences were observed in the growth habit of the different rape cultivars, and there was considerable loss of weight due to frost damage from December until early March, the most noticeable effect was the consistent lowering of the weights of the plants from the plots grazed by rabbits.

The fresh weights of both the oilseed and fodder rapes increased from March, just before the changes in the positioning of the rabbit netting. The rabbits continued to graze all unfenced plots and by mid-May, just before flowering, there were significant differences in the fresh weights of the plants subjected to the different grazing regiments, but no significant differences were observed between different cultivars.

The mean overall fresh weight of the plants continually grazed by rabbits was <6% of that of the ungrazed. The weight of those protected over winter and of those protected from March was 26% and 48% respectively of the ungrazed plants. The reduction in rape-seed yield due to rabbit grazing is shown in the table below. Where rabbits had access to the plants throughout the experiments, no seed was recovered. The overall percentage decrease in yield due to rabbit grazing between 27 August 1987 and 27 March 1988 was 51% compared with 71% for plots where rabbits had access to plants from 27 March onwards. The one exception was the fodder rape cv. Hobson which had comparable figures of 76.6% and 76.2% respectively. During the duration of the experiment no abnormal behaviour was observed and the rabbits reproduced at the same time as others within the locality, i.e. young were seen in early April.

Percentage mean yield of seed at harvest due to different periods of grazing by rabbits compared with the yield from ungrazed plots

Rabbit grazing	Oilseed rape [yield, %]		Fodder rape [yield, %]	
	Ariana	Bienvenu	Hobson	Bonar
August 87 – August 88	0	0	0	0
August 87 – March 88	42.4	52.5	76.6	32.4
March 88 - August 88	73.6	76.8	76.2	57.4
Ungrazed plots	100	100	100	100

III. CONCLUSIONS:

The results of the present investigation, apart from the initial sample in September, indicate that rabbits did not differentiate between the four rape cultivars tested. Although the winter of 1987-1988 was relatively mild, with no lying snow, the rabbits, which had access to permanent pasture within 50 m of the warren, continued to eat the rape plants throughout the winter. In the summer the rabbits also had access to spring barley in the field abutting the warren; although they grazed this to a certain extent, they continued to eat and damage all of the oilseed and fodder rape cultivars. This would suggest that at no time did the rape plants become unpalatable. The large reductions in yields of all the cultivars of rape suggests that all vulnerable rape crops must be protected from rabbit damage. Winter damage is most serious; whereas the crop is able to make compensatory growth during late spring and early summer, it is insufficient to overcome completely the damage done over winter. The effects of rabbit damage have not previously been documented but results from the present experiment suggest that substantial economic losses can be incurred if vulnerable crops are not protected throughout the growing season.

Assessment and conclusion by applicant:

This study is considered reliable and can be used for risk assessment. It confirms that rabbits eat oilseed rape plants and can be considered as focal species in the herbivorous mammal assessment.

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Data Point:	KCP 10.1.2.2/10
Report Author:	Chapuis, J. L.
Report Year:	1990
Report Title:	Comparison of the diets of two sympatric lagomorphs, Lepus europaeus (Pallas) and Orycotagus cuniculus (L.) in an agroecosystem of the Ile-de-France
Report No:	MO-01-017445
Document No:	M-074076-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

The diets of the European hare (*Lepus europaeus* Pallas) and wild rabbit (*Oryctolagus cuniculus* L.) in an agrosystem of Ile-de-France are compared. The results are based on analysis of faeces collected at least monthly at 4 sites over one or two annual cycles. Hares and rabbits had very similar diets. Grasses made up the base of their diet (50 to 100 % of the fragments found in the faeces) of which wheat was the preferred food item throughout the year. Their diet was more varied in summer and fall, and included maize, inflorescences of grasses, and various dicotyledons, as well as *Equisetum arvense* for the hare. The difference between to two species' food choices are related to the behaviour of food selection: the proximity of food resources to the warrens for the rabbits and, on a larger scale, the repartition of fields for the hare. These results show that the rabbit is a generalist compared with the hare which is more selective. Although feeding on the same plants in certain seasons, the two Lagomorphs exploit different areas, and are therefore unlikely to compete for food under these circumstances.

I. Material and Methods:

Situated 18 km southeast of Paris, the study area consists of 200 hectares of heavily cultivated land on siliceous-clay silt. The climate is oceanic with continental tendencies and some years it is marked by a 15 day period of light snow cover in January/February which does not, however, prevent access to the herb layer. The study area consists of 3 to 15 hectares fields cultivating mainly winter wheat (*Triticum sativum*) (40 to 50 % of the study area depending on the year), maize (*Zea mays*) (30 %), peas (*Pisum sativum*) (10 %) and, less commonly, oilseed rape (*Brassica napus*) (up to 10 %), sugar beet (*Beta vulgaris*) (5 %) and green beans (*Phaseolus vulgaris*) (3 %). The uncultivated zones (isolated woods, access roads, fallows) cover only small surfaces. About 15 adventice species are well represented on the cultivated plots. Roadside Vegetation is primarily graminaceous with a few dicotyledons. In the fallows, the herb layer is mainly composed of *Agropyrum repens, Phalaris arundinacea, Urtica dioica*, the shrub layer of *Salix* spp. and *Prunus spinosa*. A few fruit trees, *Pirus malus, Cerasus avium*, are also found. The wooded zones which offer protection to the warrens are dominated by trees, mainly *Fraxinus excelsior, Quercus pedunculata* and *Cerasus avium*, by *Sambucus nigra*, *Prunus spinosa, Ligustrum vulgare* and *Crataegus monogyna* for the shrub layer, and by *Urtica dioica*, *Rubus* sp., *Hedera helix* and *Galium aparine* for the herb layer.

In the first year, samples were taken from two sites in one zone; in the second year two others were added in another zone. Based on home ranges of approximately 2 ha for the rabbit and 30 ha for the hare cultivated species available per site were as follows: site 1 (hare): wheat and maize, to which were added oilseed rape, peas, and green beans in 1983; site 2 (rabbit): wheat, maize; site 3 (rabbit): peas, maize and wheat; site 4 (hare): wheat, maize, sugar beet, peas.

The hare's and rabbit's diets were analysed by a previously tested method of microscopic identification of epidermal fragments in faeces. The collection periodicity varied from 15 days to one month depending on the season, from February 1983 to February 1985 at sites 1 and 2, and from January 1984 to January 1985 at sites 3 and 4. Each sample was comprised of 15 to 20 faecal pellets taken from a maximum number of pellet groups. 350 to 400 fragments (from 0.25 to 2 mm in size) per sample were indentified, distributed among 100 microscopic fields (20 fields/slide). The results are expressed in percentage of relative abundance.

II. Results and Discussion

At site 1, starting in October, the hare's most important food source was wheat germinated from winter crops. In June the leaves were left in favour of the ears which were ingested until August. If ploughing did not immediately follow harvesting, young shoots of grains left on the ground were consumed, before the appearance of the young shoots on neighbouring fields resulting from the fall sowing. This was reflected by the predominance of this cereal in their diet during the two study years. In summer, feeding was more varied with consumption of maize during the first two months following germination (maximum 40 % in June 1983 and 30 % in August 1984) and consumption of *Equisetum arvense* (30 % maximum in July/August). Various dicotyledons (*Matricaria discoidea*, *Polygonum* spp.) and graminea (*Lolium multiflorum*, *Poa* spp.) were ingested in small amounts, especially in June and September-October, periods when the leaves of wheat had dried out or were unavailable. Even though the field was close to the site of faeces collection in 1983, pea plants were rarely consumed. A maximum of 8.6 % was recorded in May upon apparition of the young shoots and grain in October (5 %) upon germination of fallen grains. The green bean plant was ingested only in July (4.2 %) when the first leaves appeared. The hare's diet at site 4 was very similar to that in site 1. The only differences were related to a lesser consumption of *Equisetum arvense*, and to a greater proportion of various graminea at the onset of summer, essentially *Lolium multiflorum* found along roads. The leaves and roots of beet were virtually not consumed (maximum 1 % in November).

The rabbit's diet at site 2 was very similar to that of the hare: wheat was preponderant in winter, spring and fall. In summer rabbits mainly fed on inflorescences of graminea (wheat) and leaves of maize, in different proportions according to the year. Maize and its adventice plant *Equisetum arvense*, were ingested in 1984 at a time when the field was in the immediate vicinity of the warrens, but were ignored by the rabbits in 1983 because of the field's distance (approx. 100 m from the warrens). The grain teguments present in the faeces collected from October to December 1984 correspond mainly to the consumption of maize issued from cut or fallen stalks and ears left over from harvesting. Because of the drying out of wheat leaves and before the appearance of new shoots, the rabbit's diet was more varied at the beginning and end of summer. It consisted mainly of maize, wheat ears, and various grasses (*Lolium multiflorum, Phleum pratense, Poa trivialis*), of *Solanum nigrum* (foliage, flowers and seeds) and of other various dicotyledons. Fall was marked by the consumption of shrub leaves (*Prunus spinosa, Cerasus avium, Crataegus monogyna*), and in winter other underwood species (*Hedera helix, Rubus* sp.) were ingested along with small proportions of shrub bark. At site 3, the absence of wheat on fields next to the warrens explains the consumption from January to June of grasses found on the access road situated at more than 100 m from the warrens. Nevertheless, the rabbits cross the abandoned ditches, dry for most of the year, to feed on wheat. From October on, when newly sown wheat replaces the peas, the rabbits fed almost exclusively on this cereal, and did so until the end of the study. During summer the rabbits fed on maize, from its germination in June until September. At this time, various adventice dicotyledons were also ingested, especially in August and September. Among the other available species, the rabbits ate peas plants from a field adjacent to the woods (maximum 2.4 % only, in September), and in fall-winter, underwood species (bramble, ivy, shrub leaves and bark). As for site 2, consumption of gramineae grains (maize) was noted from August 1984 to January 1985.

III. CONCLUSIONS:

In the study area, as in most other habitats, hares and rabbits fed essentially on grasses (50 to 100 % of the fragments found in the faeces). Among these, the leaves of wheat constituted the base of their diet from October to May. The rest of the year they consumed ears of wheat, leaves of maize, and various dicotyledons found in the fields and along the access roads. One adventice fern, *Equisetum arvense*, was particularly sought out by the hare. Oilseed rape (variety "Bienvenu") occupied 10 % of our territory in 1983 and was completely ignored by the hare. This divergence of results could be due to difficulties in identifying rape leaf epidermis in faeces. More likely, it should be related to the presence of large surfaces of winter wheat, preferred to the crucifer. For the rabbit the oilseed rape was too far from the warrens and was therefore unaccessible. Contrary to previous studies, the hare did not use woods and thickets for feeding purposes in this study area. These results show that the two lagomorphs are selective herbivores, choosing precise foods, according to what is available in their feeding area. These choices are closely related to the phenological stage of plant species. These results strengthen Westoby (1974) and Belovsky (1978) Statement i.e. herbivores become "specialists" when food is abundant and "generalists" when food resources are limited. Because of the different behaviour of these two lagomorphs in the study area, this "specialist" tendency is more marked for the hare, whose foraging area is larger. This species also makes better use of the trophic potentialities of its habitat in adapting its foraging movements to the crops' phenology. Therefore, the hare can be more selective than the rabbit, whose feeding area is limited to the vicinity of its warren.

Assessment and conclusion by applicant: This study is considered reliable and can be used for risk assessment. It provides a large set of diet information from field samples which demonstrates that rabbit and haress are strict herbivores, with negligible portions of grain in their diet.

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Data Point:	KCP 10.1.2.2/11
Report Author:	Crawley, M. J.
Report Year:	1989
Report Title:	Rabbits as pests of winter wheat
Report No:	Lit. 8422
Document No:	M-064651-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Yes

Executive Summary

Experiments were carried out over a three-year period on the effects of the timing and duration of grazing on winter wheat, using natural and confined populations of rabbits. Experimental manipulation of grazing intensity was not attempted. Yield declined as an approximately linear function of the duration of exposure to rabbit grazing. There was no evidence of yield compensation for early defoliation, despite vigorous regrowth by the wheat after fencing. Exposure for a fixed period of 60 days was most damaging when it occured at the beginning of the growth period (November and December). Yield losses were minimal (but statistically significant) when the crop was exposed for 60 days just prior to harvest. Yield losses from 30-day exposure were roughly equal for each of the winter months from November to March. Grazing caused reductions in ear density, ear weight, seed number and individual seed weight. Indirect effects of rabbit grazing include increased weediness and increased damage from cereal aphids. Split plot trials with extra herbicide applied during the rapid growth phase, and aphicide applied at the time of ear formation, produced significant interaction effects with defoliation. Herbicide increased yields only on the grazed plots, while aphicide increased yields only on plots that were both grazed and treated with herbicide. Despite the lack of compensation for early defoliation, the wheat plants exhibited extraordinary resilience to repeated defoliation by rabbits. Even on the continuously grazed plots, some plants were able to ripen relatively large ears, containing heavy grains.

I. Material and Methods:

The research was carried out in two fields known as Pound Hill and Ashurst Warren on acid, sandy soils of the Bagshot Series at Silwood Park, Berkshire (GR 14 936692). In both experimental fields, following herbicide application in August, liming and ploughing, crops of winter wheat (variety Hustler) were sown in October at a rate of 350 seeds/m^2 and a spacing of 9 rows per meter. Fertilizer was applied at sowing. Soils were limed each year at a rate of 3 t/ha. Herbicide treatment of the crop involved a pre-ploughing application of Roundup (4 L glyphosate in 200 1 water/ha), preemergence treatment with Chandor (4 L Trifluralin and Linuron in 200 L water/ha), and a spring application of Agroxone (5 L MCPA in 200 L water/ha), with spot treatment of *Holcus mollis* with Roundup using a wick applicator.

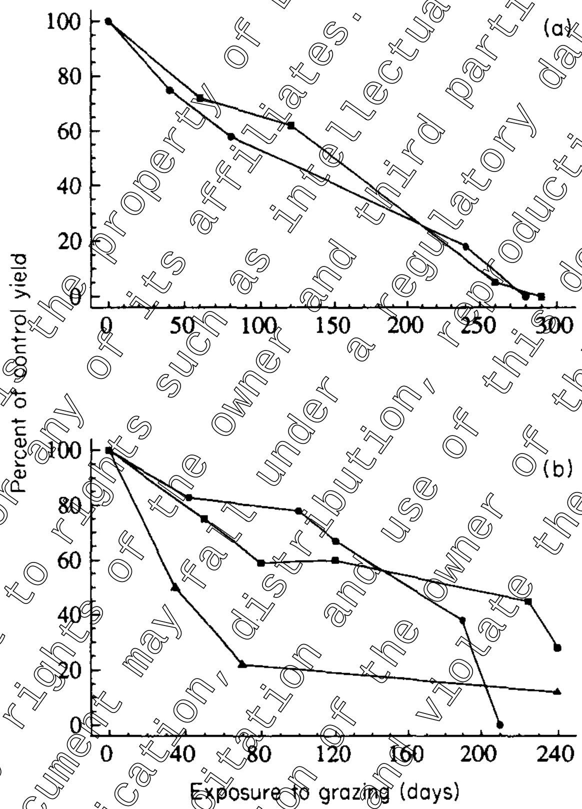
The field at Pound Hill was exposed to grazing by a natural, free-ranging population of rabbits whose harbourage was in a semi-natural woodland of oak, *Quercus robur*, and sycamore, *Acer pseudoplatanus*, with a dense undergrowth of bramble, *Rubus fruticosus*, and bracken, *Pteridium aquilinum*. The experimental area began some 25 m from the woodland boundary. As soon as the wheat crop grew clear of the soil surface, rabbits were observed in all parts of the field. Rabbits emerged from a 50 m length of harbourage to feed, up to 10 individuals could be seen on the field at any one time, and it was usual to see between four and six rabbits on the experimental area in the early evening during the winter months (i.e. approximately 3.3 rabbits/ha, or 0.1 rabbits seen per metre of harbourage).

In 1982—83 there were six different treatments: never grazed, continuously grazed and grazed for 42, 91, 121 and 183 days before fencing. In 1983-84 un-fencings were added to the design, and allowed the crop to grow for 52, 121 and 198 days before removing the fences and exposing the crop to rabbit grazing for the remainder of the growing season. As in 1982—83, a previously exposed crop was fenced after 52 and 121 days of grazing. In 1984-85 in addition to never grazed and continuously grazed, the crop were exposed for 30 or 60 day periods and then refenced. This enabled the importance of the timing of rabbit damage to be determined. The rabbit-exclusion plots used at Pound Hill measured 7 m x 3 m and were constructed of 2 cm wire mesh, using a single 10 cm fence post at each corner. Smaller vertebrates were seen (or caught in Longworth traps), including wood mice (*Apodemus sylvaticus*), harvest mice (*Micromys minutus*) and voles (*Microtus agrestis*).

The second experimental site was a plot measuring 60m x 30m situated below Ashurst Orchard, Silwood Park. This was fenced to 2 m in height with 2 cm wire mesh. A confined population of rabbits was established within the fence by introducing two male and two female wild rabbits. The rabbits were marked with plastic ear tags and released at the beginning of 1982. The rabbits caused very similar levels of damage to the wheat crop to those observed at Pound Hill (i.e. all the plants were grazed virtually to the ground for the entire period from November until April). The rabbits were provided with supplementary food throughout the winter in the form of rabbit pellets and sliced carrot. They bred successfully throughout the course of the experiment and young were removed periodically by live trapping and released outside the enclosure. Plots were laid out within the area as follows. The field was split into four blocks and, within each block, plots were allocated to five grazing treatments at random. Fences measuring 5 m x 4 m were erected to exclude rabbits at different times after sowing and to allow regrowth of the crop (durations of grazing were 0, 52, 121, 220 and 240 days). Each area was further treated with aphicide and with herbicide in a split plot, factorial design.

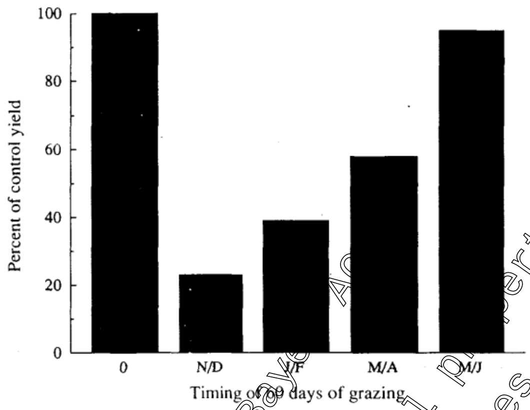
II. Results and Discussion:

The grain yields are shown in the Figure below. At Ashurst, yield measured as grain dry weight declined as a roughly linear function of duration of exposure in both years. There was no evidence of compensation for early grazing. At Pound Hill, the trend was again roughly linear, but the pattern was obscured by the fact that there was an increasingly severe problem with infestation of the grazed plots by the perennial grass weed *Holcus mollis* over the three years. The un-grazed wheat crop was able to suppress the weed effectively, but on grazed plots the grass frequently attained dominance. As before, there was no evidence of compensation for early, brief exposure to rabbit grazing.



The relationship between the duration of exposure to rabbit grazing and the yield of grain at harvest, expressed as percentage of control yield (a) Ashurst fieldin 1983 and 1984 and (b) Pound Hill Field in 1983, 1984 and 1985.

Yields suffered most from the earliest defoliation; the longer the onset of grazing was delayed, the lower the losses in yield. Close to harvest time, rabbits entered the crop and felled whole shoots, from which the ears were eaten. There was no time for the crop to recover from this kind of damage by regrowth.



The effect of exposing winter wheat to rabbit grazing for 60-day periods at different times of year, expressed as percentage of control yields.

All components of yield were affected by rabbit grazing: (1) reduced numbers of ears per unit area, due to shoot mortality (38.5% loss); (2) reduced ear size (18.4% loss), which in turn is due to (3) reduced numbers of grains per ear (7% loss); and (4) reduced individual mean grain weights (22.3% loss). In addition to these direct effects of defoliation on cereal yield, there are indirect effects of rabbit grazing that affect yields through increased weediness and increased cereal aphid numbers. Increased weediness depresses grain yield by an extra 47% on continuously grazed plots. The effect of selective defoliation of the wheat crop is to reduce the competitive ability of the cereal plants relative to less-intensively grazed weed species. In ungrazed plots, the vigorously growing wheat plants act as their own weed killers, suppressing weed growth by the dense shade they cast. In grazed plots, weeds like *Holcus mollis* and *Rutnex acetosella* that are avoided by rabbits can grow tall enough to over-top and out-compete the defoliated wheat plants. Aphids were more abundant on the grazed plots because the age structure of the leaf population was dominated by younger, more susceptible foliage. Removing the aphids with pesticide, however, only led to measurable increases in yield on split plots where weed abundance had been reduced by extra herbicide application. On the weedy plots, the increased cereal growth resulting from aphid exclusion was small compared to the reduced performance that came about from competition with those weed species that were avoided by rabbits.

III. Conclusions:

In no case was yield compensation observed, and brief early grazing led to eventual yield losses of between 17 and 30% over 3 years at two sites. Increasing the duration of exposure to rabbit grazing caused a roughly linear decline in grain yields. At one site, yield losses were exacerbated by a progressive increase in the abundance of grass weeds on the grazed plots over the 3 years. The timing of exposure had a substantial effect on yield. For a 60-day bout of feeding, losses were greatest when grazing occurred immediately after germination, in November and December. If grazing exposure lasted only 30 days, then there was less difference between the effects of different timings, with similar losses experienced for all months from November through to March. Exposure for 30 days during the rapid growth phase (April to June) was less damaging, presumably because alternative foods were available at this time, and the tall, often wet crop, was unattractive and relatively impenetrable to the rabbits. Increased weediness on grazed plots led to further yield reductions of up to 47%. There were more cereal aphids on the grazed plots, but these only added to yield losses when the extra weeds were experimentally removed

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. This study demonstrates that rabbits graze on shoots of cereals.

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Data Point:	KCP 10.1.2.2/12
Report Author:	[redacted]
Report Year:	2019
Report Title:	Relationship between magnitude of body weight effects and exposure duration in mammalian toxicology studies and implications for ecotoxicological risk assessment
Report No:	M-669217-01-1
Document No:	M-669217-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	Not applicable
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

A typical observation in long-term toxicity studies with rats is a reduction of body weight. Such reductions are generally more pronounced at the end of a study and are often used to derive an endpoint for the risk assessment. However, the exposure period in the field is rather short for most modern pesticides. Therefore, the change of the magnitude of effects over exposure time may be relevant to obtain a realistic view of effects expected in the field. Therefore, time-dependence of effects on female body weight observed in toxicity studies with rats was evaluated. 37 long-term toxicity studies conducted with 13 different active substances used as pesticides were analysed. Female body weights after 14, 21, 28, 42 and 70 days of dosing were used for BMD analysis per active substance. BMD_{10} values declined continuously with exposure duration, indicating that the longer the duration of exposure, the greater are the effects on body weights. This continuous decline was observed for all pesticide classes (i.e. herbicides, insecticides and fungicides).

I. Material and Methods

Time dependence of effects observed in toxicity studies conducted with rats was evaluated, focusing on effects on female body weight. Benchmark doses (BMD_{10}, i.e., 10 % effect) were calculated for a total of 37 long-term toxicity studies conducted with 13 different active substances used as pesticides. Female body weights after 14, 21, 28, 42 and 70 days of dosing were used for BMD analysis per active substance to evaluate time-dependent changes of BMD_{10}.

II. Results and Discussion

BMD_{10} values declined continuously with exposure duration, indicating that the longer the duration of exposure, the greater are the effects on body weights. This continuous decline was observed for all pesticide classes (i.e. herbicides, insecticides and fungicides) from the studies analyzed. After 70 days, the BMD_{10} levels were about half of the BMD_{10} at day 14.

III. CONCLUSION

The results indicate that animals respond to pesticide exposure in an exposure-time-dependent way, i.e. effects on body weight of the animals are less pronounced when the duration of exposure is short. The greatest body weight effects were observed at the end of toxicity studies (after longest exposure). The realism of the current wild mammal risk assessment for plant protection products is discussed and how it could be improved by considering an appropriate time period for the selection of endpoints in chronic toxicity studies, which reflects the exposure time of free ranging animals in the field.

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. It demonstrates that body weight effects in rats during longterm exposure typically depend on the duration of the treatment. Thus, consideration of an appropriate length of the exposure time window is essential to derive matching risk assessment endpoints for short term exposure in the field to oilseed rape seeds treated with Scenic Gold.

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Data Point:	KCP 10.1.2.2/13
Report Author:	DEFRA
Report Year:	2010
Report Title:	Dehusking of seed by small mammals - default values for use in risk assessment
Report No:	PS2349
Document No:	M-406213-01-1
Guideline(s) followed in study:	no specific guideline available
Deviations from current test guideline:	No
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Please note: This study had several scientific objectives which were addressed in several sub-studies. The following summary focuses on those parts of the study that are relevant for the risk assessment.

Executive Summary

The study aimed to generate robust generic data regarding the de-husking behavior of wood mice and voles for the use in risk assessment. Seven seed types (wheat, maize, barley, pelleted sugarbeet, peas, oilseed rape and beans) were dyed blue with 319009 Eurogran Brilliant Blue FCF food dye and presented to wood mice with a single mouse receiving a single seed type. Dyed barley and wheat seeds were additional presented to bank voles. All uneaten and hoarded seeds, remaining husk and faecal pellets were recovered. Close to 100 % of all ingested dye was eliminated in the first 48 hours following exposure. The use of a palatable dye allowed the amount of surface treatment on a seed ingested and the contribution of de-husking to removal of seed coatings to be assessed when wood mice and voles consumed treated seed under realistic worst-case conditions of food deprivation. Wood mice consumed a significant amount of the treatment from the surface of the seed during the de-husking process in the case of unpelletted oilseed rape (60 %), wheat (40 %), barley (45 %), beans (34 %) and maize (38 %). They ingested less when de-husking peas (11 %) and pelletted sugarbeet (1.4 %). Voles ingested slightly higher levels of the surface treatment than wood mice when consuming wheat (72 %) and similar amounts when consuming barley (53 %).

I. Material and Methods:

Wood mice were presented with a range of seed types dressed in 319009 Eurogran Brilliant Blue FCF food dye. In previous trials it has been shown that the blue food colourant was not unpalatable and therefore was acceptable for use in further trials. Furthermore, the mice have been shown to eliminate the dye as determined by the colour of the faeces, which showed signs of dye presence up to 48 hours post exposure to treated wheat seed. Three commercially available surfactants/stickers were used to assist in the coating process, these were; Peridiam, Bayer Crop Science, BB5, Nutriag Ltd. and Treaty, Intracrop.

One trial was conducted for 3 seed types at any given time, using a group of randomly selected wood mice and bank voles, 10 per seed type, balanced for sex (where possible). This gave a total of 30 animals for each single experimental run and a total overall of 70 wood mice and 20 bank voles for the whole trial.

Wood mice of known age were captive bred by the animal services staff. Under normal maintenance conditions they were housed in rat colony cages 530 mm × 375 mm × 160 mm. Flooring substrate was wood shavings, with paper wool for bedding and normal maintenance diet. Under test conditions the mice were acclimatised to cage paper on the floor, to facilitate ease of collection of fragments of the dressed seed samples. Water was available ad libitum from drinkers.

Although each trial consisted of 30 mice per trial, all trials were conducted under the same experimental protocol therefore the experimental conditions explained for the first trial are exactly the same for any subsequent trials and also for the bank vole trials.

Acclimatisation

The wood mice were acclimatised for at least one month prior to the commencement of the trial. The mice were maintained on a reverse daylight, light: dark regime (09:00 – 21:00 Red Light; 21:00 – 09:00 White Light) with half-light for half an hour before each change.

Pre-Trial (Day -1; 17:00)

The study animals had their normal diet removed from 17:00 (day -1).

Trial (Day 0; 09:00)

Each animal received seeds (one of seven seed types) dressed with 319009 Eurogran Brilliant Blue FCF (see Table 10.1.2.2-5 below for constituents each seed type was dressed in). Animals were monitored at regular intervals to check for consumption of the dressed seed. At 15:00 the dressed seed samples were removed. The animals did not receive their normal diet until 17:00.

Post Trial (Day 1-3)

Faeces from the test mice were collected up until day 3 post-exposure to the dressed seed samples. Faecal samples were collected and separated by colour. All traces of blue dye were eliminated by day two (Table 10.1.2.2-2).

Table 10.1.2.2- 5: Seed Dressings used in the final study

Seed type	Species	Constituents	Coating method *	Application efficiency [% of target application rate]
Wheat	wood mice and voles	100 g seed 0.4 g blue granules 0.5 ml BB5 10 ml water	Seeds were coated using Hege seed dresser.	wood mice: 95 - 116 % voles: 92 - 108 %
Barley	wood mice and voles	100 g seed 0.4 g blue granules 1 ml BB5 10 ml Water	Seeds were coated using Hege seed dresser.	wood mice: 99 - 101 % voles: 97 % - 103 %
Peas	wood mice	180 g seed 0.9 g blue granules 9 ml peridiam	Seeds were coated by stirring the mix into the peas in a glass pot.	95 - 105 %
Beans	wood mice	200 g seed 0.3 g blue granules 1 ml peridiam 5 ml water	Seeds were coated using Hege seed dresser.	92 - 102 %
Maize	wood mice	100 g seed 0.3 g blue granules 1 ml peridiam 5 ml water	Sample was coated twice and shaken in a bag. Second coating was applied after the first coating had dried.	85 - 107 %
Sugar Beet (pelleted)	wood mice	200 g seed 1.5 g blue granules 10 ml BB5 100 ml water	Seeds were coated using the Hege seed dresser.	95 - 102 %
Oilseed rape	wood mice	100 g seed 1.2 g blue granules 4 ml treaty 16 ml water	Mixed in glass pot.	96 - 102 %

*All seed samples were dried in an oven at 45°C over night (~24h) after they were dressed.

II. Results and Discussion:

Over 95 % of the dye from the faecal pellets was recovered over the first two days after exposure (Table 10.1.2.2-6). The amount of dye recovered from the faecal pellets was used to calculate the percentage of the dye which was consumed during seed ingestion (Figure 1). Due to differences in dye extraction efficiency from the husk, compared with whole seed, the dehusking efficiency was calculated by comparing the weight of husk generated with the amount of the seed consumed.

Table 10.1.2.2- 6: Recovery of dye in faecal pellets on day 1 and 2 in wood mice and bank voles

Seed type	% Mean day 1 recovered in faecal matter (n) [Range]	% Mean day 2 recovered in faecal matter (n) [Range]
Peas (wood mouse)	95.7 (8) [85.57-100]	4.33 (*4/8) [2.78-14.43]
Oilseed rape (wood mouse)	91.8 (10) [73.8-100]	6.6(*4/10) [8.3-26.2]
Beans (wood mouse)	97.3 (8) [88.6-100]	2.4 (*2/8) [7.8-11.4]
Wheat (wood mouse)	95 (9) [81.7-100]	4.47 (*3/9) [4.9-18.3]
Pelleted Sugar beet (wood mouse)	100 (10)	
Barley (wood mouse)	94.3 (10) [82.4-100]	4.2 (*4/10) [1.9-17.6]
Maize (wood mouse)	97.4 (10) [89.2-100]	2.56 (*4/10) [3.9-10.8]

Seed type	% Mean day 1 recovered in faecal matter (n) [Range]	% Mean day 2 recovered in faecal matter (n) [Range]
Barley (vole)	88.8 (8) [70.9-100]	12.85 (*4/8) [20.5-27.5]
Wheat (vole)	87.7 (7) [82.1-100]	12.25 (*6/7) [11.1-17.9]

* Number of animals still excreting blue dye in faeces until day 2.

Results for wood mice

Figure 1 shows the percentage of the dye on the seed ingested recovered in the faeces and the percentage accounted for by the remaining husk. Table 10.1.2.2-7 shows the variation between individuals in the relative importance of the two routes. This shows wide variations between seed types in ingestion of dye during the de-husking process from close to 100 % recovery of the husk in pelletted sugarbeet and peas to high levels of ingestion with unpelleted oilseed rape with ingestion of approximately 40 % of the dye available in beans, maize, wheat and barley.

Results from the data analysis for some seed types is quite variable; with correlations between the amount of blue dye consumed (based on ingestion of treated seed) and amount of blue faecal matter recovered somewhat poor for some seed types. There may be some loss in urine (based on dermal absorption during handling), which is difficult to account for as it is particularly difficult to recover. However, when an estimate of how much the animal may have handled the treated seed is taken into account (by assessing the amount taken from the dish) then there is an improvement, in some cases, of the relationship between consumption and recovery. For those seeds where levels of de-husking are high such as peas and sugar beet the correlation between handling and consumption against percent recovered in faeces was 0.217 and 0.609 respectively.

Figure 1: Percentage of the dye on the seed consumed by wood mice recovered in the faeces and the percentage accounted for by the remaining husk.

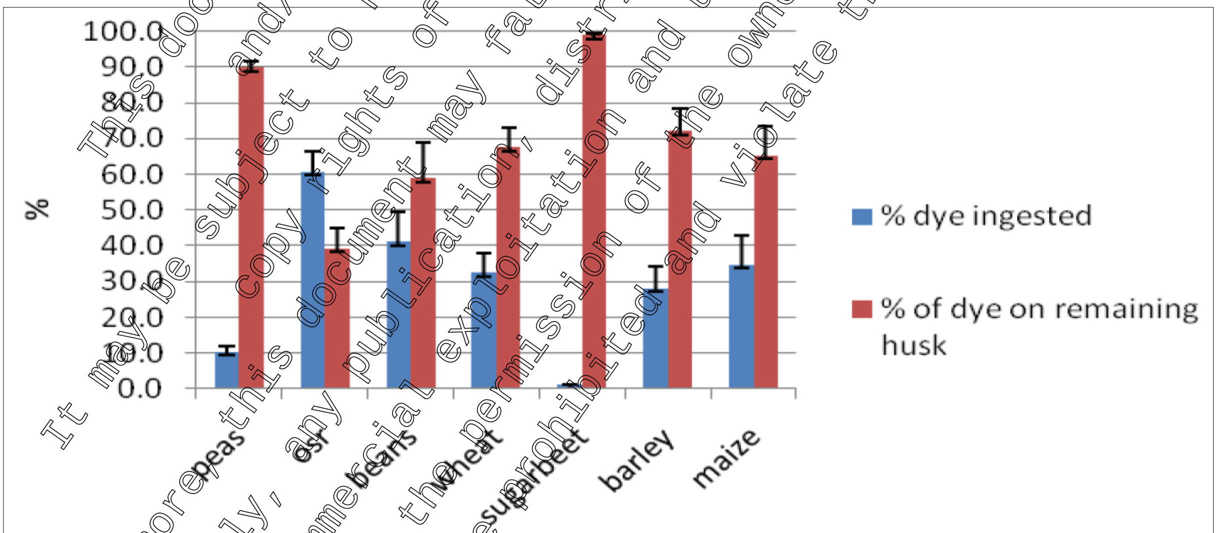

Table 10.1.2.2- 7: Percentage of consumed seed accounted for by ingestion and de-husking and percentage accounted for in wood mice

Wood mice						
Seed type	**Individual**	**% dye recovered from faeces**	**% dye on husk remaining from seed consumed**	**Total % recovered**	**% dye ingested**	**% dye de-husked**
Peas	B2988	3.6	78.5	82.2	4.4	95.6
	B2989	4.7	87.0	91.6	5.1	94.9
	B2993	9.2	71.3	80.4	11.4	88.6
	B2998	2.9	95.5	98.4	3.0	97.0
	B3000	4.7	101.6	106.3	4.4	95.6
	B3006	15.4	62.1	77.5	19.9	80.1
	B3012	17.2	49.4	66.7	25.8	74.2
				mean	**10.6**	**89.4**
				se	2.9	2.9
OSR	B2556	19.0	81.2	100.2	19.0	81.0
	B2589	66.1	12.7	78.8	83.9	16.1
	B2590	54.7	45.1	99.9	54.8	45.2
	B2608	32.2	62.9	95.2	33.9	66.1
	B2994	116.2	37.2	153.3	75.8	24.2
	B3002	21.9	69.0	90.9	24.1	75.9
	B3004	30.7	20.5	51.2	60.0	40.0
	B3005	50.2	10.3	60.5	83.0	17.0
	B3017	37.5	39.1	76.6	48.9	51.1
	B3018	71.3	0.0	71.3	100.0	0.0
				mean	**58.3**	**41.7**
				se	8.6	8.6
Beans	B2599	205.7	116.0	321.7	63.9	36.1
	B2983	10.0	80.7	90.7	11.1	88.9
	B2991	43.5	51.7	95.3	45.7	54.3
	B3001	45.5	66.3	111.8	40.7	59.3
	B3007	23.3	68.9	92.1	25.3	74.7
	B3011	25.9	89.6	115.5	22.4	77.6
	B3013	24.6	77.3	101.9	24.1	75.9
				mean	**33.3**	**66.7**
				se	6.7	6.7
Wheat	B2557	28.0	39.5	67.5	41.5	58.5
	B2560	67.2	27.2	94.5	71.2	28.8
	B2562	20.5	42.6	63.1	32.5	67.5
	B2563	0.0	73.1	73.1	0.0	100.0
	B2580	30.5	37.7	68.2	44.7	55.3
	B2587	18.2	32.3	50.5	36.0	64.0
	B2588	33.9	36.3	70.2	48.3	51.7
	B2593	14.0	11.2	25.2	55.5	44.5
	B2603	28.4	70.1	98.6	28.8	71.2
				mean	**39.8**	**60.2**
				se	6.6	6.6
Sugar Beet	B2561	0.7	100.0	100.7	0.7	99.3
	B2583	5.1	99.2	104.2	4.9	95.1
	B2607	0.7	94.9	95.6	0.7	99.3
	B2984	1.2	102.9	104.2	1.2	98.8
	B2986	3.6	101.9	105.5	3.4	96.6
	B2987	1.5	113.8	115.4	1.3	98.7
	B2990	0.5	90.7	91.1	0.5	99.5
	B3009	0.4	61.5	61.9	0.7	99.3

Wood mice						
Seed type	**Individual**	**% dye recovered from faeces**	**% dye on husk remaining from seed consumed**	**Total % recovered**	**% dye ingested**	**% dye de-husked**
	B3010	0.4	95.0	95.3	0.4	99.6
	B3020	0.4	99.7	100.2	0.4	99.6
				mean	**1.4**	**98.6**
				se	0.5	0.5
Barley	B2553	24.0	18.0	42.1	57.1	42.9
	B2558	8.4	60.5	68.8	12.2	87.8
	B2584	45.3	35.0	80.2	56.4	43.6
	B2586	45.9	30.8	76.7	59.8	40.2
	B2591	46.4	16.4	62.8	73.8	26.2
	B2596	10.5	58.2	68.7	15.3	84.7
	B2597	101.7	16.5	118.2	86.1	13.9
	B2598	22.4	24.1	46.5	48.3	51.7
	B2602	20.6	57.5	78.1	26.4	73.6
	B2605	9.6	77.3	86.9	11.0	89.0
				mean	44.6	55.4
				se	**8.5**	**8.5**
Maize	B2555	10.2	85.8	96.0	10.6	89.4
	B2559	14.1	97.6	111.7	12.6	87.4
	B2564	59.5	82.8	142.3	41.8	58.2
	B2579	35.6	62.8	98.4	36.2	63.8
	B2581	124.3	89.4	213.8	58.2	41.8
	B2585	34.9	73.8	108.7	32.1	67.9
	B2592	21.6	87.8	109.4	19.7	80.3
	B2600	17.6	0.0	17.6	100.0	0.0
	B2606	31.5	83.8	115.4	27.3	72.7
	B2934	59.0	91.6	150.6	39.2	60.8
				mean	**37.8**	**62.2**
				se	8.3	8.3

There is some variation between animals in some of the seed treatment groups above. One mouse in particular B2599 beans, consumed as little as four times his/her peers. However, this mouse hoarded 93 % of the total amount that was presented to it. The next closest mouse hoarded 65 % and the remaining wood mice hoarded from 33 - 51 %. This degree of handling may help to explain why this mouse appears to provide anomalous figures and why handling, an unmeasured variable, could contribute to the overall variability.

Results for voles

The voles ingested more dye when consuming wheat seed than the wood mice with a mean of 72 % recovered in faeces compared with 40 % in wood mice. The results for barley were more similar with ingestion accounting for 53 % in voles and 45 % in wood mice.

Table 10.1.2.2- 8: Percentage of consumed seed accounted for by ingestion and de-husking and percentage accounted for in bank voles.

Bank voles						
Seed type	**Individual**	**% dye recovered from faeces**	**% dye on husk remaining from seed consumed**	**Total % recovered**	**% dye ingested**	**% dye de-husked**
Barley	B2431	53.0	70.6	123.6	42.9	57.1
	B2433	51.6	44.0	95.6	54.0	46.0
	B2436	35.7	55.6	91.2	39.1	60.9
	B2438	38.0	17.9	55.9	68.0	32.0
	B2449	66.9	0.0	66.9	100.0	0.0
	B2765	92.1	58.3	150.4	61.3	38.7
	B2803	127.3	154.7	282.1	45.1	54.9
	B2865	5.1	27.0	32.1	15.9	84.1
				mean	**53.3**	**46.7**
				se	8.7	8.7
Wheat	B2429	70.8	0.0	70.8	100.0	0.0
	B2434	46.4	74.5	120.9	38.4	61.6
	B2450	69.2	25.6	94.8	73.0	27.0
	B2767	144.2	0.0	144.2	100.0	0.0
	B2804	64.9	79.8	144.6	44.9	55.1
	B2808	303.2	40.6	343.8	88.2	11.8
	B2809	140.8	85.6	226.3	62.2	37.8
				mean	**72.4**	**27.6**
				se	9.5	9.5

III. CONCLUSIONS:

1. The use of a palatable dye allowed the amount of surface treatment on a seed ingested and the contribution of dehusking to removal of seed coatings to be assessed when wood mice and voles consumed treated seed under realistic worst-case conditions of food deprivation.
2. Wood mice consume a significant amount of the treatment from the surface of the seed during the de-husking process in the case of unpelletted oilseed rape, wheat, barley, beans and maize. They ingest less when de-husking peas and pelletted sugarbeet.
3. Voles ingest slightly higher levels of the surface treatment to wood mice when consuming wheat and similar amounts when consuming barley.
4. The data appear robust, with very limited ingestion of the surface treatment in the case of pelletted sugarbeet as would be expected and higher levels of ingestion in the case of cereals.
5. Handling during hoarding of treated seed may contribute additional exposure over that accounted for by ingestion alone.

Following default values for ingestion rates are proposed:

Table 10.1.2.2- 9: Proposed default ingestion rates

Species	Seed type	Ingestion rate [%]
Wood mouse	Barley	45
Wood mouse	Peas	11
Wood mouse	OSR	60
Wood mouse	Beans	34
Wood mouse	Wheat	40

Species	**Seed type**	**Ingestion rate [%]**
Wood mouse	Sugar beet (pelleted)	1.4
Wood mouse	Maize	38
Bank vole	Barley	53
Bank vole	Wheat	72

Assessment and conclusion by applicant:
This study is considered reliable and can be used for risk assessment. Wood mice de-husking oilseed rape seeds reduced the ingestion of residues to 60% of the nominal rate.

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

The available and relevant data covering potential effects of fluopicolide and fluoxastrobin on terrestrial vertebrates are presented under point CP 10.1.1 for birds and CP 10.1.2 for mammals. Regarding assessment of potential effects on reptiles and amphibians neither guidance documents nor testing guidelines are available at present. Therefore, no additional data on terrestrial vertebrate wildlife is presented here.

CP 10.2 Effects on aquatic organisms

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

Table 10.2- 1: Endpoints used in risk assessment

Test substance	Test species	Endpoint		Reference
Fluopicolide	Fish, acute *Oncorhynchus mykiss*	**96 h LC_{50}** NOEC	**0.36 mg a.s./L (mm)** 0.16 mg a.s./L (mm)	[redacted] 2003; M-240806-01-1 KCA 8.2.1/01
	Fish, acute *Lepomis macrochirus*	96 h LC_{50} NOEC	0.75 mg a.s./L (mm) 0.56 mg a.s./L (mm)	[redacted] 2003; M-240805-01-1 KCA 8.2.1/02
	Fish, acute *Cyprinus carpio*	96 h LC_{50} NOEC	1.3 mg a.s./L (mm) 0.25 mg a.s./L (mm)	[redacted] 2003; M-219743-01-1 KCA 8.2.1/03
	Fish, acute *Brachydanio rerio*	96 h LC_{50} NOEC	1.8 mg a.s./L (mm) 1.0 mg a.s./L (mm)	[redacted] 2003; M-234508-01-2 KCA 8.2.1/04
	Fish, acute *Oryzias latipes*	96 h LC_{50} NOEC	0.7 mg a.s./L (mm) 0.44 mg a.s./L (mm)	[redacted] 2003; M-234510-01-2

Test substance	Test species	Endpoint		Reference
				KCA 8.2.1/05
	Fish, acute *Cyprinodon variegatus*	96 h LC_{50} NOEC	0.41 mg a.s./L (mm) 0.20 mg a.s./L (mm)	[redacted] 2003; M-223359-01-2 KCA 8.2.1/06
	Fish, acute *Pimephales promelas*	96 h LC_{50} NOEC	1.34 mg a.s./L (nom) 0.313 mg a.s./L (nom)	[redacted] 2015; M-533292-01-1 KCA 8.2.1/10
	Fish, chronic (ELS) *Pimephales promelas*	33 d NOEC **EC_{10}**	0.155 mg a.s./L (mm) **0.278 mg a.s./L (mm)**	[redacted] 2003; M-241190-01-1 KCA 8.2.2.1/01 [redacted] 2018; M-643769-01-1 Calculation of EC_{10} endpoint. KCA 8.2.2.1/02
	Fish, BCF flow through *Lepomis macrochirus*	BCFss, lipid normalised	65 L/kg (whole fish)	[redacted] 2003; M-241273-01-1 KCA 8.2.2.3/01
	Invertebrate, acute *Daphnia magna*	**48 h EC_{50}**	**>1.8 mg a.s./L (mm)**	[redacted] 2003; M-240807-01-1 KCA 8.2.4.1/01
	Invertebrate, acute *Crassostrea virginica*	96 h EC_{50}	>2.6 mg a.s./L (mm)	[redacted] 2003; M-225445-01-1 KCA 8.2.4.2/01
	Invertebrate, acute *Americamysis bahia*	96 h LC_{50}	3.2 mg a.s./L (mm)	[redacted] 2003; M-220513-01-2 KCA 8.2.4.2/02
	Invertebrate, chronic *Daphnia magna*	21 d NOEC EC_{10}	0.19 mg a.s./L (mm) Cannot be calculated	[redacted] 2003; M-241191-01-1 KCA 8.2.5.1/01 [redacted] 2018; M-617757-01-1 Endpoint recalculation. KCA 8.2.5.1/02
	Invertebrate, chronic *Americamysis bahia*	28 d NOEC **EC_{10}**	0.34 mg a.s./L (mm) **0.18 mg a.s./L (mm)**	[redacted] 2015; M-544290-02-1 KCA 8.2.5.2/01
	Sediment dweller, chronic *Lumbriculus variegatus*	**28 d NOEC**	**1.98 mg a.s./kg (nom)**	[redacted] 2020; M-671529-03-1 KCA 8.2.5.4/02

Test substance	Test species	Endpoint		Reference
	Algae *Pseudokirchneriella subcapitata* Green algae	72 h E_rC_{50} 72 h E_bC_{50} 72 h NOE_rC 72 h E_rC_{10}	> 4.3 mg a.s./L (mm) 3.0 mg a.s./L (mm) 2.4 mg a.s./L (mm) 2.6 mg a.s./L (mm)	[redacted] 2003; M-219737-01-2 KCA 8.2.6.1/01; [redacted] 2018; M-643768-01-1 Endpoint recalculation. KCA 8.2.6.1/05
	Algae, *Skeletonema costatum* (Marine diatom)	**72 h E_rC_{50}** 96 h E_yC_{50} 72 h E_rC_{05} 72 h E_rC_{10}	**0.073 mg a.s./L (nom)** 0.0612 mg a.s./L (nom) 0.0160 mg a.s./L (nom) 0.0424 mg a.s./L (nom)	[redacted] 2015; M-533278-01-1 KCA 8.2.6.2/07
	Algae, *Navicula pelliculosa* (Freshwater diatom)	72 h E_rC_{50} 72 h E_yC_{50} 72 h NOE_rC 72 h E_rC_{10}	0.121 mg a.s./L (mm) 0.067 mg a.s./L (mm) 0.043 mg a.s./L (mm) 0.064 mg a.s./L (mm)	[redacted] 2020; M-678011-01-1 KCA 8.2.6.2/08
	Aquatic macrophytes, *Lemna gibba*	**7 d E_rC_{50}** NOE_rC	**> 3.2 mg a.s./L (mm) frond number & dry weight** 3.2 mg a.s./L (mm)	[redacted] 2003; M-220201-01-2 KCA 8.2.7/01
	Amphibian larvae, acute *Xenopus laevis*	48 h LC_{50} NOEC	> 1 mg a.s./L (nom) 0.125 mg a.s./L (nom)	[redacted] 2010; M-393869-01-1 KCA 8.2.8/01
M-01 (2,6-dichloro-benzamide (BAM; BCS-AA65784))	Fish, acute *Oncorhynchus mykiss*	**96 h LC_{50}**	**240 mg p.m./L (nom)**	[redacted] 2001; M-234311-01-2 KCA 8.2.1/07
	Invertebrate, acute *Daphnia magna*	**48 h EC_{50}**	**180 mg p.m./L (nom)**	[redacted] 2001; M-234306-01-2 KCA 8.2.4.1/02
	Algae *Pseudokirchneriella subcapitata* (Green algae)	72 h E_rC_{50} 72 h E_bC_{50} 72 h NOE_rC 72 h E_rC_{10}	120 mg p.m./L (nom) 60 mg p.m./L (nom) 40 mg p.m./L (nom) 49 mg p.m./L (nom)	[redacted] 2001; M-234304-01-2 KCA 8.2.6.1/03
	Algae, *Navicula pelliculosa* (Freshwater diatom)	**72 h E_rC_{50}** 72 h E_yC_{50} 72 h NOE_rC 72 h E_rC_{10}	**92 mg p.m./L (mm)** 46 mg p.m./L (mm) 30 mg p.m./L (mm) 42 mg p.m./L (mm)	[redacted] 2020; M-678377-01-1 KCA 8.2.6.2/10

Test substance	Test species	Endpoint		Reference
	Aquatic macrophytes, *Lemna gibba*	**7 d E_rC_{50}** 7 d E_yC_{50} NOE_rC E_rC_{10}	**97.6 mg p.m./L (nom), frond number** 71.8 mg p.m./L (nom) 25.0 mg p.m./L (nom) 51.0 mg p.m./L (nom)	[redacted] 2003; M-219725-01-2 KCA 8.2.7/02 [redacted] 2018; M-664031-01-1 Endpoint recalculation. KCA 8.2.7/03
M-02 (3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid; (BCS-AB43478))	Fish, acute *Oncorhynchus mykiss*	**96 h LC_{50}**	**> 102 mg p.m./L (mm)**	[redacted] 2003; M-218631-01-2 KCA 8.2.1/08
	Algae, *Navicula pelliculosa* (Freshwater diatom)	**72 h E_rC_{50}** 72 h E_yC_{50} 72 h NOE_rC 72 h E_rC_{10}	**74 mg p.m./L (mm)** 72 mg p.m./L (mm) 42 mg p.m./L (mm) 48 mg p.m./L (mm)	[redacted] 2020; M-678012-01-1 KCA 8.2.6.2/09

Bold: Endpoints used in risk assessment
a.s.: active substance; p.m.: pure metabolite
nom = nominal concentrations; mm = mean measured concentration

Formulation studies

No studies were performed with the formulation FLC + FXA FS 350 because it is used as seed treatment. According to the regulation EU 284/2013 on data requirements for plant protection products, formulation studies are not necessary when the intended use does not include direct application on water.

The composition of the dried product which is applied in the environment (on the treated seeds) is different from the composition of the liquid formulation used for the treatment of the seeds. Therefore, tests on the liquid formulation are not deemed necessary.

Selection of algae and macrophytes endpoints for risk assessment

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.

Valid algae studies with green algae and freshwater and marine diatoms species are available for fluopicolide. In general, diatoms show a greater sensitivity to fungicides targeting oomycetes. That is the reason why tests on *Navicula* were also performed with the metabolites in order to cover the most sensitive organism group, even though diatoms do not belong to tier 1 standard species. The endpoint selected for algae risk assessment is the lowest of the 72h-E_rC_{50} (0.073 mg a.s./L), it was obtained with *Skeletonema costatum*.

Selection of endpoints for chronic risk assessment

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 0.155 mg/L based on wet weight and length, the lowest EC_{10} is 0.278 mg a.s./L based on wet weight. It is proposed to use the EC_{10} for risk assessment (refer to MCA for further explanations).

Metabolites

Metabolites M-01, M-02 and M-03 are relevant for the aquatic risk assessment. No metabolite is relevant for sediment risk assessment.

Some studies were performed with metabolites M-01 and M-02, however, M-03 cannot be tested due to its very fast degradation in water and consequently in test medium. When data are available, they are used in the metabolite risk assessment. The EFSA AGD (2013) stepwise approach is used for all metabolites when no data are available.

The decision scheme is followed step by step.

- Step 1: None of the studies with the active substance is adequate for assessing the potential effect of the metabolites: ⇨ Step 3.
- Step 3: Is it clear that the toxophore has been lost from the molecule?

M-01 and M-02 do not show any fungicidal activity (see MCA 3.6 report by [redacted] M-224842-01-1), The structure of the fluopicolide molecule is split in two to create M-01 and M-02. On this basis, it is known that the toxophore has been lost. However, data on the most sensitive organism group are available so the comparison with parent of step 4 is performed anyway.

Regarding M-03, the toxophore is considered as present because its molecular structure is very similar to the parent. ⇨ Step 4

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

Studies on *Navicula* are available for fluopicolide and its metabolites M-01 and M-02, they are used for the comparison (see table below).

Substance name	Fluopicolide	M-01	M-02
Endpoint (mg/L)	0.121	92	74
Molecular mass (g/mol)	383.59	190	225.6
Parent endpoint recalculated on a molar basis (mg/L) $10\frac{M_{met}}{M_{ai}}LC_{50_{ai}}$	NA	0.60	0.71

NA= Not applicable.

The *Navicula* endpoints for both M-01 and M-02 are much greater than 10 times the parent endpoint recalculated on a molar basis ⇨ Step 6

This comparison cannot be performed for metabolite M-03. It is proposed, as a screening step, to use parent endpoints and an additional safety factor of 10, i.e. assuming that M-03 is 10 times more toxic than the parent.

- Step 6: Assume that the acute and chronic toxicity of the metabolite is equal to the toxicity of the a.s. for all first-tier taxonomic groups.

This approach will be followed for all missing endpoints for M-01 and M-02.

Summary of the metabolite endpoints used in risk assessment:

Endpoints (mg/L)	**M-01**	**M-02**	**M-03**
Acute fish	LC_{50} = 240	LC_{50} > 102	LC_{50} = 0.036**
Acute invertebrates	EC_{50} = 180	EC_{50} > 1.8*	EC_{50} > 0.18**
Algae	E_rC_{50} = 92	E_rC_{50} = 74	E_rC_{50} = 0.0121**
Macrophyte	E_rC_{50} = 97.6	E_rC_{50} > 3.2*	E_rC_{50} > 0.32**
Chronic fish	EC_{10} = 0.278*	EC_{10} = 0.278*	EC_{10} = 0.0278**
Chronic invertebrates	NOEC = 0.19*	NOEC = 0.19*	NOEC = 0.019**

* 1st tier parent endpoint (*Skeletonema* and mysids are not considered as tier 1 species)
** 1st tier parent endpoint divided by 10

Predicted environmental concentrations used in the risk assessment

Predicted environmental concentrations of fluopicolide and its metabolites in surface water were calculated according to FOCUS Steps 1-3 for the use in winter oilseed rape.

Table 10.2- 2: Initial max PEC_{sw} values – FOCUS Steps 1 and 2 (winter oilseed rape)

Compound	**FOCUS Scenario**	**Winter oilseed rape 1 × 12 g/ha $PEC_{sw, max}$ [µg/L]**
Fluopicolide	STEP 1	2.95
	STEP 2 North	**1.45**
	STEP 2 South	1.16
M-01 (2,6-dichlorobenzamide (BAM))	STEP 1	1.31
	STEP 2 North	**0.644**
	STEP 2 South	0.515
M-02 (3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid)	STEP 1	0.574
	STEP 2 North	**0.128**
	STEP 2 South	0.103
M-03 (2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)-2-pyridinyl](hydroxy)methyl}benzamide)	STEP 1	0.387
	STEP 2 North	**0.166**
	STEP 2 South	0.133

Table 10.2- 3: Initial max PEC_{sed} values – FOCUS Steps 1 and 2 (winter oilseed rape)

Compound	FOCUS Scenario	Winter oilseed rape
		1 × 12 g/ha
		$PEC_{sed, max}$ [µg/kg]
Fluopicolide	STEP 1	7.89
	STEP 2 North	**3.89**
	STEP 2 South	3.11

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

Table 10.2- 4: Acute risk assessment based on FOCUS Step 2 for the application in winter oilseed rape (1 × 12 g a.s./ha)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	$RAC \geq PEC_{sw}$
Fluopicolide	Fish, acute *Oncorhynchus mykiss*	LC_{50} 360	3.6	1.45	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} > 1800	>18		Yes

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw,max}$ [µg/L]	RAC ≥ PEC_{sw}
M-01 (2,6-dichloro-benzamide (BAM))	Fish, acute *Oncorhynchus mykiss*	LC_{50} 240000	2400	0.644	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} 180000	1800		Yes
M-02 (3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid)	Fish, acute *Oncorhynchus mykiss*	LC_{50} > 102000	> 1020	0.128	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} > 1800*	> 18		Yes
M-03 (2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)-2-pyridinyl](hydroxy)methyl}benzamide)	Fish, acute *Oncorhynchus mykiss*	LC_{50} 36**	0.36	0.166	Yes
	Invertebrate, acute *Daphnia magna*	EC_{50} > 180**	> 1.8		Yes

* 1st tier parent endpoint
** 1st tier parent endpoint divided by 10

For fluopicolide the acute trigger was met for all aquatic organisms. Therefore, no further assessment is necessary.

CHRONIC RISK ASSESSMENT FOR AQUATIC ORGANISMS

Table 10.2- 5: Chronic risk assessment based on FOCUS Step 2 for the application in winter oilseed rape (1 × 12 g a.s./ha)

Compound	Species	Endpoint [µg/L]	RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
Fluopicolide	Fish, chronic *Pimephales promelas*	EC_{10} 278	27.8	1.45	Yes
	Invertebrate, chronic *Americamysis bahia*	EC_{10} 180	18		Yes
	Algae *Skeletonema costatum*	E_rC_{50} 73	7.3		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50} > 3200	> 320		Yes
M-01 (2,6-dichlorobenzamide (BAM))	Fish, chronic *Pimephales promelas*	EC_{10} 278*	27.8	0.644	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC 190*	19		Yes
	Algae *Pseudokirchneriella subcapitata*	E_rC_{50} 92000	9200		Yes
	Aquatic macrophytes, *Lemna gibba*	E_rC_{50} 97600	9760		Yes
M-02 (3-chloro-5-(trifluoromethyl)pyridine-2-carboxylic acid)	Fish, chronic *Pimephales promelas*	EC_{10} 278*	27.8	0.128	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC 190*	19		Yes

Compound	Species	Endpoint [µg/L]		RAC [µg/L]	$PEC_{sw.max}$ [µg/L]	RAC ≥ PEC_{sw}
	Algae *Navicula pelliculosa*	E_rC_{50}	74000	7400		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	> 3200*	> 320		Yes
M-03 (2,6-dichloro-N-{[3-chloro-5-(trifluoromethyl)-2-pyridinyl](hydroxy)methyl}benzamide)	Fish, chronic *Pimephales promelas*	EC_{10}	27.8**	2.78	0.166	Yes
	Invertebrate, chronic *Daphnia magna*	NOEC	19**	1.9		Yes
	Algae *Navicula pelliculosa*	E_rC_{50}	12.1**	1.21		Yes
	Aquatic macrophyte *Lemna gibba*	E_rC_{50}	> 320**	> 32		Yes

* 1st tier parent endpoint (*Skeletonema* and mysids are not considered as tier 1 species)
** 1st tier parent endpoint divided by 10

Table 10.2- 6: Chronic risk assessment for sediment organisms based on FOCUS Step 2 for the application in winter oilseed rape (1 × 12 g a.s./ha)

Compound	Species	Endpoint [µg/kg]		RAC [µg/kg]	$PEC_{sed.max}$ [µg/kg]	RAC ≥ PEC_{sed}
Early application						
Fluopicolide	Sediment dweller, chronic *Lumbriculus variegatus*	NOEC	1980	198	3.89	Yes

For fluopicolide the chronic trigger was met for all aquatic organisms. Therefore, no further assessment is necessary.

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

Formulation studies are not necessary based on current data requirements. As the formulation is a seed treatment, aquatic organisms will not be exposed to the formulation as such.

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.

Commission Regulations (EU) 283/2013 and 284/2013 require where bees are likely to be exposed, testing of both acute (oral and contact) and chronic toxicity, including sub-lethal effects. Consequently, in addition to the standard toxicity studies performed with adult bees (OECD 213 and 214) the following additional studies are also provided (please refer to MCA, Section 8):

- Chronic 10-day toxicity test with the solo formulation fluopicolide SC 486 on adult bees under laboratory conditions ([redacted] 2016; M-552253-01-1)
- Repeated exposure toxicity test with fluopicolide tech. on honey bee larvae under laboratory conditions (OECD guidance document 239), ([redacted] 2018; M-625695-01-1).
- Acute contact and oral toxicity of fluopicolide tech. to adult bumble bees under laboratory conditions, ([redacted] 2015; M-519981-01-1 and [redacted] 2015; M-511408-01-1)
- Brood feeding test according to Oomen *et al.* (1992) with the solo formulation fluopicolide SC 486 (using a realistic worse case spray solution concentration and covering exposure for effects on brood (eggs, young and old larvae) and their development, nurse bee on-going behaviour in brood care and colony strength), ([redacted] 2016; M-545732-01-1)
- Two semi-field brood studies following OECD guidance document 75 (using a more realistic spray scenario onto flowering *Phacelia* covering effects on mortality, foraging activity as well as general colony development) with the solo formulation fluopicolide SC 486 (these semi-field studies are presented in KCA Section 8, Point 8.3.1.3/03 and Point 8.3.1.3/04), ([redacted] 2016; M-547124-01-1 and [redacted] 2020; M-685049-01-1)
- Semi-field studies for the determination of residues of fluopicolide and fluoxastrobin in bee relevant plant matrices (nectar and pollen) of winter oilseed rape after seeding of seeds treated with the representative formulation fluopicolide + fluoxastrobin FS 350. Studies were conducted in Germany (C-EU) and Italy (S-EU); these semi-field residue studies are presented in MCP Section 10, Point 10.3.1.5 ([redacted] 2020; M-689241-01-1)

The toxicity tests conducted with the representative formulation fluopicolide + fluoxastrobin FS 350 are presented in this MCP document. The toxicity tests conducted with fluopicolide tech., its bee relevant metabolites M-01 (AE C653711) and M-02 (AE C657188) and the solo formulation fluopicolide SC 486 are presented in MCA, Section 8, Point 8.3.1.

A summary of the critical endpoints of fluopicolide tech., its metabolites M-01 (AE C653711) and M-02 (AE C657188), the solo formulation fluopicolide SC 486 and the representative formulated product Fluopicolide + Fluoxastrobin FS 350 are provided in the following tables. Endpoints shown in bold are considered relevant for risk assessment.

Table 10.3.1- 1: Critical endpoints for Fluopicolide tech. – acute toxicity to adult honey and bumble bees

Test substance	Test species/ study type	Endpoint		References
Fluopicolide tech.	Honeybee, adult, acute, 72 h	LD_{50} – oral	> 241 µg a.s./bee	[redacted] 2012; M-200452-03-1 KCA 8.3.1.1.1/01
	Honeybee, adult, acute, 72 h	LD_{50} – contact	> 100 µg a.s./bee	[redacted] 2012; M-200506-03-1 KCA 8.3.1.1.2/01
	Honeybee, adult, acute, 48 h	**LD_{50} – oral** **LD_{50} – contact**	**> 107.3 µg a.s./bee** **> 100 µg a.s./bee**	[redacted] 2015; M-539964-01-1 KCA 8.3.1.1.1/02
	Bumble bee, adult, acute, 48 h	LD_{50} – oral	> 87.3 µg a.s./bumble bee	[redacted] 2015; M-519981-01-1 KCA 8.3.1.1.1/03
	Bumble bee, adult, acute 48 h	LD_{50} - contact	> 100 µg a.s./bumble bee	[redacted] 2015; M-511408-01-1 KCA 8.3.1.1.2/02

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

Acute toxicity to adult bumble bees

Currently there are no testing requirements for any bee other than for the honey bee within Regulation EU 1107/2009. Nevertheless, acute oral and contact bumble bee studies were conducted with Fluopicolide tech. and the representative formulation Fluopicolide + Fluoxastrobin FS 350 which is presented as additional information (Table 10.3.1-2).

At time of study conduct, both guidelines for testing bumble bees (OECD 246 and OECD 247) were still undergoing the OECD validation process. However, the bumble bee oral and contact toxicity studies with Fluopicolide + Fluoxastrobin FS 350 were performed considering the latest version of the draft OECD guidelines at that point in time. The findings for the formulation indicate comparable or even higher endpoints compared to the acute oral and contact bumble bee study or even compared to the honey bee acute endpoints performed with the active ingredient Fluopicolide tech. Hence, the findings indicate that the bumble bee is not more sensitive to Fluopicolide + Fluoxastrobin FS 350 or Fluopicolide tech. compared to the honey bee.

Table 10.3.1- 2: Critical endpoints for Fluopicolide + Fluoxastrobin FS 350 – acute toxicity to adult honey and bumble bees

Test substance	Test species/ study type	Endpoint		References
Fluopicolide + Fluoxastrobin FS 350	Honeybee, adult, acute, 48 h	LD_{50} – oral LD_{50} – contact	**> 221.0 µg product/bee** **> 200 µg product/bee**	[redacted] 2015; M-524962-01-1 KCP 10.3.1.1.1/01
	Bumble bee, adult, acute, 48 h	LD_{50} – oral LD_{50} – contact	> 470.2 µg product/bumble bee > 400 µg product/bumble bee	[redacted] 2017; M-591409-01-1 KCP 10.3.1.1.1/02

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

Acute toxicity to adult honey bees for bee relevant metabolites

According to Regulation EU 1107/2009 testing of metabolites should be driven by an examination of existing data on other organisms and biological screening. Moreover, the higher exposure level of the parent will compensate for any higher toxicity of the metabolite and therefore the risk will already be covered in the majority of cases. When referring to the EFSA Bee Guidance Document (2013), metabolites exceeding a total radioactive residue (TRR) of 10% or identified as > 0.01 mg/kg in plant metabolism studies should be assessed for risk assessment to bees. The same parameter was chosen to identify the relevant metabolites of fluopicolide in the present case. Moreover, the focus is on metabolites that may occur in pollen and nectar, as these are defined as the major route of exposure.

Several plant metabolism studies were performed with the active fluopicolide and its metabolites using seed, foliar or soil application methods conducted on three crop groups (fruit, leafy and root) (see MCA 6.2.1). In addition, confined rotational crop studies (CRC) performed with the active fluopicolide and its metabolites as soil application were also conducted (see MCA 6.6.1). From these studies, the most relevant plant parts for exposure to bees were identified as oilseed rape seeds, grapes and wheat grain. In these crop parts six metabolites were found to be > 10% TRR or > 0.01 mg/kg as parent equivalents, two further metabolites were formed in other plant parts and no metabolites were unique to the least relevant crop parts for bees (i.e. roots and tubers formed underground). The metabolites found were grouped according to their chemical structures into three groups: similar to parent (meaning covered by parent), M-01 (AE C653711) and M-02 (AE C657188). Hence, the metabolites M-01 (AE C653711) and M-02 (AE C657188) were identified to be the focus for bees in relevant plant parts and were further investigated for toxicity and exposure to bees.

Both bee relevant metabolites (M-01 (AE C653711) and M-02 (AE C657188)) were tested for their acute oral and contact toxicity on honey bees (Table 10.3.1-3). The endpoints for both metabolites are of low toxicity to bees and comparable to the acute oral and contact honey bee study endpoints performed with the active ingredient fluopicolide. These findings indicate that the bee relevant metabolites M-01 (AE C653711) and M-02 (AE C657188) are not to be considered more toxic than the parent. Consequently, the risk for plant metabolites is considered to be covered by the risk assessment for the parent molecule.

Furthermore, residue trials were conducted to support the representative use Fluopicolide + Fluoxastrobin FS 350 in oilseed rape as seed treatment. For these studies residues of both bee relevant metabolites M-01 (AE C653711) and M-02 (AE C657188) were considered and are presented in Table 10.3.1-7. Details of the honey bee testing with the fluopicolide relevant metabolites M-01 (AE C653711) and M-02 (AE C657188) are presented together with the ecotoxicological endpoints in MCA, Section 8, Point 8.3.1.

Table 10.3.1- 3: Critical endpoints for metabolites M-01 (AE C653711) and M-02 (AE C657188) – acute toxicity to adult honey

Metabolite M-01 (AE C653711)	Honeybee, adult, acute, 48 h	LD_{50} – oral LD_{50} – contact	> 100 µg p.m./bee > 80.8 µg p.m./bee	[redacted] 2016; M-571897-01-1 KCA 8.3.1.1.1/04
Metabolite M-02 (AE C657188)	Honeybee, adult, acute, 48 h	LD_{50} – oral LD_{50} – contact	> 110.9 µg p.m./bee > 100.0 µg p.m./bee	[redacted] 2016; M-566365-01-1 KCA 8.3.1.1.1/05

p.m.: pure metabolite

Chronic toxicity to adult honey bees

In the year of study conduct ([redacted] 2016; M-552253-01-1) of the chronic adult honey bee study with fluopicolide SC 486 there was no finalized and adopted test guideline available. However, the study was conducted considering the latest version and recommendations according to [redacted] (2015). The final guideline OECD 245 for testing chronic oral toxicity on adult honey bees was implemented and adopted in October 2017. The performed study by [redacted] (2016) included analytical verification of the active ingredient fluopicolide in the final feeding solution which is also a requirement of the OECD 245. A simple SC formulation was chosen in place of technical material to enable chronic administration of fluopicolide in a 50% sugar solution and to overcome any solubility or palatability issues that may have occurred by using technical fluopicolide and organic solvents.

The endpoint for the solo formulation presented as a.s./bee/day is comparable to the acute oral toxicity endpoint for fluopicolide tech., indicating that there are no signs of accumulated toxicity expected after chronic exposure to the active substance fluopicolide.

Table 10.3.1- 4: Critical endpoints for Fluopicolide SC 486 – chronic toxicity to adult bees

Test substance	Test species	Endpoint		Reference
Fluopicolide SC 486	Honeybee, adult, 10 day feeding test	LDD_{50} NOEDD	> 132.68 µg a.s./bee/day = 132.68 µg a.s./bee/day	[redacted] 2016; M-552253-01-1 KCA 8.3.1.2/01

a.s. = active substance

Effects on honey bee development and other honey bee life stages

The chronic toxicity to larvae of honey bees under laboratory conditions considering emergence after 22 days was performed with fluopicolide tech. following the OECD TG 239 (2016). The findings do not indicate a risk of fluopicolide tech. after repeated feeding of contaminated food to larvae and considering emergence after 22 days. Details of the study are presented together with the ecotoxicological endpoints in MCA, Section 8, Point 8.3.1.

Table 10.3.1- 5: Critical endpoints for Fluopicolide tech. – repeated exposure to honey bee larvae

Test substance	Test species	Endpoint		Reference
Fluopicolide tech.	Honeybee larvae, chronic (emergence after 22 days follow repeated feeding)	NOED	≥ 60.1 µg a.s./larva	[redacted] 2018; M-615695-01-1 KCA 8.3.1.3/01

a.s. = active substance

In order to reveal whether fluopicolide poses a risk to immature honey bee life stages, a bee brood feeding study ([redacted] 2016; M-545732-01-1) was conducted by following the provisions/method of Oomen P.A., de Ruijter, A. & van der Steen, J. (OEPP/EPPO Bulletin 22:613-616 (1992)). Moreover, and to clarify whether fluopicolide poses a risk to honey bee brood and colony development in particular as well as on honey bees in general under realistic worst-case conditions, two higher tier semi-field honey bee brood studies (according to the provisions of the OECD Guidance Document 75) were conducted under forced/confined exposure conditions. One study was conducted in C-EU ([redacted] 2016; M-547124-01-1) and another study was conducted in S-EU ([redacted] 2020; M-685049-01-1) to cover two climatic zones within the EU. All three higher tier studies were conducted with the solo formulation fluopicolide SC 486 (Table 10.3.1-6).

It can be concluded from all three higher tier studies (Oomen et al. 1992 and OECD Guidance Document 75) performed with fluopicolide SC 486, investigating side-effects on immature honey bee life stages, that fluopicolide is of low general intrinsic toxicity to honey bees.

Table 10.3.1- 6: Critical endpoints for Fluopicolide SC 486 – toxicity to bee brood

Test substance	Test species	Endpoint	Reference
Fluopicolide SC 486	Honeybee brood feeding test (Oomen *et al.*, 1992)	No adverse effects were observed on the development of brood (eggs, young and old larvae) and on pupal mortality. Adult bee mortality in the test item treatment group appeared higher compared to the control group. However, since this observation was not consistent amongst replicates it is considered to be random and not of biological relevance. Overall, fluopicolide fed at a concentration of 1.33 g a.s./L sugar solution caused no adverse effects on honey bee colony performance including no indication for negative impacts on brood rearing success.	[redacted] 2016; M-545732-01-1 KCA 8.3.1.3/02
	Honeybee Brood – Semi-Field (OECD GD 75)	Overall, no adverse effects on brood development, adult and pupal mortality, foraging activity, behaviour, colony development and strength after application of 331.6 g product/ha (corresponding to 133 g fluopicolide/ha) onto flowering *Phacelia tanacetifolia*.	[redacted] 2016; M-547124-01-1 KCA 8.3.1.3/03
	Honeybee Brood – Semi-Field (OECD GD 75)	Overall, no adverse effects on brood development, adult and pupal mortality, foraging activity, behaviour, colony development and strength after application of 133 g fluopicolide/ha onto flowering *Phacelia tanacetifolia*.	[redacted] 2020; M-685049-01-1 KCA 8.3.1.3/04

a.s. = active substance

Plant metabolite studies to determine residues in nectar and pollen in oilseed rape

Studies to determine residues in nectar and pollen were performed in winter oilseed rape seeds treated with Fluopicolide + Fluoxastrobin FS 350. Both compounds (fluopicolide and fluoxastrobin) including the two bee relevant metabolites (M-01 (AE C653711) and M-02 (AE C657188)) were included in the analytical verification of sampled material. The studies comprised four separate semi-field residue trials conducted in Germany (C-EU) at a nominal application rate of 12 g fluopicolide/ha and 9 g fluoxastrobin/ha and in parallel, four separate semi-field residue trials conducted in Italy (S-EU) at a nominal application rate of 12 g fluopicolide/ha and 9 g fluoxastrobin/ha, respectively.

The information obtained from this set of studies shows that residue levels of fluopicolide, both bee relevant metabolites (M-01 (AE C653711) and M-02 (AE C657188)) and fluoxastrobin are below the level of detection (LOD) or below the level of quantification (LOQ) in the bee-relevant matrices nectar and pollen of oilseed rape growing from treated seeds with fluopicolide + fluoxastrobin FS 350 (please refer to Table 10.3.1-7 and to CP 10.3.1.5). The data demonstrate that no residues in pollen and nectar that are relevant for the exposure of bees are to be expected from the use of fluopicolide + fluoxastrobin FS 350 as winter oilseed rape seed treatment at a nominal application rate of 12 g fluopicolide/ha and 9 g fluoxastrobin/ha.

Table 10.3.1- 7: Residue determination of Fluopicolide + Fluoxastrobin FS 350 – residue studies in bee relevant matrices – Fluopicolide and relevant metabolites M-01 (AE C653711) and M-02 (AE C657188)

Test substance	Matrices	Residue determination [mg/kg]			Reference
Fluopicolide + Fluoxastrobin FS 350	Nectar	Fluopicolide < 0.010	M-01 (AE C653711) < 0.010	M-02 (AE C657188) < 0.010	[redacted] 2020; M-689241-01-1 KCP 10.3.1.5/01
	Pollen	Fluopicolide < 0.010	M-01 (AE C653711) < 0.010	M-02 (AE C657188) < 0.010-0.012	

LOQ (Limit of Quantification) = 0.01 mg/kg for fluopicolide and its metabolites
LOD (Limit of Detection) = 0.0030 mg/kg (= 30% of the LOQ)

Risk assessment for bees

Although the principal route of exposure to a seed-dressing product for honey bees is not via direct application on the crop (for which the hazard quotients have been validated), an indication about the hazard potential of the seed-treatment product and its individual constituents can be obtained at an initial risk characterisation step, when considering unrealistically that the seed-treatment product would be sprayed on a full-flowering crop during honey bees actively foraging.

The risk assessment for bees for fluopicolide is based on the application rates of 0.06 L prod./ha corresponding to 12 g FLC/ha for applications in winter rape using the endpoints (LD_{50} values) for the formulation FLC + FXA FS 350 and the active substance fluopicolide.

Hazard Quotients

The risk assessment is based on Hazard Quotient approach (Q_H) by calculating the ratio between the application rate (expressed in g a.s./ha or in g total substance/ha) and the laboratory contact and oral LD_{50} (expressed in µg a.s./bee or in µg total substance/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- 8: Hazard quotients for bees – oral exposure

Compound	Oral LD_{50} [µg/bee]	Max. appl. rate [g/ha]	Hazard quotient Q_{HO}	Trigger	*A-priori* acceptable risk for adult bees
FLC + FXA FS 350	> 221.0	69.84 [a)]	< 0.32	50	yes
Fluopicolide	> 107.3	12	< 0.11	50	yes

a) Based on an application rate of 60 mL prod./ha and a product density of 1.164 g/mL

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

Table 10.3.1- 9: Hazard quotients for bees – contact exposure

Compound	Contact LD_{50} [µg/bee]	Max. appl. rate [g/ha]	Hazard quotient Q_{HC}	Trigger	*A-priori* acceptable risk for adult bees
FLC + FXA FS 350	> 200	69.84 [a)]	< 0.35	50	yes
Fluopicolide	> 100	12	< 0.12	50	yes

a) Based on an application rate of 60 mL prod./ha and a product density of 1.164 g/mL

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

Further considerations for the risk assessment

Risk to bees due to systemic exposure

The risk assessment scheme for honey bees to be applied according to the Terrestrial Guidance Document (SANCO/10329/2002 rev 2) is recognized not to be fully sufficient to cover the specificities of seed treatment pesticide uses. The current validated risk assessment scheme in force at the time of the submission of this dossier is that of EPPO PP 3/10 (3) 2010[14]. The default worst case assumptions are for a worst case 95[th] percentile residue present in pollen and nectar (irrespective of the actual application or seed loading rate) being 1 mg a.s./kg (i.e. 1 µg a.s./g) that should be used for a screening level risk assessment. However, for the use of Fluopicolide + Fluoxastrobin FS 350 as a seed treatment in winter oilseed rape, measured residue values in nectar and pollen generated under worst-case experimental conditions are available and can be used in place of the default value (see Table 10.3.1-7).

Exposure estimates

Studies have been performed ([redacted] (2017); M-688587-01-1 see MCP10.3.1/02) with oilseed rape varieties measuring amongst other parameters the sugar concentration and determined the mean concentration of nectar sugars across all oilseed plant varieties as 32.4 % w/w. Moreover, a literature search ([redacted] (2019); M-688592-01-1 see MCP10.3.1/03) compiled a data set of individual measurements of sugar concentration in nectar for bee pollinated flowers. Consumption of nectar by bees is driven by sugar content with bees typically preferring nectar in the range of 35 – 65% w/w. [redacted] (2019; M-688592-01-1 see MCP10.3.1/03) determined the sugar content of oilseed rape with a mean of 40% w/w which is within this optimal range of 35 – 65% w/w sugar nectar

[14] EPPO 2010. Environmental risk assessment scheme for plant protection products. Chapter 10 Honey bees. OEPP/EPPO, Bulletin OEPP/EPPO Bulletin 40, 1–9.

concentration. Moreover, two residue studies performed in winter oilseed rape determined among residue parameters in nectar and pollen the sugar content in nectar from forager bees (M-689241-01-1). For this at least 12 forager bees were sampled from the control group on six occasions within 4 trials and the sugar content was determined in % Brix. The measured sugar content ranged from 14.3 to 63.8% w/w with a mean of 35.5% w/w supporting the concentration determined by [redacted] 2017, M-688587-01-1 see MCP 10.3.1/02) and being within the optimal range of 35 – 65% w/w cited in [redacted] (2019, M-688592-01-1 see MCP10.3.1/03).

In the following the "worst-case" concentration of 32.4% w/w for oilseed rape as cited by [redacted] (2017, M-688587-01-1 see MCP 10.3.1/02) was used to define the sugar content for forager bees.

A crop like winter oilseed rape is highly attractive to bee and produces large quantities of nectar and pollen used for food. Forager bees represent the worst-case exposure scenario for adult bees due to their high sugar consumption. In comparison to forager bees, nurse bees have a much lower sugar consumption and thus, are covered by the exposure scenario performed for foraging adult bees. Larval bees are fed worker jelly via nurse bees in the hive for their first few days and receive some nectar and pollen later. The typical diet of forager bees and larval bees is given in Table 10.3.1-10.

Table 10.3.1- 10: Worst case pollen and sugar consumption levels

Type of Honey bee	Main exposure Location	Sugar consumption	Pollen consumption	Notes
Forager	Outside and within the colony	Up to 103.7 mg /day	Negligible	320 mg nectar (assuming 32.4% sugar content)
Larva (worker)	Within the colony	59.4 mg / 5 days	1.5 – 2 mg pollen / 5 days	148.5 mg nectar (assuming 32.4% sugar content) On days 1-3 larvae are fed royal jelly. Pollen (and nectar) are fed on day 4 and 5 only

Studies measuring residues of fluopicolide and its bee relevant metabolites in nectar and pollen of winter oilseed rape were performed in C-EU and S-EU (nominal seed treated application rate of 12 g fluopicolide/ha and 9 g fluoxastrobin/ha, respectively) (M-689241-01-1). The Limit of Quantitation (LOQ) defined as the lowest validated fortification level, was 0.010 mg/kg for for fluopicolide and its metabolites M-01 (AE C653711) and M-02 (AE C657188) (please refer to CP 10.3.1.5). Residues of fluopicolide, its metabolites M-01 (AE C653711) and M-02 (AE C657188) in the treated nectar samples were below the LOQ (LOQ= 0.010 mg/kg) for all samplings in all trials. Residues of fluopicolide and its metabolites M-01 (AE C653711) in the treated pollen samples were below the LOQ (LOQ= 0.010 mg/kg) for all samplings in all trials. Residues of the metabolite M-02 (AE C657188) ranged between < 0.010 -0.012 mg/kg. In trial -01 residues of M-02 (AE C657188) were detected at the 1st and 2nd sampling (0 and 1DAS1 (DAS1= Days after Sampling 1)) with 0.012 and 0.010 mg/kg, respectively. In all subsequent sampling's residues were below LOQ (< 0.010 mg/kg).

In conclusion, the data of the residue studies show that no residues in pollen and nectar relevant for the exposure of bees, of fluopicolide and its metabolites M-01 (AE C653711) and M-02 (AE C657188) are to be expected from the use of Fluopicolide + Fluoxastrobin FS 350 as winter oilseed rape treatment at a nominal application rate of 12 g fluopicolide/ha and 9 g fluoxastrobin/ha. The two bee relevant metabolites M-01 (AE C653711) and M-02 (AE C657188) were found at equal residue levels as its parent fluopicolide and accordingly, the risk is considered to be covered by the parent fluopicolide.

The nectar and pollen consumption rates as presented in Table 10.3.1-10 are used for the calculation of the following risk assessment scenarios which cover the risk to bees due to the use of Fluopicolide + Fluoxastrobin FS 350 as a seed treatment for oilseed rape. For completeness and as honey bee acute data for both relevant metabolites M-01 (AE C653711) and M-02 (AE C657188) are available, the risk assessment on nectar and pollen consumption rates is also presented in the following tables.

Table 10.3.1- 11: Estimated worse-case exposure levels to fluopicolide M-01 (AE C653711) and M-02 (AE C657188) as oilseed rape seed treatment

Compound	Type of honey bee	Nectar consumption (g)	Pollen consumption (g)	Residue level*	Exposure (µg/bee)
Fluopicolide	Forager bees	0.32 g / day	Negligible	0.01 µg a.s./g	0.0032 µg/bee/day
	Larva (worker)	0.1485 g / 5 days	0.002 g (on day 4 and 5)		0.0015 µg /bee
M-01 (AE C653711)	Forager bees	0.32 g / day	Negligible	0.01 µg p.m./g	0.0032 µg/bee/day
M-02 (AE C657188)	Forager bees	0.32 g / day	Negligible		0.0032 µg/bee/day

a.s. = active substance; p.m. = pure metabolite

A generic risk assessment based on these exposure values is presented in the following table for the active substance fluopicolide and its metabolites M-01 (AE C653711) and M-02 (AE C657188). Appropriate endpoints are for the individual active substance and the metabolites M-01 (AE C653711) and M-02 (AE C657188) as bees will not be exposed to the formulation. According to EPPO 2010 a Toxicity Exposure Ratio trigger of 10 is applied to acute endpoints (LD_{50}) to cover both acute and chronic risks. Where a chronic endpoint is available a trigger of 1 may be used for a No Observable Effect Daily Dose (NOEDD). For larval bees the risk assessment is conducted for the NOED endpoint to cover the entire larval development period from larva to adult.

Table 10.3.1- 12: Systemic risk to bees for fluopicolide due to oilseed rape seed treatment

Type of honey bee	Substance	Risk	Endpoint (µg a.s./bee)	Exposure (µg a.s./bee)	Toxicity Exposure Ratio (TER)	EPPO (2010) Trigger
Forager bees	Fluopicolide	Acute	LD_{50} > 241 µg a.s.	0.0032	> 75313	10
		Chronic	NOED > 132.68 µg a.s.	0.0032	> 41462	1
Larvae		Dietary	NOED ≥ 60.1 µg a.s.	0.0015	> 40067	1
Forager bees	M-01 (AE C653711)	Acute	LD_{50} > 100 µg p.m.	0.0032	> 31250	10
Forager bees	M-02 (AE C657188)	Acute	LD_{50} > 110.9 µg p.m.	0.0032	> 34656	10

a.s. = active substance; p.m. = pure metabolite

The calculated TER values range from >40067 to >75313 and greatly exceed the EPPO 2010 triggers for fluopicolide indicating a high margin of safety to bees. For the metabolites M-01 (AE C653711) and M-02 (AE C657188) the calculated TER values are >31250 and >34656, respectively and also greatly exceeding the trigger of 10 for the acute risk indicating that no risk is to be expected from residues of the metabolites M-01 (AE C653711) and M-02 (AE C657188) when applied as a seed treatment using Fluopicolide + Fluoxastrobin FS 350.

The exposure of bees to residues in pollen and nectar from the seed treated product as well as to the active substance fluopicolide or its metabolites is to be expected to be negligible under natural field conditions.

Furthermore, higher tier studies with honey bees were conducted to exclude effects on colony development and honey bee brood as well as on honey bees in general under realistic worst-case conditions. In order to reveal whether fluopicolide poses a risk to immature honey bee life stages, a bee brood feeding study ([redacted] 2016; M-545732-01-1, KCA 8.3.1.3/02) has been conducted by following the provisions/method of Oomen P.A., de Ruijter, A. & van der Steen, J. (OEPP/EPPO Bulletin 22:613-616 (1992)), which require, amongst other parameters to "…use formulated products only… products are fed at a concentration recommended for high-volume use…". The honey bee brood feeding test is a worst-case screening test, by feeding the honey bees directly in the hive with a treated sugar solution which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration) and by investigating the development of eggs, young and old larvae by employing digital photo imaging technology.

This study was conducted with Fluopicolide SC 486. The administration of fluopicolide at a concentration of 1.33 g a.s./L to honey bee colonies via feeding of 1 litre spiked sucrose solution has neither resulted in adverse effects on brood performance and showed no indication for negative impacts on brood rearing success compared to a control group fed with untreated sucrose solution, respectively. Regarding brood development, the brood termination rates of the test item treatment were overall on a low level with 15.3, 9.8 and 6.5% for eggs, young larvae and old larvae, respectively, which were not statistically significant different to the control with brood termination rates of 11.5, 6.8 and 10.2% for eggs, young larvae and old larvae, respectively at the end of the brood observation period.

Moreover, and in order to clarify whether fluopicolide poses a risk to honey bee brood and colony development in particular as well as on honey bees in general under realistic worst-case conditions, two higher tier semi-field honey bee brood studies, one in C-EU and one in S-EU (according to the provisions of the OECD Guidance Document 75), were conducted under forced/confined exposure conditions using the formulation fluopicolide SC 486, by application of 133 g a.s./ha under tunnel conditions to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia* ([redacted] 2016; M-547124-01-1, KCA 8.3.1.3/03 and [redacted] 2016; M-685049-01-1, KCA 8.3.1.3/04).

Both studies included three treatment groups: Control (tap water), Test item (133 g a.s./ha) and Reference item (300 g fenoxycarb/ha) with all applications being carried out with a spray volume of 400 L water/ha. For all treatment groups, four replicates (tunnels) were set up. The application of all treatment groups was conducted during daily bee flight activity at the time of full flowering of the crop. Thereafter, the bees were kept for 7 days within the tunnels (confined exposure phase) and were then relocated out of the tunnels and transferred to a monitoring site without flowering crops and intensive agricultural area for further monitoring. Daily, throughout the confined exposure phase, mortality of worker bees, larvae and pupae was assessed along with assessments of foraging activity and behaviour. Daily mortality assessments were continued along with behaviour around the hive during the post-exposure observation period. Colony assessments (food stores, brood areas, colony strength) were made before confinement, after confinement and at the end of each study. Detailed brood assessments (brood termination rate, brood index and brood compensation index) by employing digital photo imaging technology, investigating the fate of more than 200-250 individually marked cells was performed on 5 occasions throughout the study, covering an entire brood cycle of honey bees. Overall, no adverse effects on brood development, adult and pupal mortality, foraging activity, behaviour, colony development and strength after application of 133 g fluopicolide/ha onto flowering *Phacelia tanacetifolia* were observable for both studies.

Hence, from the higher tier tests performed with the formulation Fluopicolide SC 486 investigating side-effects on immature honey bee life stages it can be concluded that fluopicolide is of low general intrinsic toxicity to honey bees.

Risk to bees due to exposure to guttation water

Honey bees are specific in their requirement for water to cool the hive and also to dilute concentrated honey stores. Other bees do not require water for these purposes and get their water from their diet (nectar). The occurrence of guttation droplets is highly dependent upon systemic properties, soil and air humidity and the type of crop. Bees use a wide variety of sources for water collection. As water does not offer an energetic reward (unlike nectar foraging) bees will optimize their collection to nearby sources such as dew located in the immediate vicinity of the fields. Also, typically only a small proportion of bees (5%) will collect water ([redacted] 2014, M-648268-01-1 see MCP10.3.1/04). Overall guttation has been shown to not be a significant route of exposure to pesticides for bees ([redacted] 2015; M-647265-01-1 see MCP 10.3.1/05).

A worst-case assessment of risk due to consumption of guttation water can be based on the water solubility of each active substance at a pH of 5 - 7. This is an appropriate range for pH of guttation water from plants ([redacted] 1966, M-329396-01-1 see MCP 10.3.1/06). These exposure estimates are also a worst-case estimate. The water solubility for fluopicolide is 2.8 mg/L (see MCA 2.5). Based on a daily water consumption of 11.4 µL/bee/day and water solubility the risk to bees can be assessed by calculating TER value according to EPPO PP 3/10 (3) 2010.

Table 10.3.1- 13: Systemic risk to bees due to guttation

Type of honey bee	Substance	Risk	Endpoint (µg a.s./bee)	Exposure (µg a.s./bee)	Toxicity Exposure Ratio (TER)	EPPO (2010) Trigger
Water bee	Fluopicolide	Water consumption	LD_{50} > 241	0.032	> 7531	10

The calculated TER value exceeds the trigger of 10 as used for other routes of exposure in EPPO 2010 and thus indicates no unacceptable to bees for fluopicolide.

<u>Risk to bees due to exposure to dust drift</u>

At present there is no validated and adopted risk assessment scheme for bees available in the EU that covers exposure to dust drift. However, the acute endpoints available for the active substance fluopicolide as well as for the representative formulation Fluopicolide + Fluoxastrobin FS 350 indicate that contact and oral toxicity to honey and bumble bees is low.

For oilseed rape seeds the exposure of bees to dust drift is generally considered to be low since seed treatment by incrustation results in an almost spherically shape that is less vulnerable to mechanical abrasion. Information supporting this fact is available from an adherence test performed with fluopicolide + fluoxastrobin FS 350 on oilseed rape seeds ([redacted] 2017; M-599012-01-1, please refer to KCP 10.3.1/01). The respective loading (contents of a.s.) on the seeds was measured before they were stressed by pouring through a funnel falling on a slide and after the seeds had undergone this procedure. The result of the test indicated that the loss due to mechanical stress was minimal with a difference in percentage of 0.4% for fluopicolide and 0.1% for fluoxastrobin. These findings support the fact of low vulnerability to mechanical abrasion during sowing and allow concluding on a negligible exposure of bees via dust drift.

Moreover, the already mentioned and available higher tier semi-field honey bee brood studies performed according to the OECD Guidance Document 75 can be used to demonstrate that a spray application rate of 133 g fluopicolide/ha under tunnel conditions to the full flowering and highly bee attractive surrogate crop *Phacelia tanacetifolia* does not pose a risk to honey bee brood and colony development in particular as well as on honey bees in general under realistic worst-case conditions ([redacted] 2016; M-547124-01-1, KCA 8.3.1.3/03 and [redacted] 2016; M-685049-01-1, KCA 8.3.1.3/04). For both studies a rate of 133 g a.s./ha was applied as spray application of the formulation fluopicolide SC 486, exceeding the seed application rate of 12 g fluopicolide/ha implemented for the residues studies in oilseed rape (M-689241-01-1). Even when considering the loss due to mechanical stress of 0.4% for fluopicolide, the spray application rate of 133 g fluopicolide/ha is much higher and represents a worst-case exposure scenario for bees applied as spray application inside confined tunnel systems.

Considering this, the risk to bees due to dust drift exposure is considered as minimal and sufficiently covered with the available higher tier and residue studies.

Overall conclusions for bees

The calculated Hazard Quotients based on the empirical exposure levels of 12.0 g a.s./ha for fluopicolide are well below the validated trigger value. Furthermore, for the formulated product at 60.0 mL product/ha for Fluopicolide + Fluoxastrobin FS 350, the calculated Hazard Quotients are also well below the validated trigger. Although not fully validated for a seed treatment these HQ calculations give an appreciation of the low risk to bees due to the use of Fluopicolide + Fluoxastrobin FS 350 in winter oilseed rape.

However, this risk assessment was considered too simplistic to fully cover all concerns and consequently a risk assessment for systemic products as provided in EPPO PP 3/10 (3) 2010 was conducted which included potential routes of exposure such as via systemic residues and guttation water and indicated that there was no unacceptable risk to bees due to the use of Fluopicolide + Fluoxastrobin FS 350 as a seed treatment for winter oilseed rape.

Overall, it can be concluded that fluopicolide when used as a seed treatment in winter oilseed rape in the product Fluopicolide + Fluoxastrobin FS 350 at the maximum application rate of 12.0 g a.s./ha for fluopicolide does not pose an unacceptable risk to honey bees and honey bee colonies.

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Data Point:	KCP 10.3.1/01
Report Author:	[redacted]
Report Year:	2017
Report Title:	Determination of seed loading, adherence test and uniformity of distribution of fluopicolide + fluoxastrobin FS 350 (200+150 g/L) on rape seed before and after storage - Packaging material: paper
Report No:	FM0247(SSF94)N01
Document No:	M-599012-01-1
Guideline(s) followed in study:	Regulation (EC) No 1107/2009, Commission Regulation (EU) 284/2013
Deviations from current test guideline:	Data reported met the Regulation (EC) No 1107/2009 and Commission Regulation (EU) 284/2013 requirements and no deviations were made.
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

An adherence test was performed with FLC+FXA FS 350 on oilseed rape seeds. The respective loading (contents of a.s.) on the seeds was measured before they were stressed by pouring through a funnel falling on a slide and after the seeds had undergone this procedure. The result of the test indicated that the loss due to mechanical stress was minimal with a difference in percentage of 0.4% for fluopicolide and 0.1% for fluoxastrobin.
Based on these results the treated seed batches are assessed to withstand normal handling, storage and sowing and to deliver appropriate efficacy data.

I. MATERIAL AND METHODS

Test item: fluopicolide + fluoxastrobin FS 350 (200+150 g/L); Specification number: 102000028578, Material number: 84095370, Lot: 2014-014396-01; Certificate of analysis: TOX10774-00.

The determination of the shelf life was carried out by means of a storage test. In this method the seeds were stored 18 months in paper bags in a locker at ambient temperature. The treatment, loading, adherence and uniformity of distribution in this study were performed with rape seeds as this is a typical crop proposed for use of this preparation.

3 kg of rape seeds were treated with 35.1 g of the formulation [corresponding to 200 g fluopicolide and 150 g fluoxastrobin / dt (1000 mL formulation / dt) (1 dt = 100 kg)] according to the treatment procedure described in CIPAC MT 175. The treatment procedure was carried out in a laboratory seed treatment machine type "Niklas WN5". The seeds were taken for the determination of seed loadings and adherence tests.

The determination of the adhesion to seeds was carried out by means of a standardised test procedure. In this method the ratio of the seed loading before and after the adherence test was determined. Before the seeds were stressed under standardised conditions the seed loading with fluopicolide and fluoxastrobin was determined. For the stress test, 90 g of the treated seeds were poured through a funnel falling on a slide. When the slide was opened, the seeds fell a further distance onto a sieve and loose material was separated. The seeds were again stressed in the same manner for a total of five passes through the apparatus. Following this procedure three stressed samples were obtained. A seed loading analysis was performed according to method AM023414MF1 LC/ESTD (sample preparation: approximately 12 g seeds + 100 mL acetone/water (8/2, v/v) extraction time 30 min in an ultrasonic bath) and the retention of the seed treatment formulation on the stressed samples was compared with that of the unstressed sample. The ratio of the seed loading before and after the adherence test was calculated.

II. RESULTS AND DISCUSSION

Test / Method	Initial	18 months at ambient temperature
adherence CIPAC MT 194	on rape seeds	
fluopicolide	99.6 %	93.9 %
fluoxastrobin	99.9 %	94.1 %
distribution to seeds CIPAC MT 175	on rape seeds	
distribution	acceptable	acceptable

Adherence of fluopicolide on rape seeds (initial)	
Seed loading before adherence test:	102.9 % (mean value of double determination)
Seed loading after adherence test:	102.5 % (mean value of triple determination)
Adherence (ratio before and after adherence test): 99.6 %	

Adherence of fluoxastrobin on rape seeds (initial)	
Seed loading before adherence test:	102.7 % (mean value of double determination)
Seed loading after adherence test:	102.6 % (mean value of triple determination)
Adherence (ratio before and after adherence test): 99.9 %	

Adherence of fluopicolide on rape seeds (after 18 months storage)	
Seed loading before adherence test:	103.5 % (mean value of double determination)
Seed loading after adherence test:	97.2 % (mean value of triple determination)
Adherence (ratio before and after adherence test): 93.9 %	

Adherence of fluoxastrobin on rape seeds (after 18 months storage)	
Seed loading before adherence test:	104.2 % (mean value of double determination)
Seed loading after adherence test:	98.1 % (mean value of triple determination)
Adherence (ratio before and after adherence test): 94.1 %	

A content greater than 100 % could be observed. This is due to a slightly different distribution of the formulation on the seeds as a result of the treatment procedure. Nevertheless, all values were found in their acceptable limits.

III. CONCLUSION

In an adherence test was performed with FLC+FXA FS 350 on oilseed rape seeds the loss due to mechanical stress was minimal with a difference in percentage of 0.4% for fluopicolide and 0.1% for fluoxastrobin. Based on these results the treated seed batches are assessed to withstand normal handling, storage and sowing and to deliver appropriate efficacy data.

Assessment and conclusion by applicant:

Treated seed batches with FLC+FXA FS 350 (corresponding to 200 g fluopicolide and 150 g fluoxastrobin / dt (1000 mL formulation / dt) (1 dt = 100 kg)) on oilseed rape seeds withstand normal handling, storage and sowing and deliver appropriate efficacy data.

Data Point:	KCP 10.3.1/02
Report Author:	[redacted]
Report Year:	2017
Report Title:	Oilseed rape (brassica napus) as a resource for farmland insect pollinators: quantifying floral traits in conventional varieties and breeding systems
Report No:	M-688587-01-1
Document No:	M-688587-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The study investigated the 24-h nectar secretion rate of oilseed rape (OSR) representing open-pollinated (OP), genic male sterility (GMS) hybrid and cytoplasmic male sterility (CMS) hybrid breeding systems. In March 2013, seeds of all varieties were sown in trays containing a standard compost mix. Plants were inspected daily, in June, to record the day on which each began to flower. 24-h secretion rate was used for analysis. Flowers produced nectar with a mean of 2.38 (geometric mean 241.7 µg sugar) ± 0.020 (N = 146) in 24 h. The mean volume of nectar secreted by the inner nectaries per flower across all OSR varieties over 24 h was 0.90 (0.82 µL) ± 0.021 (N = 150). The mean concentration of nectar sugars across all plants was 324 ± 6.6 µg/µL (32.4% w/w; N = 148).

I. MATERIAL AND METHODS:

A. Material

No test item used as nectar secretion rate was measured.

B. Study design and methods

1. Test procedure

Test system (study type):	Twenty-four commercially available, certified varieties of winter OSR, comprising eight open-pollinated (OP), seven cytoplasmic male sterility (CMS) hybrids and nine (genic male sterility) GMS hybrids.
Test conditions:	In March 2013, seeds of all varieties were sown in trays containing a standard compost mix. Seedlings were vernalized at the 3–4 leaf stage for 8 weeks at 5 °C, and seven plants of each variety were individually re-potted to 21 cm diameter (4 L) pots, containing fresh standard compost mix. The potted plants were then evenly arranged in a randomized complete block (RCB) design, with seven blocks, in a glasshouse at a mean density of 8.5 pots m^2.
Environmental conditions:	An automated system watered plants twice daily, while supplementary lighting and heating were provided to ensure irradiance of at least 100 µmol $m^{-2}s^{-1}$ from 05:00 to 21:00 and temperatures of at least 18 °C during the day, and 14 °C at night.

2. Observations and measurements

Biological parameters measured:	Nectar production

3. Sampling

Sampling technique:	Nectar was sampled from flowers of the same age. Plants were inspected daily, in June, to record the day on which each began to flower. On each day, petals of all open flowers were marked with a permanent ink pen to ensure these older flowers were not used for nectar sampling. The plants were visited 24 h later, and the nectar was carefully removed from any flowers that had opened since the previous day by draining the inner nectaries using microcapillary tubes. As outer (median) nectaries only secrete c. 5% nectar due to reduced phloem vascularization, nectar production was quantified from the inner (lateral) nectaries only. Nectar was then allowed to accumulate in these flowers for 24 h, prior to being sampled to measure 24-h secretion rate.
Sampling material:	24-h secretion rate
Transport/ storage if samples:	Microcapillary tubes were immediately stored in 1.5 mL Eppendorf tubes placed on ice inside a cool box before being transferred to a freezer set at - 20 °C.

4. Chemical analysis

Method:	High-performance liquid chromatography (HPLC).

5. Statistical analysis

Method:	The mean secretion of nectar per flower in 24 h, expressed as total sugar mass, volume, total sugar concentration and fructose/ glucose ratio, for all varieties except DK Sequoia, was compared among varieties using a linear mixed model (LMM) fitted using restricted maximum likelihood (REML), with block and sample date as the (crossed) random factors, to allow for environmental differences among sampling dates as well as differences associated with plant location in the glasshouse.

II. RESULTS AND DISCUSSION:

Nectar was secreted by flowers of all 23 varieties included in this analysis. Across these varieties, flowers produced nectar with a mean of 2.38 (geometric mean 241.7 µg sugar) ± 0.020 (N = 146) in 24 h. Per flower sugar mass differed among breeding systems, with more produced by GMS hybrid varieties than by the CMS hybrid and OP varieties. There were no differences in the mass of sugar per flower within any of the breeding systems. The mean volume of nectar secreted by the inner nectaries per flower across all OSR varieties over 24 h was 0.90. The mean concentration of nectar sugars across all plants was 324 ± 6.6 µg/µL (32.4% w/w; N =148), and differences were not found among or within any of the breeding systems. The majority of the sugar detected in OSR nectar was glucose (57.7% by mass), followed by fructose (41.7%) and sucrose (0.7%).

III. CONCLUSION:

The mean concentration of nectar sugars across all plants was 32.4% w/w.

Assessment and conclusion by applicant:
This study is considered supportive for use in risk assessment. The mean concentration of nectar sugars across all plants was 32.4% w/w

Data Point:	KCP 10.3.1/03
Report Author:	[redacted]
Report Year:	2019
Report Title:	The nectar report: quantitative review of nectar sugar concentrations offered by bee visited flowers in agricultural and non-agricultural landscapes
Report No:	M-688592-01-1
Document No:	M-688592-01-1
Guideline(s) followed in study:	not applicable
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	No, not conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Supportive only

Executive Summary

The literature search compiled a data set of individual measurements of sugar concentration in nectar for bee pollinated flowers. Overall nectar concentration in all regions were comparable around a median value of 40% sugar concentration and no significant differences between crop, weed or wild plant communities were found globally or within the different geographic regions.

I. MATERIAL AND METHODS:

A. Material

No test item used data of literature research were compiled.

B. Study design and methods

1. Test procedure

Data collection and categorization: In late 2017 and early 2018 the literature research for records on nectar quality in bee pollinated flowers using ISI web of knowledge and google scholar was done. Search terms: flower and nectar and sugar concentration adding either pollinator or bee as additional search term were used.

Plant selection: Plant species were categorized as bee visited if either bee pollination was directly observed or the flowers were explicitly classified as "melittophily" based on their floral characteristics by the study authors. In addition, we used the USDA pollinator manual (McGregor, 1976[15]) and the expertise of plant experts for cross validation of the derived classifications.

Categorization: Cultivated crops and non-cultivated crops (classified as weeds or wild plants)

2. Observations and measurements

Biological parameters measured: sugar content

3. Statistical analysis

Method: Statistical analysis and graphs generation were conducted in R v. 3.3.3.

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[15] McGregor SE. 1976. Insect pollination of cultivated crop plants. Washington, D.C.: Agricultural Research Service, US Department of Agriculture.

II. RESULTS AND DISCUSSION:

Overall nectar concentration in all regions were comparable around a median value of 40% sugar concentration and no significant differences between crop, weed or wild plant communities were found globally or within the different geographic regions. In contrast to the median concentrations the three plant communities differed in the variability of nectar quality. This effect is mainly driven by an increased variability of the wild community which differs significantly from the crop community on a global level, with a similar trend in the same direction when compared to the weed community. In contrast crop and weed species clearly do not differ in terms of their variability. Comparing the variability of nectar quality on a species level only a limited number of species with multiple nectar measurements (N >2) was recorded. No indication of intrinsic difference in variability (measured as SD) of plant species belonging to the three different plant communities was found.

III. CONCLUSION:

Overall nectar concentration in all regions were comparable around a median value of 40% sugar concentration and no significant differences between crop, weed or wild plant communities were found globally or within the different geographic regions.

Assessment and conclusion by applicant:

This study is considered supportive for use in risk assessment. Overall nectar concentration in all regions were comparable around a median value of 40% sugar concentration.

Data Point:	KCP 10.3.1/04
Report Author:	Couvillon, M.; Schuerch, R.; Ratnieks, F.
Report Year:	2014
Report Title:	Waggle dance distances as integrative indicators of seasonal foraging challenges
Report No:	M-648268-01-1
Document No:	M-648268-01-1
Guideline(s) followed in study:	-
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Supportive only

Executive Summary

The study investigated 5097 waggle dances to track seasonal changes in foraging, as indicated by the distance to which the bees as economic foragers will recruit, over a representative rural-urban landscape. The nectar sugar concentration was also determined. The mean foraging distance/area significantly increased from springs (493 m, 0.8 km^2) to summers (2156 m, 15.2 km^2), even though nectar is not better quality, before decreasing in autumns (1275 m, 5.1 km^2). As bees will not forage at long distances unnecessarily, this suggests summer is the most challenging season, with bees utilizing an area 22 and 6 times greater than spring or autumn.

I. MATERIAL AND METHODS:

- **A. Material**

1. Test material

No test material used waggle dance distances were monitored.

2. Test area

Location: observation hives at the University of Sussex

3. Test organism(s)

Species: *Apis mellifera mellifera*

B. Study design and methods

1. Test procedure

Duration of study: August 2009 to July 2010 and August 210 to July 2011

Test conditions: The bees were allowed to forage naturally, and the potential foraging range contained a wide diversity of land types. Within a 4 km radius of the hives, these included agricultural land (62%, including both arable, improved grassland), urban and suburban areas (21%, including gardens, allotments, and built-up areas), broadleaved woodlands (10%), and unimproved grassland (7%).

Individuals per replicate: 5000 worker bees

Feeding: Hives sometimes require food supplementation with sugar solution during periods of nectar dearth (e.g., July or in early spring when bad weather precludes foragers from collecting food for several consecutive days).

2. Observations and measurements

Biological parameters measured: - Determining nectar sugar content:
During 2012, 10 returning foragers from two observation hives on days when the bees were actively foraging (113 days from March to October) were collected and chilled. The immobile bees had gentle pressure applied to their abdomens to cause them to regurgitate some of the nectar in their crop. Using a pipette, this was transferred to a handheld refractometer designed for small volumes to determine total sugar concentration (% w/w, °Brix). Readings of 0% indicate water collection, which were not included in the analysis. Water was a rare occurrence (< 5%), as English summers are not overwarm.
- Determining the effect of temperature on foraging distance:
To determine if temperature affected foraging distance, the daily maximum temperature for all study days (August 2009–August 2011) from Herstmonceux, which is the nearest weather station (approximately 27 km) and situated in a meteorologically similar location were used.
- Plotting dances as probability distributions

II. RESULTS AND DISCUSSION:

The mean foraging distances communicated by the dances vary significantly with month in both years. This variation shows a general pattern with significantly greater distances in summers than in early springs and autumns. Summer is also the warmest season, but temperature was a non-significant predictor of distance. The calculated foraging area used by the bees in the summer (August 2009) was 22 and 26 times greater than early spring (March 2010) at the 90th and 50th percentiles, respectively. In July 2010, the calculated foraging area was 14 and 26 times greater than early spring (March 2011) at the same percentiles. Together, this gives a 22-fold average ratio in foraging area for summer vs. early spring over the two years. The calculated foraging area used by colonies in summer (August 2009) was also 2 and 3 times greater than autumn (October 2009) at the 90th and 50th percentiles, respectively. In July 2010, the calculated foraging area was 6 and 14 times greater than autumn (October 2010) at the same percentiles. Together, this gives a 6-fold average ratio in foraging area for summer vs. autumn over the two years. The data also show that summer is also a season when nectar sugar content is not significantly higher. Sugar content (%) is a correlative measure of nectar quality, as sweeter nectar contains more energy, and bees have evolved great sensitivity to this metric. In June, July and August, the median and range of sugar content is low. The median sugar content is also low in March and April. However, spring sugar concentration range is wide, showing that better quality nectar is also available (and at closer distances) to foragers. Taken together, the data show that in summer compared to spring or autumn, the bees fly further to bring back nectar that is not better in quality.

III. CONCLUSION:

The mean foraging distance/area significantly increase from springs (493 m, 0.8 km^2) to summers (2156 m, 15.2 km^2), even though nectar is not better quality, before decreasing in autumns (1275 m, 5.1 km^2). As bees will not forage at long distances unnecessarily, this suggests summer is the most challenging season, with bees utilizing an area 22 and 6 times greater than spring or autumn.

<u>**Assessment and conclusion by applicant:**</u>
This study is considered supportive for use in risk assessment.

Data Point:	KCP 10.3.1/05
Report Author:	Nikolakis, A.; Keppler, J.; Miles, M.; Schoening, R. E. O. P. A.; Pistorius, J.
Report Year:	2015
Report Title:	Neonicotinoid seed treatment products - occurrence and relevance of guttation for honeybee colonies [Conference poster]
Report No:	M-647265-01-1
Document No:	M-647265-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Supportive only

Executive Summary

Field studies to determine the occurrence and effect of exposure to guttation water from neonicotinoid seed treatment products were conducted over a number of years in Germany and France. Studies focused on winter cereals, winter oil seed rape, sugar beet, maize and potato seed. Seeds were treated with a combination of imidacloprid (IMD) + clothianidin (CTD) at a rate of 55 g total neonicotinoid a.s./100 kg seeds. In this experiment fields were sown so that there was about 110 g total neonicotinoid/ha via seed-treated winter cereals, about 30 g CTD/ha via seed-treated winter oil seed rape, about 120 g total neonicotinoid/ha via treated sugar beet pills and about 50 g CTD/ha via seed-treated maize. For potato, IMD was applied at the rate corresponding to about 180 g a.s./ha via an in-furrow treatment at planting. At control sites seeds of the same crop variety as at the treated sites were

sown but were not treated with neonicotinoid seed - or soil treatment products. In the studies with winter barley, winter oil seed rape and maize, honeybee colonies were present directly adjacent at the edge of fields at the time of sowing and were as such also exposed to seed - treatment dust, generated during the sowing operation.
It can be concluded that exposure of honeybee colonies to guttation fluid, did not pose an unacceptable acute or chronic risk to honeybee colony development or survival. Overall, guttation water from seed-treated crop plants was found not to be a significant exposure route for honeybees.

I. MATERIAL AND METHODS:

- **A. Material**

1. Test material

Test item:	imidacloprid (IMD) + clothianidin (CTD)
Active substance(s):	imidacloprid (IMD) + clothianidin (CTD)

2. Test area

Location:	Germany and France
Crops:	Winter cereals, winter oil seed rape, sugar beet, potato and maize
Crop growth stage at treatment:	Seed treatment

3. Test organism(s)

Species:	Honeybee colonies

B. Study design and methods

1. Test procedure

Test system (study type):	Field study
Treatments:	Cereal seeds were seed-treated with a combination of imidacloprid (IMD) + clothianidin (CTD) at a rate of 55 g total neonicotinoid a.s./100 kg seeds. Winter oil seed rape seeds were treated with CTD at a rate of 7 g a.s./kg. Sugar beet seeds were prepared as pills with a combination of IMD + CTD corresponding to a rate of 0.9 mg total neonicotinod/pill. For maize, the seeds were seed-treated with CTD at a rate of 0.5 mg a.s./seed.
Number of replication and colonies:	The winter cereal study was replicated five times with five honey bee colonies (in total, 25 colonies in treatment and control, respectively). Studies in sugar beet and potatoes consisted of two neonicotinoid treated and untreated plots, each with eight honey bee colonies per site, so conclusions are based on in total 16 colonies in treatment and control for each crop, respectively. Winter oil seed rape trials were set up so that there were three replicated study plots each for neonicotinoid treated and untreated plots. Five honey bee colonies were placed at each winter oil seed rape location, so conclusions are based on in total 15 colonies in treatment and control. Maize studies were placed at four different regions in France each containing a single neonicotinoid-treated and untreated field with six honey bee colonies each, so conclusions are based on in total 24 colonies in treatment and control.
Control:	Seeds of the same crop variety as at the treated sites were sown, but not treated with neonicotinoid seed - or soil treatment products.

2. Observations and measurements

Biological parameters measured:	relevance of guttation for honeybee colonies

II. RESULTS AND DISCUSSION:

At all test locations and for each of the five crops guttation was observed. In winter cereals and winter oil seed rape, guttation was a common occurrence in both the autumn and spring exposure periods.

Exposure of honey bees to guttation fluid

Crop	% of days where guttation was observed	Guttation coincides with be flight	% of total bees observed that were seen collecting guttation fluid
Cereals (winter wheat and barley)	90 % (autumn) 86 % (spring)	64 % (autumn) 63 % (spring)	1.2 % (autumn) 14 % (spring)
Winter oil seed rape	80 % (autumn) 76 % (spring)	76 % (autumn) 54 % (spring)	0.5 % (autumn) 5.0 % (spring)
Sugar beet	25 % (spring only)	yes	0 %
Potato	50 % (spring only)	yes	0 %
Maize	68 % (spring only)	yes	0 %

III. CONCLUSION:

It can be concluded that exposure of honeybee colonies to guttation fluid, did not pose an unacceptable acute or chronic risk to honeybee colony development or survival, and does not adversely interfere with bee keeping practices. Overall, guttation water from seed-treated crop plants was found not to be a significant exposure route for honeybees.

Assessment and conclusion by applicant:

This study is considered supportive for use in risk assessment.

Data Point:	KCP 10.3.1/06
Report Author:	Goatley, J. L.; Lewis, R. W.
Report Year:	1965
Report Title:	Composition of guttation fluid from rye, wheat, and barley seedlings
Report No:	Lit. 9095
Document No:	M-329396-01-1
Guideline(s) followed in study:	--
Deviations from current test guideline:	Not applicable
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	not applicable
Acceptability/Reliability:	Supportive only

Executive Summary

The composition of guttation fluid from rye, wheat and barley seedlings was analysed. Seeds were germinated and when the seedlings were about 3 cm tall guttation fluid was collected drop by drop into a suction flask. The fluid was collected twice a day and frozen immediately. Collections for 5 days were pooled and used for analysis of amino acids, carbohydrates, organic acids, elements, ions, vitamins and pH.
Total sugar content is about equal in rye and barley, but considerably lower in wheat. Most of the amino acid in all 3 fluids is aspartic acid or asparagine. Total amino acid is considerably higher in barley fluid than in the other two. Nitrate, phosphate and ammonium ions did not vary greatly. Most elements are found to be highest in barley fluid and lowest in wheat. The pH was 5.0 in rye fluid, 5.5 in wheat fluid and 6.7 in barley fluid. These results show that this is an appropriate range for pH of

guttation water from plants.

Goatley and Lewis. (1966). Composition of Guttation Fluid from Rye, Wheat, and Barley Seedlings. Plant Physiol. 41, 373-375. https://www.ncbi.nlm.nih.gov/pubmed/16656266, reference: M-329396-01-1

Assessment and conclusion by applicant:

This study is considered supportive for use in risk assessment.

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:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150) G: Effects (Acute contact and oral) on honey bees (Apis mellifera L.) in the laboratory
Report No:	99591035
Document No:	M-524962-01-1
Guideline(s) followed in study:	OECD 213 and 214 (1998)
Deviations from current test guideline:	Current Guidelines: OECD 213 (1998) and OECD 214 (1998) A 5 µL droplet was chosen in the contact toxicity test in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item and allowed testing a higher application dose. The relative humidity was 38 – 70%, below the 50 – 70% recommended in the guideline. These deviations are not expected to have impacted the study results.
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 fluopicolide + fluoxastrobin FS 350 (200+150) G to the honey bee *(A. mellifera L.)*. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behavior, were also assessed. Therefore, under laboratory conditions *Apis mellifera* 50 worker bees were exposed for 48 hours to a single dose of 200.0 µg product/bee by topical application and 50 worker bees were exposed for 48 hours to a single dose of 221.0 µg product/bee by feeding. At the end of the contact toxicity test, there was no mortality at 200.0 µg product/bee. There was 6.0 % mortality in the contact control group (water + 0.5 % Adhäsit). In the oral toxicity test an actual intake of 221.0 µg product/bee led to 4.0 % mortality after 48 hours. No mortality occurred in the oral control group (50 % w/v sucrose solution = 500 g sucrose/L tap water). The LD_{50} of the reference item was calculated to be 0.23 and 0.12 µg/bee in the contact and oral test, respectively. All validity criteria of the test were met. The contact LD_{50} (48 h) was > 200.0 µg product/bee. The oral LD_{50} (48 h) was > 221.0 µg product/bee.

I. Material and Methods:

Test item: Fluopicolide + Fluoxastrobin FS 350 (200+150) G: fluopicolide (AE C638206): 17.0 % w/w, 198.7 g/L, fluoxastrobin (HEC 5725 E-iso): 13.1 % w/w, 148.4 g/L (all analytical values); Supplier Batch No.: 2014-014396; Sample Description: TOX10774-00; Specification No.: 102000028578; density:1.164 g/mL.
Under laboratory conditions *Apis mellifera* 50 worker bees were exposed for 48 hours to a single dose of 200.0 µg product per bee by topical application (contact limit test) and 50 worker bees were exposed for 48 hours for feeding to a single dose of 221.0 µg product per bee (oral limit test, value based on the actual intake of take of the test item).

Dates of experimental work: April 13, 2015 to April 15, 2015

II. Results and Discussion:

Toxicity to Honey Bees; laboratory tests

Test Item	Fluopicolide + Fluoxastrobin FS 350 (200+150) G	
Test Species	*Apis mellifera* L.	
Exposure	contact (solution in Adhäsit (0.5 %)/water)	oral (sucrose solution)
Application rate µg product/bee	200.0	221.0
LD_{50} µg product/bee	> 200.0	> 221.0
LD_{20} µg product/bee	> 200.0	> 221.0
LD_{10} µg product/bee	> 200.0	> 221.0
NOED µg product/bee*	≥ 200.0	≥ 221.0

* The NOED was estimated using Fisher's Exact Test (pairwise comparison, one-sided greater, α = 0.05).

The contact and oral LD_{50} (24 h) values of the reference item (dimethoate) were calculated to be 0.23 and 0.12 µg a.s./bee, respectively.

Observations:

Contact Test:
At the end of the contact toxicity test (48 hours after application), no mortality occurred at 200.0 µg product/bee. There was 6.0 % mortality in the control group (water + 0.5 % Adhäsit). No test item induced behavioural effects were observed at any time in the contact toxicity test.

Dosage [µg prod./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 200	0.0	0.0	0.0	0.0	0.0	0.0
Water	0.0	0.0	0.0	0.0	6.0	0.0
Reference item						
0.30	22.0	6.0	74.0	2.0	78.0	2.0
0.20	6.0	0.0	36.0	4.0	48.0	6.0
0.15	2.0	0.0	20.0	2.0	30.0	6.0
0.10	0.0	0.0	8.0	0.0	8.0	0.0

Results are averages from three replicates (ten bees each) per dosage / control
Water = CO_2/water-treated control

Oral Test:
In the oral toxicity test, the maximum nominal test level of Fluopicolide + Fluoxastrobin FS 350 (200+150) G (*i.e.* 200 µg product/bee) corresponded to an actual intake of 221.0 µg product/bee (after a feeding period of 1 hour and 5 minutes for the test item treatments). This dose level led to 4.0 % mortality after 48 hours. In the control group (50 % w/v sucrose solution = 500 g sucrose/L tap water), no mortality occurred. No test item induced behavioural effects were observed at any time in the oral toxicity test.

Dosage [µg prod./bee]	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 221.0	0.0	0.0	0.0	0.0	4.0	0.0
Water	0.0	0.0	0.0	0.0	0.0	0.0
Reference item						
0.32	86.0	14.0	100.0	0.0	100.0	0.0
0.16	4.0	20.0	80.0	0.0	82.0	4.0
0.08	0.0	0.0	10.0	28.0	18.0	26.0
0.06	0.0	0.0	0.0	0.0	4.0	0.0

Results are averages from three replicates (ten bees each) per dosage / control
Water = water control

Validity criteria:
All validity criteria of the test were met.

Validity criteria (OECD 213 and 214, 1998)	Obtained in this study
Control mortality should not exceed 10 % at test end	Contact test: 6 % Oral test: 0 %
LD_{50} of the reference item should be in the specified range (contact test: 0.10 – 0.30 µg a.s./bee, oral test: 0.10 – 0.35 µg a.s./bee)	Contact test: 0.23 µg a.s./bee Oral test: 0.12 µg a.s./bee

III. CONCLUSIONS:

The toxicity of Fluopicolide + Fluoxastrobin FS 350 (200+150) G was tested in both, an acute contact and an acute oral toxicity test on honey bees. The contact LD_{50} (48 h) was > 200.0 µg product/bee. The oral LD_{50} (48 h) was > 221.0 µg product/bee.

Assessment and conclusion by applicant: This study is considered reliable for risk assessment and the endpoints are: LD_{50} contact (48 h) > 200.0 µg product/bee LD_{50} oral (48 h) > 221.0 µg product/bee

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Data Point:	KCP 10.3.1.1.1/02
Report Author:	[redacted]
Report Year:	2017
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150) G: Effects (acute contact and oral) on bumble bees (Bombus terrestris L.) in the laboratory
Report No:	120801105
Document No:	M-591409-01-1
Guideline(s) followed in study:	Regulation (EC) No. 1107/2009 Directive 2003-01 (Canada/PMRA) US EPA OCSPP 850.SUPP based on OECD 213 and OECD 214 (1998), Van der Steen (2001) and ICPPR non-apis group (2015 and 2016)
Deviations from current test guideline:	Current Guidelines: OECD 246 (2017) and 247 (2017) A 5 µL droplet was chosen in the contact toxicity test in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item and allowed testing a higher application dose. Analytical determination of the test item was not conducted, but the study was conducted before guideline implementation and no analytical dose verification was foreseen at that point in time. Moreover, since it is a limit test with a single dosing of the test item this deviation is not expected to have impacted the study results. The exposure duration in the oral toxicity test was 6 hours and thus greater than the maximum 4 hours recommended by the guideline. The test was conducted before implementation of the guideline and the exposure duration of the ring test discussed at the time was 6 hours. This deviation is not expected to have impacted the study results.
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 oral and contact toxicity of Fluopicolide + Fluoxastrobin FS 350 to the bumble bee (*Bombus terrestris* L.) in the laboratory. Mortality of the bumble bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed. Therefore, under laboratory conditions 50 bumble bees (*Bombus terrestris*) were exposed for 48 hours to a single dose of 400 µg prod./bumble bee, by topical application of 5 µL, in a contact limit test and to a single dose of 470.2 µg prod./bumble bee by feeding in an oral limit test. At the end of the contact toxicity test (48 hours after application) 2% mortality occurred in the 400-µg prod./bumble bee treatment group. No mortality occurred in the water contact control group (48 hours). After 48 hours there was no mortality in the 470.2 µg prod./bumble bee test item group. No mortality occurred also in the water oral control group. The mortality in the reference item group in the contact and oral test was 70% at rates of 10 µg dimethoate/bumble bee (contact) and 4 µg dimethoate/bumble bee (oral). All validity criteria of the test were met. The contact LD_{50} (48 h) was > 400 µg product/bee. The oral LD_{50} (48 h) was > 470.2 µg product/bee.

I. Material and Methods:

Test item: Fluopicolide + Fluoxastrobin FS 350 (200 + 150) G: 17.2% w/w (201.3 g/L) fluopicolide (AE C638206) (analytical), 12.9 % w/w (151.1 g/L) fluoxastrobin (hEC 5725 E-iso) (analytical), Supplier batch No: 2016-006417, Sample description: TOX20466-00, Specification No.: 102000028578.
Test organism: female worker bumble bees (*B. terrestris*), obtained from a healthy and queen-right colony, bred by a commercial bumble bee breeding company (Koppert B.V.).
Under laboratory conditions worker bumble bees were exposed for 48 hours to a single dose of 400 µg prod./bumble bee, by topical application of 5 µL, in a contact limit test and to a single dose of 470.2 µg prod./bumble bee by feeding in an oral limit test (value based on the actual intake of the test item).
Furthermore, each test consisted of a control and a reference item group. In the contact limit test, tap water containing 0.1% v/v Triton X-100 was used as control. In the oral limit test a 50 % w/v sucrose solution (500 g sucrose/L tap water) was used as control. In both limit tests, BAS 152 11 I (active ingredient 420.3 g/L dimethoate, batch No: FRE-001226) was used as reference item. Each treatment group consisted out of 50 bumble bees and each reference group out of 30 bumble bees with 1 bumble bee per test unit (replicate). The measured food uptake in the acute oral toxicity test ranged between 10 and 51 mg after a maximum contaminated feeding period of 6 hours. For this reason, individual bumble bees which did not take up at least 80 % of the mean food uptake per treatment group were excluded from the evaluation. For the 470.2 µg prod./bumble bee test item treatment group 47 bumble bees were considered for the evaluation. For the water control (50 % w/v sucrose solution) and the reference item treatment groups 49 and 26 bumble bees were considered for the evaluation.
Test units were cylindrical, latticed plastic cages with a length of approximately 7 cm and a diameter of 2.2 cm at the large and 1.7 cm at the small opening.
The test was conducted in darkness, exposure temperature was 23-25°C and humidity was 50-62 % during exposure. Bumble bees used for the contact toxicity test were acclimatized for 22 hours and 35 minutes and bumble bees used for the oral toxicity test were acclimatized for 20 hours and 30 minutes, respectively. The bumble bees implemented for the oral toxicity test were starved for 150 – 170 minutes prior to the oral feeding exposure. Biological observations, including mortality and sub-lethal effects were recorded 4, 24 and 48 h after application. The software used to perform the statistical analysis was ToxRat Professional Version 3.2.1.

Dates of experimental work: February 21, 2017 to February 22, 2017

II. RESULTS AND DISCUSSION:

Biological findings:

Test item	Fluopicolide + Fluoxastrobin FS 350	
Test object	*Bombus terrestris*	
Exposure	Contact (CO_2 / tap water containing 0.1% v/v Triton X-100)	Oral (50% w/v sucrose solution) (based on recorded consumption considering bumble bees with food uptake of ≥ 80% of the mean uptake per treatment group[3])
Dose rate [µg prod./bumble bee]	400	470.2
LD_{50} [µg prod./bumble bee][1]	24 hours: > 400	24 hours: > 470.2
LD_{20} [µg prod./bumble bee][1]	24 hours: > 400	24 hours: > 470.2
LD_{10} [µg prod./bumble bee][1]	24 hours: > 400	24 hours: > 470.2
NOED [µg prod./bumble bee][2,4]	24 hours: ≥ 400	24 hours: ≥ 470.2
LOEC [µg prod./bumble bee][2,4]	24 hours: > 400	24 hours: > 470.2

[1] As the test item treatment groups did not show mortality above 50% no statistical evaluation on the LD_{50}, LD_{20} and LD_{10} was carried out.
[2] The NOED/LOED was determined using Fisher's Exact Test after Bonferroni-Holm (pairwise comparison, one-sided greater, α = 0.05).
[3] For the 470.2 µg prod./bumble bee test item treatment group 47 bumble bees were considered for the evaluation.
[4] Results obtained from test item treated group were compared to those obtained from the water control treated group.

Observations

Contact test

At the end of the contact toxicity test (48 hours after application) 400 µg prod./bumble bee led to 2.0 % mortality. No mortality occurred in the water control group (tap water containing 0.1 % v/v Triton X-100). No test item related behavioural effects were observed in the contact toxicity test. The mortality in the reference item treatment group was 70.0 % (48 hours after application).

Treatment group	After 4 hours		After 24 hours		After 48 hours	
	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 400 µg product/ bumble bee	2.0	0.0	2.0	0.0	2.0	0.0
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Reference item 10 µg dimethoate/ bumble bee	13.3	7.7	50	33.3	70	22.2

Test item: Fluopicolide + Fluoxastrobin FS 350 (200+150) G
Mortality mean = Mean of 50 individuals per test item and control, mean of 30 individuals per reference
Behav. abnorm mean = Mean of living individuals per treatment group
Water control = CO_2 / tap water containing 0.1% Triton X-100

Oral test

The actual oral dose of 470.2 µg prod./bumble bee resulted in no mortality. No mortality occurred also in the water control group (50 % w/v sucrose solution). No test item related behavioural effects were observed in the oral toxicity test. The mortality in the reference item treatment group was 100.0 %.

Treatment	After 4 hours	After 24 hours	After 48 hours

	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %	Mortality mean %	Behavioral abnormalities mean %
Test item 470.2 µg product/ bumble bee	2.0	0.0	2.0	0.0	2.0	0.0
Water control	0.0	0.0	0.0	0.0	0.0	0.0
Reference item 4.9 µg dimethoate/ bumble bee	13.3	7.7	50	33.3	70	22.2

Test item: Fluopicolide + Fluoxastrobin FS 350 (200+150) G
Mortality mean = Mean of 50 individuals per test item and control, mean of 30 individuals per reference
Behav. abnorm mean = Mean of living individuals per treatment group
Water control = 50 % w/v sucrose solution

Validity criteria:

All validity criteria of the test were met.

Validity criteria (OECD 246 and 247, 2017)	Obtained in this study
Control mortality should not exceed 10 % at test end	Contact test: 0.0 % Oral test: 0.0%
Mortality of the reference item should be ≥ 50 % at test end	Contact test: 70% Oral test: 70%

III. CONCLUSIONS:

The 48-h contact LD_{50} of fluopicolide + fluxastrobin FS 350 (200 + 150 G) was estimated to be > 400 µg prod./bumble bee.
The 48 oral LD_{50} of fluopicolide + fluoxastrobin FS 350 (200 + 150 G) was estimated to be > 470.2 µg prod./bumble bee.

Assessment and conclusion by applicant:

This study is considered reliable for risk assessment and the endpoints are:

LD_{50} contact (48 h) > 400 µg product/bumble bee
LD_{50} oral (48 h) > 470.2 µg product/bumble bee

CP 10.3.1.1.2 Acute contact toxicity to bees

Please refer to CP 10.3.1.1.1.

CP 10.3.1.2 Chronic toxicity to bees

The formulation fluopicolide + fluoxastrobin FS 350 (200 + 150 G) is applied as a single seed treatment in winter oilseed rape (corresponding to 200 g fluopicolide/100 kg seeds and 150 g fluoxastrobin/100 kg seeds at minimum 2.5 to maximum 6 kg seeds/ha at BBCH 00).

Fluopicolide (as well as fluoxastrobin) is of low acute toxicity to honey bees, with an LD_{50} (oral and contact) above the highest tested dose rates. In addition, the formulated product fluopicolide + fluoxastrobin FS 350 was also subjected to acute laboratory studies with adult honey bees ([redacted] 2015; M-524962-01-1, KCP 10.3.1.1/01) and to acute bumble bee testing ([redacted] 2017; M-591409-01-1, KCP 10.3.1.1/02). The studies resulted in LD_{50} values of > 200 µg product/bee and > 400 µg product/bumble bee, respectively, and did not reveal sensitivity differences between honey bee and bumble bee foragers.

Moreover, a solo formulation fluopicolide SC 486 was further subjected to chronic laboratory testing with adult honey bees ([redacted] 2016; M-552253-01-1, KCA 8.3.1.2/01). After exposing honey bees for ten consecutive days exclusively to sugar solution containing fluopicolide SC 486 (corresponding to a daily mean dose of 132.68 µg a.s./bee/day), the 10- day LC_{50} (Lethal Concentration) was determined to be > 3000 mg a.s./kg, which corresponds to a LDD_{50} (Lethal Dietary Dose) of > 132.68 µg a.s./bee/day.
Regarding potential side effects of fluopicolide on immature honey bee life stages, fluopicolide tech. was tested on first instar honey bee larva by repeated oral exposure ([redacted] 2018; M-615695-01-1, KCA 8.3.1.3/01). After a test duration of 22 days the NOED (emergence) was determined to be ≥ 60.1 µg a.s./larva, equivalent to a NOEC of ≥ 390 mg a.s./kg food. In addition, the solo formulation fluopicolide SC 486 was further tested for potential risk to immature honey bee life stages in a bee brood feeding study ([redacted] 2016; M-545732-01-1, KCA 8.3.1.3/02) and subjected to two higher tier semi-field honey bee brood studies considering realistic worst-case conditions ([redacted] 2016; M-547124-01-1, KCA 8.3.1.3/03 and [redacted] 2020; M-685049-01-1, KCA 8.3.1.3/04).

The study from [redacted] 2016 (M-545732-01-1, KCA 8.3.1.3/02) was conducted following the provisions/method of Oomen P.A., de Ruijter, A. & van der Steen, J. (OEPP/EPPO Bulletin 22:613-616 (1992)), which require, amongst other parameters to "…use formulated products only… products are fed at a concentration recommended for high-volume use…". The honey bee brood feeding test is a worst-case screening test, by feeding the honey bees directly in the hive with a treated sugar solution which contains the test substance at a concentration typically present in the spray tank (and as such at a very high concentration) and by investigating the development of eggs, young and old larvae by employing digital photo imaging technology. Fluopicolide SC 486 was administered at a concentration of 1.33 g fluopicolide/L to honey bee colonies via feeding of 1 litre spiked sucrose solution. This concentration has neither resulted in adverse effects on brood performance and showed no indication for negative impacts on brood rearing success compared to a control group fed with untreated sucrose solution, respectively. Regarding brood development, the brood termination rates of the test item treatment were overall on a low level with 15.3, 9.8 and 6.5% for eggs, young larvae and old larvae, respectively, which were not statistically significant different to the control with brood termination rates of 11.5, 6.8 and 10.2% for eggs, young larvae and old larvae, respectively at the end of the brood observation period.

Moreover, the two higher tier semi-field studies [redacted] 2016 (M-547124-01-1, KCA 8.3.1.3/03) and [redacted] 2020 (M-685049-01-1, KCA 8.3.1.3/04) were performed according to the provisions of the OECD Guidance Document 75. In these particular studies fluopicolide SC 486 was applied in order to clarify whether fluopicolide poses a risk to honey bee brood and colony development in particular as well as on honey bees in general under realistic worst-case conditions. One study was performed in C-EU (Schmitzer, S.; 2016) and one study was conducted in S-EU (Roig, J; 2020) under forced/confined exposure conditions using the formulation fluopicolide SC 486, by application of 133 g a.s./ha under tunnel conditions to the full flowering and highly bee attractive surrogate crop *Phacelia* *tanacetifolia*. Both studies included three treatment groups: control (tap water), test item (133 g a.s./ha) and reference item (300 g fenoxycarb/ha) with all applications being carried out with a spray volume of 400 L water/ha. For all treatment groups, four replicates (tunnels) were set up. The application of all treatment groups was conducted during daily bee flight activity at the time of full flowering of the crop. Thereafter, the bees were kept for 7 days within the tunnels (confined exposure phase) and were then relocated out of the tunnels and transferred to a monitoring site without flowering crops and intensive agricultural area for further monitoring. Daily, throughout the confined exposure phase, mortality of worker bees, larvae and pupae was assessed along with assessments of foraging activity and behaviour. Daily mortality assessments were continued along with behaviour around the hive during the post-exposure observation period. Colony assessments (food stores, brood areas, colony strength) were made before confinement, after confinement and at the end of each study. Detailed brood assessments (brood termination rate, brood index and brood compensation index) by employing digital photo imaging technology, investigating the fate of more than 200-250 individually marked cells was performed on 5 occasions throughout the study, covering an entire brood cycle of honey bees.

Overall, no adverse effects on brood development, adult and pupal mortality, foraging activity, behaviour, colony development and strength after application of 133 g fluopicolide/ha onto flowering *Phacelia tanacetifolia* were observable for both studies.

In addition, and to clarify whether residues of fluopicolide and its metabolites (as well as fluoxastrobin) would occur after systemic uptake from the treated seed into the plant potentially leading to residues in bee-relevant matrices (pollen and nectar), investigations in oilseed rape were carried out. For fluopicolide systemic activity is known, while fluoxastrobin does not show systemic activity. Nevertheless, both compounds including bee relevant metabolites of fluopicolide were included in the analytical verification of sampled material from a residue study (please refer to [redacted] 2020; M-689241-01-1, CP 10.3.1.5). The study was conducted in Germany (C-EU) and Italy (S-EU) using winter oilseed rape seeds treated with fluopicolide + fluoxastrobin FS 350 (nominal application rate of 12 g fluopicolide/ha and 9 g fluoxastrobin/ha).
The information obtained from this study showed residues of fluopicolide and its metabolites M-01 (AE C653711) in the treated pollen samples below the LOQ (LOQ = 0.010 mg/kg) for all samplings in all trials. Residues of the metabolite M-02 (AE C657188) ranged between < 0.010 -0.012 mg/kg. In trial -01 residues of M-02 (AE C657188) were detected at the 1st and 2nd sampling with 0.012 and 0.010 mg/kg, respectively. In all subsequent sampling's residues were below LOQ (< 0.010 mg/kg) (please refer to Mack, P.; 2020; M-689241-01-1, CP 10.3.1.5). The data demonstrate that no residues in pollen and nectar that are relevant for the exposure of bees are to be expected from the use of fluopicolide + fluoxastrobin FS 350 as oilseed rape seed treatment.

Taking into consideration this large study package of acute and chronic studies with the active substance as well as solo formulation and given that fact that chronic exposure of bees to the seed treatment product, as well as to the active substance fluopicolide or its metabolites (or fluoxastrobin) is not expected, chronic toxicity studies with the formulation fluopicolide + fluoxastrobin FS 350 were not considered necessary.

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

Please refer to CP 10.3.1.2.

CP 10.3.1.4 Sub-lethal effects

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

CP 10.3.1.5 Cage and tunnel tests

Data Point:	KCP 10.3.1.5/01
Report Author:	[redacted]
Report Year:	2020
Report Title:	Determination of residues of fluopicolide (FLC) + fluoxastrobin (FXA) in nectar and pollen of winter oilseed rape after seeding of seeds treated with fluopicolide + fluoxastrobin FS 200 + 150 G/L in a semi-field residue study in Germany and Italy in 2019/2020
Report No:	S19-05083
Document No:	M-689241-01-1
Guideline(s) followed in study:	Commission Regulation (EU) No 283/2013 and 284/2013 (Mar. 2013) in accordance with Regulation (EC) No 1107/2009 (Oct. 2009); SANCO/825/00 (2010), SANCO/3029/99 rev. 4 (2000), EC (2018) Technical guidelines for determining the magnitude of pesticide residues in honey and setting Maximum Residue Levels in honey (SANTE/11956/2016 rev. 9)
Deviations from current test guideline:	None
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive summary

The objective of this study was to determine potential residues of fluopicolide (FLC) + fluoxastrobin (FXA) in nectar, and pollen from winter oilseed rape plants under field conditions after sowing of seeds treated with fluopicolide + fluoxastrobin FS 200 + 150 G/L. The study comprised four separate semi-field residue trials conducted in Germany and Italy in 2019/2020.

On each trial site one tunnel was established on each the control (C) and the treated plot (T). Two honeybee hives were set up per tunnel.

Forager bees and pollen from pollen traps were collected in the untreated control (C) and in test item treatment (T) six times during the study period starting at BBCH 62-63 with sampling S1 (0DAS1).

For pollen samples, on every sampling day a pooled sample of at least 0.2 g was collected using pollen traps (A-sample) and, if possible, a retain sample (R-sample) of 0.2 g. For nectar samples, on every sampling day approximately 150 forager bees were collected for the preparation of nectar from their honey stomachs (A-sample) and, if possible, a retain sample (R-sample) of 150 forager bees. In addition, forager bees for the determination of sugar content (at least 50 forager bees) were sampled in the control at each sampling day.

Residues were verified by HPLC-MS/MS. Residues of fluopicolide, its metabolites M-01 (AE C653711) and M-02 (AE C657188) in the treated nectar samples were below the LOQ (LOQ= 0.010 mg/kg) for all samplings in all trials.

Residues of fluoxastrobin (HEC 5725 E-isomer (AE1228646) and HEC 5725 Z-isomer (AE 1302951)) in the treated nectar samples were below the LOQ (LOQ= 0.0090 mg/kg for HEC 5725 E-isomer (AE1228646) and 0.0010 mg/kg for HEC 5725 Z-isomer (AE 1302951)) for all samplings in all trials.

Residues of fluopicolide and its metabolites M-01 (AE C653711) in the treated pollen samples were below the LOQ (LOQ= 0.010 mg/kg) for all samplings in all trials. Residues of the metabolite M-02 (AE C657188) ranged between < 0.010 -0.012 mg/kg. In trial -01 residues of M-02 (AE C657188) were detected at the 1st and 2nd sampling (0 and 1DAS1 (DAS1= Days after Sampling 1)) with 0.012 and 0.010 mg/kg, respectively. In all subsequent sampling's residues were below LOQ (< 0.010 mg/kg).

Residues of fluoxastrobin (HEC 5725 E-isomer (AE1228646) and HEC 5725 Z-isomer (AE 1302951)) in the treated pollen samples were below the LOQ (LOQ= 0.0090 mg/kg for HEC 5725 E-isomer (AE1228646) and 0.0010 mg/kg for HEC 5725 Z-isomer (AE 1302951)) for all samplings in all trials.

I. MATERIAL AND METHODS

Test item: LEONARDO KWS 2+1.5 g a.s./kg FLC+FXA FS 200+150 G), Batch No: 4240/I.1: fluopicolide: Content of a.s. nominal 2.00 g/kg seed, fluoxastrobin: Content of a.s. nominal 1.50 g/kg seed.

Test species: Honeybee colonies (*Apis mellifera* L.) were used. The honey bees were used as sampling device only (i.e. collection of nectar and pollen). Colonies were either from Eurofins in-house beehive stock keeping or were obtained from a commercial supplier. Colonies kept in one brood chamber with a sufficient number of forager bees were used.

Test design: The honey bee colonies were placed in the tunnels at the beginning of flowering (3-1DBS1). Two colonies per tunnel were set up. The colonies were equipped with pollen traps. Residue trials were carried out at four independent locations, two in Germany and two in Italy. Each trial comprised 2 plots (one untreated and one treated with FLC+FXA FS 200+150 G). The drilling of the control and test item treatment in all trials was conducted using a calibrated drilling machine for commercial sowing. Drilling was performed with a target drilling rate of 6 kg seeds/ha (corresponding to 12 g FLC/ha + 9 g FXA/ha) for all trials. Sampling was conducted six times during the study period starting at BBCH 62-63. On each sampling day forager bees were collected for the preparation of nectar from their honey stomachs for residue analysis. On each sampling day an A-sample of at least 150 bees was collected. If possible, an R-sample of at least 150 bees was taken on each sampling day, too. For the preparation of nectar from honey stomachs for determination of sugar content forager bees were sampled in the control on each sampling day. One sample of at least 50 bees was taken per sampling day. No R-sample was taken. On each sampling day an A-sample and an R-sample of at least 0.2 g pollen was collected.

Application scheme:

Trial No. Country	Plot	Appl. mode	Date	Drilling				
				Target rate (kg/ha)	**Actual rate (kg/ha)**	**a.s.**	**Target Drilling rate (g a.s./ha)**	**Actual Drilling rate (g a.s./ha)**
S19-05083-01 Germany	C	DRIL	2019-08-28	6.0	6.50	-	-	-
	T			6.0	6.14	FLC+ FXA	12.0 + 9.0	12.28 + 9.21
S19-05083-02 Germany	C	DRIL	2019-09-04	6.0	6.03	-	-	-
	T			6.0	5.77	FLC+ FXA	12.0 + 9.0	11.54 + 8.65
S19-05083-03 Italy	C	DRIL	2019-10-10	6.0	4.88	-	-	-
	T			6.0	5.28	FLC+ FXA	12.0 + 9.0	10.57 + 7.93
S19-05083-04 Italy	C	DRIL	2019-10-10	6.0	5.15	-	-	-
	T			6.0	5.78	FLC+ FXA	12.0 + 9.0	11.56 + 8.67

Analytics: Residues of fluopicolide and its metabolites M-01 (AE C653711) and M-02 (AE C657188) and fluoxastrobin ((HEC 5725 E-isomer (AE1228646) / HEC 5725 Z-isomer (AE 1302951)) in nectar and pollen were analysed. Two aliquots were analyzed by high performance liquid chromatography, chromatographed under reversed phase gradient conditions and detected by Tandem Mass Spectrometry with electrospray ionisation. Residues were quantified with internal standards and solvent calibration standards were used.

Full details and acceptable validation data to support this method are presented within document M-CA 4, which comply with the EU regulatory requirements outlined within SANCO/3029/99 rev 4.

Dates of work: August 28, 2019 to April 27, 2020.

II. RESULTS AND DISCUSSION

Analytical methods were validated for the determination of fluopicolide, its metabolites M-01 (AE C653711) and M-02 (AE C657188) with a limit of quantification of 0.010 mg/kg and for residues of fluoxastrobin (HEC 5725 E-isomer (AE1228646) and HEC 5725 Z-isomer (AE 1302951)) with a limit of quantification of 0.010 mg/kg as total residue of both analytes ((HEC 5725 E-isomer(AE1228646) 0.0090 mg/kg/ HEC 5725 Z-isomer (AE 1302951) 0.0010 mg/kg)) in nectar and pollen.

The mean recovery values (concurrent recoveries) of the analytes in nectar for the quantifier mass transition ranged between 85 % and 100% with relative standard deviations between 2.1% and 9.3%.

The overall mean recoveries of the analytes ranged between 86% and 98% and the corresponding overall relative standard deviation (RSD) ranged between 2.6% and 7.1% (n = 12 for each analyte).

The mean recovery values (concurrent recoveries) of the analytes in pollen for the quantifier mass transition ranged between 89% and 105% with relative standard deviations between 1.9% and 11%. The overall mean recoveries of the analytes ranged between 91% and 105% and the corresponding overall relative standard deviation (RSD) ranged between 3.0% and 8.5% (n = 12 for each analyte).

Summary for Fluopicolide and its Metabolites in Nectar

Sample Type	Residues [mg/kg]		
	Fluopicolide	**M-01 (AE C653711)**	**M-02 (AE C657188)**
C	< 0.010	< 0.010	< 0.010
T	< 0.010	< 0.010	< 0.010

Summary for Fluoxastrobin in Nectar

Sample Type	Residues [mg/kg]	
	HEC 5725 E-isomer (AE1228646)	**HEC 5725 Z-isomer (AE 1302951)**
C	< 0.0090	< 0.0010
T	< 0.0090	< 0.0010

Summary for Fluopicolide and its Metabolites in Pollen

Sample Type	Residues [mg/kg]		
	Fluopicolide	**M-01 (AE C653711)**	**M-02 (AE C657188)**
C	< 0.010	< 0.010	< 0.010
T	< 0.010	< 0.010	< 0.010 -0.012

Summary for Fluoxastrobin in Pollen

Sample Type	Residues [mg/kg]	
	HEC 5725 E-isomer (AE1228646)	**HEC 5725 Z-isomer (AE 1302951)**
C	< 0.0090	< 0.0010
T	< 0.0090	< 0.0010

III. CONCLUSION

Residues of fluopicolide, its metabolites M-01 (AE C653711) and M-02 (AE C657188) in the treated nectar samples were below the LOQ (LOQ= 0.010 mg/kg) for all samplings in all trials.

Residues of fluoxastrobin (HEC 5725 E-isomer (AE1228646) and HEC 5725 Z-isomer (AE 1302951)) in the treated nectar samples were below the LOQ (LOQ= 0.0090 mg/kg for HEC 5725 E-isomer (AE1228646) and 0.0010 mg/kg for HEC 5725 Z-isomer (AE 1302951)) for all samplings in all trials.

Residues of fluopicolide and its metabolites M-01 (AE C653711) in the treated pollen samples were below the LOQ (LOQ= 0.010 mg/kg) for all samplings in all trials. Residues of the metabolite M-02 (AE C657188) ranged between < 0.010 -0.012 mg/kg. In trial -01 residues of M-02 (AE C657188) were detected at the 1st and 2nd sampling (0 and 1DAS1 (DAS1= Days after Sampling 1)) with 0.012 and 0.010 mg/kg, respectively. In all subsequent sampling's residues were below LOQ (< 0.010 mg/kg).

Residues of fluoxastrobin (HEC 5725 E-isomer (AE1228646) and HEC 5725 Z-isomer (AE 1302951)) in the treated pollen samples were below the LOQ (LOQ= 0.0090 mg/kg for HEC 5725 E-isomer (AE1228646) and 0.0010 mg/kg for HEC 5725 Z-isomer (AE 1302951)) for all samplings in all trials.

Assessment and conclusion by applicant:

The study is considered reliable.

Residues of fluopicolide, its metabolites M-01 (AE C653711) and M-02 (AE C657188) in the treated nectar samples were below the LOQ (LOQ= 0.010 mg/kg).

Residues of fluopicolide and its metabolite M-01 (AE C653711) in the treated pollen samples were below the LOQ (LOQ= 0.010 mg/kg). Residues of the metabolite M-02 (AE C657188) in the treated pollen samples ranged between < 0.010-0.012 mg/kg.

CP 10.3.1.6 Field tests with honeybees

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

CP 10.3.2 Effects on non-target arthropods other than bees

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

Table 10.3.2- 1: Ecotoxicological endpoints for non-target arthropods

Test species, Dossier-file-No. Reference	Tested Formulation, study type, exposure	Ecotoxicological Endpoint
Aphidius rhopalosiphi M-528905-01-1 Rep.No: 15 10 48 029 A KCP 10.3.2.1/01	Fluopicolide + Fluoxastrobin FS 350 Laboratory, glass plates 20 mL product/ha 36 mL product/ha 63 mL product/ha 112 mL product/ha 200 mL product/ha	LR_{50} > 200 mL prod./ha ER_{50} > 200 mL prod./ha Corr. Mortality [%] 0 0 -2.6 [A] 0 0
Typhlodromus pyri M-528984-01-1 Rep.No: 15 10 48 030 A KCP 10.3.2.1/02	Fluopicolide + Fluoxastrobin FS 350 Laboratory, glass plates 20 mL product/ha 36 mL product/ha 63 mL product/ha 112 mL product/ha 200 mL product/ha	LR_{50} > 200 mL prod./ha ER_{50} > 200 mL prod./ha Corr. Mortality [%] 0 0 0 0 -1.0 [A]

[A] A negative value indicates a lower mortality in the treatment than in the control

Risk assessment for non-target arthropods

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

The LR_{50} and the ER_{50} values of tier 1 laboratory studies with *Aphidius rhopalosiphi* and *Typhlodromus pyri* exceed 200 mL product/ha. Since the maximum in-field application rate is equivalent to 60 mL product/ha, it can be concluded that no unacceptable adverse effects on non-target arthropods are to be expected in the in-field area.

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

The LR_{50} and the ER_{50} values of tier 1 laboratory studies with *Aphidius rhopalosiphi* and *Typhlodromus pyri* exceed 200 mL product/ha. Since the maximum in-field application rate is equivalent to 60 mL product/ha, it can be concluded that also in the off-field area no unacceptable adverse effects on non-target arthropods are to be expected.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

Data Point:	KCP 10.3.2.1/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Effects of fluopicolide + fluoxastrobin FS 350 (200+150 g/L) on the parasitic wasp Aphidius rhopalosiphi (DESTEFANI-PEREZ) in a laboratory test
Report No:	15 10 48 029 A
Document No:	M-528905-01-1
Guideline(s) followed in study:	EU Directive 91/414/EEC Regulation (EC) No. 1107/2009 US EPA OCSPP not applicable IOBC: Mead-Briggs et al. 2000
Deviations from current test guideline:	Current Guideline: Mead-Briggs et al. (2000) No deviations.
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 laboratory study was to investigate the lethal and sublethal effects of fluopicolide + fluoxastrobin FS 350 (200+150 g/L) on the parasitoid wasp *Aphidius rhopalosiphi* when exposed on a glass surface to dried spray residues. The test substance was applied at rates of 20, 36, 63, 112 and 200 mL product/ha in 200 L deionised water/ha. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (0.3 mL product/ha, nominally equivalent to 0.12 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item. Adults of the parasitic wasp *Aphidius rhopalosiphi* were exposed in 4 replicates per treatment group and 7 females and 3 males per replicate to the residues of the test item, reference item and control treatments, respectively. During the exposure phase the wasps were fed with 25 % w/w aqueous fructose solution. The number of surviving, affected, moribund and dead wasps was recorded over a period of 48 hours. From these data the endpoint mortality was calculated. Additionally, effects on reproduction were investigated (number of parasitised aphids, assessed 11 days after parasitisation). All validity criteria were met. The LR_{50} was estimated to be > 200 mL product/ha. The NOER for mortality was ≥ 200 mL product/ha. The ER_{50} was estimated to be > 200 mL product/ha. The NOER for reproduction was ≥ 200 mL product/ha.

I. MATERIAL AND METHODS:

Test item: fluopicolide + fluoxastrobin FS 350 (200+150 g/L) G: 17.0 % w/w fluopicolide (analytical), 13.1 % w/w fluoxastrobin (analytical); Supplier batch No: 2014-014396, Sample description: TOX10774-00, Specification No.: 102000028578, density (20 °C): 1.164 g/mL (according to Certificate of Analysis)].

The test took place under laboratory conditions after contact exposure of adults of the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) to dried spray residues of the test item with rates of 20 – 36 – 63 – 112 – 200 mL product/ha in 200 L deionised water/ha applied on glass plates. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (0.3 mL product/ha, nominally equivalent to 0.12 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item.

Adults of the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) were exposed in 4 replicates per treatment group and 7 females and 3 males per replicate to the residues of the test item, reference item and control treatments, respectively. During the exposure phase the wasps were fed with 25 % w/w aqueous fructose solution. The number of surviving, affected, moribund and dead wasps was recorded over a period of 48 hours. From these data the endpoint mortality was calculated. Additionally, effects on reproduction were investigated (number of parasitised aphids, assessed 11 days after parasitisation).

II. Results and Discussion:

Test item	**Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L)**			
Test object	***Aphidius rhopalosiphi* (DeStefani-Perez)**			
Exposure	**Dried spray deposits on glass plates**			
Treatment	**Mortality [2] (Day 7) [%]**	**Corrected mortality [3] [%]**	**Reproduction [Mean number of mummies per female][4]**	**Effect on reproduction[5] [%]**
Control	2.5	-	22.3	-
Application rate[1] [mL product/ha]				
20	2.5 (n.s.)	0	21.6 (n.s.)	3.1
36	2.5 (n.s.)	0	22.0 (n.s.)	1.3
63	0 (n.s.)	-2.6	20.6 (n.s.)	7.6
112	2.5 (n.s.)	0	22.7 (n.s.)	-1.8
200	2.5 (n.s.)	0	20.9 (n.s.)	6.3
LR_{50}	**> 200 mL product/ha**	-	-	-
ER_{50}	-	-	**> 200 mL product/ha**	
Reference item dimethoate EC 400 15 mL product/ha	100*	100	n.d.	-

[1] Application rate in 200 L water/ha
[2] Mortality after 48-hour exposure to residues on treated glass plates. The results for mortality in individual treatments were compared to that in the control using Fisher's Exact Binomial test (α = 0.05).
[3] Corrected mortality according to Abbott (1925)
[4] Reproduction: mean number of parasitised aphids (mummies)/female. The results for the test item treatments and control were compared by Williams-t-test (α = 0.05).
[5] Change in mean number of mummies per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase relative to the control. (n.s.) not statistically significantly different compared to the control
* Statistically significantly different compared to the control
n.d. not determined

In a laboratory study with fluopicolide + fluoxastrobin FS 350 (200+150 g/L) mortality was 0 % (corrected mortality: -2.6 %) at 63 mL product/ha and 2.5 % (corrected mortality: 0 %) at 20, 36, 112 and 200 mL product/ha.
The NOER (no observed effect rate) for pre-imaginal mortality was ≥ 200 mL product/ha.
The LR_{50} is empirically estimated to exceed the highest tested application rate, 200 mL product/ha.
The effects on reproduction were lower than or equal to 7.6 % at all test rates and the ER_{50} was estimated to be > 200 mL product/ha.
The NOER (no observed effect rate) for reproduction was ≥ 200 mL product/ha.
The results of the control group indicated that the test organisms were in a good condition (mortality: 2.5 %, reproduction: 22.3 mummies per female).
The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 100 %).
No unusual observations were noted in the control and all test item treatment groups at any observation point during the test.

Validity criteria:

Validity criteria (Mead-Briggs et al., 2000)	Guideline	Test result
Control mortality	Not more than 5 out of 40 wasps (12.5%)	2.5 %
Toxic reference mortality (according to study protocol)	>50%	100 %
Reproduction rate	≥ 5 mummies/female ≤ 2 females producing 0 mummies	22.3 mummies/female 2 female with 0 mummies

III. CONCLUSIONS:

The LR_{50} was estimated to be > 200 mL product/ha. The NOER (no observed effect rate) for mortality was ≥ 200 mL product/ha. The ER_{50} was estimated to be > 200 mL product/ha. The NOER (no observed effect rate) for reproduction was ≥ 200 mL product/ha. All validity criteria according to Mead-Briggs *et al.* (2000) for conducting the laboratory test with *Aphidius rhopalosiphi* were met.

Assessment and conclusion by applicant:

The study is considered reliable. The LR_{50} and ER_{50} of >200 mL/ha are the relevant endpoints for the risk assessment.

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Data Point:	KCP 10.3.2.1/02
Report Author:	[redacted]
Report Year:	2015
Report Title:	Effects of fluopicolide + fluxastrobin FS 350 (200+150 g/L) on the predatory mite Typhlodromus pyri Scheuten in a laboratory tes
Report No:	151048030A
Document No:	M-528984-01-1
Guideline(s) followed in study:	IOBC Guideline (Blümel et al. 2000)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The objective of this laboratory study was to investigate the lethal and sublethal effects of fluopicolide + fluoxastrobin FS 350 (200+150 g/L) on the predatory mite *Typhlodromus pyri*. Mites were exposed to dried spray residues of different application rates of 20, 36, 63, 112- and 200-mL product/ha in 200 L deionised water/ha on glass plates. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (15 mL product/ha, nominally equivalent to 6 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item. Protonymphs of the predatory mite *Typhlodromus pyri* Scheuten exposed in 5 replicates per treatment group and 20 mites per replicate to the residues of the test item, reference item and control treatments, respectively. During the assessments the mites were fed with a mix of pollen pine (Pinus nigra) and birch (Betula pendula), 1:1. The number of surviving, dead, trapped and escaped predatory mites was recorded over a period of 7 days. From these data the endpoint mortality was calculated. Additionally, effects on reproduction were investigated (number of eggs per surviving female, assessed 9, 11 and 14 days after application). All validity criteria were met. The LR_{50} was estimated to be > 200 mL product/ha. The NOER for pre-imaginal mortality was ≥ 200 mL product/ha. The ER_{50} was estimated to be > 200 mL product/ha. The NOER for reproduction was ≥ 200 mL product/ha.

I. Material and Methods:

Test item: fluopicolide + fluoxastrobin FS 350 (200+150 g/L) [analysed active substances: 17.0 % w/w (198.7 g/L) fluopicolide (AE C638206); 13.1 % w/w (148.4 g/L) fluoxastrobin (HEC 5725 E-iso); Specification No.: 102000028578; Supplier batch No.: 2014-014396; Sample description: TOX10774-00; density (20 °C): 1.164 g/mL (according to Certificate of Analysis)] was tested under laboratory conditions after contact exposure of protonymphs of the predatory mite *Typhlodromus pyri* Scheuten to dried spray residues of the test item with rates of 20 – 36 – 63 – 112 – 200 mL product/ha in 200 L deionised water/ha applied on glass plates. The control was treated with deionised water (200 L/ha). Dimethoate EC 400 (15 mL product/ha, nominally equivalent to 6 g a.s./ha, in 200 L deionised water/ha) was used as a toxic reference item.
Protonymphs of the predatory mite *Typhlodromus pyri* Scheuten were exposed in 5 replicates per treatment group and 20 mites per replicate to the residues of the test item, reference item and control treatments, respectively. During the assessments the mites were fed with a mix of pollen pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1. The number of surviving, dead, trapped and escaped predatory mites was recorded over a period of 7 days. From these data the endpoint mortality was calculated. Additionally, effects on reproduction were investigated (number of eggs per surviving female, assessed 9, 11 and 14 days after application).

II. Results and Discussion:

Test item	**Fluopicolide + Fluoxastrobin FS 350 (200+150 g/L)**			
Test object	***Typhlodromus pyri***			
Exposure	**Dried spray deposits on glass plates**			
Treatment	**Mortality [2] (Day 7) [%]**	**Corrected mortality [3] [%]**	**Reproduction [Mean number of eggs per female][4] (7-14 Day)**	**Effect on reproduction[5] [%]**
Control	2.0	-	7.03	-
Application rate[1] [mL product/ha]				
20	2.0 (n.s.)	0	6.51 (n.s.)	7.4
36	2.0 (n.s.)	0	6.92 (n.s.)	1.6
63	2.0 (n.s.)	0	7.01 (n.s.)	0.3
112	2.0 (n.s.)	0	6.62 (n.s.)	5.8
200	1.0 (n.s.)	-1.0	7.04 (n.s.)	-0.1
LR_{50}	**> 200 mL product/ha**	-	-	-
ER_{50}	-	-	**> 200 mL product/ha**	-
Reference item dimethoate EC 400 15 mL product/ha	77.0*	76.5	n.d.	-

[1] Application rate in 200 L water/ha
[2] Mortality after exposure to residues on treated glass plates. The results for mortality in individual treatments were compared to that in the control using Fisher´s Exact Binomial test (α = 0.05).
[3] Corrected mortality according to Abbott (1925)
[4] Results for reproduction compared by Williams-t-test (α = 0.05)
[5] Change in mean numbers of eggs per female, relative to control. A positive value indicates a decrease relative to the control.
(n.s.) not statistically significantly different compared to the control
* statistically significantly different compared to the control
n.d. not determined

In a laboratory study with fluopicolide + fluoxastrobin FS 350 (200 + 150 g/L) mortality was 2.0 % (corrected mortality: 0 %) at 20, 36, 63- and 112 mL product/ha and 1.0 % (corrected mortality: -1.0 %) at 200 mL product/ha.
The NOER (no observed effect rate) for pre-imaginal mortality was ≥ 200 mL product/ha. The LR_{50} is empirically estimated to exceed the highest tested application rate, 200 mL product/ha in 200 L water/ha.
The effects on reproduction were lower than or equal to 7.4 % at all test rates and the ER_{50} was estimated to be > 200 mL product/ha.
The NOER (no observed effect rate) for reproduction was ≥ 200 mL product/ha.
The results of the control group indicated that the test organisms were in a good condition (mortality: 2.0 %, reproduction: 7.03 eggs per female per day).
The results of the reference item group indicated that the test system was sensitive to harmful substances (corrected mortality: 76.5 %).
No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Validity criteria:

Validity criteria (Blümel et al., 2000)	Guideline	Test result
Mortality rate	Mean mortality (dead + escaped) ≤ 20% at day 7	2 %
Toxic reference mean mortality of protonymphs at day 7 (control corrected)	Between 50 and 100 %	77 %
Reproduction (number of eggs per female in the control from day 7 to 14)	≥ 4	7.03

III. CONCLUSIONS:

The LR_{50} was estimated to be > 200 mL product/ha. The NOER (no observed effect rate) for pre-imaginal mortality was ≥ 200 mL product/ha. The ER_{50} was estimated to be > 200 mL product/ha. The NOER (no observed effect rate) for reproduction was ≥ 200 mL product/ha. All validity criteria according to BLÜMEL *et al.* (2000) for conducting the laboratory test with *Typhlodromus pyri* were met.

Assessment and conclusion by applicant:

The study is considered reliable. The LR_{50} and ER_{50} of >200 mL/ha are the relevant endpoints for the risk assessment.

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

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

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.

Table 10.4- 1: Maximum PEC_{soil} values for fluopicolide, its metabolites, fluoxastrobin and the product FLC + FXA FS 350 in winter oilseed rape (for details see MCP Section 9, Point 9.1.3)

Compound	**Winter oilseed rape, 1 × 0.06 L prod./ha**		
	$PEC_{soil, initial}$ [mg/kg]	**$PEC_{soil, plateau, 20 cm}$ [mg/kg]**	**$PEC_{soil, accu}$* [mg/kg]**
1 × 12 g a.s./ha			
Fluopicolide	0.016	0.005	0.021
M-01 (AE C653711)	0.004	<0.001	0.005
M-02 (AE C657188)	0.002	<0.001	0.002
M-03 (AE 0608000)	0.002	0.002	0.003
1 × 0.06 L prod./ha			
FLC + FXA FS 350	0.093 1)		

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

1) The PEC_{soil} value for the product FLC + FXA FS 350 is calculated based on the initial rate of the product (0.06 L/ha) in a single seed treatment application, the portion reaching soil (BBCH 00, no interception for oil seed rape), the standard soil density (1.5 g/cm³), the standard soil depth (5 cm) and the density of the formulation (1.164 g/mL).

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.

Table 10.4.1- 1: Endpoints used in risk assessment

Test item	**Test species, test design**	**Ecotoxicological endpoint**		**Reference**
FLC + FXA FS 350	*Eisenia fetida* reproduction 56 d, mixed into soil	NOEC EC_{10} EC_{20}	**158 mg prod./kg dws** a) 181 mg prod./kg dws a) 256 mg prod./kg dws a)	[redacted] 2015; M-528042-01-1 KCP 10.4.1.1/01
Fluopicolide	*Eisenia fetida* reproduction 56 d, mixed into soil	NOEC $EC_{10/20}$	**31.25 mg a.s./kg dws** a) not calculable	[redacted] 2003; M-218270-01-1 KCA 8.4.1/05
M-01 (AE C653711)	*Eisenia fetida* reproduction 56 d, mixed into soil	NOEC $EC_{10/20}$	**250 mg p.m./kg dws** Calculation not possible	[redacted] 2003; M-218219-01-1 KCA 8.4.1/06
M-02 (AE C657188)	*Eisenia fetida* reproduction 56 d, mixed into soil	NOEC $EC_{10/20}$	**≥ 100 mg p.m./kg dws** Calculation not possible	[redacted] 2016; M-558329-01-1 KCA 8.4.1/08
M-03 (AE0608000)	*Eisenia fetida* reproduction 56 d, mixed into soil	NOEC $EC_{10/20}$	**≥ 50 mg p.m./kg dws** a) Calculation not possible	[redacted] 2016; M-557757-01-1 KCA 8.4.1/07

Bold values used in risk assessment
dws = dry weight soil; prod.= product; a.s. = active substance; p.m.= pure metabolite
a) Endpoint corrected by a factor of 2 due to lipophilic substance (log P_{OW} > 2)

Risk assessment for earthworms

Table 10.4.1- 2: TER calculation for earthworms for the product FLC + FXA FS 350

Compound	Species, study type	Endpoint [mg prod./kg]		PEC_{soil} [mg prod./kg]	TER_{LT}	Trigger
Winter oilseed rape, 1 × 0.06 L prod./ha						
FLC + FXA FS 350	Earthworm, reproduction	NOEC	158	0.093	1699	5

Table 10.4.1- 3: TER calculations for earthworms for fluopicolide and its metabolites

Compound	Species, study type	Endpoint [mg/kg]		PEC_{soil} [mg/kg]	TER_{LT}	Trigger
Winter oilseed rape, 1 × 0.06 L prod./ha						
Fluopicolide	Earthworm, reproduction	NOEC	31.25	0.021	1488	5
M-01 (AE C653711)	Earthworm, reproduction	NOEC	250	0.005	50000	5
M-02 (AEC657188)	Earthworm, reproduction	NOEC	> 100	0.002	>50000	5
M-03 (AE0608000)	Earthworm, reproduction	NOEC	≥ 50	0.003	≥16667	5

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 FLC + FXA FS 350 in winter oilseed rape.

CP 10.4.1.1 Earthworms sub-lethal effects

Data Point:	KCP 10.4.1.1/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150) G: Sublethal toxicity to the earthworm Eisenia fetida in artificial soil
Report No:	15 10 48 129 S
Document No:	M-528042-01-1
Guideline(s) followed in study:	OECD 222 (2004), ISO 11268-2 (1998)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to assess the effect of FLC + FXA FS 350 (200 + 150) G on survival, growth, and reproduction of the earthworm *Eisenia fetida* during an exposure into an artificial soil at 8 different application rates. Adult earthworms (3 months old, 8 × 10 animals per control and 4 × 10 animals per test item group) were exposed in an artificial soil to concentrations of 0 (control), 18, 32, 56, 100, 178, 316, 562 and 1000 mg test item/kg dry weight (mixed into soil). After 28 days, the number of surviving animals and their weight alteration were determined. After further 28 days, the numbers of juveniles were determined. All validity criteria were met. No significantly adverse effects on mortality of the earthworm *Eisenia fetida* in artificial soil up to and including 1000 mg test item/kg soil dry weight were observed. The test item showed statistically significantly adverse effects on growth and reproduction at 562 and 1000 mg test item/kg soil d.w. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 316 mg test item/kg soil d.w., and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 562 mg test item/kg soil d.w. The EC_{10} and EC_{20} (reproduction) were calculated being 361 and 512 mg/kg dry weight soil, respectively.

I. MATERIAL AND METHODS:

Test item: Fluopicolide + Fluoxastrobin FS 350 (200+150) G, Short name: FLC + FXA FS 350 (200 + 150) G, Supplier batch No.: 2014-014396, Sample description: TOX10774-00, Specification No.: 102000028578, active ingredients (analysed content): 17.0 % w/w (198.7 g/L) fluopicolide (AE C638206), 13.1 % w/w (148.4 g/L) fluoxastrobin (HEC 5725 E-iso), Density (20 °C): 1.164 g/mL, water solubility: dispersible.
Adult earthworms (*Eisenia fetida,* about 3 months old) were exposed to 18 - 32 - 56 - 100 - 178 – 316 – 562 - 1000 mg test item/kg dry weight (d.w.) of soil containing 69.5 % quartz sand, 20 % kaolin clay, 10 % sphagnum peat and 0.5 % $CaCO_3$, at 19.1 – 22.0 °C and a photoperiod: light : dark = 16 h : 8 h (550 lux) and were fed with horse manure. Mortality and biomass change were determined after 4 weeks and reproduction was determined after 8 weeks.

II. RESULTS AND DISCUSSION:

Effects on mortality, growth and reproduction of the earthworms

Test item **Test object** **Exposure**	**Fluopicolide + Fluoxastrobin FS 350 (200+150) G** ***Eisenia fetida*** **Artificial soil**		
	Mortality	**Growth**	**Reproduction**
	[mg test item/kg d.w.]		
NOEC LOEC	≥ 1000 > 1000	316 562	316 562
EC_{10} [1)] (95% confidence limits)	-	-	361 (258 – 506)
EC_{20} [1)] (95% confidence limits)	-	-	512 (411 – 637)

[1)] based on Probit analysis

Observations:

	Fluopicolide + fluoxastrobin FS 350 (200+150) G [mg test item/kg d.w.]								
	Control	**18**	**32**	**56**	**100**	**178**	**316**	**562**	**1000**
Mortality of adult worms after 4 weeks									
Mortality (%)	2.5	0.0	0.0	0.0	5.0	0.0	0.0	2.5	2.5
Biomass change (change in fresh weight after 4 weeks relative to initial fresh weight)									
Mean (mg)	84.5	81.9	79.1	88.2	84.8	80.5	76.7	47.5*	29.5 *
Mean (%)	24.4	23.7	23.4	25.4	24.5	23.1	22.2	13.7	8.6
Number of juveniles per surviving adult worm after 8 weeks									
Mean	13.2	12.7	13.0	12.9	14.2	13.5	12.8	9.2	6.9
Number of juveniles per replicate after 8 weeks									
Mean	128.6	127.0	129.5	128.5	135.3	135.3	128.0	89.8*	67.5 *
Reproduction compared to control (%)									
% to control	100	98.7	100.7	99.9	105.2	105.2	99.5	69.8	52.5

No statistically significant differences between the control and test item were calculated for mortality (Chi² 2 x 2 Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater)
* statistically significantly different compared to control for biomass and reproduction
(Williams-t-test, $\alpha = 0.05$, one-sided smaller)

In a reference test, the number of juveniles was reduced by 46 and 100 % by the toxic standard Nutdazim 50 FLOW (Carbendazim, SC 500) at concentrations of 5 and 10 mg/kg d.w. in comparison to the control. Therefore, the observed effects assure a high sensitivity of the test system.

Validity criteria:

Validity criteria (OECD 222, 2004)	Recommended	Obtained
Adult control mortality	≤ 10%	2.5 % (after 4 weeks)
Number of juveniles per control replicate	≥ 30	129 (mean)
Coefficient of variation of reproduction in the control	≤ 30%	11.4 %

III. CONCLUSIONS:

Fluopicolide + fluoxastrobin FS 350 (200+150) G showed no statistically significantly adverse effects on mortality of the earthworm *Eisenia fetida* in artificial soil up to and including 1000 mg test item/kg soil dry weight, i.e. the highest concentration tested. The test item showed statistically significantly adverse effects on biomass and reproduction and at 562 and 1000 mg test item/kg soil d.w. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be 316 mg test item/kg soil d.w., and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be 562 mg test item/kg soil d.w. The EC_{10} and EC_{20} (reproduction) were calculated being 361 and 512 mg/kg dry weight soil, respectively.

Assessment and conclusion by applicant:

The study is considered reliable. The NOEC = 316 mg product/kg dws should be used in the risk assessment.

CP 10.4.1.2 Earthworms field studies

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

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: Endpoints used in risk assessment

Test item	Test species, test design	Ecotoxicological endpoint		Reference
FLC + FXA FS 350	*Folsomia candida* reproduction 28 d, mixed into soil	NOEC EC_{10}	**≥ 500 mg prod./kg dws** [a)] calculation not possible	[redacted] 2015; M-525666-01-1 KCP 10.4.2.1/01
FLC + FXA FS 350	*Hypoaspis aculeifer* reproduction 14 d, mixed into soil	NOEC EC_{10}	**≥ 500 mg prod./kg dws** [a)] calculation not possible	[redacted] 2015; M-528918-01-1 KCP 10.4.2.1/02
Fluopicolide	*Folsomia candida* reproduction 28 d, mixed into soil	NOEC EC_{10}	31.25 mg a.s./kg dws [a)] **16.44 mg a.s./kg dws** [a)]	[redacted] 2003; M-241194-01-1 KCA 8.4.2.1/01 EC_{10} calculation: [redacted] 2020; M-679537-01-1
Fluopicolide	*Hypoaspis aculeifer* reproduction 14 d, mixed into soil	NOEC EC_{10}	**≥ 500 mg a.s./kg dws** [a)] calculation not possible	[redacted] 2016; M-548042-01-1 KCA 8.4.2.1/05
M-01 (AE C653711)	*Folsomia candida* reproduction 28 d, mixed into soil	NOEC EC_{10}	**25 mg a.s./kg dws** calculation not possible	[redacted] 2003; M-241193-01-1 KCA 8.4.2.1/02
M-01 (AE C653711)	*Hypoaspis aculeifer* reproduction 14 d, mixed into soil	NOEC EC_{10}	**≥ 100 mg p.m./kg dws** calculation not possible	[redacted] 2015; M-538626-01-1 KCA 8.4.2.1/06
M-02 (AE C657188)	*Folsomia candida* reproduction 28 d, mixed into soil	NOEC EC_{10}	**≥ 100 mg p.m./kg dws** calculation not possible	[redacted] 2016; M-558332-01-1 KCA 8.4.2.1/04
M-02 (AE C657188)	*Hypoaspis aculeifer* reproduction 14 d, mixed into soil	NOEC EC_{10}	**≥ 100 mg p.m./kg dws** calculation not possible	[redacted] 2016; M-557987-01-1 KCA 8.4.2.1/07
M-03 (AE 0608000)	*Folsomia candida* reproduction 28 d, mixed into soil	NOEC EC_{10}	**≥ 50 mg p.m./kg dws** [a)] calculation not possible	[redacted] 2016; M-558337-01-1 KCA 8.4.2.1/03
M-03 (AE 0608000)	*Hypoaspis aculeifer* reproduction 14 d, mixed into soil	NOEC EC_{10}	**≥ 50 mg p.m./kg dws** [a)] calculation not possible	Calculated endpoint [b)]

Bold values used in risk assessment
dws = dry weight soil; prod. = product; a.s. = active substance; p.m.= pure metabolite
a) Endpoint corrected by a factor of 2 due to lipophilic substance (log P_{OW} > 2)
b) calculated endpoint assuming a 10-fold higher toxicity of M-03 (AE 0608000) compared to the parent active substance (see KCA 8.4.2.1/05)

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

Table 10.4.2- 2: TER calculations for the product FLC + FXA FS 350 for other non-target soil meso- and macrofauna

Compound	Species	Endpoint [mg prod./kg]		PEC_{soil} [mg prod./kg]	TER_{LT}	Trigger
Winter oilseed rape, 1 × 0.06 L prod./ha						
FLC + FXA FS 350	*Folsomia candida*	NOEC	≥ 500	0.093	5376	5
FLC + FXA FS 350	*Hypoaspis aculeifer*	NOEC	≥ 500	0.093	5376	5

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

Compound	Species	Endpoint [mg/kg]		PEC_{soil} [mg/kg]	TER_{LT}	Trigger
Winter oilseed rape, 1 × 0.06 L prod./ha						
Fluopicolide	*Folsomia candida*	EC_{10}	16.44	0.021	783	5
Fluopicolide	*Hypoaspis aculeifer*	NOEC	≥ 500	0.021	23810	5
M-01 (AE C653711)	*Folsomia candida*	NOEC	25	0.005	5000	5
M-01 (AE C653711)	*Hypoaspis aculeifer*	NOEC	≥ 100	0.005	20000	5
M-02 (AE C657188)	*Folsomia candida*	NOEC	≥ 100	0.002	50000	5
M-02 (AE C657188)	*Hypoaspis aculeifer*	NOEC	≥ 100	0.002	50000	5
M-03 (AE 0608000)	*Folsomia candida*	NOEC	≥ 50	0.003	16667	5
M-03 (AE 0608000)	*Hypoaspis aculeifer*	NOEC	≥ 50 [a)]	0.003	16667	5

[a)] calculated endpoint for *Hypoaspis aculeifer* assuming a 10-fold higher toxicity compared to the parent active substance

A *Hypoaspis aculeifer* reproduction study is not available for the metabolite M-03 (AE 0608000). However, the toxicity of the parent active substance fluopicolide and of all other metabolites to *Hypoaspis aculeifer* is very low. Even if a 10-fold higher toxicity compared to the parent active substance would be assumed, the tier 1 risk assessment would still indicate a low risk to soil mites with a high margin of safety (TER ≥ 16667). Hence, no unacceptable risk can be concluded for the metabolite M-03 (AE 0608000) in the risk assessment for soil mites.

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 FLC + FXA FS 350 in winter oilseed rape.

CP 10.4.2.1 Species level testing

Data Point:	KCP 10.4.2.1/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150) G: Effects on the reproduction of the collembolan Folsomia candida
Report No:	15 10 48 127 S
Document No:	M-525666-01-1
Guideline(s) followed in study:	OECD 232 (2009), ISO 11267 (1999)
Deviations from current test guideline:	Current Guideline: OECD 232 (2016) No deviations
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 the study was to determine the effects of FLC+FXA FS 350 (200+150) G on reproduction of the Collembola *Folsomia candida* in artificial soil. 10 (age of 10-12 days) collembolans per replicate were exposed to control (water treated) and treatments with 100, 178, 316, 562 and 1000 mg/kg dry soil. After 28 days the mortality and reproduction were assessed. All validity criteria were met. The test item showed no statistically significantly ad-verse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil up to and including 1000 mg test item/kg d.w. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg d.w.

I. Material and Methods:

Test item: fluopicolide + fluoxastrobin FS 350 (200+150) G, Short name: FLC+FXA FS 350 (200+150) G, Supplier batch No.: 2014-014396, Sample description: TOX10774-00, Specification No.: 102000028578, active ingredients (analysed content): 17.0 % w/w (198.7 g/L) fluopicolide (AE C638206), 13.1 % w/w (148.4 g/L) fluoxastrobin (HEC 5725 E-iso), Density (20 °C): 1.164 g/mL, water solubility: dispersible.
10 Collembola (9-12 days old) were exposed to concentrations of 100 – 178 – 316 – 562 - 1000 mg test item/kg dry weight soil (mixed into soil) containing 74.7 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.3 % $CaCO_3$, at 19.1 – 22.0 °C and a photoperiod: light : dark = 16 h : 8 h (550 lux) and were fed weekly with granulated dry yeast. Mortality and reproduction were determined after 28 days.
Toxic standard: 44 – 67 – 100 - 150 - 225 mg boric acid/kg soil d.w; control: untreated, solvent control: none.

II. Results and Discussion:

Effects on mortality and reproduction of *Folsomia candida* Test item Test object Exposure		**Fluopicolide + Fluoxastrobin FS 350 (200+150) G *Folsomia candida* Artificial soil**		
mg test item/kg soil dry weight nominal concentration	**Adult mortality (%)**	**Mean number of juveniles per test vessel ± standard deviation**	**Reproduction (% of control)**	**Significance (*)**
Control	3.8	773 ± 110	-	
100	5.0	756 ± 48	98	-
178	0.0	777 ± 118	101	-
316	0.0	772 ± 103	100	-
562	2.5	785 ± 121	102	-
1000	2.5	769 ± 61	100	-
Reproduction				
$NOEC_{reproduction}$ (mg test item/kg soil dry weight)		≥ 1000		
$LOEC_{reproduction}$ (mg test item/kg soil dry weight)		> 1000		

The calculation of an ECx-curve was not possible due to the lack of a significant dose-response relationship.

In a separate study (BioChem project No. R14 10 48 003 S, dated July 30, 2014), the EC_{50} (reproduction) of the reference item boric acid was calculated to be 104 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system.

Validity criteria:

Validity criteria (OECD 232, 2016)	**Required**	**Achieved**
Control Mortality	≤ 20 %	3.8 %
Control Reproduction (Juveniles per Container)	≥ 50	773
Coefficient of Variation of the Control Reproduction	< 30%	14.2 %

III. Conclusions:

The test item fluopicolide + fluoxastrobin FS 350 (200+150) G showed no statistically significantly adverse effects on adult mortality and reproduction of the collembolan *Folsomia candida* in artificial soil up to and including 1000 mg test item/kg d.w. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg d.w., and the Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg d.w.

Assessment and conclusion by applicant:

The study is considered reliable. The NOEC ≥ 1000 mg product/kg dry weight soil should be used in the risk assessment.

Data Point:	KCP 10.4.2.1/02
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150) G: Effects on the reproduction of the predatory mite Hypoaspis aculeifer
Report No:	15 10 48 128 S
Document No:	M-528918-01-1
Guideline(s) followed in study:	OECD 226 (2008)
Deviations from current test guideline:	Current Guideline: OECD 226 (2016) No deviations
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine potential effects of FLC+FXA FS 350 (200+150) G on the mortality and the reproductive output of the soil mite species *Hypoaspis aculeifer* as a representative of soil micro-arthropods during a test period of 14 days. Ten adults, fertilized, female *Hypoaspis aculeifer* per replicate (8 replicates for the control group and for each treatment group) were exposed to control and limit concentration of 1000 mg test item/kg dry weight (d.w.) mixed into soil. After a period of 14 days, the surviving adults and the living juveniles were extracted by applying a temperature gradient using a MacFadyen-apparatus. All *Hypoaspis aculeifer* were counted. All validity criteria were met. The test item showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 1000 mg test item/kg soil dry weight. Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight, and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg soil dry weight.

I. MATERIAL AND METHODS:

Test item: fluopicolide + fluoxastrobin FS 350 (200+150) G, Short name: FLC+FXA FS 350 (200+150) G, Supplier batch No.: 2014-014396, Sample description: TOX10774-00, Specification No.: 102000028578, active ingredients (analysed content): 17.0 % w/w (198.7 g/L) fluopicolide (AE C638206), 13.1 % w/w (148.4 g/L) fluoxastrobin (HEC 5725 E-iso), Density (20 °C): 1.164 g/mL, water solubility: dispersible.
10 adult soil mites (females) were exposed to 1000 mg test item/kg dry weight (d.w.) of soil containing 74.8 % quartz sand, 20 % kaolin clay, 5 % sphagnum peat and 0.2 % $CaCO_3$, at 19.7 - 21.7 °C and a photoperiod: light : dark = 16 h : 8 h (503 lx) and were fed every 2 - 3 days with *Tyrophagus putrescentiae*. Mortality and reproduction were determined after 14 days of exposure.
Reference item (Dimethoate): 1.00 – 1.60 – 2.56 – 4.10 – 6.55 – 10.5 mg/kg soil d.w.; control: untreated, solvent control: none.

II Results and Discussion:

Effects on mortality and reproduction of *Hypoaspis aculeifer*

Test item **Test object** **Exposure**	**Fluopicolide + Fluoxastrobin FS 350 (200+150) G** ***Hypoaspis aculeifer*** **Artificial soil**	
	Adult mortality	**Reproduction**
	[mg test item/kg d.w.]	
NOEC	≥ 1000	≥ 1000
LOEC	> 1000	> 1000
EC_{10}	> 1000	> 1000
EC_{20}	> 1000	> 1000

Observations:

Endpoint	**Fluopicolide + Fluoxastrobin FS 350 (200+150) G** **[mg test item/kg d.w.]**	
	Control	**1000**
Mortality of soil mites after 14 days (%)	0.0	1.3
Mean number of juveniles after 14 days	188.0	201.3
CV (%)	21.4	22.4
Reproduction (% to control)	100	107

Not statistically significantly different compared to control (Fisher´s Exact Binomial Test for mortality, α = 0.05, one-sided greater; Student t-test for reproduction, α = 0.05, one-sided smaller)
Calculations were done using non-rounded values
Percent reproduction: (Rt / Rc) * 100 %
Rt = mean number of juvenile mites in the treated group(s)
Rc = mean number of juvenile mites in the control group.

An EC_x curve could not be calculated as only one concentration was tested and no effects were observed.

In a separate study (BioChem project No. R 14 10 48 001 S, dated June 10, 2014), the EC_{50} (reproduction) of the reference item Dimethoate was calculated to be 6.2 mg/kg soil d.w.
The results of the reference test demonstrate the sensitivity of the test system.

Validity criteria:

Validity criteria (OECD 226, 2016)	**Required**	**Archieved**
Control Mortality	≤ 20 %	0 %
Control Reproduction (Juveniles per Container)	≥ 50	188
Coefficient of Variation of the Control Reproduction:	≤ 30%	21.4 %

III. Conclusions:

The test item fluopicolide + fluoxastrobin FS 350 (200+150) G showed no statistically significantly adverse effects on adult mortality and reproduction of the predatory mite *Hypoaspis aculeifer* in artificial soil at 1000 mg test item/kg soil dry weight.
Therefore, the overall No-Observed-Effect-Concentration (NOEC) was determined to be ≥ 1000 mg test item/kg soil dry weight, and the overall Lowest-Observed-Effect-Concentration (LOEC) was determined to be > 1000 mg test item/kg soil dry weight.

Assessment and conclusion by applicant:

The study is considered reliable. The NOEC ≥ 1000 mg product/kg dry weight soil should be used in the risk assesment.

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: Endpoints used in risk assessment

Test item	Test design	Endpoint		Reference
FLC + FXA FS350	Study duration: 28 days	No unacceptable effects at an appl. rate of:	**0.47 mg prod./kg dws**	[redacted] 2015; M-527525-01-1 KCP 10.5/01
Fluopicolide	Study duration: 28 days	No unacceptable effects at an appl. rate of:	**1.77 mg a.s./kg dws**	[redacted] 2003; M-230023-01-1 KCA 8.5/01
M-01 (AE C653711)	Study duration: 28 days	No unacceptable effects at an appl. rate of:	**0.92 mg p.m./kg dws**	[redacted] 2004; M-235991-01-1 KCA 8.5/03
M-01 (AE C653711)	Study duration: 14 days	No unacceptable effects at an appl. rate of:	3.8 mg p.m./kg dws	[redacted] 1996; M-234312-01-1 KCA 8.5/02
M-02 (AE C657188)	Study duration: 28 days	No unacceptable effects at an appl. rate of:	**1.89 mg p.m./kg dws**	[redacted] 2016; M-557910-01-1 KCA 8.5/07
M-03 (AE 0608000)	Study duration: 28 days	No unacceptable effects at an appl. rate of:	**2.78 mg p.m./kg dws**	[redacted] 2016; M-555852-01-1 KCA 8.5/06

Bold values: endpoints used for risk assessment
dws = dry weight soil; prod. = product; a.s. = active substance; p.m. = pure metabolite

Risk assessment for Soil Nitrogen Transformation

Table 10.5-2: Risk Assessment for the product FLC + FXA FS 350 for soil micro-organisms

Compound	Species	Endpoint [mg prod./kg]	$PEC_{soil,max}$ [mg prod./kg]	Refinement required
Winter oilseed rape 1 × 0.06 L prod./ha				
FLC + FXA FS 350	Soil micro-organisms	0.47	0.093	No

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

Compound	Species	Endpoint [mg/kg]	$PEC_{soil,max}$ [mg/kg]	Refinement required
Winter oilseed rape 1 × 0.06 L prod./ha				
Fluopicolide	Soil micro-organisms	1.77	0.021	No
M-01 (AE C653711)	Soil micro-organisms	0.92	0.005	No
M-02 (AE C657188)	Soil micro-organisms	1.89	0.002	No
M-03 (AE 0608000)	Soil micro-organisms	2.78	0.003	No

According to regulatory requirements, the risk is acceptable if the effect on nitrogen transformation at the maximum PEC_{soil} values is < 25% after 100 days. In no case, deviations from the control exceeded 25% at concentrations which were clearly higher than the PECs in soil, indicating low risk to soil micro-organisms.

Data Point:	KCP 10.5/01
Report Author:	[redacted]
Report Year:	2015
Report Title:	Fluopicolide + fluoxastrobin FS 350 (200+150) G: Effects on the activity of soil microflora (Nitrogen transformation test)
Report No:	15 10 48 047 N
Document No:	M-527525-01-1
Guideline(s) followed in study:	OECD 216 (2000)
Deviations from current test guideline:	none
Previous evaluation:	No, not previously submitted
GLP/Officially recognised testing facilities:	Yes, conducted under GLP/Officially recognised testing facilities
Acceptability/Reliability:	Yes

Executive Summary

The purpose of this study was to determine the effects of FLC + FXA FS 350 (200+150) G on the activity of soil microflora with regard to nitrogen transformation in a laboratory test. A loamy sand soil was exposed for 28 days to concentrations of 0.09 mg test item/kg soil dry weight and 0.47 mg test item/kg soil dry weight. Each treatment consisted of 3 replicates. Application rates were equivalent to 0.061 L test item/ha and 0.305 L test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment). A reference item is not required by the guideline. Nevertheless, Dinoterb was used as a reference. Fluopicolide + fluoxastrobin FS 350 (200+150) G caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformation (expressed as NO_3-N-production) at the end of the 28-day incubation period.

I. MATERIAL AND METHODS:

Fluopicolide + fluoxastrobin FS 350 (200 + 150) G [short name: FLC + FXA FS 350 (200+150) G], Supplier batch No.: 2014-014396, Specification No.: 102000028578, Sample description: TOX10774-00, analytical findings: 17.0 % w/w (198.7 g/L) fluopicolide (AE C638206); 13.1 % w/w (148.4 g/L) fluoxastrobin (HEC 5725 E-iso), Density (20 °C): 1.164 g/mL, water solubility: dispersible.

A loamy sand soil (DIN 4220) was exposed for 28 days to 0.09 mg test item/kg soil dry weight and 0.47 mg test item/kg soil dry weight. Application rates were equivalent to 0.061 L test item/ha and 0.305 L test item/ha. The nitrogen transformation was determined in soil enriched with lucerne meal (concentration in soil 0.5 %). NH_4-nitrogen, NO_3- and NO_2-nitrogen were determined by an Autoanalyzer at different sampling intervals (0, 7, 14 and 28 days after treatment).

The coefficients of variation in the control (NO_3-N) were maximum 4.3 % and thus fulfilled the demanded range (≤15 %).

II. RESULTS AND DISCUSSION:

Effects on nitrogen transformation in soil after treatment with Fluopicolide + Fluoxastrobin FS 350 (200 + 150) G

Time Interval (days)	Control			0.09 mg test item/kg soil dry weight equivalent to 0.061 L test item/ha				0.47 mg test item/kg soil dry weight equivalent to 0.305 L test item/ha			
	Nitrate-N[1]			Nitrate-N[1]			% difference to control	Nitrate-N[1]			% difference to control
0-7	5.86	±	0.69	5.46	±	0.28	-6,8 n.s.	5.32	±	0.39	-9.2 n.s.
7-14	1.86	±	0.34	1.78	±	0.20	-4.4 n.s.	2.34	±	0.54	+25.9 n.s.
14-28	1.70	±	0.38	1.94	±	0.12	+13.7 n.s.	1.83	±	0.05	+7.3 n.s.

The calculations were performed with unrounded values
1) Rate: Nitrate-N in mg/kg soil dry weight/time interval/day, mean of 3 replicates and standard deviation
n.s. = No statistically significant difference to the control (Student-t-test for homogeneous variances, 2-sided, $p \leq 0.05$)

In a separate study the reference item Dinoterb caused stimulations of the nitrogen transformation of +39.1 %, +62.5 % and +112.0 % at 6.80 mg, 16.00 mg and 27.00 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28).

Observations:
The test item fluopicolide + fluoxastrobin FS 350 (200+150) G caused a temporary stimulation of the daily nitrate rate at the tested concentration of 0.47 mg test item/kg soil dry weight at time interval 7-14 days after application.
However, no adverse effects of fluopicolide + fluoxastrobin FS 350 (200+150) G on nitrogen transformations in soil could be observed at both tested concentrations at the end of the test, 28 days after application (time interval 14-28). Differences from the control of +13.7 % (test concentration 0.09 mg test item/kg soil dry weight) and +7.3 % (test concentration 0.47 mg test item/kg soil dry weight) were measured at the end of the 28-day incubation period (time interval 14-28).

Validity criteria:
All validity criteria were met in this study.

Validity criteria (OECD 216, 2000)	Obtained in this study
The coefficient of variation in the control for NO_3-N ≤ 15 %	4.3%

III. CONCLUSIONS:

Fluopicolide + fluoxastrobin FS 350 (200+150) G caused no adverse effects (difference to control < 25 %, OECD 216) on the soil nitrogen transformations (expressed as NO_3-N-production) at the end of the 28-day incubation period. The study was performed in a field soil at concentrations up to 0.47 mg test item/kg soil dry weight, which are equivalent to application rates up to 0.305 L test item/ha.

Assessment and conclusion by applicant:
The study is considered reliable. No adverse effects on the nitrate formation rate were seen up to a concentration of 0.47 mg product/kg dry weight soil (highest concentration tested), which is considered being the relevant endpoint for the risk assessment.

CP 10.6 Effects on terrestrial non-target higher plants

The provision of data on the formulation is not considered necessary because FLC + FXA FS 350 is used as seed treatment, and exposure of non-target plants in adjacent fields due to spray drift will not occur. Therefore, a risk assessment and tests on non-target plants are not needed.

CP 10.6.1 Summary of screening data

Not necessary under current data requirements.

CP 10.6.2 Testing on non-target plants

Not necessary under current data requirements.

CP 10.6.3 Extended laboratory studies on non-target plants

Not necessary under current data requirements.

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

Not necessary under current data requirements.

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

No further tests on other terrestrial organism deemed to be necessary due to the low to moderate acute and chronic ecotoxicity of fluopicolide + fluoxastrobin FS 350 as presented under the Points CP 10.1 to CP 10.6 in this MCP Summary.

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. Due to the low to moderate acute and chronic ecotoxicity of fluopicolide + fluoxastrobin FS 350 as presented under the Points CP 10.1 to CP 10.7, no monitoring of non-target organism is deemed to be necessary.