REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: SAP2101F

Product name(s): ZELORA START

Chemical active substance(s):

Prothioconazole 120 g/L

Folpet 300 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: Selectis Produtos para a Agricultura, S.A.

Submission date: December 2023, update: March 2024 April 2024

MS Finalisation date: June 2024 (initial Core Assessment)

August 2024 (final Core Assessment)

Version history

When	What
December 2023	V0 - Initial version submitted by the Selectis Produtos para a Agricultura, S.A. for submission to Poland in the frame of new PPP registration (Acording Art. 33 of Regulation EC No 1107/2009)
March 2024	V1 - Updated version submitted by the Selectis Produtos para a Agricultura, S.A. to address the data gaps received by zRMS PL. All changes are highlighted in green.
April 2024	V2 – Upated version from Applicant Selectis Produtos para a Agricultura, S.A. following data gaps identified by zRMS Poland. All the changes are highlighted in yellow.
June 2024	Initial zRMS assessment The report in the dRR format has been prepared by the Applicant, therefore all comments, additional evaluations and conclusions of the zRMS are presented in grey commenting boxes. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency. Following the evaluation and before sending the document for commenting, all coloured highlighting was removed, from the parts updated by the Applicant, for better legibility.
August 2024	Final report (Core Assessment updated following the commenting period) Additional information/assessments included by the zRMS in the report in response to comments received from the Applicant are highlighted in yellow. Not agreed or not relevant information are ~~struck through~~ and shaded for transparency.

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F. Fn. Fpn G. Gn. Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses. certain types of protected crops)																				
1	PL ~~SEU: FR. ES. IT. PT. GR. BG. HR~~	Wheat	F	*Septoria*	Tractor mounted spray	BBCH 32-61	a) 2 b) 2	14 days	a) 1.5 L/ha b) 3 L/ha	a) 180 g ai/ha + 450 g ai/ha b) 360 g ai/ha + 900 g ai/ha	150-400	42	Range: 1 L/ha - 1.5 L/ha	A	A	R [1)] (R1 scenario)	A	A	A	A
2	PL ~~SEU: FR. ES. IT. PT. GR. BG. HR~~	Barley	F	*Helminstorporium*	Tractor mounted spray	BBCH 30-61	a) 2 b) 2	14 days	a) 1.5 L/ha b) 3 L/ha	a) 180 g ai/ha + 450 g ai/ha b) 360 g ai/ha + 900 g ai/ha	150-400	42	Range: 1 L/ha - 1.5 L/ha	A	A	R [1)] R1 scenario)	A	A	A	A

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

[1)] Risk mitigation measures refered to Polnad PECsw scenarios

Explanation for column 15 – 21 "Conclusion"

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

Remarks table:

(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
(6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(8) The maximum number of application possible under practical conditions of use must be provided
(9) Minimum interval (in days) between applications of the same product.
(10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
(13) PHI - minimum pre-harvest interval
(14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-4 are the same as indicated in EFSA Scientific Report (2009) 297, 70-80.
The maximum occurrence is relevant for exposure evaluation, for information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of PEC_{soil} and $PEC_{sw/sed}$ values, considered further in the risk assessment.
As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1-4.

9.1.1.1 Effects on birds (KC 10.1.1) Effects on terrestrial vertebrates other than birds (KCP 10.1.2), Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

The risk assessment to birds and mammals was conducted according to the EFSA Guidance Document for the Risk Assessment for Birds and Mammals.
An acceptable risk was obtained for Prothioconazole at the screening phase for acute and long-term exposure. For the prothioconazole metabolite JAU 6476-desthio and for Folpet, an acceptable risk was obtained at the first-tier for acute and long-term exposure. The acute and long-term risk to birds exposed to SAP2101F via drinking water is acceptable for the intended uses. An acceptable risk was also obtained for the secondary poisoning scenarios. Overall, birds present an acceptable risk towards SAP2101F when used according to the proposed application patterns.
An acceptable risk was obtained for Prothioconazole and Folpet at the screening phase for acute and long-term exposure. For the prothioconazole metabolite JAU 6476-desthio, an acceptable risk was obtained at the higher-tier for long-term exposure. The acute and long-term risk to mammals exposed to SAP2101F via drinking water is acceptable for the intended uses. An acceptable risk was also obtained for the secondary poisoning scenarios. Overall, mammals present an acceptable risk towards SAP2101F when used according to the proposed application patterns. No further data for to reptiles and amphibians.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

Regarding the active substance **prothioconazole** and its respective metabolites, the max PECsw are below the RAC with FOCUS Step 1-2 calculations with the exception for the metabolite **prothioconazole-desthio**, for which Step 4 calculations were needed (i.e. mitigation measures).
The detailed results of FOCUS Step 3 and Step 4 calculations for ~~both~~ maximum ~~and minimum~~ dose demonstrate a safe use to aquatic organisms in the following mitigated scenarios:

Maximum dose:

Winter cereals:

- a non-spray buffer zone of 20 m of vegetated filter strip, for multiple applications for R1, R3 and R4

scenarios;

- a non-spray buffer zone of 10 m of vegetated filter strip, for single application for R3, R4 scenarios;

Spring cereals:

- a non-spray buffer zone of 20 m of vegetated filter strip, for multiple application for D1, R4 scenario and **R1, R3** scenarios (from winter cereals)
- a non-spray buffer zone of 10 m of vegetated filter strip, for single application for R4 scenario and **R3** scenarios (from winter cereals)

Regarding **the folpet** active substance, FOCUS Step 3 and Step 4 were simulated. For the folpet metabolites the max PECsw are below the RAC with Step 1-2 calculations. The detailed results of the FOCUS Step 3 and 4 calculations demonstrate a safe use to aquatic organisms in the following mitigated scenarios:

Maximum dose:

Winter cereals:
- a non-spray buffer zone of 10 m of vegetated filter strip, for multiple application for R1, R3 and R4 scenarios.
- a non-spray buffer zone for single application to winter cereals

Spring cereals:
- a non-spray buffer zone of 20 m of vegetated filter strip, for multiple application for R1, R3 and R4 scenario;
- a non-spray buffer zone of 10 m of vegetated filter strip, for single application for R4 scenario

It should be noted that the risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.
For remained scenarios, which are not relevant for Poland such as (D1, D2, R3 and R4) the risk mitigation measures for aquatic organism are left for decision at MSs.
Please note that additional aquatic risk assessment may be required by the concerned Member States that do not accept simulations performed according to FOCUS recommendations.

~~***Maximum dose***~~
~~Winter cereals:~~
- ~~a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple applications for R4 scenario;~~
- ~~a non-spray buffer zone of *15 m of vegetated filter strip*, for multiple applications for R1and R3 scenario;~~
- ~~a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R4 scenario;~~
- ~~a non-spray buffer zone of 5 *m of vegetated filter strip*, for single application for R3 scenario;~~

~~Spring cereals:~~
- ~~a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple application for D1 and R4 scenario;~~
- ~~a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R4 scenario;~~

~~Regarding the folpet active substance, FOCUS Step 3 and Step 4 were simulated. For the folpet metabolites the max PECsw are below the RAC with Step 1-2 calculations.~~
~~The detailed results of the FOCUS Step 3 and 4 calculations demonstrate a safe use to aquatic organisms in the following mitigated scenarios:~~
~~***Maximum dose***~~
~~Winter cereals:~~
- ~~a non-spray buffer zone of 5 *m of vegetated filter strip*, for multiple application for R3 scenario;~~

~~Spring cereals:~~
- ~~a non-spray buffer zone of *10 m of vegetated filter strip*, for multiple application for R4 scenario;~~
- ~~a non-spray buffer zone of *5 m of vegetated filter strip*, for single application for R4 scenario;~~

9.1.1.3 Effects on bees (KCP 10.3.1)

The risk assessment for bees was conducted according to SANCO/10329/2002 rev 2 final and according to EFSA Journal 2013;11(7):3295 for illustrative purposes only as the last-mentioned guidance document is not yet noted. The risk assessment performed for both the active substances and the formulated product derived hazard quotients lower than 50, indicating that the active substance prothioconazole, folpet and the formulation SAP2101F pose an acceptable risk to bees from oral and contact, both acute and chronic exposure, according to the proposed use.

9.1.1.4 **The risk assessment for bees was conducted according to SANCO/10329/2002 rev 2 final and according to EFSA Journal 2013;11(7):3295 for illustrative purposes only as the last-mentioned guidance document is not yet noted. The risk assessment performed for both the active substances and the formulated product derived hazard quotients lower than 50, indicating that the active substance prothioconazole, folpet**

and the formulation SAP2101F pose an acceptable risk to bees from oral and contact, both acute and chronic exposure, according to the proposed use.

9.1.1.5 Effects on arthropods other than bees (KCP 10.3.2)

No unacceptable risks are expected to the non-target arthropods (*T. pyri, A. rhopalosiphi, C. septempunctata* L. *and C. carnea*) due to exposure to SAP2101F formulation. The assessed risk in- and off-field at extended exposure showed acceptable risk, according to SAP2101F proposed uses, with Hazard Quotients below the trigger value of 1.

9.1.1.6 Effects on non-target soil meso- and macrofauna (KCP 10.4), No unacceptable long-term risks are expected for earthworms and other non-target soil organisms (meso- and macrofauna) due to exposure to either prothioconazole, folpet, relevant metabolites and SAP2101F formulation on its intended uses based on the TER values significantly higher than 5 trigger.

9.1.1.7 Effects on soil microbial activity (KCP 10.5)

No unacceptable long-term risks are expected for earthworms and other non-target soil organisms (meso- and macrofauna) due to exposure to either prothioconazole, folpet, relevant metabolites and SAP2101F formulation on its intended uses based on the TER values significantly higher than 5 trigger.

The use of prothioconazole, folpet, respective metabolites and SAP2101F according to the proposed use patterns, will not have unacceptable effects on soil micro-organisms. The applied maximum concentration used in RA did not cause any significant effects on soil nitrogen transformation.

9.1.1.8 Effects on non-target terrestrial plants (KCP 10.6)

The worst-case ER_{50} values are greater than the maximum single application dose rate and therefore it is considered that risks to non-target plants after SAP2101F formulation applications are acceptable according to its proposed use.

9.1.1.9 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

Table 9.1-2: Critical use pattern of SAP2101F grouped according to application pattern

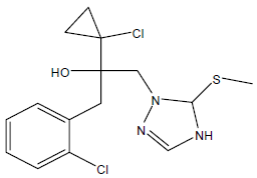
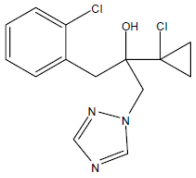
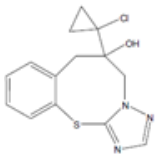
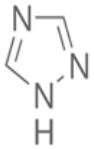
Grouping according to application pattern			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Effects on birds and terrestrial vertebrates (9.2 and 9.3)			
Bulbs and onion like crops. **cereals**. fruiting vegetables. leafy vegetables. legume forage. maize.	Wheat BBCH 32-61	Crop group according to EFSA/2009/1438	Prothioconazole: 2 x 180 g a.s./ha. Folpet: 2 x 450 g a.s./ha

oilseed rape. potatoes. pulses. root and stem vegetables. strawberries. sugar beet. and sunflower	Barley BBCH 30-61		Interval 14 days BBCH 30-61
Effects on aquatic organisms (9.5)			
Grouped according to Environmental Fate section 8	Wheat BBCH 32-61	Crop group according to application pattern	Prothioconazole: 2 x 180 g a.s./ha. Folpet: 2 x 450 g a.s./ha Interval 14 days BBCH 30-61
	Barley BBCH 30-61		
Effects on bees (9.6)			
Field corps	Wheat BBCH 32-61	Crop group according to application pattern	Prothioconazole: 2 x 180 g a.s./ha. Folpet: 2 x 450 g a.s./ha Interval 14 days BBCH 30-61
	Barley BBCH 30-61		
Effects on non-target arthropods (9.7)			
Field crops	Wheat BBCH 32-61	Crop group according to application pattern	Prothioconazole: 2 x 180 g a.s./ha. Folpet: 2 x 450 g a.s./ha Interval 14 days BBCH 30-61
	Barley BBCH 30-61		
Effects on non-target soil meso- and macrofauna (9.8)			
Grouped according to Environmental Fate section 8	Wheat BBCH 32-61	Crop group according to application pattern	Prothioconazole: 2 x 180 g a.s./ha. Folpet: 2 x 450 g a.s./ha Interval 14 days BBCH 30-61
	Barley BBCH 30-61		
Effects on soil microbial activity (9.9)			
Grouped according to Environmental Fate section 8	Wheat BBCH 32-61	Crop group according to application pattern	Prothioconazole: 2 x 180 g a.s./ha. Folpet: 2 x 450 g a.s./ha Interval 14 days BBCH 30-61
	Barley BBCH 30-61		
Effects on non-target terrestrial plants (9.10)			
Field crops	Wheat BBCH 32-61	Crop group according to application pattern	Prothioconazole: 2 x 180 g a.s./ha. Folpet: 2 x 450 g a.s./ha Interval 14 days BBCH 30-61
	Barley BBCH 30-61		

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of SAP2101F is indicated in the table.

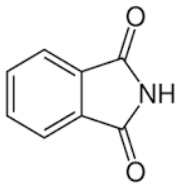
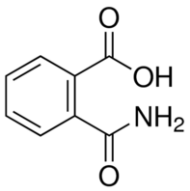
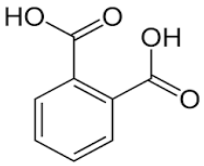
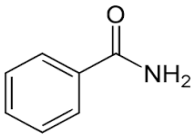
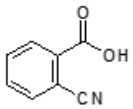
Table 9.1-3 Metabolites of prothioconazole

Metabolite	Chemical structure	Molar mass (g/mol)	~~Maximum occurrence in compartments~~	Risk assessment required?
M01: JAU 6476-S methyl Prothioconazole-S-Methyl CAS 178928-71-7		358.281	~~Soil: 14.6 %~~ ~~Water: 8.6 %~~ ~~(anaerobic water/sediment study)~~ ~~Sediment: 77 %~~ ~~(anaerobic water/sediment study)~~	Yes, soil and aquatic organisms
M04 : JAU 6476-desthio Prothioconazole-desthio CAS 120983-64-4		312.2	~~Soil: 49.4% (57.1% conversion (field))~~ ~~Water: 32.3% (55.7% aqueous photolysis)~~ ~~Sediment: 26.9%~~	Yes, soil and aquatic organisms
M12: Prothioconazole-thiazocine		307.8	~~Soil: –~~ ~~Water: 14.1% (aqueous photolysis)~~ ~~Sediment: –~~	Yes, aquatic organisms
M13: 1.2.4-triazole		69.07	~~Soil: –~~ ~~Water: 37.2 % (11.9% aqueous photolysis)~~ ~~Sediment: 4.6%~~	Yes, aquatic organisms

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-3 are the same as indicated in EFSA Scientific Report (2007) 106. It is noted that in the course of the EU review of prothioconazole metabolite JAU 6476-thiazocine was formed at >10% in photodegradation study in water, however according to EFSA Scientific Report (2007) 106, it was considered to be not relevant for evaluation in area of ecotoxicology.
The maximum occurrence is relevant for exposure evaluation, for information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of PEC_{soil} and $PEC_{sw/sed}$ values, considered further in the risk assessment.
As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1-3.

Table 9.1-4 Metabolites of folpet

Metabolite	Chemical structure	Molar mass (g/mol)	~~Maximum occurrence in compartments~~	Risk assessment required?
Phthalimide		147.13	~~Soil: 64.9 %*~~ ~~Water: 26.0 %~~ ~~Sediment: 5.9 %~~	Yes, soil and aquatic organisms
Phthalamic acid		165.15	~~Soil: 16.7 %*~~ ~~Water: 13.3 %~~ ~~Sediment: –~~	Yes, soil and aquatic organisms
Phthalic acid		166.14	~~Soil: 16.6 %*~~ ~~Water: 37.5 %~~ ~~Sediment: 3.8 %~~	Yes, soil and aquatic organisms
Benzamide		121.14	~~Soil: –~~ ~~Water: 10.2 %~~ ~~Sediment: –~~	Yes, aquatic organisms
2-cyanobenzoic acid		147.13	~~Soil: –~~ ~~Water: 39.7 %~~ ~~Sediment: –~~	Yes, aquatic organisms

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-4 are the same as indicated in EFSA Scientific Report (2009) 297, 70-80.
The maximum occurrence is relevant for exposure evaluation, for information agreed in this area please refer to the Core Assessment, Part B, Section 8, where all respective data are provided and used in calculation of PEC_{soil} and $PEC_{sw/sed}$ values, considered further in the risk assessment.
As the information on the maximum occurrence was not checked in detail, it was struck through in Table 9.1-4.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with prothioconazole, folpet and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.
Effects on birds of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet.
However, the provision of further data on the SAP2101F is not considered essential, because the risk assessment can be reliably and conservatively performed with the data of the separate active substances.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.2-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Prothioconazole				
Bobwhite quail	Prothioconazole	Acute	LD_{50} > 2000 mg a.s./kg bw	EFSA Scientific Report (2007)
Bobwhite quail	Prothioconazole	5d, dietary	LC_{50} > 5000 mg a.s./kg diet **Calc. LD_{50} > 1413 mg a.s./kg bw/day**	EFSA Scientific Report (2007)
Mallard Duck	Prothioconazole	5d, dietary	LC_{50} > 5000 mg a.s./kg diet Calc. LD_{50} > 2457 mg a.s./kg bw/day	EFSA Scientific Report (2007)
Bobwhite quail	Prothioconazole	Reproduction 22w, dietary	NOEC ≥ 1000 mg a.s./kg diet Calc. NOEL ≥ 86 mg a.s./kg bw/day	EFSA Scientific Report (2007)
Mallard Duck	Prothioconazole	Reproduction 21w, dietary	NOEC = 700 mg a.s./kg diet **Calc. NOEL = 78 mg a.s./kg bw/day**	EFSA Scientific Report (2007)
Bobwhite quail	JAU 6476-desthio	Acute	LD_{50} > 2000 mg p.m./kg b.w.	EFSA Scientific Report (2007)
Bobwhite quail	JAU 6476-desthio	5d, dietary	LC_{50} = 4090 mg p.m./kg diet **Calc. LD_{50}[1] > 297 mg p.m./kg bw/d**	EFSA Scientific Report (2007)
Bobwhite quail	JAU 6476-desthio	Reproduction 20w, dietary	NOEC = 173 mg p.m./kg diet **Calc. NOEL = 14.8 mg p.m./kg bw/day**	EFSA Scientific Report (2007)
Mallard Duck	JAU 6476-desthio	Reproduction 20w, dietary	NOEC **≥** 500 mg p.m./kg diet Calc. NOEL = 63 mg p.m./kg bw/day	EFSA Scientific Report (2007)
Folpet				
Bobwhite quail (*Colinus virginianus*)	Folpet	Acute toxicity	**LD_{50} > 2510 mg/kg bw**	EFSA Scientific Report No.297 (2009)
Bobwhite quail (*Colinus virginianus*)	Folpet	Dietary toxicity (short-term)	LC_{50} > 5000 ppm (≈ 1127 mg/kg bw/day)	EFSA Scientific Report No.297 (2009)
Mallard duck (*Anas platyrhynchos*)	Folpet	Dietary toxicity (short-term)	LC_{50} > 5000 ppm (**≈ 746 mg/kg bw/day**)	EFSA Scientific Report No.297 (2009)
Bobwhite quail (*Colinus virginianus*)	Folpet	Reproductive toxicity (long-term)	NOEC = 1000 ppm (≈ 78.3 mg/kg bw/day)	EFSA Scientific Report No.297 (2009)
Mallard duck (*Anas platyrhynchos*)	Folpet	Reproductive toxicity (long-term)	NOEC = 1000 ppm (≈ 90.0 mg/kg bw/day)	EFSA Scientific Report No.297 (2009)

Bold indicates endpoints used in risk assessment.

[1] value represents the dose converted from the test group in which No Effect on mortality or food consumption was reported (1243 mg/kg diet/d multiplied by the mean daily food consumption (6.4 g/d for the 5 day exposure period) divided by the mean body-weight (26.75 g for the 5 day exposure period). A more precise conversion of the LC_{50} value requires reanalysis of data using the converted daily dietary doses for each test group.

Prothioconazole-desthio (JAU 6476-desthio) was considered to be the only major metabolite in cereal foliage. A total conversion of prothioconazole to the desthio metabolite was assumed in the risk assessment as a worst-case approach.

zRMS comments:

Prothioconazole

Avian toxicity data for prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.2-1 were confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2007) 106.

Folpet

Avian toxicity data for folpet provided in Table 9.2-1 above has been confirmed that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

9.2.1.1 Justification for new endpoints

Prothioconazole:
In line with the EFSA Guidance Document on Risk Assessment for Birds and Mammals (2009) Section 2.2. where it is lower than the acute LD_{50}, the dietary LD_{50} divided by 10 should be used in the acute risk assessment. Therefore, the dietary LD_{50} values for Prothioconazole and its metabolite JAU 6476-desthio have been considered in the risk assessment instead of the acute LD_{50} EU Agreed endpoints according to EFSA Scientific Report (2007), especially as the metabolite LC_{50} is an unbounded value.

Folpet:
Since the dietary endpoint divided by 10 (74.6 mg/kg bw/d) is lower than the reproductive endpoint (78.3 mg/kg bw/d), the lowest will be used for the long-term risk assessment below.

zRMS comments:

The following endpoints should be used in the acute and long-term risk assessment for birds:

Prothioconazole

LD_{50} >1413 mg a.s./kg bw
NOEC = 78 mg a.s/kg bw

Metabolite JAU 6476-desthio

LC_{50} >297 mg met./kg bw
NOEC=14.8 mg met/kg bw
In the risk assessment for prothioconazole, the short-term endpoint of LD_{50} > 1413 mg/kg bw/d instead of the EU agreed acute endpoint of LD_{50} > 2000 mg/kg bw/d was used. It is noted that both values represent the highest dose tested and that in both studies no mortalities were observed at this level. In zRMS's opinion the Applicant's approach is acceptable since it is more conservative.

Folpet

LD_{50}>2510 mg a.s./kg bw/d
LD_{50} >746 mg a.s./kg bw/d
NOEC=78.3 mg a.s./kg bw/d
NOEC = 74.63 mg/kg bw/dw/d*

*In zRMS's opinion the short - term endpoint LD_{50} > 746 mg/kg bw/d should be used in the acute risk assessment according to recommendation given in EFSA GD 2009 for B&M. For this reason, the risk assessment with LD_{50}/10 > 74.6 mg/kg bw/d is added by zRMS in the calculation of the risk assessment.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

9.2.2.1 First-tier assessment (screening/generic focal species)

For the screening step risk assessment of prothioconazole and its metabolite, it has been assumed that 100% of the parent becomes the metabolite. The application rate calculation for the metabolite was calculated as the respective maximum occurrence transformation, multiplying by a conversion factor (metabolite molecular weight (312.2) ÷ parent molecular weight (344.3)) to correct the molecular weight. This is a worst-case assumption and therefore appropriate as a screening/first-tier assessment.

The results of the acute and reproductive screening/first-tier risk assessments are summarised in the following tables.

Table 9.2-2: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of SAP2101F in cereals - Prothioconazole

Intended use	**Cereals**				
Active substance/product	Prothioconazole				
Application rate (g/ha)	2 x 180 - Prothioconazole 2 x 163.2 – JAU 6476-desthio				
Acute toxicity (mg/kg bw)	1413 (Prothioconazole) / 297 (JAU 6476-desthio)				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals (Prothioconazole)	Small omnivorous bird	158.8	1.2	34.30	41.2
Cereals (JAU 6476-desthio)	Small omnivorous bird	158.8	1.2	31.10	**9.6**
Reprod. toxicity (mg/kg bw/d)	78 (Prothioconazole) / 14.8 (JAU 6476-desthio)				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals (Prothioconazole)	Small omnivorous bird	64.8	1.4 x 0.53	8.65	9.0
Cereals (JAU 6476-desthio)	Small omnivorous bird	64.8	1.4 x 0.53	7.85	**1.9**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

No unacceptable risks of birds exposure the active substance prothioconazole are expected after application of SAP2101F in the intended uses. However, for the metabolite JAU 6476-desthio this is not the case. Therefore, a first-tier risk assessment for the metabolite is presented below in Table 9.2-3.

zRMS comments:

Screening step in the risk assessment

The screening step risk assessment for prothioconazole is validated by zRMS.
TER_A and TER_{LT} values for the exposure to prothioconazole are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds.

In case of risk assessment for metabolite JAU 6476 desthio an application rate of 0.1632 kg a.s./ha has been used. The application rate was calculated by multiplying the appl. rate of Prothioconazole by the ratio of the molecular weight of the metabolite (312.2 g/mol) and the molecular weight of the parent (344 g/mol), i.e. 312.2 / 344.26 = 0.907.

Based on the results of the acute and long-term risk assessment for metabolite JAU 6476-desthio the TER_A and TER_{LT} values are below the trigger of 10 and 5, respectively and Tier - 1 acute and long-term risk assessment for the metabolite of concern was required.

Table 9.2-3: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of SAP2101F in cereals – JAU 6476-desthio (metabolite)

Intended use	**Cereals**
Active substance/product	JAU 6476-desthio
Application rate (g/ha)	2 x 163.2 – JAU 6476-desthio
Acute toxicity (mg/kg bw)	297
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1.2	1.410048	210.6
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	1.2	2.35008	126.4

Reprod. toxicity (mg/kg bw/d)	14.8
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	1.4 x 0.53	0.39961152	37.0
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	5.4	1.4 x 0.53	0.65390976	22.6

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

After first-tier risk assessment, the risk of birds exposure to the metabolite JAU 6476-desthio is acceptable. Therefore, the risk of birds exposure to both the active substance prothioconazole and its metabolite JAU 6476-desthio is considered sufficiently addressed.

zRMS comments:

Tier 1 risk assessment

The evaluation of the acute and long-term risk at Tier 1 for birds for JAU 6476-desthio presented in Table 9.2-3 indicated acceptable risk for birds in cereals as TER_A and TER_{LT} are above trigger values of 10 and 5, respectively.

zRMS remark:

Due to that for combined risk assessment the Tier 1 for Folpet is considered, zRMS added Tier 1 for prothioconazole in the Table below:

First-tier assessment long-term/reproductive risk for birds due to the use of SAP2101F in cereals – prothiocanazole.

Intended use		**Cereals**			
Active substance/product		Prothioconazole			
Application rate (g/ha)		2 x 180 g a.s./ha			
Reprod. toxicity (mg/kg bw/d)		78 mg/kg bw/d			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	1.4 x 0.53	0.44	177.3
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	5.4	1.4 x 0.53	0.72	108.3

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The evaluation of long-term risk at Tier 1 for birds for prothioconazole presented in Table above indicated acceptable risk for birds in cereals as TER_{LT} are above trigger values 5.

Table 9.2-4: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of SAP2101F in cereals - Folpet

Intended use		**Cereals**			
Active substance/product		Folpet			
Application rate (g/ha)		2 x 450			
Acute toxicity (mg/kg bw) Short tem toxicity (mg/kg bw)		2 510,			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	85.75	29.3 ~~25.1~~
Reprod. toxicity (mg/kg bw/d)		74.6			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small omnivorous bird	64.8	1.4 x 0.53	21.64	**3.4**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

An acceptable acute risk for omnivorous birds can be identified at the screening step but the same was not observed for the long-term risk for which a first-tier risk assessment is presented below in Table 9.2-5.

zRMS comments:

Screening step in the risk assessment

The screening step risk assessment for folpet presented in the Table above has been amended by zRMS using for acute risk assessment the correct LD_{50} >2510 mg a.s./kg bw value. It should be noted that when LD_{50}>2510 mg a.s./kg bw is considered TER_A value for the exposure to folpet is above the trigger of 10, indicating acceptable acute risk for birds.
In addition, the lower dietary endpoint LD_{50}>746 mg a.s./kg bw for acute risk assessment has been considered by zRMS in the calculations.

The additionally calculations are presented below:

Screening assessment of the acute risk for birds due to the use of SAP2101F in cereals - Folpet

Intended use		**Cereals**			
Active substance/product		Folpet			
Application rate (g/ha)		2 x 450			
Acute toxicity (mg/kg bw)		746			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	85.75	**8.7**

Based on the results with lower value LD_{50} of 746 mg a.s./kg bw/d, the acute risk needs Tier 1 risk assessment.

First-tier assessment of the acute risk for birds due to the use of SAP2101F in cereals - Folpet

Intended use		**Cereals**			
Active substance/product		Folpet			
Application rate (g/ha)		2 x 450			
Acute toxicity (mg/kg bw)		746			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1.2	3.888	191.9
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	1.2	6.48	115.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Overall, acceptable acute risk to birds may be concluded when lower LD_{50} of 746 mg a.s./kg bw is considered.

Table 9.2-5: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of SAP2101F in cereals - Folpet

Intended use	**Cereals**
Active substance/product	Folpet
Application rate (g/ha)	2 x 450
Reprod. toxicity (mg/kg bw/d)	74.6
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	1.4 x 0.53	1.10187	67.7
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	5.4	1.4 x 0.53	1.80306	41.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

No unacceptable risks of birds exposure to the active substance folpet are expected after application of SAP2101F in the intended uses.

zRMS comments:

Long-term risk assessment:

The evaluation of the long-term risk at Tier - 1 for birds presented in Table 9.2-5 indicated acceptable risk for birds in cereals as TER_{LT} is above trigger values 5.

Overall, based on Applicants' and zRMS calculations, an acceptable acute and long-term risk to birds from active substances and metabolite JAU 6476-desthio may be concluded from the intended uses of SAP2101F.

Combined acute toxicity of the formulation SAP2101F

According to the EFSA Guidance on Birds and Mammals (2009), the mixed effects of a formulation containing more than one active substance should be assessed. For that, the calculation of a surrogate LD_{50} should be done following the equation:

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

With:

$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture; (please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

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

SAP2101F contains 120 g/L of Prothioconazole and 300 g/L of Folpet, i.e. 29% of Prothioconazole and 71% of Folpet.
Therefore,
$LD_{50\,(mix)} = ((0.29/1413) + (0.71/2510))^{-1} = 1867.5$ mg/kg bw/d
LD50 (mix) = ((0.29/1413) + (0.71/746))-1 = 864.3 mg/kg bw/d
The expected LD_{50} of SAP1240H is of 1867.5 mg/kg bw/d.
The expected LD50 of SAP1240H is of 864.3 mg/kg bw/d.

In order to analyze if one of the active substances is leading the toxicity of the mixture, a "tox per fraction" quotient can be calculated, using the following equation:

$$\text{tox per fraction (a.s.)} = \frac{LD_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{tox per fraction (mix)} = \frac{LD_{50}(\text{mix})}{\sum_i X(\text{a.s.}_i)}$$

Therefore,

Tox per fraction (Prothioconazole) = 1413/0.29 = 4872.4 mg/kg bw/d
Tox per fraction (Folpet) = 2510/0.71 = 3535.2 mg/kg bw/d
Tox per fraction (SAP2101F) = 1867.5/1 = 1867.5 mg/kg bw/d
Tox per fraction (Folpet) = 746/0.71 = 1050.7 mg/kg bw/d
Tox per fraction (SAP2101F) = 864.3/1 = 864.3 mg/kg bw/d

The quotient for Prothioconazole is: 1867.5/4872.4 = 0.38 (38%; deviation of 62% > 10%)
The quotient for Folpet is: 1867.5/3535.2 = 0.53 (53%; deviation of 47% > 10%)
The quotient for Prothioconazole is: 864.3/4872.4 = 0.18 (18%; deviation of 82% > 10%)
The quotient for Folpet is: 864.3/1050.7 = 0.82 (82%; deviation of 18% > 10%)

Therefore, the toxicity of the mixture is not lead by any of the active substance alone. An acute risk assessment is presented below with the calculated LD_{50} for the mixture (1867.5 mg/kg bw/d).
Therefore, the toxicity of the mixture is not lead by any of the active substance alone.
An acute risk assessment is presented below with the calculated LD_{50} for the mixture (864.3 mg/kg bw/d).

Table 9.2-6: Screening assessment of the acute risk for birds ~~mammals~~ due to the use of SAP2101F in cereals

Intended use		**Cereals**			
Active substance/product		SAP2101F			
Application rate (g/ha)		2 x 1710*			
Acute toxicity (mg/kg bw) LD_{50mix}		1867.5			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	325.86	**5.7**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Considering a density of the formulation of 1.14 g/mL.

Table 9.2-7: First-tier assessment of the acute risk for birds due to the use of SAP2101F in cereals

Intended use		**Cereals**			
Active substance/product		SAP2101F			
Application rate (g/ha)		2 x 1710*			
Acute toxicity (mg/kg bw) LD_{50mix}		1867.5			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**

Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1.2	14.7744	126.4
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	1.2	24.624	74.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Considering a density of the formulation of 1.14 g/mL.

zRMS comments:

The acute mixture risk assessment presented in the Tables 9.2-6 and 9.2-7 has been validated by zRMS.

In addition, conservative approach based on lower LD_{50mix} of 864.3 mg/kg bw/d value is also presented below:

Screening assessment of the acute risk for birds due to the use of SAP2101F in cereals

Intended use	**Cereals**
Active substance/product	SAP2101F
Application rate (g/ha)	2 x 1710*
Acute toxicity (mg/kg bw)	864.3
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	325.86	**2.7**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Considering a density of the formulation of 1.14 g/mL.

Based on the results presented in the Table above for the mixture toxicity assessment with LD_{50} of 864.3 mg a.s./kg bw the Tier 1 calculations are required.

First-tier assessment of the acute risk for birds due to the use of SAP2101F in cereals

Intended use	**Cereals**
Active substance/product	SAP2101F
Application rate (g/ha)	2 x 1710*
Acute toxicity (mg/kg bw)	864.3
TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals BBCH ≥ 40	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1.2	14.7744	58.5
Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	1.2	24.624	35.1

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Considering a density of the formulation of 1.14 g/mL.

The Tier 1, TER_A value is above trigger of 5, indicating an acute acceptable risk due to the use of SAP2101F in

cereals.

Overall, based on Applicants' and zRMS calculations of acute mixture toxicity, acceptable risk to birds from compounds active substances may be concluded from the intended uses of SAP2101F.

Combined long-term risk assessment:

TER_{combi} approach relevant for Central Zone has been used for combined risk assessment by zRMS and presented below:

The relevant calculations are provided below:

TER_{mix} assessment of long-term/reproductive risk for birds due to the use of SAP2101F in cereals for mixture prothioconazole +folpet and JAU6476-desthio is presented below:

Prothioconazole		**Folpet**		**Σ1/TER**	**Σ1/TER^{-}**	**Trigger**
108.3	0.0092	41.4	0.02	0.0292	34.24	5

[1)] TER_{LT} values calculated at Tier 1, lark BBCH 30-39

JAU 6476-desthio		**Folpet**		**Σ1/TER**	**Σ1/TER^{-}**	**Trigger**
22.6	0.044	41.4	0.02	0.064	15.62	5

[1)] TER_{LT} values calculated at Tier 1, lark BBCH 30-39

Overall, based on performed calculations of TER_{mix} values an acceptable combined long-term risk may be concluded for birds from combined exposure of the active substances or active substances and metabolite JAU 6476- deshio.

9.2.2.2 Higher-tier risk assessment

Not relevant.

9.2.2.3 Drinking water exposure

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/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

Since SAP2101F is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

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

With a K(f)oc of 1765 and 575.4 respectively for Prothioconazole and JAU 6476-desthio, both belong to the group of more sorptive substances. And Folpet, with a K(f)oc of 304, belongs to the group of less sorptive substances.

The effective application rate was calculated following the equation presented below:

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1-e^{-nki}}{1-e^{-ki}}$$

Where
k = $\ln(2)/DT_{50}$ (rate constant)
n = number of applications
i = application interval (days) = 12 days

Table 9.2-8: Effective application rate

Crop	Substance	DT_{50}	Max application rate (AR) (g as/ha)	No. of applications	$1-e^{-nki}$	$1-e^{-ki}$	MAF_{mean}	AR_{eff}
Cereals	Prothioconazole	1.2	180	2	1.000	1.000	1.000	180.06
	Folpet	1.38	450	2	1.000	0.999	1.001	450.40

Table 9.2-9: Ratio of AReff to acute/long term toxicity endpoint

					No concern if ratio
Prothioconazole: Effective application rate (g/ha) =	180.06				
Prothioconazole					
Koc (l/kg) =	1765				
Acute toxicity (mg/kg bw) =	1413	quotient =	0.13		≤ 3000
Reprod. toxicity (mg/kg bw/d) =	78	quotient =	2.32		≤ 3000
JAU 6476-desthio: Effective application rate (g/ha) =	180.06 (worst-case approach)				
JAU 6476-desthio					
Koc (l/kg) =	575.4				
Acute toxicity (mg/kg bw) =	297	quotient =	0.61		≤ 3000
Reprod. toxicity (mg/kg bw/d) =	14.8	quotient =	12.20		≤ 3000
Folpet: Effective application rate (g/ha) =	450.40				
Folpet					
Koc (l/kg) =	304				
Acute toxicity (mg/kg bw) =	746*	quotient =	0.60		≤ 50
Reprod. toxicity (mg/kg bw/d) =	74.6*	quotient =	6.04		≤ 50
Acute toxicity (mg/kg bw) =	>2510	quotient =	0.17		≤ 50
Reprod. toxicity (mg/kg bw/d) =	78.3	quotient =	5.75		≤ 50

* The lowest values

The acute and long-term risk to birds exposed to SAP2101F via drinking water is therefore acceptable for the intended uses.

zRMS comments:

zRMS agrees with calculation of acute and long-term risk to birds exposed to SAP2101F via drinking water
In order to apply consistent approach, the drinking water risk assessment was performed also for metabolite JAU 6476-S-methyl. Calculations were performed with assumption of 10 times toxicity of the parent.

The relevant calculations are presented below:

Effective application rate (g/ha) =	277*			
JAU 6476-S-methyl				
Koc (l/kg) =	2525.9			
Acute toxicity (mg/kg bw) =	141.3**	quotient =	1.96	≤ 3000
Reprod. toxicity (mg/kg bw/d) =	7.82**	quotient =	35.54	≤ 3000

*MAF= 1.54, based on DT_{50}soil= 15.7 (geomean, used for PECsw and PCEgw calculations).
**Parent/10: LD_{50} > 1413/10= 141.3 mg/kg bw and NOEC= 78.2/10= 7.82 mg/kg bw, respectively.

Prothioconazole-thiazocine and 1.2.4-triazole**,** these metabolites are not relevant for the soil compartment, thus they are not expected to be formed in puddle water.

The acute and long-term risk to birds exposed to SAP2101F via drinking water is acceptable for the intended uses.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of prothioconazole, JAU 6476-desthio and folpet amounts to 4.05, 3.04 and 3.017, respectively and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.2-10: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	comments
PEC_{soil} (mg/kg soil)	0.050	Worst-case initial PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	4.05 / 11220	-
Koc	1765	Aged soil column leaching study; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	3.83802	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.19190	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.20150	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78	-
TER_{lt}	387.1	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table 9.2-11: Assessment of the risk for earthworm-eating birds due to exposure to JAU 6476-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	comments
PEC_{soil} (mg/kg soil)	0.047	Worst-case PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	3.04 / 1096	-
Koc	575.4	Geomean, n=4; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	1.21635	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.05717	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.06003	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	-
TER_{lt}	246.6	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table 9.2-12: Assessment of the risk for earthworm-eating birds due to exposure to folpet via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Folpet	comments
PEC_{soil} (mg/kg soil)	0.198	Worst-case initial PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	3.017 / 1040	-
Koc	304	Worst-case assumption; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	2.191	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.433745	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.45543	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	74.6	-
TER_{lt}	163.8	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

zRMS comments:

zRMS agrees with the risk for earthworm-eating birds due to exposure to prothioconazole, metabolite JAU 6476-desthio and folpet via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals.
In case of prothioconazole-S-methyl, according to EFSA conclusion 2007, this metabolite has a log P_{ow}= 4.19, thus the risk assessment from secondary poisoning is triggered (logPow ≥ 3).
The relevant calculations for earthworm-eating birds from exposure of Prothioconazole-S-methyl are presented below:

Parameter	Prothioconazole-S-methyl	comments
PEC_{soil} (mg/kg soil)	0.02885	Worst-case PECsoil calculated in cereals
log P_{ow} / P_{ow}	4.19 / 15488.2	-
Koc	2525.9	Geomean used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	3.696	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.10	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.111	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	7.8*	-
TER_{lt}	70.3*	>5, no further refinement

* NOEC= 78/10= 7.8 mg/kg bw

Overall, the risk assessment to earthworm-eating birds from exposure SAP2101F is acceptable for the intended uses.

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water as a limit value for admissible concentrations of prothioconazole and folpet in water.

Table 9.2-13: Assessment of the risk for fish-eating birds due to exposure to prothioconazole via bio-accumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	comments
PEC_{sw} (mg/L)	0.01955	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	19.7	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.385135	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.061	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	78	-
TER_{lt}	1278.69	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table 9.2-14: Assessment of the risk for fish-eating birds due to exposure to JAU 6476-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	comments
PEC_{sw} (mg/L)	0.06649	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	65	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	4.32185	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.687	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	14.8	-
TER_{lt}	21.54	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table 9.2-15: Assessment of the risk for fish-eating birds due to exposure to folpet via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Folpet	comments
PEC_{sw} (mg/L)	0.11087	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	56	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	6.20872	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.99	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	74.6* 78.3	-
TER_{lt}	75.35* 79.1	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

zRMS comments:

Assessment of the risk for fish-eating birds due to exposure to prothioconazole, metabolite JAU 6476 and folpet via bioaccumulation in fish (secondary poisoning) for the intended use in cereals has been accepted by zRMS.
It is noted that in EFSA conclusion 2007 is specified that bioconcentration of prothioconazole-S-methyl should be considered at Member State level in case the surface water exposure assessment show that this metabolite may contaminate surface water from drainage and/or run-off. No bioconcentration study was available in EFSA 2007 since the concentration of this metabolite in surface water was predicted to be low.
In case of 1.2.4-triazole metabolite, according to EFSA 2007 it has a log Pow ≤ 3 thus no secondary poisoning

assessment is triggered.
In case of prothioconazole-thiazocine, according to DAR 2004, Vol.1, under environmental conditions, this metabolite is unlikely to be formed at >10% in natural surface water systems.

Overall, the risk assessment to earthworm-eating birds from exposure SAP2101F is acceptable for the intended uses.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.2.4 Overall conclusions

The risk assessment to birds was conducted according to the EFSA Guidance Document for the Risk Assessment for Birds and Mammals.
An acceptable risk was obtained for prothioconazole at the screening phase for acute and long-term exposure. For the prothioconazole metabolite JAU 6476-desthio and for Folpet, an acceptable risk was obtained at the first-tier for acute and long-term exposure.
The acute and long-term risk to birds exposed to SAP2101F via drinking water is acceptable for the intended uses.
An acceptable risk was also obtained for the secondary poisoning scenarios.
Overall, birds present an acceptable risk towards SAP2101F when used according to the proposed application patterns.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with prothioconazole, folpet and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet. However, the provision of further data on the formulation SAP2101F is not considered essential, because the risk assessment can be reliably and conservatively performed with the data of the separate active substances.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.3-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Prothioconazole				
Rat	Prothioconazole	Oral Acute	**$LD_{50(male, female)}$ > 6200 mg a.s./kg bw/d**	EFSA Scientific Report (2007)
Rat	EC 250	Oral Acute	$LD_{50(male, female)}$ > 2500 mg a.s./kg bw/d	EFSA Scientific Report (2007)
Rat	FS 100	Oral Acute	$LD_{50(male, female)}$ > 2500 mg a.s./kg bw/d	EFSA Scientific Report (2007)
Rat	Prothioconazole	Long-term (2-generation), gavage	$NOEL_{parental}$ = 9.7 mg a.s./kg bw/d **$NOEL_{reproduction}$ = 95.6**	EFSA Scientific Report (2007)

Species	Substance	Exposure System	Results	Reference
			mg a.s./kg bw/d	
Rat	JAU 6476-desthio	Oral Acute	$LD_{50(female)}$ = 2506 mg p.m./kg bw/d $LD_{50(male)}$ = 2806 mg p.m./kg bw/d	EFSA Scientific Report (2007)
Mouse	JAU 6476-desthio	Oral Acute	$LD_{50(female)}$ = 3459 mg p.m./kg bw/d **$LD_{50(male)}$ = 2235 mg a.s./kg bw/d**	EFSA Scientific Report (2007)
Rat	JAU 6476-desthio	Long-term (2-generation), oral	$NOEL_{parental}$ = 2.5 mg p.m./kg bw/d **$NOEL_{reproduction}$ = 10 mg p.m./kg bw/d**	EFSA Scientific Report (2007)
Folpet				
Rat	Folpet	Acute Oral	LD_{50} > **2000** mg/kg bw	EFSA Scientific Report (2009), 297, 1-80
-	Folpet	Long-term Reproductive	NOEC = **150** mg/kg bw/day	EFSA Scientific Report (2009), 297, 1-80

Bold indicates endpoints used in risk assessment.

Prothioconazole-desthio (JAU 6476-desthio) was considered to be the only major metabolite in cereal foliage. A total conversion of prothioconazole to the desthio metabolite was assumed in the risk assessment as a worst-case approach.

zRMS comments:

Prothioconazole

Mammalian toxicity data for prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.3-1 were confirmed that they are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106.

Folpet

Mammalian toxicity data for folpet provided in Table 9.3-1 above has been confirmed that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84.

9.3.1.1 Justification for new endpoints

Not relevant.

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

9.3.2.1 First-tier assessment (screening/generic focal species)

For the screening step risk assessment, it has been assumed that 100% of the parent becomes the me-tabolite. The application rate calculation for the metabolite was calculated as the respective maximum occurrence transformation, multiplying by a conversion factor (metabolite molecular weight (312.2) ÷ parent molecular weight (344.3)) to correct the molecular weight. This is a worst-case assumption and therefore

ap-propriate as a screening/first-tier assessment.

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables.

Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of SAP2101F in cereals - Prothioconazole

Intended use	**Cereals**				
Active substance/product	Prothioconazole				
Application rate (g/ha)	2 x 180 - Prothioconazole 2 x 163.2 – JAU 6476-desthio				
Acute toxicity (mg/kg bw)	6200 (prothioconazole) / 2235 (JAU 6476-desthio)				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals (Prothioconazole)	Small herbivorous mammal	118.4	1.2	25.57	242.4
Cereals (JAU 6476-desthio)	Small herbivorous mammal	118.4	1.2	23.19	96.4
Reprod. toxicity (mg/kg bw/d)	95.6 (prothioconazole) / 10 (JAU 6476-desthio)				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals (Prothioconazole)	Small herbivorous mammal	48.3	1.4 x 0.53	6.45	14.82
Cereals (JAU 6476-desthio)	Small herbivorous mammal	48.3	1.4 x 0.53	5.85	**1.71**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

No unacceptable risks of mammals exposure to the active substance prothioconazole are expected after application of SAP2101F in the intended uses. However, for the metabolite and in long-term scenario this is not the case. Therefore, a first-tier long-term risk assessment for the metabolite is presented below.

Table 9.3-3: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of SAP2101F in cereals – JAU 6476-desthio

Intended use	**Cereals**				
Active substance/product	JAU 6476-desthio				
Application rate (g/ha)	2 x 163.2 – JAU 6476-desthio				
Reprod. toxicity (mg/kg bw/d)	10				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	1.9	1.4 x 0.53	0.23007936	43.5
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.4 x 0.53	2.62774848	**3.8**
Cereals BBCH ≥ 40	Small omnivorous mammal “mouse” Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	1.4 x 0.53	0.27851712	35.9

Cereals BBCH 30-39	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	3.9	1.4 x 0.53	0.47226816	21.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

After first-tier risk assessment, the risk of mammals exposure to the metabolite JAU6476-desthio is still unacceptable. Therefore, a higher-tier for the long-term risk assessment of the risk of small herbivorous mammal "Vole" exposure to the metabolite is presented below (point 9.3.2.2).

Table 9.3-4: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of SAP2101F in cereals - Folpet

Intended use	**Cereals**				
Active substance/product	Folpet				
Application rate (g/ha)	2 x 450				
Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small herbivorous mammal	118.4	1.2	63.94	31.3
Reprod. toxicity (mg/kg bw/d)	150				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small herbivorous mammal	48.3	1.4 x 0.53	16.13	9.30
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Tier 1					
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	1.9	1.4 x 0.53	0.63	238.1
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.4 x 0.53	7.24	20.71
Cereals BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	1.4 x 0.53	0.77	194.80
Cereals BBCH 30-39	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	3.9	1.4 x 0.53	1.30	38.47

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

No unacceptable risks of mammals exposure to the active substance folpet are expected after application of SAP2101F in the intended uses.

zRMS comments:

The screening step risk assessment for both active substances and prothioconazole metabolite JAU 6476-desthio has been validated by zRMS.TER_A and TER_{LT} values for the exposure to prothioconazole are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for mammals.

Based on the calculations provided above in the above, the TER_A for acute exposure for prothioconazole metabolite JAU 6476-desthio is above trigger value of 10 but long-term exposure from this metabolite Tier 1 risk assessment has been provided in the Table 9.3-3. Based on these calculations trigger value of 5 was still not achieved for Cereals BBCH ≥ 40 for small herbivorous mammal "vole". Further refinement for this species has been performed using higher tier approach.

For the second active substance - folpet, TER_A and TER_{LT} values are above the trigger of 10 and 5 for acute and long-term exposure at screening level, indicating acceptable risk for mammals.

zRMS remark:

Due to that for combined risk assessment, the Tier 1 for metabolite JAU 6476 - destho is considered, zRMS added Tier 1 for folpet in the Table 9.3-4 above.

Combined acute toxicity of the formulation

According to the EFSA Guidance on Birds and Mammals (2009), the mixed effects of a formulation containing more than one active substance should be assessed. For that, the calculation of a surrogate LD_{50} should be done following the equation:

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

With:

$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture; (please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

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

SAP2101F contains 120 g/L of Prothioconazole and 300 g/L of Folpet, i.e. 29% of Prothioconazole and 71% of Folpet.
Therefore,

$LD_{50\,(mix)} = ((0.29/6200) + (0.71/2000))^{-1} = 2489$ mg/kg bw/d
The expected LD_{50} of SAP2101F is of 2489 mg/kg bw/d.

In order to analyse if one of the active substances is leading the toxicity of the mixture, a "tox per fraction" quotient can be calculated, using the following equation:

$$\text{tox per fraction (a.s.)} = \frac{LD_{50}(\text{a.s.}_i)}{X(\text{a.s.}_i)}$$

$$\text{tox per fraction (mix)} = \frac{LD_{50}(\text{mix})}{\sum_i X(\text{a.s.}_i)}$$

Tox per fraction (Prothioconazole) = 6200/0.29 = 21379.3 mg/kg bw/d
Tox per fraction (Folpet) = 2000/0.71 = 2816.9 mg/kg bw/d
Tox per fraction (SAP2101F) = 2489/1 = 2489 mg/kg bw/d

The quotient for Prothioconazole is: 2489/21379.3 = 0.12 (12%; deviation of 88% > 10%)
The quotient for Folpet is: 2489/2816.9 = 0.88 (88%; deviation of 12% > 10%)

Therefore, the toxicity of the mixture is not lead by any of the active substance alone. An acute risk assessment is presented below with the calculated LD_{50} for the mixture (2489 mg/kg bw/d).

Table 9.3-5: Screening assessment of the acute ~~and long-term/reproductive~~ risk for mammals due to the use of SAP2101F in cereals – SAP2101F

Intended use		**Cereals**			
Active substance/product		SAP2101F			
Application rate (g/ha)		2 x 1710*			
Acute toxicity (mg/kg bw)		2489			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	118.4	1.2	242.96	10.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Considering a density of the formulation of 1.14 g/mL.
*Considering a density of the formulation of 1.14 g/mL

zRMS comments:

The screening acute risk assessment based on LD_{50mix} of 2489 mg /kg/bw value has been validated by zRMS. The acute combined risk assessment is considered acceptable, TERA is above trigger of 5.

9.3.2.2 Higher-tier risk assessment

The first-Tier risk assessment after SAP2101F application indicates a long-term risk for ~~the mixture chronic toxicity and risk of~~ the metabolite JAU 6476-desthio for Small herbivorous mammal (Common vole). Thus, a refined risk assessment for long-term exposure for mammals must be performed. For the chronic toxicity risk assessment, the refinement of deposition factor (DF) value is presented and two independent approaches for the metabolite assessment are presented below (please be aware that each refinement proposal is independent, thus not connected between them).

Tier 2a – Refinement of Deposition Factor (DF) for long-term toxicity exposure

Considering the dated Birds and Mammals Guidance (EFSA Journal 2009; 7(12):1438), the presented deposition factors (DF) may not reflect the currently knowledge so far. The updated "Generic Guidance for Tier 1 FOCUS Ground Water Assessments (2021)" provides new updated DF values. The interception by crops reduces the amount of the PPP that reaches the ground underneath the crop, therefore the amount of PPP reaching the soil depends highly on crops BBCH stage. According to the aforementioned Generic Guidance, in table 1.5 for spring and winter cereals with a BBCH ≥40, the interception of the of the PPP from the plant would be 80% which translates in 20% (DF of 0.2) of the PPP that may reach the soil. In this more realistic exposure scenario condition and, highlighting that "vole" focal species diet is constituted by 100% grass (based on Appendix A of EFSA Journal 2009; 7(12):1438) the interception from the cereals plus at BBCH ≥40 would contribute as a barrier for "vole" exposure. The refinement proposed for the higher tier risk assessment was follow accordingly.

~~**Table 9.3-5.3: First-tier assessment of the long-term/reproductive risk for mammals due to the use of SAP2101F in cereals**~~

~~**Intended use**~~ ~~**Active substance/product**~~ ~~**Application rate (g/ha)**~~	~~**Cereals**~~ ~~SAP2101F~~ ~~2 x 1710*~~						
~~**Reprod. toxicity (mg/kg bw/d)**~~ ~~**TER criterion**~~	~~129~~ ~~5~~						
~~**Crop scenario**~~ ~~**Growth stage**~~	~~**Indicator/generic focal species**~~	~~**FIR/bw**~~	~~**RUD_m × DF (mg/kg food)**~~	~~**MAF_m × TWA**~~	~~**PT**~~	~~**DDD_m (mg/kg bw/d)**~~	~~**TER_{lt}**~~
~~Cereals BBCH ≥ 40~~	~~Small herbivorous mammal "vole Grass + cereals 100% grass~~	~~1.33~~	~~54.2 × 0.2**~~	~~1,4x0.53~~	~~1~~	~~18.29~~	~~7.1~~

~~SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.~~
~~*Considering a density of the formulation of 1.14 g/mL. **refined value from "Generic Guidance for Tier 1 FOCUS Ground Water Assessments (2021)"~~

Acceptable risks to mammal group were found for SAP2101F application to its proposed use on cereals crops.

zRMS comments:

The long-term risk assessment presented above is not validated by zRMS as it is not clear for zRMS from the refined endpoint used. For this reason, the Table has been crossed out.

Tier 2a – Refinement of f_{TWA}

The following information was considered for a more realistic estimation of the residue decline in and on plant material as the measured residue decline of JAU 6476-desthio on plant material can be considered to calculate a refined MAF and/or fTWA. A total of 8 trials were conducted to determine the residue of Prothioconazole-desthio in wheat and the DT_{50} after a spray application of 200 g a.s./ha. The overall mean DT_{50} in wheat considering foliar residues is considered to be 3.2 days, with a measured maximum residue of 3.7 mg/kg (EFSA, 2007). This DT_{50} was proposed to be used as refinement in the EFSA Scientific Report (2007) 106, 1-98.

It can be therefore considered consistent/robust the use of a DT_{50} of 3.2 days in foliage and assuming first-order kinetics, to calculate the time-weighted-average factor (fTWA) according to the following formula:
$f_{TWA} = (1-e^{-kt})/kt$
where:
k velocity constant (ln2/DT50)
t average time (21 days)

The refined **time-weighted-average factor (f_{TWA}) is 0.22.**

Table 9.3-6: Refinement of the long-term/reproductive risk for mammals due to the use of SAP2101F in Cereals (JAU 6476-desthio) – fTWA

Intended use **Active substance/product** **Application rate (g/ha)**	**Cereals** JAU 6476-desthio 2 x 163.2 – JAU 6476-desthio					
Reprod. toxicity (mg/kg bw/d) **TER criterion**	10 5					
Crop scenario **Growth stage**	**Indicator/generic focal species**	**SV_m**	**MAF_m × fTWA***	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**

Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.4 x 0.22*	1.0	1.09076352	9.17

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*refined parameter

After refinement of of f_{TWA} values, no unacceptable risks were found for mammals after application of SAP2101F in the intended uses.

Tier 2b – Refinement of parent-metabolite transformation portion

The risk assessment for the metabolite JAU 6476-desthio in the previous steps was performed assuming a total transformation of Prothioconazole into such metabolite.
Haas and Bornatsch (2000), Haas (2001b) and Haas (2001c), referred in DAR (2004; sections B7.1.1 and B.7.1.5), studied the residue formation after spray application of Prothioconazole in wheat, peanut or confined rotational crops (wheat, chard and turnip seedlings were planted at 28, 146 and 269 days after Prothioconazole application, simulating three rotations), respectively.
Various crop matrices were collected for residue measurement in all studies. For the metabolite JAU 6476-desthio, the maximum residue content measured was 35.49% in wheat fodder. Therefore, and as a worst-case approach, the following refinement was made assuming a 40% transformation rate from the parent to the metabolite in foliar matrices. From these results, the total amount of Prothioconazole to be applied, 180g as described in the GAP, would correspond to 163.2g of JAU 6476-desthio which would be transformed into 65.3g for cereals of JAU 4676-desthio (40%). The refinement with these more realistic values was considered and is presented in Table 9.3-9.

Table 9.3-7: Transformation amount of JAU 6476-desthio measured in plant matrices after Prothioconazole foliar application (results taken from DAR, 2004)

Reference	Crop	Matrices
Haas, Bornatsch, 2000	Wheat	Fodder 35.4 % Hay 18.5 % Straw 22.3 % Grain 15.9 %
Haas, 2001b	Peanut	Peanut hay 28.2 % Nutmeat n.d.
Haas, 2001c	Wheat	Wheat hay (rotation 3) 19.9% Wheat straw (rotation 1) 15.1 %

n.d. – not determined

Table 9.3-8: Refinement of the long-term/reproductive risk for mammals due to the use of SAP2101F in Cereals (JAU 6476-desthio)

Intended use	**Cereals**
Active substance/product	JAU 6476-desthio
Application rate (g/ha)	2 × 65.3 - JAU 6476-desthio
Reprod. toxicity (mg/kg bw/d)	10
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × fTWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.4 x 0.53	1.0	1.05142142	9.51

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

After refinement of parent-metabolite transformation portion in foliar matrices, no unacceptable risks were found for mammals after application of SAP2101F in the intended uses.

zRMS comments:

The first-Tier risk assessment after SAP2101F application indicates a long-term risk for JAU 6476-desthio for small herbivorous mammal (Common vole). Thus, a refined risk assessment for long-term exposure for mammals is needed. For the long-term risk assessment, the refinement of deposition factor (DF) value is used by zRMS and presented below:

Refinement of Deposition Factor (DF) for long-term toxicity exposure from JAU 6476-desthio.

Intended use	**Cereals**
Active substance/product	JAU 6476-desthio
Application rate (g/ha)	2 x 163.2 – JAU 6476-desthio
Reprod. toxicity (mg/kg bw/d)	10
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**RUD x DF**	**MAF_m × fTWA***	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	54.2 x 0.12	1.4 x 0.53	1.0	0.873~~1.31~~	11.45 ~~7.62~~

Based on the calculations above the TER_{LT} value achieved the trigger value of 5, indicating an acceptable risk for small herbivorous mammals.

In addition, two other approaches for refining the risk from exposure to metabolite JAU 6476-desthio has been performed by the Applicant:

<u>The first approach is based on DT_{50} = 3.2 days in plant material</u>

The refined DT_{50} value of 3.2 d derives from residues of prothioconazole-desthio on feed and is included in DAR (October, 2005).
Based on DT_{50}= 3.2 d the Applicant calculated a fTWA of 0.22 assuming an averaging time of 21 days which is the default value of EFSA GD 2009.

To be consistent with DAR 2005 and EFSA conclusion 2007, where an averaging time of 14 days (shortest spray interval) was used in the risk assessment, the following calculation were performed by zRMS:

Refinement of ftwa parameter for long-term toxicity exposure from JAU 6476-desthio

Intended use	**Cereals**
Active substance/product	JAU 6476-desthio
Application rate (g/ha)	2 x 163.2 – JAU 6476-desthio
Reprod. toxicity (mg/kg bw/d)	10
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × fTWA***	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.4 x **0.31***	1.0	1.537	6.5

***using DT_{50}= 3.2 d and averaging time of 14 days (DAR, October 2005).

Based on the calculations above the TER_{LT} value achieved the trigger value of 5, indicating an acceptable risk for small herbivorous mammals.

The second approach is based on refined application rate presented in the Table 9.3-9.
The Applicant refines the application rate of prothioconazole-desthio metabolite based on distribution data (highest value: 35.4% compared to parent) following spray application on wheat. The studies used are included in EFSA 2007. This approach can be used as additional information.

Based on refined calculations presented above the long-term risk for vole is considered be acceptable from exposure of metabolite JAU 6476-desthio.

Combined long-term risk assessment to mammals.

TER_{mix} approach was taken by zRMS to combined risk assessment with regard the active substances folpet + prothioconazole and folpet + JAU 6476-desthio.

The relevant calculations are provided below:

TER_{mix} assessment of long-term/reproductive risk for mammals due to the use of SAP2101 F in cereals for mixture prothioconazole +folpet and JAU6476-desthio is presented below:

				Σ1/TER	Σ1/TER^{-}	Trigger
Prothioconazole		**Folpet**				
14.82	0.067	9.30	0.107	0.17	5.88	5

[1)] TER_{LT} values calculated at screening Step

				Σ1/TER	Σ1/TER^{-}	Trigger
JAU 6476-desthio		**Folpet**				
3.8 [1)]	0.26	20.71 [1)]	0.048	0.308	**3.24**	5
11.45-~~7.63~~	0.08-~~0.12~~	20.71 [1)]	0.048	0.128 ~~0.179~~	7.81 ~~5.6~~	

[1)] TER_{LT} values calculated at Tier 1, vole BBCH >40
[2)] refined DF for vole

Overall, based on performed calculations of TER_{mix} values with an acceptable combined long-term risk may be concluded for mammals from combined exposure of the active substances or active substances and metabolite JAU 6476- deshio (with refined TER_{Lt} value) ad TER value is abobe trigger of 5.

Overall, no unacceptable acute and long - term risk to mammals are expected following application of SAP2101F according to the proposed use pattern.

Overall conclusion

~~For voles, risk assessment is considered to be covered through the assessment of other small mammalian species for the following reasons:~~

- ~~High fecundity and population recuperation~~
- ~~Primary source of food outside crops fields~~
- ~~Necessity of population control measures since the vole is considered a crop pest when high population levels are reached~~
- ~~Other agricultural techniques being also means of population control~~

~~This may not apply in some Member States where the "vole" scenario is considered as relevant. In such cases considering~~ The refinement options described above, ~~the risk assessment~~ for herbivorous mammals " vole" is considered sufficiently addressed**.** In conclusion, the risk assessment is considered acceptable for mammals.

9.3.2.3 Drinking water exposure

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/d (*cf.* Appendix K of EFSA/2009/1438).

Puddle scenario

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

substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

With a K(f)oc of 1765 and 575.4 respectively for prothioconazole and JAU 6476-desthio, both belong to the group of more sorptive substances. And folpet, with a K(f)oc of 304, belongs to the group of less sorptive substances.

The effective application rate was calculated following the equation presented below:

$$AR_{eff} = AR \times MAF_m = AR \times \frac{1-e^{-nki}}{1-e^{-ki}}$$

Where
k = $\ln(2)/DT_{50}$ (rate constant)
n = number of applications
i = application interval (days) = 12 days

Table 9.3-1: Effective application rate

Crop	Substance	DT_{50}	Max application rate (AR) (g as/ha)	No. of applications	$1-e^{-nki}$	$1-e^{-ki}$	MAF_{mean}	AR_{eff}
Cereals	Prothioconazole	1.2	180	2	1.000	1.000	1.000	180.06
	Folpet	1.38	450	2	1.000	0.999	1.001	450.40

Table 9.3-2: Ratio of AReff to acute/long term toxicity endpoint

No concern if ratio					
Prothioconazole: Effective application rate (g/ha)	=	180.06			
Prothioconazole					
Koc (l/kg)	=	1765			
Acute toxicity (mg/kg bw)	=	6200	quotient =	0.03	≤ 3000
Reprod. toxicity (mg/kg bw/d)	=	95.6	quotient =	1.88	≤ 3000
JAU 6476-desthio: Effective application rate (g/ha) =		180.06 (worst-case approach)			
JAU 6476-desthio					
Koc (l/kg)	=	575.4			
Acute toxicity (mg/kg bw)	=	2235	quotient =	0.08	≤ 3000
Reprod. toxicity (mg/kg bw/d)	=	10	quotient =	18.01	≤ 3000
Folpet: Effective application rate (g/ha)	=	450.40			
Folpet					
Koc (l/kg)	=	304			
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.23	≤ 50
Reprod. toxicity (mg/kg bw/d)	=	150	quotient =	3.00	≤ 50

The acute and long-term risk to mammals exposed to SAP2101F via drinking water is therefore acceptable for the intended uses.

zRMS comments:

zRMS agrees with calculation of acute and long-term risk to mammals exposed to SAP2101F via drinking water
In order to apply consistent approach, the drinking water risk assessment was performed also for metabolite JAU 6476-S-methyl. Calculations were performed with assumption of 10 times toxicity of the parent.
The risk assessment is presented below:

Effective application rate (g/ha) Prothioconazole S-methyl =	277*			
Prothioconazole S-methyl				
Koc (l/kg) =	2525.9			
Acute toxicity (mg/kg bw) =	620*	quotient =	0.45	≤ 3000
Reprod. toxicity (mg/kg bw/d) =	9.56**	quotient =	28.98	≤ 3000

MAF= 1.54, based on DT_{50soil}= 15.7 (geomean, used for PEC_{sw} and PEC_{gw} calculations).
**Parent/10: LD_{50} > 6200/10= 620 mg/kg bw and NOEC= 95.6= 9.56 mg/kg bw, respectively.

In case of, Prothioconazole-thiazocine and 1.2.4-triazole**,** these metabolites are not relevant for the soil compartment, thus they are not expected to be formed in puddle water.

The acute and long-term risk to mammals exposed to SAP2101F via drinking water is acceptable for the intended uses.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of prothioconazole, JAU 6476-desthio and folpet amounts to 4.05, 3.04 and 3.017, respectively and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Table 9.3-3: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	Comments
PECsoil (mg/kg soil)	0.050	Worst-case initial PECsoil calculated for multiple applications in cereals
log Pow / Pow	4.05 / 11220	-
Koc	1765	Aged soil column leaching study; value used for PECgw and PECsw simulations
foc	0.02	Default
BCFworm	3.83802	BCFworm/soil = (PECworm,ww/PECsoil,dw) = (0.84 + 0.012 × Pow) / foc × Koc
PECworm	0.19190	PECworm = PECsoil × BCFworm/soil
Daily dietary dose (mg/kg bw/d)	0.24563	DDD = PECworm × 1.28
NOEL (mg/kg bw/d)	95.6	-
TERlt	389.2	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table 9.3-4: Assessment of the risk for earthworm-eating mammals due to exposure to JAU 6476-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	Comments
PEC_{soil} (mg/kg soil)	0.047	Worst-case PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	3.04 / 1096.5	-
Koc	575.4	Geomean, n=4; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	1.21635	$BCF_{worm/soil}$ = ($PEC_{worm,ww}$/$PEC_{soil,dw}$) = (0.84 + 0.012 × P_{ow}) / foc × Koc
PEC_{worm}	0.05717	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.07318	DDD = PEC_{worm} × 1.28

Parameter	JAU 6476-desthio	Comments
NOEL (mg/kg bw/d)	10	-
TER_{lt}	136.7	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table 9.3-5: Assessment of the risk for earthworm-eating mammals due to exposure to folpet via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Folpet	Comments
PEC_{soil} (mg/kg soil)	0.198	Worst-case PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	3.017 / 1039.9	-
Koc	304	Worst-case assumption; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	2.191	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.433745	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.55519	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	150	-
TER_{lt}	270.2	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water / is based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of prothioconazole and folpet in water.

Table 9.3-6: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	Comments
PECsw (mg/L)	0.01955	Worst-case Step 1 PECsw calculated for multiple application in winter and spring cereals
BCFfish	19.7	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PECfish	0.385135	PECfish = PECwater × BCFfish
Daily dietary dose (mg/kg bw/d)	0.055	DDD = PECfish × 0.142
NOEL (mg/kg bw/d)	95.6	-
TERlt	1748.1	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table 9.3-7: Assessment of the risk for fish-eating mammals due to exposure to JAU 6476-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	Comments
PEC_{sw} (mg/L)	0.06649	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	65	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)

Parameter	JAU 6476-desthio	Comments
PEC_{fish}	4.32185	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.6137	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	10	-
TER_{lt}	16.3	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table 9.3-8: Assessment of the risk for fish-eating mammals due to exposure to folpet via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Folpet	Comments
PEC_{sw} (mg/L)	0.11087	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	56	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	6.20872	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.8816	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	150	-
TER_{lt}	170.1	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

zRMS comments:

zRMS agrees with the risk for earthworm-eating mmmals due to exposure to prothioconazole, metabolite JAU 6476-desthio and folpet via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals.
In case of prothioconazole-S-methyl, according to EFSA conclusion 2007, this metabolite has a log P_{ow}= 4.19, thus the risk assessment from secondary poisoning is triggered ($logP_{ow} \geq 3$).
The relevant calculations for earthworm-eating mammals from exposure of Prothioconazole-S-methyl are presented below:

Parameter	Prothioconazole-S-methyl	comments
PEC_{soil} (mg/kg soil)	0.02885	Worst-case PECsoil calculated in cereals
log P_{ow} / P_{ow}	4.19 / 15488.2	-
Koc	2525.9	Geomean used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	3.696	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.11	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.14	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	9.56*	-
TER_{lt}	68.3*	>5, no further refinement

* NOEC= 95.610 = 9.56 mg/kg bw

In case of 1.2.4-triazole metabolite, according to EFSA 2007 it has a logPow ≤ 3 thus no secondary poisoning assessment is triggered.
The metabolite prothioconazole-thiazocine**,** according to DAR 2004, Vol.1, this metabolite is unlikely to be formed at >10% in natural surface water systems under environmental conditions.

Overall, no unacceptable effects to mammals through secondary poisoning are expected following application of SAP2101F according to the proposed use pattern.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant.

9.3.4 Overall conclusions

The risk assessment to mammals was conducted according to the EFSA Guidance Document for the Risk Assessment for Birds and Mammals.
An acceptable risk was obtained for prothioconazole and Folpet at the screening phase for acute and long-term exposure. For the prothioconazole metabolite JAU 6476-desthio, an acceptable risk was obtained at the higher-tier for long-term exposure.
The acute and long-term risk to mammals exposed to SAP2101F via drinking water is acceptable for the intended uses.
An acceptable risk was also obtained for the secondary poisoning scenarios.
Overall, mammals present an acceptable risk towards SAP2101F when used according to the proposed application patterns.

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

No data available.

zRMS comments:

As currently there are no agreed rules or criteria for evaluation of the risk to other terrestrial vertebrates like reptiles and amphibians, this issue should be addressed once respective guidance is available and EU agreed endpoints concluded.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with prothioconazole, folpet and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, as well as in Appendix 2 of this document (new studies).

Effects on aquatic organisms of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – prothioconazole and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	Prothioconazole	Acute	**LC_{50} = 1.83 mg a.s./L**	EFSA Scientific Report (2007)

Oncorhynchus mykiss	Prothioconazole (EC 250)	Acute	LC_{50} = 1.00 mg a.s./L	EFSA Scientific Report (2007)
Lepomis macrochirus	Prothioconazole	Acute	LC_{50} = 4.59 mg a.s./L	EFSA Scientific Report (2007)
Cyprinus carpio	Prothioconazole	Acute	LC_{50} = 6.91 mg a.s./L	EFSA Scientific Report (2007)
Cyprinus carpio	Prothioconazole (EC 250)	Acute	LC_{50} = 3.72 mg a.s./L	EFSA Scientific Report (2007)
Oncorhynchus mykiss	Prothioconazole	Chronic. ELS	**NOEC = 0.308 mg a.s./L**	EFSA Scientific Report (2007)
Oncorhynchus mykiss	JAU 6476-desthio	Acute	LC_{50} = 6.63 mg p.m./L	EFSA Scientific Report (2007)
Leuciscus idus melanotus	JAU 6476-desthio	Acute	LC_{50} =13.2 mg p.m./L	EFSA Scientific Report (2007)
Oncorhynchus mykiss	JAU 6476-desthio	Chronic. ELS	NOEC = 3.34 µg p.m./L	EFSA Scientific Report (2007)
Oncorhynchus mykiss	JAU 6476-S-methyl	Acute	LC_{50} = 1.8 mg p.m./L	EFSA Scientific Report (2007)
Oncorhynchus mykiss	1.2.4-Triazole	Acute	LC_{50} = 498 mg p.m./L	EFSA Scientific Report (2007)
Oncorhynchus mykiss	1.2.4-Triazole	Chronic	NOErC = 3.2 mg a.s./L	EFSA Scientific Report (2007)
Invertebrates				
Daphnia magna	Prothioconazole	Acute	**EC_{50} = 1.3 mg a.s./L**	EFSA Scientific Report (2007)
Daphnia magna	Prothioconazole (EC 250)	Acute	EC_{50} = 0.71 mg a.s./L	EFSA Scientific Report (2007)
Daphnia magna	Prothioconazole	Chronic	**NOEC = 0.56 mg a.s./L**	EFSA Scientific Report (2007)
Daphnia magna	JAU 6476-desthio	Acute	EC_{50} > 10 mg p.m./L	EFSA Scientific Report (2007)
Daphnia magna	JAU 6476-desthio	Chronic	NOEC = 0.10 mg p.m./L	EFSA Scientific Report (2007)
Daphnia magna	JAU 6476-S-methyl	Acute	EC_{50} = 2.8 mg p.m./L	EFSA Scientific Report (2007)
Daphnia magna	1.2.4-Triazole	Acute	EC_{50} = 900 mg p.m./L	EFSA Scientific Report (2007)
Freshwater Algae				
Pseudokirchneriella subcapitata	Prothioconazole	Subchronic	E_bC_{50} = 1.10 mg a.s./L **E_rC_{50} = 2.18 mg a.s./L**	EFSA Scientific Report (2007)
Pseudokirchneriella subcapitata	Prothioconazole (EC 250)	Subchronic	EbC_{50} = 2.92 mg a.s./L ErC_{50} = 1.11 mg a.s./L	EFSA Scientific Report (2007)
Scenedesmus subspicatus	JAU 6476-desthio	Subchronic	EbC_{50} = 0.073 mg p.m./L ErC_{50} = 0.55 mg p.m/L	EFSA Scientific Report (2007)
Pseudokirchneriella subcapitata	JAU 6476-S-methyl	Subchronic	EbC_{50} = 3.77 mg p.m./L ErC_{50} = 47.4 mg p.m./L	EFSA Scientific Report (2007)
Pseudokirchneriella subcapitata	1.2.4-Triazole	Subchronic	EbC_{50} = 8.2 mg p.m./L* ErC_{50} = 22.5 mg p.m./L*	EFSA Scientific Report (2007)
Sediment organisms				
Chironomus riparius	Prothioconazole	Chronic	**NOEC = 9.14 mg a.s./L**	EFSA Scientific Report (2007)

Chironomus riparius	JAU 6476-desthio	Chronic	NOEC = 2.0 mg p.m./L	EFSA Scientific Report (2007)
Fish. Bioconcentration				
Lepomis macrochirus	Prothioconazole	BCF parent = 19.7 Clearance time (CT_{50} days): 0.8 Level of residues (%) after 14 day depuration phase: 9%		EFSA Scientific Report (2007)
Lepomis macrochirus	JAU 6476-desthio	BCF parent = 65 Clearance time (CT_{50} days): 0.4-05 Level of residues (%) after 14 day depuration phase: 4%		EFSA Scientific Report (2007)
Higher-tier studies (micro- or mesocosm studies)				
-				

*Endpoint value according to agreement in PRAPeR expert meeting on triazole metabolites (PRAPeR 13, January 2007).

Table 9.5-2 Endpoints and effect values relevant for the risk assessment for aquatic organisms – folpet and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish				
Rainbow trout	Folpet	96-h, s	LC_{50} = 233 µg/L	EFSA Scientific Report (2009); Addendum to DAR, Folpet (2005)
Brown trout*	Folpet	96-h, s	LC_{50} = 98 µg/L*	EFSA Scientific Report (2009); Addendum to DAR, Folpet (2005)
Common carp	Folpet	96-h, s	LC_{50} = 1012 µg/L	Addendum to DAR, Folpet (2005)
3-spined stickleback	Folpet	96-h, s	LC_{50} = 229 µg/L	Addendum to DAR, Folpet (2005)
Roach	Folpet	96-h, s	LC_{50} = 211 µg/L	Addendum to DAR, Folpet (2005)
Bream	Folpet	96-h, s	LC_{50} = 114 µg/L	Addendum to DAR, Folpet (2005)
Rainbow trout	Folpet	28-day, ss	**NOEC = 39 µg/L**	Addendum to DAR, Folpet (2005)
Rainbow trout	Folpan 500 SC	28-day, ss	24-h LC_{50} > 156 µg folpet/L 96-h LC_{50} = 133 µg folpet/L 28-day LC_{50} = 110 µg folpet/L	EFSA Scientific Report (2009)
Bluegill sunfish	Phthalimide	96-h, ss	LC_{50} = 38000 µg/L	EFSA Scientific Report (2009)
Rainbow trout	Phthalic acid	96-h, s	LC_{50} > 100000 µg/L	EFSA Scientific Report (2009)
Rainbow trout	Phthalamic acid	96-h, s	LC_{50} > 100000 µg/L	EFSA Scientific Report (2009)
Rainbow trout	Benzamide	96-h, s	LC_{50} > 100000 µg/L	EFSA Scientific Report (2009)
Rainbow trout	2-cyanobenzoic acid	96-h, s	LC_{50} > 100000 µg/L	EFSA Scientific Report (2009)
Daphnia				
Daphnia magna	Folpet	28-d, f	NOEC = 1.8 µg a.s./L (**320 µg a.s./L**)**	Section B9 of Folpet DAR (2005)

Daphnia magna	Folpan 80 WDG	48-h, ss	**24-h EC_{50} = 680 µg folpet/L**	EFSA Scientific Report (2009)
Daphnia magna	Phthalimide	48-h, s	EC50 = 39000 µg/L	EFSA Scientific Report (2009)
Daphnia magna	Phthalic acid	48-h, s	EC50 ≥ 100000 µg/L	EFSA Scientific Report (2009)
Daphnia magna	Phthalamic acid	48-h, s	EC50 ≥ 100000 µg/L	EFSA Scientific Report (2009)
Daphnia magna	Benzamide	48-h, s	EC50 ≥ 102000 µg/L	EFSA Scientific Report (2009)
Daphnia magna	2-cyanobenzoic acid	48-h, s	EC50 > 100000 µg/L	EFSA Scientific Report (2009)
Freshwater Algae				
Scenedesmus subspicatus	folpet	72-h, s	**E_rC_{50} > 10000 µg a.s./L** E_bC_{50} > 10000 µg a.s./L	EFSA Scientific Report (2009)
Selenastrum capricornutum	Phthalic acid	72-h , s	**E_bC_{50} > 100000 µg/L**	EFSA Scientific Report (2009)
Selenastrum capricornutum	Phthalamic acid	72-h, s	**E_bC_{50} > 100000 µg/L**	EFSA Scientific Report (2009)
Selenastrum capricornutum	Benzamide	72-h, s	**E_bC_{50} > 100000 µg/L**	EFSA Scientific Report (2009)
Selenastrum capricornutum	2-cyaznobenzoic acid	72-h, s	**E_bC_{50} > 100000 µg/L**	EFSA Scientific Report (2009)
Higher-tier studies (micro- or mesocosm studies)				
-				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations
* Six species of fish were tested. Brown trout (*Salmo trutta*) was the most sensitive species tested.
**In line with the recommendations in the PRAPER conclusion a new semi-static study on folpet technical ([redacted], 2007, ref. 33881221, submitted with post Annex I compliance dossier under IIA 8.3.2/02) was also conducted. The 21-day EC_{50} of the test item for *Daphnia magna* under semi-static conditions with renewal every 2 to 3 days was determined to be 1.4 mg/L for mortality and 0.85 mg/L for reproduction. The NOECs were 1.0 mg/L and 0.32 mg/L for survival of adults and reproduction, respectively.

zRMS comments:

Prothioconazole
Aquatic toxicity data for prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.5-1 were confirmed that they are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106.
Folpet
Aquatic toxicity data for folpet and relevant metabolites provided in Table 9.5-2 above has been confirmed that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84.

Table 9.5-3: Endpoints and effect values relevant for the risk assessment for aquatic organisms – SAP2101F

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	SAP2101F	96 h, ss	LC_{50} = 0.401 mg/L $_{nom}$ LC_{50} = 0.311 mg/L $_{mm}$ LC_{50} = 0.0492 mg/L $_{mm}$ LC_{50}= 0.124 mg/L geomean*	KCP 10.2.1/03, [redacted]023, S23-100707
Daphnia magna	SAP2101F	48 h, ss	EC_{50} = 4.69 mg/L $_{nom}$ NOEC= 0.854 mg/L $_{nom}$ EC_{50} = 0.484 mg/L $_{mm}$ NOEC = 0.131 mg/L $_{mm}$	KCP 10.2.1/01, Schuler L., 2022, S21-05200

Species	Substance	Exposure System	Results	Reference
Pseudokirchneriella subcapitata	SAP2101F	72 h, s	E_rC_{50} = 11.9 mg/L $_{nom}$ E_yC_{50} = 6.94 mg/L $_{nom}$ $NOEC_r$ = 3.05 mg/L $_{nom}$ E_rC_{50} = 0.134 mg/L $_{mm}$ E_yC_{50} = 0.121mg/L $_{mm}$ $NOEC_r$ = 0.0879 mg/L $_{mm}$	KCP 10.2.1/02, Schuler L., 2022, S21-05199
Higher-tier studies (micro- or mesocosm studies)				
-				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

* Test-item geomean mean measured concentration of mean measured concentrations of prothioconazole (LC_{50} = 0.311 mg/L and mean measured concentrations of folpet (LC_{50} = 0.0492 mg/L).

zRMS comments:

Studies on effects of the formulated product on aquatic organisms listed in Table 9.5-3 were evaluated by the zRMS and considered acceptable. Summaries of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.5.1.1 Justification for new endpoints

- Folpet fish acute endpoint for risk assessment

Species Sensitivity Distribution – SSD – Tier 2b approach

The fish acute endpoint previously presented and used for risk assessment as a higher tier RAC in EFSA Scientific Report for folpet (2009) was derived by using the lowest available LC_{50} of 98 µg/L and reducing the Assessment Factor (AF) to 10. This approach is outdated after the entry into force of the EFSA Aquatic Guidance (2013) - "Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters". The mentioned guidance document states that when data is available for more than five vertebrate species, then a tier 2b approach (i.e. Species Sensitivity Distribution – SSD) should be applied.
Looking at all the data available for folpet, six species were tested (see table below) and the SSD approach applies.

Table 17: Folpet: acute fish toxicity studies on 6 species. Results of analytical measurements in relation to the derived LC50 from each study.

Species	Analysis of stocks compared to intended conc. (%)	Range of nominal test concs.	Analysis of test media		LC50 (nominal)	Is LC50 in range where nominals confirmed by analysis?	Ref:
			Measured concs in test media compared to nominal	Nominal tests concs. for which % nominal range applies			
rainbow trout	78 – 96%	24.3 - 568 µg/L	87 – 103%	117 – 568 µg/L	233 µg/L	YES	[redacted] 2002a
brown trout	91 – 100%	13.7 – 320 µg/L	139%	320 µg/L	98 µg/L	NO	[redacted] 2002b
common carp	85 – 94%	64 – 1500 µg/L	67 – 76%	320 – 1500 µg/L	1012 µg/L	YES	[redacted] 2002c
3-spined stickleback	97 – 121%	42.7 – 1000 µg/L	86 – 92%	207 – 1000 µg/L	229 µg/L	YES	[redacted] 2002d
roach	93 – 101%	42.7 – 1000 µg/L	71 – 83%	207 – 1000 µg/L	211 µg/L	YES	[redacted] 2002e
bream	97 – 121%	19.4 – 456 µg/L	92 – 101%	207 – 456 µg/L	114 µg/L	NO*	[redacted] 2002f

* LC50 was outside the range where analytical measurement of test media was possible. However, from the concentration-response at nominal 207 µg/L (71% mortality) and 465 µg/L (100% mortality) it is possible to judge that an LC50 of 114 µg/L is likely to be accurate.

However, SSD with the LC_{50} reported as measured concentrations instead of nominal values can be conducted. From the table above, the analysis of stock solutions resulted in acceptable (80-120%) recovery values for all tests with the exception of rainbow trout which failed by 2% (78%). This proves the correct preparation of the application solutions. The LC_{50} values reported as nominal values are also inside the range where nominal concentrations were confirmed by analysis for almost all species (only brown trout and bream LC_{50} did not fall inside the referred range). Therefore, only these two endpoints should be corrected accounting for the measured concentrations.
For brown trout, 139% recovery was observed in the test media when compared to nominal, resulting in a LC_{50} of 136.22 µg a.s./L (98 µg a.s./L x 139%). Thus, using the nominal value for the derivation of the SSD would be conservative. Nevertheless, both values were used, and two different SSD-curves were determined.
For bream, and as stated in the footnote of the table, "from the concentration-response at nominal 207 µg/L (71% mortality) and 465 µg/L (100% mortality) it is possible to judge that an LC_{50} of 114 µg/L is likely to be accurate". Therefore, no further correction is applicable in the Applicant's point of view.
Both statistical tools - MOSAIC and ETX - were used for both set of data: endpoints derived using the measured recovery values or using the nominal recovery values. All derived SSD-curves and consequent Tier 2b-RACs are presented below.

1) Species Sensitivity Distribution curves derived using endpoints based on measured recovery values (LC_{50})

As mentioned above, all LC_{50} were inside the nominal range confirmed by analysis with the exception of brown trout and bream (for which no correction will be applied). For brown trout, an LC_{50} of 136.2 µg/L would be obtained when considering 139% recovery of the 98 µg/L LC_{50}. The SSD's are presented below, using both programs for the statistical analyses and the data described in the table below.

Table 1-1 – Measured values used in the SSD

Species	LC_{50} (derived with the measured recovery, µg/L)
Rainbow trout	233
Brown trout	136.2
Common carp	1012
3-spined stickleback	229
Roach	211
Bream	114

1.a) MOSAIC tool approach
(available at: https://mosaic.univ-lyon1.fr/ssd)

The SSD curve obtained is presented below alongside with both distribution characteristics (log- normal and log-logistic) and the derived Hazard Concentration (HC_x) values:

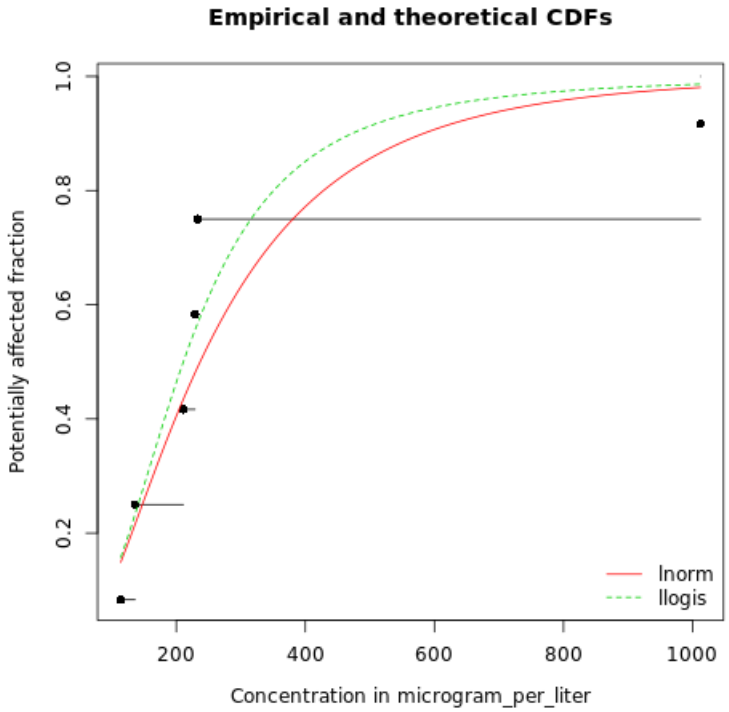


Figure 1-1 – SSD for fish lethal concentration endpoint (LC_{50}) derived based on measured recovery values of folpet using MOSAIC tool.

Respective SSD statistical parameters:
Log normal distribution (log-likelihood = -39.2)
meanlog: 5.5 [4.9; 6]
sdlog: 0.7 [0.27; 1]

Log logistic distribution (log-likelihood = -39.0)
shape: 2.1e+02 [1.3e+02; 3.6e+02]
scale: 2.7 [1.7; 7.9]

Table 1-2-Reported values from MOSAIC tool ran with LC_{50} derived based on measured recoveries for the six fish species

HC	Log-normal	Log-logistic
HC_5	**75 [35; 2e02]**	**72 [31; 1.7e02]**
HC_{10}	96 [49; 2.2e02]	94 [46; 1.9e02]
HC_{20}	1.3e02 [73; 2.7e02]	1.3e02 [68; 2.3e02]

HC_{50}	2.4e02 [1.4e02; 4.2e02]	2.1e02 [1.2e02; 3.5e02]

Based on the log-likelihood of both distributions used in MOSAIC tool (log-normal and log-logistic), the one that best suits the data, i.e. that presents the lowest result, is the log-logistic (-39.0). This is the distribution that results in the lowest HC_5 from the two tested and so, this would be the chosen endpoint to be used in the risk assessment.

1.b) ETX tool approach

(*available at*: Vlaardingen PLA, Traas TP, Wintersen AM, Aldenberg T. 2004. ETX 2.0. A program to calculate hazardous concentrations and fraction affected, based on normally distributed toxicity data. Bilthoven, the Netherlands: National Institute for Public Health and the Environment (RIVM). Report no. 601501028/2004, 68 pp)

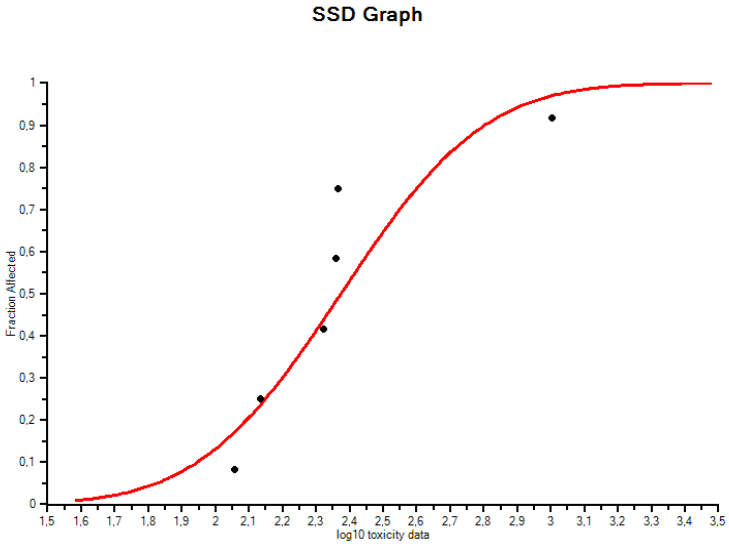


Figure 1-2 – SSD for fish lethal concentration endpoint (LC_{50}) derived based on measured recovery values of folpet using ETX tool.

Respective SSD statistical parameters:

Parameters of the normal distribution		
Mean	2.37e0	mean of the log toxicity values
s.d.	3.34e-1	Sample standard deviation
n	6.00e0	Sample size
Anderson-Darling test for normality		
Sign. level	Critical	Normality
0.1	0.631	Rejected
0.05	0.752	Accepted
0.025	0.873	Accepted
0.01	1.035	Accepted
Kolmogorov-Smirnov test for normality		
0.1	0.819	Rejected
0.05	0.895	Rejected
0.025	0.995	Accepted
0.01	1.035	Accepted
Cramer von Mises test for normality		
0.1	0.104	Accepted
0.05	0.126	Accepted
0.025	0.148	Accepted
0.01	0.179	Accepted

Table 2-Reported values from ETX tool ran with LC_{50} derived based on measured recoveries for the six fish species.

HC	Value
HC_5	**6.155e1**
HC_{50}	2.369e2

As the goodness-of-fit indicates, the normal distribution derived by the ETX tool does not fit well the data available. Thus, the derived endpoints should not be used for the risk assessment.

2) Species Sensitivity Distribution curves derived using endpoints based on nominal recovery values (LC_{50})

For this second set of SSD curves, only the nominal values collected from the Addendum DAR, 2005 for folpet, for the six fish species presented in the table below will be used.

Table 2-1 – Nominal values used in the SSD

Species	LC_{50} (nominal, µg/L)
Rainbow trout	233
Brown trout	**98**
Common carp	1012
3-spined stickleback	229
Roach	211
Bream	114

2.a) MOSAIC tool approach

The SSD curve obtained is presented below alongside with both distribution characteristics (log- normal and log-logistic) and the derived Hazard Concentration (HC_x) values:

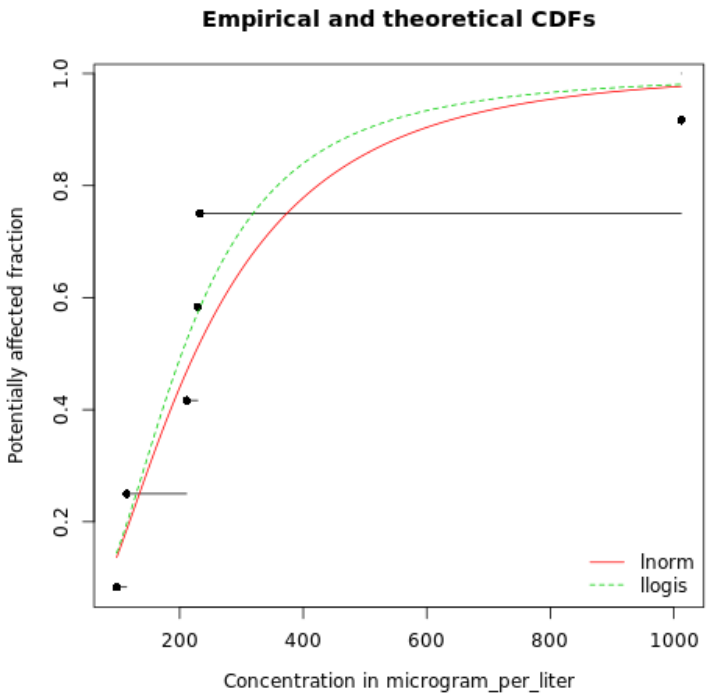


Figure 2-1 – SSD for fish lethal concentration endpoint (LC_{50}) of folpet using MOSAIC tool with nominal values from the Addendum DAR, 2005 for folpet.

Respective SSD statistical parameters:
Log normal distribution (log-likelihood = -39.3)
meanlog: 5.4 [4.8; 6]
sdlog: 0.75 [0.28; 1.1]

Log logistic distribution (log-likelihood = -39.2)
shape: 2e+02 [1.1e+02; 3.6e+02]
scale: 2.4 [1.5; 7]

Table 2-2-Reported values from MOSAIC tool ran with nominal values of LC_{50} for the six fish species

HC	Log-normal	Log-logistic
HC_5	**65 [30; 1.9e02]**	**61 [23; 1.6e02]**
HC_{10}	85 [42; 2.2e02]	83 [37; 1.9e02]
HC_{20}	1.2e02 [64; 2.6e02]	1.2e02 [58; 2.3e02]
HC_{50}	2.2e02 [1.2e02; 4.2e02]	2e02 [1.1e02; 3.6e02]

Based on the log-likelihood of both distributions used in MOSAIC tool (log-normal and log-logistic), the one that best suits the data, i.e. that presents the lowest result, is the log-logistic (-39.2). This is the distribution that results in the lowest HC_5 from the two tested and so, this would be the chosen endpoint to be used in the risk assessment.

2.b) ETX tool approach

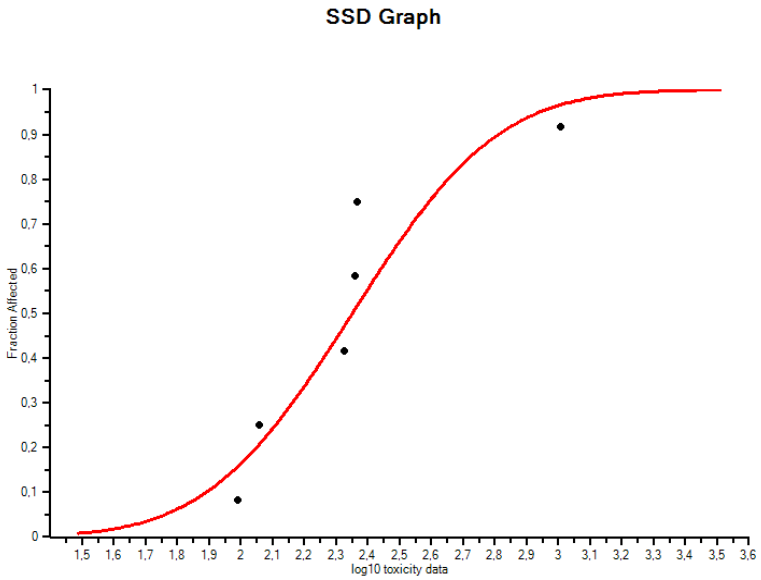


Figure 2-2 – SSD for fish lethal concentration endpoint (LC_{50}) of folpet using ETX tool with nominal values from the Addendum DAR, 2005 for folpet.

Respective SSD statistical parameters:

Parameters of the normal distribution		
Mean	2.35e0	mean of the log toxicity values
s.d.	3.59e-1	Sample standard deviation
n	6.00e0	Sample size
Anderson-Darling test for normality		
Sign. level	**Critical**	**Normality**
0.1	0.631	Accepted
0.05	0.752	Accepted
0.025	0.873	Accepted
0.01	1.035	Accepted
Kolmogorov-Smirnov test for normality		
0.1	0.819	Rejected
0.05	0.895	Accepted
0.025	0.995	Accepted
0.01	1.035	Accepted

Cramer von Mises test for normality		
0.1	0.104	Accepted
0.05	0.126	Accepted
0.025	0.148	Accepted
0.01	0.179	Accepted

Table 2-3-Reported values from ETX tool ran with nominal values of LC_{50} for the six fish species.

HC	Value
HC5	**5.274e1**
HC50	2.243e2

The goodness-of-fit indicates of the normal distribution derived by the ETX tool fails for the significance level of 10% for only one statistical test. For the remaining significance levels and tests, the derived SSD curve is accepted. Therefore, this will be considered for the risk assessment.

Conclusion:
Using a Tier 2b-approach (SSD) according to the EFSA Aquatic Guidance (2013), the following HC_5 values that could be considered for risk assessment were derived: 72 (measured), 61 (nominal) and 52.7 (nominal) µg a.s./L. Using a conservative approach and since all these derived curves are fitting the data quite well, the lowest Tier 2b approach (SSD) derived HC_5 of 52.7µg/L for fish acute endpoint will be used. This HC_5 will be coupled with an assessment factor of 9 with a final tier 2b-RAC of 5.9 µg/L for acute fish exposure (the most conservative approach).

zRMS comments:

zRMS agrees with Tier 2b approach (SSD) and derived HC_5 = 52.7 µg/L value with 9 giving RAC = 5.9 µg a.s./L for the acute risk assessment to fish.

~~**Model Deviation Ratio (MDR)**~~

~~The scientific opinion from EFSA Journal 2013;11(7):3290 – Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters, states that: "The Regulation (EC) No 1107/2009, in Article 29, requires that interaction between the a.s. safeners, synergists and co-formulants shall be taken into account' in the evaluation and authorisation.(…) Note, this mixture RA scheme has to be carried out for each endpoint and exposure scenario separately unless it is evident that one specific endpoint/exposure scenario combination clearly drives the risk."~~

~~SAP2101F endpoints derived based on measured concentrations for O. mykiss, D. magna and P. subcapitata were lower when compared to reported endpoints of prothioconazole and folpet. It must be taken into account that the lower measured values of SAP2101F for aquatic organisms are the result of the degradation behaviour and chemical properties of folpet and are thus not due to the degradation/instability of the formulated product SAP2101F. Due to folpet fast reaction when in contact with water the derived endpoints of O. mykiss, D. magna and P. subcapitata are conditioned by the effect of this a.s. solely. This is proven by the recovery values observed in both studies for prothioconazole.~~

~~On this note, the Model Deviation Ratio (MDR) will be performed with the endpoints derived with measured concentrations for O. mykiss, D. magna and P. subcapitata. This is a highly conservative approach for the aquatic organisms exposed to SAP2101F attending at the arguments presented above.~~

~~**Model Deviation Ratio**~~

~~Step 1: There are sufficient data for three aquatic organisms (O. mykiss, D. magna and P. subcapitata) from the active substance ($ECx_{a.s.}$) and the formulated product (ECx_{ppp})~~

~~**Endpoints data for O. mykiss:**~~

~~EC_{50} SAP2101F: 0.0492 mg/L, LC_{50} SAP2101F: 0.124 mg/L (geomean)~~
~~EC_{50} Prothioconazole: 1.83 mg/L~~
~~EC_{50} Folpet: 0.098 mg/L~~

Confirmation of the plausibility of the measured formulation toxicity against the calculated mixture toxicity:

Equation 13: $$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where:

n:	number of mixture components
i:	index from 1…n mixture components
p_i:	the i^{th} component as a relative fraction of the mixture composition (note: Σp_i must be 1)
ECx_i:	concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

Total pi : 120 $_{prothioconazole}$ + 300 $_{folpet}$ = 420 g/L
pi $_{prothioconazole}$ = 28.6 %
pi $_{folpet}$ = 71.4%

- Eq 13: ECx_{mix-CA} = 1/ [(0.286/1.83) + (0.714/0.098)] = **0.134 mg/L** Step 2

MDR= ECx_{mix-CA}/ECx_{PPP}

- MDR = 0.134 / 0.0492 = 2.7

If MDR = 0.2 – 5: Go to 3
ECx_{mix-CA} (a.s. in PPP)/ECx_{mix-CA} (a.s. in PEC_{mix})

PEC_{mix} = $PEC_{prothioconazole}$ + PEC_{folpet};
Total pi: 1.48 $_{prothioconazole, FOCUS step 2}$ + 8.38 $_{folpet, FOCUS step 3}$ = 9.86
pi $_{prothioconazole}$ = 15.0%
pi $_{folpet}$ = 85.0 %
(Section 8 for PEC values)

- Eq 13: ECx_{mix-CA} (a.s. in PEC_{mix}) =1/ [(0.15/1.83) + (0.85/0.098)] = **0.114 mg/L**

ECx_{mix-CA} (a.s. in PPP)/ECx_{mix-CA} (a.s. in PEC_{mix}) = 0.8 -1.2 (**mixture similar**) Step 3

0.134/0.114 = **1.17 mg/L**

If not: Go to 4

ETR_{mix} = PEC_{mix}/ECx_{PPP} = 9.86/49.2 = 0.2

If ETR_{mix} < trigger (100 for fish acute): **Low risk**

Therefore, it can be concluded, based on measured values of SAP2101F for O. mykiss that the mixture is similar and acceptable risk or low risk (If ETR_{mix} < trigger :100) for the aquatic organisms is expected.

Endpoints data for D. magna:
EC_{50} SAP2101F: 0.484 mg/L
EC_{50} Prothioconazole: 1.3 mg/L
EC_{50} Folpet: 0.680 mg/L

Confirmation of the plausibility of the measured formulation toxicity against the calculated mixture toxicity:

Equation 13: $$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where:

n:	number of mixture components
i:	index from 1…n mixture components
p_i:	the i^{th} component as a relative fraction of the mixture composition (note: Σp_i must be 1)
ECx_i:	concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

Total pi : 120 $_{prothioconazole}$ + 300 $_{folpet}$ = 420 g/L
pi $_{prothioconazole}$ = 28.6 %
pi $_{folpet}$ = 71.4%

- Eq 13: $ECx_{mix\ CA}$ = 1/ [(0.286/1.3) + (0.714/0.680)] = **0.79 mg/L** Step 2

MDR= $ECx_{mix\ CA}/ECx_{PPP}$

- MDR = 0.79 / 0.484 = 1.63

If MDR = 0.2 – 5: Go to 3
$Ecx_{mix\ CA}$ (a.s. in PPP)/$Ecx_{mix\ CA}$ (a.s. in PEC_{mix})

PEC_{mix} = $PEC_{prothioconazole}$ + PEC_{folpet};
Total pi: 1.48 $_{prothioconazole,\ FOCUS\ step\ 2}$ + 8.38 $_{folpet,\ FOCUS\ step\ 3}$ = 9.86
pi $_{prothioconazole}$ = 15.0%
pi $_{folpet}$ = 85.0 %
(Section 8 for PEC values)

- Eq 13: ECx_{mix} CA (a.s. in PEC_{mix}) =1/ [(0.15/1.3) + (0.85/0.680)] = **0.73 mg/L**

$ECx_{mix\ CA}$ (a.s. in PPP)/$ECx_{mix\ CA}$ (a.s. in PEC_{mix}) = 0.8 -1.2 (**mixture similar**) Step 3

0.79/0.73 = **1.08 mg/L**

If $ECx_{mix\ CA}$ (a.s. in PPP)/$ECx_{mix\ CA}$ (a.s. in PECmix) = 0.8 -1.2: Go to 4

ETR_{mix} = PECmix/ECx_{ppp} = 9.86/484 = 0.020

If ETR_{mix} < trigger (100 for invertebrates acute): **Low risk**

Therefore, it can be concluded, based on measured values of SAP2101F for D. magna that the mixture is similar and acceptable risk or low risk (If ETR_{mix} < trigger :100) for the aquatic organisms is expected.

Endpoints data for P. subcapitata:
EC_{50} SAP2101F: 0.134 mg/L
EC_{50} Prothioconazole: 2.18 mg/L
EC_{50} Folpet: 10 mg/L

Total pi: 120 $_{prothioconazole}$ + 300 $_{folpet}$ = 420 g/L
pi $_{prothioconazole}$ = 28.6 %
pi $_{folpet}$ = 71.4%

- Eq 13: $ECx_{mix\ CA}$ = 1/ [(0.286/2.18) + (0.714/10)] = **4.936 mg/L** Step 2

MDR= $ECx_{mix\ CA}/ECx_{PPP}$

- MDR = 4.936 / 0.134= 36.84 mg/L

If MDR > 5 (mixture more toxic than CA): Go to 10

This is not considered a case of synergism, instead, due to the low measured value, the effect is related to such lower concentrations observed for folpet alone (see explanation above), then, the following step is 3.

$ECx_{mix\ CA}$ (a.s. in PPP)/$ECx_{mix\ CA}$ (a.s. in PEC_{mix}) Step 3

PEC_{mix} = $PEC_{prothioconazole}$ + PEC_{folpet};
Total pi: 1.48 $_{prothioconazole}$ + 8.38 $_{folpet}$ = 9.86
pi $_{prothioconazole}$ = 15.0%
pi $_{folpet}$ = 85.0 %
(Section 8 for PEC values)

- Eq 13: $ECx_{mix\ CA}$ (a.s. in PEC_{mix}) =1/ [(0.15/2.18) + (0.85/10)] = 6.50 mg/L

4.936/6.5 = **0.76 mg/L**

If $ECx_{mix\ CA}$ (a.s. in PPP)/$ECx_{mix\ CA}$ (a.s. in PEC_{mix}) ≠ 0.8–1.2, (mixture not similar): Go to 5

Equation 14:
$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

Step 5

- Eq. 14: (1.48/2.18) + (8.38/10) = 1.516899
- Prothioconazole TU: ((1.48/2.18)*100)/1.516899 = 45%
- Folpet TU: ((8.38/10)*100)/1.516899 = 55%

No single "driver of mixture" toxicity identified: Go to 8

$ETRx_{mix\ CA}$ = (PEC_{mix})/ $ECx_{mix\ CA}$ = 9.86/4.936= 1.99 Step 8

If ETR_{mix} < trigger (10 for algae): **Low risk**

Therefore, it can be concluded, based on measured values of SAP2101F for P. subcapitata that for the mixture there is not a driver substance (Equation 14 < 90%) and acceptable risk or low risk (If ETR_{mix} < trigger = 10) is expected for the aquatic organisms.
The risk assessment provided below for both aquatic organisms based on each a.s. data is conservative and reliable in the Applicant's point of view to cover SAP2101F toxicity.

1. Folpet + prothioconazole

- Based on formulation nominal endpoints

Final conclusions				
Steps	Conclusion on the steps			
	Fish	Invetrates	Algae	Macrophytes
Step 1: data available?	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	
Step 2: apparent synergism or antagonism?	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	
Step 3: mixture similar or not?	Different assessment factor or additional data available, go to 8b.	Mixture similar every scenario. All scenarios can be assessed via product test, go to 4.	Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.	
Step 4: ETRmix assessment (ECxPPP)		Acceptable risk have been found in all scenarios in FOCUS step 1-3.	Acceptable risk have been found for some FOCUS scenarios. Others were not acceptable even with risk mitigation.	
Step 5: driver available?			There is no driver for algae in all scenarios. Go to 8.	
Step 6: driver assessment				
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment			Risk acceptable for all scenarios in FOCUS step 1-3.	
Step 8b: RQmix assessment	Risk acceptable for all scenarios in FOCUS step 1-3.			
Step 9: anatagonism assessment				
Step 10: synergism assessment				

(File name: AGD_AquaMix_v1.15_SAP2101F_nominalPPP updated by zRMS)

— Based on formulation mean measured endpoints

Final conclusions				
Steps	Conclusion on the steps			
	Fish	Invetrates	Algae	Macrophytes
Step 1: data available?	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	
Step 2: apparent synergism or antagonism?	The MDR is >5. Thus, synergism is indicated, go to 10.	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	The MDR is >5. Thus, synergism is indicated, go to 10.	
Step 3: mixture similar or not?	Different assessment factor or additional data available, go to 8b.	Mixture similar every scenario. All scenarios can be assessed via product test, go to 4.	Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.	
Step 4: ETRmix assessment (ECxPPP)		Acceptable risk have been found in all scenarios, if risk mitigation is applied (FOCUS Step 4).	Acceptable risk have been found for some FOCUS scenarios. Others were not acceptable even with risk mitigation.	
Step 5: driver available?			There is no driver for algae in all scenarios. Go to 8.	
Step 6: driver assessment				
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment			Risk acceptable for all scenarios in FOCUS step 1-3.	
Step 8b: RQmix assessment	Risk acceptable for all scenarios in FOCUS step 1-3.			
Step 9: anatagonism assessment				
Step 10: synergism assessment	Measured mixture toxicity plausible: Go to 3		Measured mixture toxicity plausible: Go to 3	

(File name: AGD_AquaMix_v1.15_SAP2101F_mean measured PPP)

For invertebrates, in step 4 of the AquaMix the following conclusion is reached "Acceptable risk have been found in all scenarios, if risk mitigation is applied (FOCUS Step 4)". However, no calculations have been presented in the excel file with PECsw at FOCUS step 4. This conclusion is reached based on the fact that for invertebrates in step 5, a driver of toxicity is clearly identified for all scenarios (which is folpet) and the risk assessment presented below in this section clearly demonstrates that with appropriate mitigation measures, an acceptable risk is obtained.

1. Mixture toxicity for folpet + prothioconazole-desthio

Regarding the mixture toxicity with the combination folpet+prothioconazole desthio, it should be noted that the aquatic guidance indicates that "In order to determine if the a.s. may act more (i.e. synergistically) or less (i.e. antagonistically) than expected by CA, a comparison of the calculated ECxmix CA for the mixture composition of a.s. in the formulation versus measured ECxPPP endpoints is informative." So, in the Applicant's point of view, metabolites should not be considered for the mixture toxicity calculation.
Nevertheless, it also states that "This comparison may also indicate that relevant toxicity contributions of co-formulants not included in the calculation do occur, which might be included in a refined calculation (if the respective single substance toxicity data are available)." For this reason, the Applicant provides the requested comparison as a refinement, although no risk has been identified above in point 1.

For this, prothioconazole and its endpoints has been replaced by prothioconazole-desthio and the following endpoints were used:

	prothio-desthio	folpet
	120	300
	0.29	0.71
	6.63	0.098
	10	0.68

	0.55	10

The conclusions of such are presented here and are exactly the same as with prothioconzole:

- Based on formulation nominal endpoints

Final conclusions

Steps	Conclusion on the steps			
	Fish	Invetrates	Algae	Macrophytes
Step 1: data available?	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	
Step 2: apparent synergism or antagonism?	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	
Step 3: mixture similar or not?	Different assessment factor or additional data available, go to 8b.	Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.	Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.	
Step 4: ETRmix assessment (ECxPPP)		Acceptable risk have been found in all scenarios, if risk mitigation is applied (FOCUS Step 4).	Acceptable risk have been found in all scenarios, if risk mitigation is applied (FOCUS Step 4).	
Step 5: driver available?		There is a driver for invertebrates in all scenarios. Assess driver, go to 6.	There is a driver for algae in some scenarios. Go either to 6 or 8.	
Step 6: driver assessment		Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).	
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment			Risk acceptable for all scenarios in FOCUS step 1-3.	
Step 8b: RQmix assessment	Risk acceptable for all scenarios in FOCUS step 1-3.			
Step 9: anatagonism assessment				
Step 10: synergism assessment				

(File name: AGD_AquaMix_v1.15_SAP2101F_nominalPPP_folpet+desthio)

- Based on formulation mean measured endpoints

Final conclusions

Steps	Conclusion on the steps			
	Fish	Invetrates	Algae	Macrophytes
Step 1: data available?	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	Endpoints available for a.s. and the ppp, go to 2.	
Step 2: apparent synergism or antagonism?	The MDR is >5. Thus, synergism is indicated, go to 10.	The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.	The MDR is >5. Thus, synergism is indicated, go to 10.	
Step 3: mixture similar or not?	Different assessment factor or additional data available, go to 8b.	Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.	Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.	
Step 4: ETRmix assessment (ECxPPP)		Acceptable risk have been found in all scenarios, if risk mitigation is applied (FOCUS Step 4).	Acceptable risk have been found in all scenarios, if risk mitigation is applied (FOCUS Step 4).	
Step 5: driver available?		There is a driver for invertebrates in all scenarios. Assess driver, go to 6.	There is a driver for algae in some scenarios. Go either to 6 or 8.	
Step 6: driver assessment		Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).	Risk acceptable for all scenarios, if risk mitigation is applied (FOCUS Step 4).	
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment			Risk acceptable for all scenarios in FOCUS step 1-3.	
Step 8b: RQmix assessment	Risk acceptable for all scenarios in FOCUS step 1-3.			
Step 9: anatagonism assessment				
Step 10: synergism assessment	Measured mixture toxicity plausible: Go to 3		Measured mixture toxicity plausible: Go to 3	

(File name: AGD_AquaMix_v1.15_SAP2101F_mean measuredPPP_folpet+desthio)

~~For invertebrates and algae, in step 4 of the AquaMix the following conclusion is reached "Acceptable risk have been found in all scenarios, if risk mitigation is applied (FOCUS Step 4)". However, no calculations have been presented in the excel file with PECsw at FOCUS step 4.~~
~~The conclusion for invertebrates is reached based on the fact that in step 5, a driver of toxicity is clearly identified for all scenarios (which is folpet) and the risk assessment presented below in this section clearly demonstrates that with appropriate mitigation measures, an acceptable risk is obtained.~~

~~The conclusion for algae is reached based on the fact that, although no driver of toxicity is clearly identified for all scenarios, the risk assessment presented below in this section clearly demonstrates that with appropriate mitigation measures, an acceptable risk is obtained for all active substances and metabolites.~~
~~The FOCUS Step 4 calculations were not included in the excel file as different mitigation measures and for different scenarios were calculated to prove an acceptable risk for the metabolite (prothioconazole-desthio, 15 to 20 m VFS) or the active substance (folpet, 5 m VFS) and thus no mixture calculations would be possible.~~

zRMS comments:

The mixture toxicity assessment has been updated by the Applicant in April 2024 for request of zRMS with consideration of the PEC_{sw} values accepted by e-fate expert in Section 8 and with consideration of toxicity endpoints for aquatic organism.
The combination folpet + prothioconazole has been performed and the calculations are provided in the AquaMix excel documents (with nominal and mean measured PPP endpoints).

The relevant Excel files are presented below:

The combination of folpet + prothioconazole based on PPP nominal concentration are included in the following Excel files:

- **AGD_AquaMix_v1.15_1.1:** nominal PPP, prothioconazole and folpet, winter cereals, single applications

AGD_AquaMix_v1.15_1.1.xlsm

- **AGD_AquaMix_v1.15_1.2:** nominal PPP, prothioconazole and folpet, winter cereals, multiple applications

AGD_AquaMix_v1.15_1.2.xlsm

- **AGD_AquaMix_v1.15_1.3:** nominal PPP, prothioconazole and folpet, spring cereals, single applications

AGD_AquaMix_v1.15_1.3.xlsm

- **AGD_AquaMix_v1.15_1.4:** nominal PPP, prothioconazole and folpet, spring cereals, multiple applications

AGD_AquaMix_v1.15_1.4.xlsm

Based on the nominal endpoints, an acceptable risk has been identified for all organisms with the information provided in all Excel files above. No mitigation measures are required for scenarios relevant for Central Zone.

The final conclusion for the worst - case scenario - winter cereals - multiple application (Exel Files: AGD_Aqua-Mix_v1.15_1.2:) is summarized below:

Final conclusions				
	Conclusion on the steps			
Steps	**Fish**	**Invetrates**	**Algae**	**Macrophytes**
Step 1: data available?	**Endpoints available for a.s. and the ppp, go to 2.**	**Endpoints available for a.s. and the ppp, go to 2.**	**Endpoints available for a.s. and the ppp, go to 2.**	
Step 2: apparent synergism or antagonism?	**The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.**	**The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.**	**The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.**	
Step 3: mixture similar or not?	**Different assessment factor or additional data available, go to 8b.**	**Mixture similar every scenario. All scenarios can be assessed via product test, go to 4.**	**Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.**	
Step 4: ETRmix assessment (ECxPPP)		**Acceptable risk have been found in all scenarios in FOCUS step 1-3.**	**Acceptable risk have been found in all scenarios in FOCUS step 1-3.**	
Step 5: driver available?			**There is no driver for algae in all scenarios. Go to 8.**	
Step 6: driver assessment				
Step 7: synergism assessment (few data)				
Step 8a: ETRmix assessment			**Risk acceptable for all scenarios in FOCUS step 1-3.**	
Step 8b: RQmix assessment	**Risk acceptable for all scenarios in FOCUS step 1-3.**			
Step 9: anatagonism assessment				
Step 10: synergism assessment				

The combination of folpet + prothioconazole based **on PPP <u>mean measured concentration</u>** are included in the following Excel files:

- **AGD_AquaMix_v1.15_2.1:** mean measured PPP, prothioconazole and folpet, winter cereals, single applications

AGD_AquaMix_v1.15_2.1.xlsm

- **AGD_AquaMix_v1.15_2.2:** mean measured PPP, prothioconazole and folpet, winter cereals, **multiple applications**

AGD_AquaMix_v1.15_2.2.xlsm

- **AGD_AquaMix_v1.15_2.3:** mean measured PPP, prothioconazole and folpet, spring cereals, single applications

AGD_AquaMix_v1.15_2.3.xlsm

- **AGD_AquaMix_v1.15_2.4:** mean measured PPP, prothioconazole and folpet, spring cereals, multiple applications

AGD_AquaMix_v1.15_2.4.xlsm

The final conclusion for mean measured concentration PPP for the worst - case scenario - winter cereals – multiple application (Excel File: AGD_AquaMix_v1.15_2.2) is presented below:

Final conclusions				
	Conclusion on the steps			
Steps	**Fish**	**Invetrates**	**Algae**	**Macrophytes**
Step 1: data available?	**Endpoints available for a.s. and the ppp, go to 2.**	**Endpoints available for a.s. and the ppp, go to 2.**	**Endpoints available for a.s. and the ppp, go to 2.**	
Step 2: apparent synergism or antagonism?	**The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.**	**The MDR is between 0.2-5. No antagonism or synergism is indicated. Thus, the "concentration addition" concept holds, go to 3.**	**The MDR is >5. Thus, synergism is indicated, go to 10.**	
Step 3: mixture similar or not?	**Different assessment factor or additional data available, go to 8b.**	**Mixture similar every scenario. All scenarios can be assessed via product test, go to 4.**	**Mixture sometimes similar. Some scenario can be assessed via product test, go either to 4 or 5.**	
Step 4: ETRmix assessment (ECxPPP)		**Acceptable risk have been found in all scenarios, if risk mitigation is applied (FOCUS Step 4).**	**Acceptable risk have been found in all scenarios in FOCUS step 1-3.**	
Step 5: driver available?			**There is no driver for algae in all scenarios. Go to 8.**	
Step 6: driver assessment				
Step 7: synergism assessment (few data)				

Step 8a: ETRmix assessment			**Risk acceptable for all scenarios in FOCUS step 1-3.**	
Step 8b: RQmix assessment	**Risk acceptable for all scenarios in FOCUS step 1-3.**			
Step 9: anatagonism assessment				
Step 10: synergism assessment			**Measured mixture toxicity plausible: Go to 3**	

For the mean measured endpoints, an acceptable risk has been identified for fish and algae with the information provided, while for invertebrates a Step 4 analysis is required.
In the same time the Applicant recalculated toxicity endpoints for invertebrates and algae as follows:

	Invertebrates	**Algae**
Nominal	4.69 mg/L	11.9 mg/L
Mean measured	0.484 mg/L	0.134 mg/L
Geomean	1.507 mg/L	1.263 mg/L

It should be noted that there are no mean measured concentrations calculated based on prothioconazole recoveries as its concentration was maintained between 80-120% of nominal. However, if a geomean of the two mean measured endpoints derived for fish (based on folpet and prothioconazole recoveries) is used, the same can be applied to invertebrates and algae by assuming the prothioconazole recoveries as the nominal concentrations.

The calculations of mixture toxicity for **aquatic invertebrates and algae based on geomean measured concertation PPP** are included in the following excel files:

- AGD_AquaMix_v1.15_3.1: geomean mean measured PPP, prothioconazole and folpet, winter cereals, single applications


AGD_AquaMix_v1.15_3.1.xlsm

o Based on the results an acceptable risk for invertebrates for all scenarios.

- AGD_AquaMix_v1.15_3.2: geomean mean measured PPP, prothioconazole and folpet, winter cereals, multiple applications


AGD_AquaMix_v1.15_3.2.xlsm

o Based on the results unacceptable risk for R1/stream, R3/stream and R4/stream with TER of 0.01 (in the trigger); in Step 5 folpet is shown as the driver for these 3 scenarios and an acceptable risk for folpet alone has been proved for invertebrates;

- AGD_AquaMix_v1.15_3.3: geomean mean measured PPP, prothioconazole and folpet, spring cereals, single applications


AGD_AquaMix_v1.15_3.3.xlsm

o Based on the results unacceptable risk for R4/stream with TER of 0.01 (in the trigger); in Step 5 folpet is

shown as the driver for this scenario and an acceptable risk for folpet alone has been proved for invertebrates;

- AGD_AquaMix_v1.15_3.4: geomean mean measured PPP, prothioconazole and folpet, spring cereals, multiple applications

AGD_AquaMix_v1.15_3.4.xlsm

o Based on the results unacceptable risk for R4/stream with TER of 0.02; in Step 5 folpet is shown as the driver for this scenario and an acceptable risk for folpet alone has been proved for invertebrates.

No change is observed for algae conclusions, i.e. risk acceptable for all scenarios,

Conclusion based on PPP geomean mean measured concentration:

All scenarios for which an acceptable risk is not proven with the geomean mean measured endpoints have folpet as a driver of the PPP toxicity. For this active substance, an acceptable risk has been proven for invertebrates' exposure with risk mitigation measures indicated in STEP 4PEC_{sw}:

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-off density-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080. 15 January 2015).
The relevant global maximum FOCUS Step 1, 2, 3 and 4 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1, 2 calculations for the use of SAP2101F

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. sub-capitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC
(µg/L)		1830	308	1300	560	2180	9140
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	218	914
Winter cereals							
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	19.55	**1.07**	0.63	**1.50**	0.35	0.09	0.02
Step 2							
N-Europe	1.66	0.09	Resolved at Step 1	0.13	Resolved at Step 1		
S-Europe	1.66	0.09		0.13			
Spring cereals							
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	19.55	**1.07**	0.63	**1.50**	0.35	0.09	0.02
Step 2							
N-Europe	1.66	0.09	Resolved at Step 1	0.13	Resolved at Step 1		
S-Europe	1.66	0.09		0.13			

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of SAP2101F

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 6630	NOEC 3.34	EC_{50} 10000	NOEC 100	E_rC_{50} 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55	200
Winter cereals							
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	66.49	**1.002**	**199.07**	0.66	**6.65**	**1.21**	0.33
Step 2							
N-Europe	11.92 ~~10.56~~	0.179 ~~0.16~~	**35.68** ~~31.62~~	Resolved at Step 1	**1.192** ~~1.06~~	0.21 ~~0.19~~	Resolved at Step 1
S-Europe	8.64	0.13	**25.87**		0.86	0.16	
Step 3 – Multiple application							
D1/ditch	0.1446	Resolved at Step 2	0.43	Resolved at Step 1	0.01	Resolved at Step 2	Resolved at Step 1
D1/stream	0.04557		0.14		0.00		
D2/ditch	0.1753		0.52		0.02		
D2/stream	0.1719		0.51		0.02		
D3/ditch	0.06053		0.18		0.01		
D4/pond	0.01118		0.03		0.00		
D4/stream	0.02906		0.09		0.00		
D5/pond	0.01352		0.04		0.00		
D5/stream	0.04129		0.12		0.00		
D6/ditch	0.07893		0.24		0.01		

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
R1/pond	0.1028		0.31		0.01		
R1/stream	0.9081		**2.72**		0.09		
R3/stream	0.8642		**2.59**		0.09		
R4/stream	1.395		**4.18**		0.14		
Step 3 – Single application							
D1/ditch	0.03664	Resolved at Step 2	0.11	Resolved at Step 1	0.00	Resolved at Step 2	Resolved at Step 1
D1/stream	0.04348		0.13		0.00		
D2/ditch	0.07467		0.22		0.01		
D2/stream	0.05085		0.15		0.01		
D3/ditch	0.03627		0.11		0.00		
D4/pond	0.007217		0.02		0.00		
D4/stream	0.03247		0.10		0.00		
D5/pond	0.008345		0.02		0.00		
D5/stream	0.04174		0.12		0.00		
D6/ditch	0.01962		0.06		0.00		
R1/pond	0.03565		0.11		0.00		
R1/stream	0.3153		0.94		0.03		
R3/stream	0.3948		**1.18**		0.04		
R4/stream	0.5744		**1.72**		0.06		
<u>Spring cereals</u>							
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	66.49	**1.002**	**199.07**	0.66	**6.65**	**1.21**	0.33
Step 2							
N-Europe	5.34 ~~4.81~~	0.08 ~~0.07~~	**15.98** ~~**14.40**~~	Resolved at Step 1	0.543 ~~0.48~~	0.534 ~~0.09~~	Resolved at Step 1

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
S-Europe	8.64	0.13	**25.87**		0.86	0.16	
Step 3 – Multiple application							
D1/ditch	0.3484	Resolved at Step 2	**1.04**	Resolved at Step 1	Resolved at Step 2		Resolved at Step 1
D1/stream	0.08887		0.27				
D3/ditch	0.06289		0.19				
D4/pond	0.01384		0.04				
D4/stream	0.03699		0.11				
D5/pond	0.01331		0.04				
D5/stream	0.0412		0.12				
R4/stream	0.9841		**2.95**				
Step 3 – Single application							
D1/ditch	0.2055	Resolved at Step 2	0.62	Resolved at Step 1 and 2			
D1/stream	0.08223		0.25				
D3/ditch	0.07005		0.21				
D4/pond	0.009042		0.03				
D4/stream	0.038		0.11				
D5/pond	0.008413		0.03				
D5/stream	0.04395		0.13				
R4/stream	0.5207		**1.56**				

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-5a: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 4 calculations for the use of SAP2101F

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}	NOEC
(µg/L)		6630	3.34	10000	100	550	2000

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55	200
Winter cereals							
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
~~**Step 4 – Multiple application, 15 meters of VFS**~~							
~~R1/stream~~	~~0.3164~~	~~Resolved at previous Steps~~	~~0.95~~	Resolved at previous Steps			
~~R3/stream~~	~~0.2924~~		~~0.88~~				
Step 4 – Multiple application, 20 meters of VFS							
R1/stream	0.2159	Resolved at previous Steps1	0.65	Resolved at previous Steps			
R3/stream	0.1997		0.60				
R4/stream	0.3323		0.99				
~~**Step 4 – Single applications, 5 meters of VFS**~~							
R3/stream	~~0.2581~~	~~Resolved at previous Steps~~	~~0.77~~	Resolved at previous Steps			
Step 4 –Single application, 10 meters of VFS							
R3/stream	0.1802	Resolved at previous Steps	0.54	Resolved at previous Steps			
R4/stream	0.2612		0.78				
Spring cereals							
Step 4 – Multiple application, 20 meters of VFS							
D1/ditch	0.02912	Resolved at previous Steps	0.09	Resolved at previous Steps			
R4/stream	0.2311		0.69				
Step 4 – Single application, 10 meters of VFS							
R4/stream	0.2368	Resolved at previous Steps	0.71	Resolved at previous Steps			

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-6 Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-S-methyl (M01) for each organism group based on FOCUS Steps 1, 2 calculations for the use of SAP2101F

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		1800	2800	47400
AF		100	100	10
RAC (µg/L)		18	28	4740
Winter cereals				
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	28.85	**1.60**	**1.03**	0.01
Step 2				
N-Europe	2.23	0.12	0.08	Resolved at Step 1
S-Europe	1.93	0.11	0.07	
Spring cereals				
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	28.85	**1.60**	**1.03**	Resolved at Step 1
Step 2				
N-Europe	1.53	0.09	0.05	Resolved at Step 1
S-Europe	1.93	0.11	0.07	

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole (M13) for each organism group based on FOCUS Steps 1, 2 calculations for the use of SAP2101F

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint		LC_{50}	NOEC	EC_{50}	E_rC_{50}

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**
(µg/L)		498000	3200	900000	22500
AF		100	10	100	10
RAC (µg/L)		4980	320	9000	2250
Winter cereals					
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	3.37	0.001	0.011	0.000	0.001
Step 2					
N-Europe	0.15	Resolved at Step 1			
S-Europe	0.13				
Spring cereals					
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	3.37	0.001	0.011	0.000	0.001
Step 2					
N-Europe	0.11	Resolved at Step 1			
S-Europe	0.13				

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole- thiazocine (M12) for each organism group based on FOCUS Steps 1, 2 calculations for the use of SAP2101F

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
(µg/L)		183	130	218
AF		100	100	10

Group		**Fish acute**	**Inverteb. acute**	**Algae**
RAC (µg/L)		1.83*	1.3*	21.8*
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**			
Step 1				
	2.464	**1.35**	**1.90**	0.11
Step 2				
S-Europe	0.21	0.11	0.16	Resolved at Step 1

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold. *Endpoint values estimated assuming metabolite 10 times more toxic than a.s.10

zRMS comments:

Based on the calculations of the risk assessment for aquatic organism for the following conclusions has been derived:

Regarding the active substance prothioconazole and its respective metabolites, the max PECsw are below the RAC with FOCUS Step 1-2 calculations with the exception for the metabolite prothioconazole-desthio, for which Step 4 calculations were needed (i.e. mitigation measures).
The detailed results of FOCUS Step 3 and Step 4 calculations for maximum dose demonstrate a safe use to aquatic organisms in the following mitigated scenarios:

Maximum dose

Winter cereals:

- a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple applications for R1, R3 and R4 scenarios

- a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R3, R4 scenarios

Spring cereals:

- a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple application for D1, R4 scenarios and **R1, R3 scenarios** (from winter cereals)

- a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R4 scenario and **R3 scenarios** (from winter cereals)

It should be noted that the risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of SAP2101F

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
Test species		-	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*
Endpoint (µg/L)		HC_5 52.7	NOEC 39	EC_{50} 680	NOEC 320	E_rC_{50} 10000
AF		9	10	100	10	10
RAC (µg/L)		5.9	3.9	6.8	32	1000
Winter cereals						
FOCUS Scenario	$PEC_{gl\ max}$ (µg/L)					
Step 1						
-	110.87	**18.79**	**28.43**	**16.30**	**3.46**	0.11
Step 2						
N-Europe	7.48	**1.27**	**1.92**	**1.10**	0.23	Resolved at Step 1
S-Europe	6.34	**1.07**	**1.63**	0.93	0.20	
Step 3 – Multiple application						
D1/ditch	2.517	0.43	0.65	0.37	Resolved at Step 2	Resolved at Step 1
D1/stream	2.126	0.36	0.55	0.31		
D2/ditch	2.523	0.43	0.65	0.37		
D2/stream	2.206	0.37	0.57	0.32		
D3/ditch	2.493	0.42	0.64	0.37		
D4/pond	0.1198	0.02	0.03	0.02		
D4/stream	1.884	0.32	0.48	0.28		
D5/pond	0.1387	0.02	0.04	0.02		
D5/stream	2.174	0.37	0.56	0.32		
D6/ditch	2.505	0.42	0.64	0.37		
R1/pond	0.2274	0.04	0.06	0.03		

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
R1/stream	3.337	0.57	0.86	0.49		
R3/stream	4.464	0.76	**1.14**	0.66		
R4/stream	2.54	0.43	0.65	0.37		
Step 3 – Single application						
D1/ditch	2.861	0.48	0.73	0.42	Resolved at Step 2	Resolved at Step 1
D1/stream	2.223	0.38	0.57	0.33		
D2/ditch	2.879	0.49	0.07	0.42		
D2/stream	2.445	0.41	0.63	0.36		
D3/ditch	2.851	0.48	0.73	0.42		
D4/pond	0.09837	0.02	0.03	0.01		
D4/stream	2.106	0.36	0.54	0.31		
D5/pond	0.09838	0.02	0.03	0.01		
D5/stream	2.276	0.39	0.58	0.33		
D6/ditch	2.818	0.48	0.72	0.41		
R1/pond	0.09838	0.02	0.03	0.01		
R1/stream	1.878	0.32	0.48	0.28		
R3/stream	2.638	0.45	0.68	0.39		
R4/stream	1.886	0.32	0.48	0.28		
			Spring cereals			
FOCUS Scenario	**$PEC_{gl\ max}$ (µg/L)**					
Step 1						
-	110.87	**18.79**	**28.43**	**16.30**	**3.46**	0.11
Step 2						
N-Europe	**4.14**	0.70	**1.06**	0.61	0.12	Resolved at Step 1
S-Europe	6.34	**1.07**	**1.63**	0.93	0.20	
Step 3 –Multiple application						

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
D1/ditch	3.366	0.57	0.86	Resolved at Step 2		Resolved at Step 1
D1/stream	2.183	0.37	0.56			
D3/ditch	2.495	0.42	0.64			
D4/pond	0.1323	0.02	0.03			
D4/stream	2.083	0.35	0.53			
D5/pond	0.1232	0.02	0.03			
D5/stream	2.152	0.36	0.55			
R4/stream	8.38	**1.42**	**2.15**			
Step 3 - Single application						
D1/ditch	2.886	0.49	0.74	Resolved at Step 2		Resolved at Step 1
D1/stream	2.524	0.43	0.65			
D3/ditch	2.854	0.48	0.73			
D4/pond	0.09842	0.02	0.03			
D4/stream	2.333	0.40	0.60			
D5/pond	0.09841	0.02	0.03			
D5/stream	2.396	0.41	0.61			
R4/stream	4.662	0.79	**1.20**			

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-9.1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of SAP2101F

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
Test species			-	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*
Endpoint (µg/L)			HC_5 52.7	NOEC 39	EC_{50} 680	NOEC 320	E_rC_{50} 10000
AF			9	10	100	10	10

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
RAC (µg/L)			5.9	3.9	6.8	32	1000
	Winter cereals						
FOCUS Scenario		$PEC_{gl\text{-}max}$ (µg/L)					
	Step 3 – Multiple application						
D1/ditch		2.516	0.4	0.6	0.4	Resolved at Step 2	Resolved at Step 1
D1/stream		2.125	0.4	0.5	0.3		
D2/ditch		2.523	0.4	0.6	0.4		
D2/stream		2.205	0.4	0.6	0.3		
D3/ditch		2.493	0.4	0.6	0.4		
D4/pond		0.107	0.0	0.0	0.0		
D4/stream		1.882	0.3	0.5	0.3		
D5/pond		0.130	0.0	0.0	0.0		
D5/stream		2.173	0.4	0.6	0.3		
D6/ditch		2.504	0.4	0.6	0.4		
R1/pond		0.178	0.0	0.0	0.0		
R1/stream		2.615	0.4	0.7	0.4		
R3/stream		3.213	0.5	0.8	0.5		
R4/stream		1.872	0.3	0.5	0.3		
	Step 3 – Single application						
D1/ditch		2.860	0.5	0.7	0.4	Resolved at Step 2	Resolved at Step 1
D1/stream		2.224	0.4	0.6	0.3		
D2/ditch		2.878	0.5	0.7	0.4		
D2/stream		2.445	0.4	0.6	0.4		
D3/ditch		2.850	0.5	0.7	0.4		
D4/pond		0.098	0.0	0.0	0.0		

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
D4/stream		2.107	0.4	0.5	0.3		
D5/pond		0.098	0.0	0.0	0.0		
D5/stream		2.275	0.4	0.6	0.3		
D6/ditch		2.818	0.5	0.7	0.4		
R1/pond		0.098	0.0	0.0	0.0		
R1/stream		1.878	0.3	0.5	0.3		
R3/stream		2.638	0.4	0.7	0.4		
R4/stream		1.886	0.3	0.5	0.3		
	Spring cereals						
FOCUS Scenario		PEC $_{gl\text{-}max}$ (µg/L)					
	Step 3 –Multiple application						
D1/ditch		3.044	0.5	0.8	Resolved at Step 2		Resolved at Step 1
D1/stream		2.182	0.4	0.6			
D3/ditch		2.494	0.4	0.6			
D4/pond		0.125	0.0	0.0			
D4/stream		2.082	0.4	0.5			
D5/pond		0.113	0.0	0.0			
D5/stream		2.152	0.4	0.6			
R4/stream		6.499	**1.1**	**1.7**			
	Step 3 –Single application						
D1/ditch		2.885	0.5	0.7	Resolved at Step 2		Resolved at Step 1
D1/stream		2.523	0.4	0.6			
D3/ditch		2.853	0.5	0.7			
D4/pond		0.098	0.0	0.0			
D4/stream		2.332	0.4	0.6			

Group			Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
D5/pond		0.098	0.0	0.0			
D5/stream		2.395	0.4	0.6			
R4/stream		3.410	0.6	0.9			

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 9.5-9a: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for each organism group based on FOCUS Steps 4 calculations for the use of SAP2101F

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Pseudokirchn. subcapitata*
Endpoint (µg/L)		HC_5 52.7	NOEC 39	EC_{50} 680	NOEC 320	E_rC_{50} 10000
AF		9	10	100	10	10
RAC (µg/L)		5.9	3.9	6.8	32	1000
Winter cereals						
FOCUS Scenario	$PEC_{gl\ max}$ (µg/L)					
Step 4 – Multiple application, 5 meters of VFS						
R3/stream	1.658	Resolved at previous Steps	0.43	Resolved at previous Steps		
Spring cereals						
FOCUS Scenario	$PEC_{gl\ max}$ (µg/L)					
Step 4 – Multiple application, 10 meters of VFS						
R4/stream	3.790	Resolved at previous Steps	0.97	Resolved at previous Steps		
Step 4 – Single application, 5 meters of VFS						
R4/stream	3.027	Resolved at previous Steps	0.78	Resolved at previous Steps		

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

~~**Table 9.5-9a.1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for each organism group based on FOCUS Steps 4 calculations for the use of SAP2101F**~~

~~**Group**~~		~~**Fish acute**~~	~~**Fish prolonged**~~	~~**Inverteb. acute**~~	~~**Inverteb. prolonged**~~	~~**Algae**~~
~~**Test species**~~		~~***Oncorhynchus mykiss***~~	~~***Oncorhynchus mykiss***~~	~~***Daphnia magna***~~	~~***Daphnia magna***~~	~~***Pseudokirchn. subcapitata***~~
~~**Endpoint (µg/L)**~~		~~HC_5 52.7~~	~~NOEC 39~~	~~EC_{50} 680~~	~~NOEC 320~~	~~E_rC_{50} 10000~~
~~**AF**~~		~~9~~	~~10~~	~~100~~	~~10~~	~~10~~
~~**RAC (µg/L)**~~		~~5.9~~	~~3.9~~	~~6.8~~	~~32~~	~~1000~~
~~**Spring cereals**~~						
~~**FOCUS Scenario**~~	~~**PEC $_{gl-max}$ (µg/L)**~~					
~~**Step 4 – Multiple application, 10 meters of VFS**~~						
~~R4/stream~~	~~2.937~~	~~Resolved at previous Steps~~	~~0.8~~	~~Resolved at previous Steps~~		

~~AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.~~

For the Step 3 calculations, the additional PEC_{SW} values (performed on April 2024 by the Applicant) agreed in e -fate Section 8 has been performed. The Applicant's relevant calculations are presented below.

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for each organism group based on FOCUS Steps 4 calculations for the use of SAP2101F

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**
Test species		-	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		HC_5	NOEC	EC_{50}	NOEC	E_rC_{50}
(µg/L)		52.7	39	680	320	10000
AF		9	10	100	10	10
RAC (µg/L)		5.9	3.9	6.8	32	1000
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Winter cereals						
Step 3 – Multiple applications (worst-case value between sets 1 and 2, further details refer to B8)						
D1/ditch	2,522	0.4	0.6	0.4	0.1	0.0

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
D1/stream	2,129	0.4	0.5	0.3	0.1	0.0
D2/ditch	3,341	0.6	0.9	0.5	0.1	0.0
D2/stream	2,212	0.4	0.6	0.3	0.1	0.0
D3/ditch	2,493	0.4	0.6	0.4	0.1	0.0
D4/pond	0,107	0.0	0.0	0.0	0.0	0.0
D4/stream	1,882	0.3	0.5	0.3	0.1	0.0
D5/pond	0,130	0.0	0.0	0.0	0.0	0.0
D5/stream	2,173	0.4	0.6	0.3	0.1	0.0
D6/ditch	2,504	0.4	0.6	0.4	0.1	0.0
R1/pond	0,443	0.1	0.1	0.1	0.0	0.0
R1/stream	6,840	**1.2**	**1.8**	**1.01**	0.2	0.0
R3/stream	7,645	**1.3**	**2.0**	**1.1**	0.2	0.0
R4/stream	6,974	**1.2**	**1.8**	**1.03**	0.2	0.0
Step 3 – Single application (worst-case value between sets 1 and 2, further details refer to B8)						
D1/ditch	2.864	0.5	0.7	0.4	0.1	0.0
D1/stream	2.227	0.4	0.6	0.3	0.1	0.0
D2/ditch	3.335	0.6	0.9	0.5	0.1	0.0
D2/stream	2.445	0.4	0.6	0.4	0.1	0.0
D3/ditch	2.850	0.5	0.7	0.4	0.1	0.0
D4/pond	0.098	0.0	0.0	0.0	0.0	0.0
D4/stream	2.107	0.4	0.5	0.3	0.1	0.0
D5/pond	0.098	0.0	0.0	0.0	0.0	0.0
D5/stream	2.275	0.4	0.6	0.3	0.1	0.0
D6/ditch	2.818	0.5	0.7	0.4	0.1	0.0
R1/pond	0.131	0.0	0.0	0.0	0.0	0.0
R1/stream	1.878	0.3	0.5	0.3	0.1	0.0

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
R3/stream	2.638	0.4	0.7	0.4	0.1	0.0
R4/stream	1.886	0.3	0.5	0.3	0.1	0.0
Spring cereals						
Step 3 – Multiple applications (worst-case value between sets 1 and 2, further details refer to B8)						
D1/ditch	3.057	0.5	0.8	0.4	0.1	0.0
D1/stream	2.182	0.4	0.6	0.3	0.1	0.0
D3/ditch	2.494	0.4	0.6	0.4	0.1	0.0
D4/pond	0.125	0.0	0.0	0.0	0.0	0.0
D4/stream	2.082	0.4	0.5	0.3	0.1	0.0
D5/pond	0.113	0.0	0.0	0.0	0.0	0.0
D5/stream	2.152	0.4	0.6	0.3	0.1	0.0
R4/stream	9.871	**1.7**	**2.5**	**1.5**	0.3	0.0
Step 3 – Single application (worst-case value between sets 1 and 2, further details refer to B8)						
D1/ditch	2.888	0.5	0.7	0.4	0.1	0.0
D1/stream	2.523	0.4	0.6	0.4	0.1	0.0
D3/ditch	2.853	0.5	0.7	0.4	0.1	0.0
D4/pond	0.098	0.0	0.0	0.0	0.0	0.0
D4/stream	2.332	0.4	0.6	0.3	0.1	0.0
D5/pond	0.098	0.0	0.0	0.0	0.0	0.0
D5/stream	2.395	0.4	0.6	0.4	0.1	0.0
R4/stream	6.020	**1.02**	**1.5**	0.9	0.2	0.0

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

For the Step 4 calculations, the additional PEC_{SW} values agreed in e - fate Section 8 (performed on April 2024 by the Applicant) with the lowest RAC of 3.9 µg/L value has been required and the updated risk is presented below:

Table 9.5 9.1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for each organism group based on FOCUS Steps 4 calculations for the use of SAP2101F

Intended use	Cereals
Active substance	folpet
Application rate (g/ha)	2 x 450
Winter Cereals - Multiple applications –10 meters of vegetated filter strip	
R1/stream	3.107
R3/stream	3.489
R4/stream	3.173
RAC (µg/L)	
3.9	**PEC/RAC ratio**
R1/stream	0.8
R3/stream	0.9
R4/stream	0.8
Spring Cereals - Multiple applications –20 m of vegetated filter strip	
R4/strean	2.332
RAC (µg/L)	
3.9	**PEC/RAC ratio**
R4/strean	0.6
Spring Cereals - Single application –10 meters of vegetated filter strip	
R4/strean	2.717
RAC (µg/L)	
3.9	**PEC/RAC ratio**
R4/strean	0.7

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Maximum dose

Winter cereals:

- a non-spray buffer zone of *10 m of vegetated filter strip*, for multiple application for R1, R3 and R4 scenarios.
- *No risk mitigation for single application*

Spring cereals:

-
- a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple application for R4 scenario;
- a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R4 scenario.

For folpet metabolites, the FOCUS step 1-2 calculations were not addressed since they are much less toxic than the parent as demonstrated by the table below. The Applicant considers that there is no need to provide the PEC/RAC ratios in this situation.

Species	Substance	Results
Rainbow trout	Folpet	LC_{50} = 98 µg/L
Bluegill sunfish	Phthalimide	LC_{50} = 38000 µg/L
Rainbow trout	Phthalic acid	LC_{50} > 100000 µg/L
Rainbow trout	Phthalamic acid	LC_{50} > 100000 µg/L
Rainbow trout	Benzamide	LC_{50} > 100000 µg/L
Rainbow trout	2-cyanobenzoic acid	LC_{50} > 100000 µg/L
Daphnia magna	Folpet	EC_{50} = 680 µg/L
Daphnia magna	Phthalimide	EC_{50} = 39000 µg/L
Daphnia magna	Phthalic acid	EC_{50} ≥ 100000 µg/L
Daphnia magna	Phthalamic acid	EC_{50} ≥ 100000 µg/L
Daphnia magna	Benzamide	EC_{50} ≥ 102000 µg/L
Daphnia magna	2-cyanobenzoic acid	EC_{50} > 100000 µg/L
Scenedesmus subspicatus	folpet	E_rC_{50} > 10000 µg/L; E_bC_{50} > 10000 µg/L
Scenedesmus subspicatus	Phthalic acid	EbC_{50} > 100000 µg/L
Scenedesmus subspicatus	Phthalamic acid	EbC_{50} > 100000 µg/L
Scenedesmus subspicatus	Benzamide	EbC_{50} > 100000 µg/L
Selenastrum capricornutum	2-cyaznobenzoic acid	EbC_{50} > 100000 µg/L

zRMS comments:

An updated risk assessment for Folpet (performed by the Applicant on April 2024) to aquatic organism with regard to PEC_{sw} values agreed by e-fate expert in Section 8 has been validated by zRMS.

Based on the calculations of the risk assessment for aquatic organism for folpet the following conclusions has been derived:

Winter cereals:

- a non-spray buffer zone of 10 m of vegetated filter strip, for multiple application for R1, R3 and R4 scenarios.
- no risk mitigation measures are required for single application rate

Spring cereals:
- a non-spray buffer zone of 20 m of vegetated filter strip, for multiple application for **R1, R3** R4 scenarios
- a non-spray buffer zone of 10 m of vegetated filter strip, for single application for R4 scenario

It should be noted that the risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.

9.5.3 Overall conclusions

Regarding the active substance **prothioconazole and its respective metabolites**, the max PECsw are below the RAC with FOCUS Step 1-2 calculations with the exception for the metabolite prothioconazole-desthio, for which Step 4 calculations were needed (i.e. mitigation measures).
The detailed results of FOCUS Step3 and Step 4 calculations for both maximum and minimum dose demonstrate a safe use to aquatic organisms in the following mitigated scenarios:

Maximum dose

Winter cereals:

- a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple applications for R4 scenario;
- a non-spray buffer zone of *15 m of vegetated filter strip*, for multiple applications for R1and R3 scenario;
- a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R4 scenario;
- a non-spray buffer zone of 5 *m of vegetated filter strip*, for single application for R3 scenario;

Spring cereals:

- a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple application for D1 and R4 scenario;
- a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R4 scenario;

Minimum dose (Annex 3)

Winter cereals:

- a non-spray buffer zone of *15 m of vegetated filter strip*, for multiple application for R4 scenario;
- a non-spray buffer zone of 10 *m of vegetated filter strip*, for multiple application for R1 and R3 scenario;
- a non-spray buffer zone of *5 m of vegetated filter strip*, for single application for R4 scenario;

Spring cereals:

- a non-spray buffer zone of *10 m of vegetated filter strip*, for multiple application for R4 scenario;

Regarding **the folpet** active substance, FOCUS Step 3 and Step 4 were simulated. For the folpet metabolites the max PECsw are below the RAC with Step 1-2 calculations.

The detailed results of the FOCUS Step 3 and 4 calculations demonstrate a safe use to aquatic organisms in the following mitigated scenarios:

Winter cereals:

- a non-spray buffer zone of 10 m of vegetated filter strip, for multiple application for R1, R3 and R4 scenarios.

Spring cereals:

- a non-spray buffer zone of 20 m of vegetated filter strip, for multiple application for R4 scenario;
- a non-spray buffer zone of 10 m of vegetated filter strip, for single application for R4 scenario.

Maximum dose

Winter cereals:

- a non-spray buffer zone of 5 *m of vegetated filter strip*, for multiple application for R3 scenario;

Spring cereals:

- a non-spray buffer zone of *10 m of vegetated filter strip*, for multiple application for R4 scenario;
- a non-spray buffer zone of *5 m of vegetated filter strip*, for single application for R4 scenario;

Minimum dose (Annex 3)

Spring cereals:

- a non-spray buffer zone of 5 *m of vegetated filter strip*, for multiple application for R4 scenario.

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Maximum dose

Winter cereals:

- none;

Spring cereals:

- a non-spray buffer zone of *10 m of vegetated filter strip*, for multiple application for R4 scenario.

zRMS comments:

Prothiconazole and prothioconazole metabolite JAU 6476-desthio:

Regarding the active substance prothioconazole and its respective metabolites, the max PECsw are below the RAC with FOCUS Step 1-2 calculations with the exception for the metabolite prothioconazole-desthio, for which Step 4 calculations were needed (i.e. mitigation measures).
The following risk mitigations measures are required for prothioconazole-desthio for aquatic organism:

Maximum dose:

Winter cereals:
- a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple applications for R1, R3 and R4 scenarios
- a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R3, R4 scenarios

Spring cereals:
- a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple application for D1, R4 scenarios and **R1, R3 scenarios** (from winter cereals)
- a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R4 scenario and **R3 scenario** (from winter cereals)

Folpet:
Regarding the active substance prothioconazole and its respective metabolites, the max PECsw are below the RAC with FOCUS Step 1-2 calculations with the exception for the metabolite prothioconazole-desthio, for which Step 4 calculations were needed (i.e. mitigation measures).

The detailed results of FOCUS Step 3 and Step 4 calculations for maximum dose demonstrate a safe use to aquatic organisms in the following mitigated scenarios:

Maximum dose:
Winter cereals:
- a non-spray buffer zone of 10 m of vegetated filter strip, for multiple application for R1, R3 and R4 scenarios
- a non-spray buffer zone for single application to winter cereals

Spring cereals:
- a non-spray buffer zone of 20 m of vegetated filter strip, for multiple application for **R1, R3** and R4 scenario
- a non-spray buffer zone of 10 m of vegetated filter strip, for single application for R4 scenario

It should be noted that the risk from R scenarios not defined for spring cereals is covered by the risk assessment performed for these scenarios available for winter cereals.
The calculation for maximum dose cover lower dose.
The final risk mitigation measures to aquatic organism are left at MSs level.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with prothioconazole, folpet and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet. New data submitted with this application are listed in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.6-1: Endpoints and effect values relevant for the risk assessment for bees relevant for active substances.

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Prothioconazole	Acute, oral	LD_{50} > 71 µg a.s./bee	EFSA Scientific Report (2007)
Apis mellifera	Prothioconazole	Acute, contact	**LD_{50} >200 µg a.s./bee**	EFSA Scientific Report (2007)
Apis mellifera	Prothioconazole EC250	Acute, oral	**LD_{50} > 48.7 µg a.s./bee**	EFSA Scientific Report (2007)
Apis mellifera	Prothioconazole EC250	Acute, contact	LD_{50} > 200 µg a.s./bee	EFSA Scientific Report (2007)
Apis mellifera	Folpet	Acute, oral	**LD_{50} > 236 µg a.s. /bee**	EFSA Scientific Report (2009)
Apis mellifera	Folpan 80 WDG	Acute, oral	LD_{50} > 179 µg a.s./bee	EFSA Scientific Report (2009)
Apis mellifera	Folpet	Acute, contact	**LD_{50} > 200 µg /bee**	EFSA Scientific Report (2009)
Apis mellifera	Folpet 80 WDG	Acute, contact	LD_{50} > 160 µg a.s./bee	EFSA Scientific Report (2009)
Apis mellifera	Folpet 80 WG	Acute, oral	LD_{50} > 104.8 µg a.s./bee	KCP 10.3.1.1/01, Schmitzer S. and Pavic, B., 2007, 33893035
Apis mellifera	Folpet 80 WG	Acute, contact	LD_{50} > 100 µg a.s./bee	
Apis mellifera	Folpet technical	Chronic, oral	LDD_{50} > 16.29 µg a.s./bee/day	KCP 10.3.1.2/01, Ansaloni, 2015, TRC14-246BA
Apis mellifera	Folpet technical	Acute, larvae	LDD_{50} = 4.846 µg a.s./larvae/dayevelopmental period NOED = 0.89 µg a.s./larvae/dayevelopmental period [redacted]	KCP 10.3.1.3/01, Ansaloni, 2015, TRC14-245BA
Bombus sp.	Folpet technical	Acute oral	LD_{50} > 100 µg a.s./bee	KCP 10.3.1.1/02, Fauser-Misslin, 2015, 20140156
Bombus sp.	Folpet technical	Acute contact	LD_{50} > 100 µg a.s./bee	

zRMS comments:

Bees' toxicity data for prothioconazole, prothioconazole metabolite JAU 6476-desthio and folpet provided in Table 9.6-1 were confirmed that they are in line with EU agreed endpoints reported in EFSA EFSA Scientific Report (2007) 106 and in EFSA Journal (2007) 124, 1-84 respectively.

9.7 Effects on bees (KCP 10.3.1)

9.7.1 Toxicity data

Studies on the toxicity to bees have been carried out with prothioconazole, folpet and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on bees of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for bees for a.s and formulation SAP2101F

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Prothioconazole	Acute, oral	LD_{50} > 71 µg a.s./bee	EFSA Scientific Report (2007)
Apis mellifera	Prothioconazole	Acute, contact	**LD_{50} >200 µg a.s./bee**	EFSA Scientific Report (2007)
Apis mellifera	Prothioconazole EC250	Acute, oral	**LD_{50} > 48.7 µg a.s./bee**	EFSA Scientific Report (2007)
Apis mellifera	Prothioconazole EC250	Acute, contact	LD_{50} > 200 µg a.s./bee	EFSA Scientific Report (2007)
Apis mellifera	Folpet	Acute, oral	**LD_{50} > 236 µg a.s. /bee**	EFSA Scientific Report (2009)
Apis mellifera	Folpan 80 WDG	Acute, oral	LD_{50} > 179 µg a.s./bee	EFSA Scientific Report (2009)
Apis mellifera	Folpet	Acute, contact	**LD_{50} > 200 µg /bee**	EFSA Scientific Report (2009)
Apis mellifera	Folpet 80 WDG	Acute, contact	LD_{50} > 160 µg a.s./bee	EFSA Scientific Report (2009)
Apis mellifera	Folpet 80 WG	Acute, oral	LD_{50} > 104.8 µg a.s./bee	KCP 10.3.1.1/01, Schmitzer S. and Pavic, B., 2007, 33893035
Apis mellifera	Folpet 80 WG	Acute, contact	LD_{50} > 100 µg a.s./bee	
Apis mellifera	Folpet technical	Chronic, oral	LDD_{50} > 16.29 µg a.s./bee/day	KCP 10.3.1.2/01, Ansaloni, 2015, TRC14-246BA
Apis mellifera	Folpet technical	Acute, larvae	LDD_{50} = 4.846 µg a.s./larvae/dayevelopmental period NOED = 0.89 µg a.s./larvae/dayevelopmental period	KCP 10.3.1.3/01, Ansaloni, 2015, TRC14-245BA
Bombus sp.	Folpet technical	Acute oral	LD_{50} > 100 µg a.s./bee	KCP 10.3.1.1/02,

Species	Substance	Exposure System	Results	Reference
~~*Bombus* sp.~~	~~Folpet technical~~	~~Acute contact~~	~~LD_{50} > 100 µg a.s./bee~~	~~Fauser-Misslin, 2015, 20140156~~
~~*Bombus terrestris L*~~	~~Folpet 80 WG~~	~~Acute oral~~	~~LD_{50} > 389.3 µg a.s./bee~~	~~KCP 10.3.1.1/03, Amsel, 2015, 15 10 48 167 B~~
~~*Bombus terrestris L*~~	~~Folpet 80 WG~~	~~Acute contact~~	~~LD_{50} > 199.5 µg a.s./bee~~	
~~*Osmia bicornis L.*~~	~~Folpet 80 WG~~	~~Acute, oral~~	~~LD_{50} > 104.1 µg a.s./bee~~	~~KCP 10.3.1.1/04, Schurr A. 2015, 15 10 48 114 B~~
~~*Osmia bicornis L.*~~	~~Folpet 80 WG~~	~~Acute, contact~~	~~LD_{50} > 199.5 µg a.s./bee~~	
Apis mellifera L.	SAP2101F	Acute, oral	**LD_{50} = 986 µg t.i./bee**	KCP 10.3.1.1/05, Ansaloni, T., 2022, S21-05005
Apis mellifera L.	SAP2101F	Acute, contact	**LD_{50} > 2340 µg t.i./bee**	
Apis mellifera L.	SAP2101F	Chronic, oral	LDD_{50} = 99.21 µg t.i./bee/day	KCP 10.3.1.2/02, Ansaloni, T., 2022, S21-05006
Apis mellifera L.	SAP2101F	Larvae	NOED = 9.05 µg t.i./larva	KCP 10.3.1.3/02, Ansaloni, T., 2022, S21-05007
Bombus terrestris	SAP2101F	Acute, oral	LD_{50} > 1083.88 µg t.i./bee	KCP 10.3.1.1/06, Aguilar-Alberola, J. A., 2023, S22-108802
		Acute, contact	LD_{50} > 1153.09 µg t.i./bee	
Higher-tier studies (tunnel test. field studies)				
-				

Values shown in bold were used for QH calculations

zRMS comments:

The acute risk assessment for bees has been based on the active substances data which were evaluated at EU level and data for formulation SAP2101F.
To fulfil the data requirements as set by Commission Regulation (EU) No 284/2013, studies on acute toxicity to adult bees and chronic larvae toxicity to bees for formulated product have been performed and have been used in the risk assessment. In addition, the acute data for formulation SAP2101F have been available for bumble bees and are considered in the acute risk assessment.

Studies on acute and chronic effects of the formulated product SAP2101F to bees listed in Table above were evaluated by the zRMS and considered acceptable. The reported endpoints are confirmed.
Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.
The data not relevant for product authorisation at zonal level has been crossed out by zRMS in the Table 9.6-1.
The new chronic endpoints for technical a.s. – folpet are not considered by zRMS.
Instead of these studies, the chronic SAP2101F formulation studies have been used in the chronic risk assessment.

9.7.1.1 Justification for new endpoints

Not relevant.

9.7.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

To achieve a concise risk assessment the risk envelope approach is applied. Here. the assessment for the use group cereals covers the risk for bees from all intended uses (see 9.1.2).

9.7.2.1 Hazard quotients for bees

Risk assessment according to SANCO/10329/2002 rev.2 (final), October 17, 2002)

Table 9.7-2: First-tier assessment of the risk for bees due to the use of SAP2101F in cereals

Intended use **Active substance** **Application rate (g/ha)**	Cereals Prothioconazole 2 x 180		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}. Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>48.7	180	3.7
Contact toxicity	>200		0.9
Product **Application rate (g/ha)**	Folpet 2 x 450		
Test design	**LD_{50} (lab.)** (µg/bee)	**Single application rate** (g/ha)	**Q_{HO}. Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>236	450	1.9
Contact toxicity	>200		2.3
Product **Application rate (g/ha)**	SAP2101F 2 x 1710*		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}. Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	986	1710	1.7
Contact toxicity	2340		0.7

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
*Calculated accounting for the formulation density of 1.14 g/mL.

zRMS comments:

The acute risk assessment for bees presented in Table 9.7-2 is validated by the zRMS. $HQ_{oral, contact}$ values for the active substances and the formulated product SAP2101F are below the trigger of 50, indicating a low acute risk for bees.
Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final.

Overall, acceptable risk to bees may be concluded from the intended uses of SAP2101F.

Chronic risk to honey bees

~~According to the new data requirements (Commission regulation No 283/2013 and 284/2013), a chronic toxicity test with honeybees is required where exposure to honeybees cannot be excluded.~~
~~The EPPO 2010 scheme does not recommend a chronic assessment for adults for foliar spray applications. However, as an approach, it is proposed an assessment based on the refinement for seed coatings/soil treatments (point 7 on the scheme). This approach can be adapted to provide a worst-case assessment for foliar sprays.~~
~~A worst-case of potential exposure via residues in pollen / nectar can be estimated based on the default worst-case value of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts as a surrogate for nectar and pollen.~~
~~The default residues can then be combined with a measure of consumption in order to estimate the exposure of bees. Worst case data from Rortais *et al*., 2005 as proposed in the EPPO 2010 scheme have been used to estimate the consumption by bee foragers:~~

~~Worst case: forager consuming 898.8 mg sugar for 7 days (= 128 mg sugar /day).~~

Assuming 40% sugar content of nectar: (898.8 * 2.5)/7 = 321 mg nectar/day
Thus, considering residues of 1 mg a.s./kg sugar x consumption of 321 mg nectar/bee/day
Total exposure ETE = 0.32 µg a.s./bee/day

This can be compared to the adult LDD_{50} of 99.21 µg t.i./bee/day.
TER = NOED (µg/bee/day)/ ETE (µg/bee/day) (EPPO 2010 trigger = 1)
The chronic TER values are given in the table below.

Table 9.7-3: Chronic adult bees risk due to the use of SAP2101F

Uses	Exposure Route	LDD_{50} [µg a.s./bee/day]	Maximum nectar consumption [mg nectar/bee/day]	Generic worst case residue intake of folpet [µg a.s./bee/day]	$TER_{chronic}$	Trigger
Cereals	Oral	99.21	321	0.32	**310**	1

Values in **bold** are above the trigger and indicate an acceptable risk to honey bees

The chronic TER based on worst case generic residue assumptions is 310 and higher than the respective trigger of 1.
The TER value of 310 is above the trigger (1), indicating that the proposed uses of SAP2101F pose an acceptable chronic risk to adult bees.

Risk to honey bee larvae

According to the new data requirements (Commission regulation No 283/2013 and 284/2013), a honey bee brood study is required where exposure to honey bee brood cannot be excluded.

The toxicity of SAP2101F to honeybee larvae (KCP 10.3.1.3/02 was determined with a NOED of 9.05 µg a.s./larva. Since this study lasted for the entire developmental period of the larvae, the derived endpoints are considered valid as chronic ones.
A worst case risk assessment to honeybee larvae can be conducted through the calculation of a TER value as set out in the EPPO 2010 scheme (point 5 on the scheme). A worst case potential exposure via residues in pollen / nectar can be estimated based on the default worst case residue of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6). The default residues can then be combined with a measure of consumption in order to estimate the exposure of bees. Worst case data from Rortais *et al*., 2005 as proposed in the EPPO scheme have been used to estimate the consumption by bee larvae:

Worst case: drone larvae consuming 98.2 mg sugar during 6.5 days (= 15.1 mg sugar /day).
Assuming a mean sugar content of 40% in nectar: (98.2 * 2.5)/6.5 = **37.8 mg nectar/larva/day**

Thus, considering residues of 1 mg a.s./kg x consumption of 37.8 mg nectar/larva/day:
Total exposure ETE = 0.0378 µg a.s./larva/day

This can be compared to the larval NOED of 9.05 µg t.i./larva
TER = NOED (µg/larva)/ ETE (µg/larva) (EPPO 2010 trigger = 1)

According to the parameters above, the worst case consumption of nectar for a honey bee larvae values 37.8 mg nectar/larvae/day during its whole development and the generic worst case residue value is set to 1 mg a.s./kg in nectar as given in the revised EPPO scheme (2010).

Therefore, the maximum amount of folpet residues, a honeybee larvae could ingest by consumption of nectar is 0.0378 µg a.s./ larvae/day.

Table 9.6-4: Chronic larvae risk for bees due to the use of SAP2101F

Use	Scenario	NOED [µg a.s./larvae]	Maximum consumption of pollen/nectar [mg/larvae/day]	Generic worst case residue intake of folpet [µg a.s./larvae/day]	TERlarvae	Trigger

Cereals	Nectar	9.05	37.8	0.0378	**239**	1

The TER based on the combined residues of worst case generic residue assumptions for the whole developmental period is higher than the trigger of 1 (TER=239).

Risk assessment according to EFSA Journal 2013; 11(7):3295

The Applicant would like to highlight that the guidance document used for this risk assessment is not yet noted and is currently under update. Therefore, the risk assessment is only presented for illustrative reasons and no conclusion should be drawn on the basis of these results until the updated and noted document is available.

Table 9.6-5: Screening step of the risk for bees due to the use of prothioconazole, folpet and SAP2101F in cereals according to EFSA Journal 2013; 11(7):329

Intended use	Cereals				
Active substance	Prothioconazole				
Application rate (g a.s./ha)	2 x 180				
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**"Calculation factor"**	**HQ/ETR**	**Trigger**	**Risk indicator**
Acute oral toxicity	>48.7	7.6	0.03	0.2	Ok!
Acute contact toxicity	>200	1	0.9	42	Ok!
Intended use	Cereals				
Active substance	Folpet				
Application rate (g a.s./ha)	2 x 450				
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**"Calculation factor"**	**HQ/ETR**	**Trigger**	**Risk indicator**
Acute oral toxicity	>236	7.6	0.01	0.2	Ok!
Acute contact toxicity	>200	1	2.3	42	Ok!
Chronic oral toxicity	16.29	7.6	0.210	0.03	**Not Ok!**
Chronic larvae toxicity	2.16	4.4	0.92	0.2	**Not Ok!**
Intended use	Cereals				
Active substance	SAP2101F				
Application rate (g/ha)	2 x 1710				
Test design	**LD_{50} (lab.)/NOED (µg a.s./bee)**	**"Calculation factor"**	**HQ/ETR**	**Trigger**	**Risk indicator**
Acute oral toxicity	986	7.6	0.01	0.2	Ok!
Acute contact toxicity	2340	1	0.7	42	Ok!
Chronic oral toxicity	99.21	7.6	0.131	0.03	**Not Ok!**
Chronic larvae toxicity	9.05	4.4	0.83	0.2	**Not Ok!**

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Table 9.6-5a: First tier of the risk for honey bees oral toxicity due to the use of folpet and SAP2101F in cereals according to EFSA Journal 2013; 11(7):329

Intended use	Cereals								
Active substance	Folpet								
Application rate (g a.s./ha)	2 × 450								
Application	**BBCH**	**Category**	**Scenario**	**Ef**	**SV HB**	**TWA HB**	**ETR**	**trigger**	**Risk indicator**

~~Spray DW~~	~~30 – 39~~	~~chronic~~	~~treated crop~~	~~1~~	~~0.92~~	~~0.72~~	~~0.018~~	~~0.03~~	~~Ok!~~
	~~40 – 69~~	~~chronic~~	~~treated crop~~	~~1~~	~~0.92~~	~~0.72~~	~~0.018~~	~~0.03~~	~~Ok!~~
	~~30 – 39~~	~~chronic~~	~~weeds~~	~~0.5~~	~~2.9~~	~~0.72~~	~~0.029~~	~~0.03~~	~~Ok!~~
	~~40 – 69~~	~~chronic~~	~~weeds~~	~~0.3~~	~~2.9~~	~~0.72~~	~~0.017~~	~~0.03~~	~~Ok!~~
	~~30 – 39~~	~~chronic~~	~~field margin~~	~~0.0092~~	~~2.9~~	~~0.72~~	~~0.001~~	~~0.03~~	~~Ok!~~
	~~40 – 69~~	~~chronic~~	~~field margin~~	~~0.0092~~	~~2.9~~	~~0.72~~	~~0.001~~	~~0.03~~	~~Ok!~~
	~~30 – 39~~	~~chronic~~	~~adjacent crop~~	~~0.0033~~	~~5.8~~	~~0.72~~	~~0.000~~	~~0.03~~	~~Ok!~~
	~~40 – 69~~	~~chronic~~	~~adjacent crop~~	~~0.0033~~	~~5.8~~	~~0.72~~	~~0.000~~	~~0.03~~	~~Ok!~~
	~~30 – 39~~	~~chronic~~	~~next crop~~	~~1~~	~~0.54~~	~~0.72~~	~~0.011~~	~~0.03~~	~~Ok!~~
	~~40 – 69~~	~~chronic~~	~~next crop~~	~~1~~	~~0.54~~	~~0.72~~	~~0.011~~	~~0.03~~	~~Ok!~~
	~~30 – 39~~	~~larva~~	~~treated crop~~	~~1~~	~~0.15~~	~~0.85~~	~~0.03~~	~~0.2~~	~~Ok!~~
	~~40 – 69~~	~~larva~~	~~treated crop~~	~~1~~	~~0.15~~	~~0.85~~	~~0.03~~	~~0.2~~	~~Ok!~~
	~~30 – 39~~	~~larva~~	~~weeds~~	~~0.5~~	~~2.2~~	~~0.85~~	~~0.19~~	~~0.2~~	~~Ok!~~
	~~40 – 69~~	~~larva~~	~~weeds~~	~~0.3~~	~~2.2~~	~~0.85~~	~~0.12~~	~~0.2~~	~~Ok!~~
	~~30 – 39~~	~~larva~~	~~field margin~~	~~0.0092~~	~~2.2~~	~~0.85~~	~~0.00~~	~~0.2~~	~~Ok!~~
	~~40 – 69~~	~~larva~~	~~field margin~~	~~0.0092~~	~~2.2~~	~~0.85~~	~~0.00~~	~~0.2~~	~~Ok!~~
	~~30 – 39~~	~~larva~~	~~adjacent crop~~	~~0.0033~~	~~4.4~~	~~0.85~~	~~0.00~~	~~0.2~~	~~Ok!~~
	~~40 – 69~~	~~larva~~	~~adjacent crop~~	~~0.0033~~	~~4.4~~	~~0.85~~	~~0.00~~	~~0.2~~	~~Ok!~~
	~~30 – 39~~	~~larva~~	~~next crop~~	~~1~~	~~0.4~~	~~0.85~~	~~0.07~~	~~0.2~~	~~Ok!~~
	~~40 – 69~~	~~larva~~	~~next crop~~	~~1~~	~~0.4~~	~~0.85~~	~~0.07~~	~~0.2~~	~~Ok!~~

Intended use	Cereals
Active substance	SAP2101F
Application rate (g/ha)	2 × 1710

Application	**BBCH**	**Category**	**Scenario**	**Ef**	**SV HB**	**TWA HB**	**ETR**	**trigger**	**Risk indicator**
	30 - 39	chronic	treated crop	1	0.92	0.72	0.011	0.03	Ok!
	40 - 69	chronic	treated crop	1	0.92	0.72	0.011	0.03	Ok!
	30 - 39	chronic	weeds	0.5	2.9	0.72	0.018	0.03	Ok!
	40 - 69	chronic	weeds	0.3	2.9	0.72	0.011	0.03	Ok!
	30 - 39	chronic	field margin	0.0092	2.9	0.72	0.000	0.03	Ok!
	40 - 69	chronic	field margin	0.0092	2.9	0.72	0.000	0.03	Ok!
	30 - 39	chronic	adjacent crop	0.0033	5.8	0.72	0.000	0.03	Ok!
	40 - 69	chronic	adjacent crop	0.0033	5.8	0.72	0.000	0.03	Ok!
	30 - 39	chronic	next crop	1	0.54	0.72	0.007	0.03	Ok!
	40 - 69	chronic	next crop	1	0.54	0.72	0.007	0.03	Ok!
	30 - 39	larva	treated crop	1	0.15	0.85	0.02	0.2	Ok!
	40 - 69	larva	treated crop	1	0.15	0.85	0.02	0.2	Ok!
	30 - 39	larva	weeds	0.5	2.2	0.85	0.18	0.2	Ok!
	40 - 69	larva	weeds	0.3	2.2	0.85	0.11	0.2	Ok!
	30 - 39	larva	field margin	0.0092	2.2	0.85	0.00	0.2	Ok!
	40 - 69	larva	field margin	0.0092	2.2	0.85	0.00	0.2	Ok!
	30 - 39	larva	adjacent crop	0.0033	4.4	0.85	0.00	0.2	Ok!
	40 - 69	larva	adjacent crop	0.0033	4.4	0.85	0.00	0.2	Ok!

	30 - 39	larva	next crop	1	0.4	0.85	0.06	0.2	Ok!
	40 - 69	larva	next crop	1	0.4	0.85	0.06	0.2	Ok!

Regarding the EFSA guidance on bees risk assessment, an acceptable risk was obtained for all scenarios at Tier 1. Therefore, no unacceptable risk is expected for honeybees exposed to SAP2101F under its proposed uses application.

zRMS comments:

The chronic adult and larvae bees risk assessment is not required according to SANCO/10329/2002 rev 2 final.

Based on Tier 1 based on the EFSA Journal 2013; 11(7):329 (not approved at EU level) an acceptable chronic risk was obtained for all scenarios at Tier 1 for formulation SAP2101F.
Therefore, no unacceptable risk to adult bees and larvae is expected for honeybees exposed to SAP2101F under its proposed uses application.

The chronic risk assessment for adults and larvae bees for PL registration of the product is not required yet until Bee GD document will be approved at EU level.

9.7.2.2 Higher-tier risk assessment for bees (tunnel test, field studies)

Not relevant.

9.7.3 Effects on bumble bees

When looking to the results obtained for honeybee (acute oral LD_{50} > 236 µg/bee; acute contact LD_{50} > 200 µg/bee) and bumble bee (acute oral LD_{50} > 389.3 µg/bee; acute contact LD_{50} > 199.5 µg/bee) with the active substance folpet, it is possible to see that bumble bee is not the most sensitive. Therefore, the risk assessment performed with the honeybee (*Apis melifera* L.) for prothioconazole, folpet and formulation SAP2101F covers the effects on bumble bees and an acceptable risk is to be expected. Nevertheless, a study was conducted with SAP2101F and a risk assessment is presented below.

- ***Risk assessment according to SANCO/10329/2002 rev 2 final***

Table 9.7-6: First-tier assessment of the risk for bumble bees due to the use of SAP2101F in cereals

Intended use	Cereals		
Active substance	SAP2101F		
Application rate (g a.s./ha)	2 × 1500		
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>1083.88	1500	<1.4
Contact toxicity	>1153.09		<1.3

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

zRMS comments:

The acute risk assessment for bees presented in Table 9.6-6 is agreed by the zRMS. $HQ_{oral,contact}$ values for formulation product SAP2101F are below the trigger of 50, indicating a low acute risk for bumble bees.
Please note that the evaluation has been performed in line with SANCO/10329/2002 rev 2 final.

Overall, acceptable risk to bumble bees may be concluded from the intended uses of SAP2101F.

- ***Risk assessment according to EFSA Journal 2013; 11(7):3295***

Table 9.6-7: Screening step of the risk for bumble bees due to the use of SAP2101F in cereals according to EFSA Journal 2013; 11(7):329

Intended use	Cereals
Active substance	SAP2101F
Application rate (g a.s./ha)	2 × 1500

Test design	**LD_{50} (lab.) (µg a.s./bee)**	**"Calculation factor"**	**HQ/ETR**	**Trigger**	**Risk indicator**
Acute oral toxicity	>1083.88	11.2	0.02	0.036	OK
Acute contact toxicity	>1153.09	1	1.3	7	OK

zRMS comments:

The acute risk assessment for bumble bees presented in Table 9.6-7 is validated by the zRMS.
$HQ_{oral, contact}$ values for formulation SAP2101F are below the trigger values, indicating a low acute risk for bumble bees.

Overall, acceptable risk to bumble bees may be concluded from the intended uses of SAP2101F according to EFSA Journal 2013; 11(7):3295 (not approved at EU level).

9.7.4 Effects on solitary bees

No data is available with the formulation for solitary bees. However, when looking to the results obtained for honeybee (acute oral LD_{50} > 236 µg/bee; acute contact LD_{50} > 200 µg/bee) and solitary bee (acute oral LD_{50} > 104.1 µg/bee; acute contact LD_{50} > 199.5 µg/bee) with the active substance folpet, it is possible to see that solitary bee is not the most sensitive. Therefore, the risk assessment performed with the honeybee (*Apis melifera* L.) for prothioconazole, folpet and formulation SAP2101F covers the effects on bumble bees and an acceptable risk is to be expected.

zRMS comments:

The risk assessment for solitary bees for the active substance or formulated product is not required yet at zonal registration of plant protection products in the Central Zone.

9.7.5 Overall conclusions

The risk assessment for bees was conducted according to SANCO/10329/2002 rev 2 final and according to EFSA Journal 2013;11(7):3295 for illustrative purposes only as the last-mentioned guidance document is not yet noted. The risk assessment performed for both the active substances and the formulated product derived hazard quotients lower than 50, indicating that the active substance prothioconazole, folpet and the formulation SAP2101F pose an acceptable risk to bees from oral and contact, both acute and chronic exposure, according to the proposed use.

9.8 Effects on arthropods other than bees (KCP 10.3.2)

9.8.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with prothioconazole and folpet. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target arthropods of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results		Reference
Prothioconazole					
Predatory mites					
Typhlodromus pyri	Prothiocona-zole EC250	Lab., coffin cells, 14 d	LR_{50} = 18.7 g a.s./ha		EFSA Scientific Report (2007)
			Corrected mor-tality [%] 11.2 18.5 4.0 27.5* 52.9*	Effect on reprod. [%] 4 -14 -6 19 n.a.a)	
Typhlodromus pyri	Prothiocona-zole EC250	Ext. laboratory test bean leaves, 14 d	LR_{50} = 445.5 g a.s./ha		EFSA Scientific Report (2007)
			Corrected mor-tality [%] -2.3 1.5 9.1 45.1* 67.8*	Effect on reprod. [%] 14 9 47* 40 n.a.[a)]	
Typhlodromus pyri	Prothiocona-zole EC250	Ext. lab., aged residues (1 and 15 days), bean leaves, 300 g a.s./ha	Corrected mor-tality [%] 14.5 6.4	Effect on reprod. [%] 7.5 -28.8	EFSA Scientific Report (2007)
Parasitoids					
Aphidius rhopalosiphi (adults)	Prothiocona-zole EC250	Laboratory test glass plates, 14 d	LR_{50} = 139.9 g a.s./ha		EFSA Scientific Report (2007)
			Corrected mor-tality [%] 1st run 3.5 6.9 3.5 3.5 100 2nd run 13.3 13.3 33.3* 33.3* 96.7*	Effect on reprod. [%] 1st run 21 10 39 34 n.a.a) 2nd run 17 36 44* 53* n.a.[a)]	
Aphidius rhopalosiphi (adults)	Prothiocona-zole EC250	Ext. laboratory test wheat plants, 14 d	48 h mortality <5% in any of test concentrations. No significant effect on reproduction in any treatment		EFSA Scientific Report (2007)
Foliage dwelling predators					
Coccinella septempunctata (larvae)	Prothiocona-zole EC250	Lab., glass plates, 46 d	LR_{50} = 229.8 g a.s./ha		EFSA Scientific Report (2007)
			Corrected mor-tality [%] - -9.6 -5.3 22.4 30.7 73.7*	Effect on reprod. [%] 147 54 0 84 549 n.a.[a)]	
			effects on reproduction are not considered to be treatment related (no adverse effects on reproduction at the highest tested treatment rate).		

Chrysoperla carnea (larvae)	Prothiocona-zole EC250	Lab., glass plates, 23 d	Corrected mortality [%] 15.2* 28.3* 41.3* no adverse effects on reproduction	EFSA Scientific Report (2007)
Ground dwelling predators				
Poecilus cupreus (adults)	Prothiocona-zole EC250	Quartz sand, 14 d	Corrected mortality [%] 0.0 3.3 no adverse effects on feeding rate	EFSA Scientific Report (2007)
Aleochara bilineata (adults/larvae)	Prothiocona-zole EC250	Quartz sand, 87 d	Corrected mortality [%] 2.5 9.9 24.6*	EFSA Scientific Report (2007)
Poecilus cupreus (adults)	FS 100	Ext. lab., 14 d, soil (Lufa 2.1), dressed seeds	Corrected mortality [%] 0 Effect on feeding rate [%] 5.6 - 9.6	EFSA Scientific Report (2007)
Aleochara bilineata (adults/larvae)	FS 100	Ext. lab., 82 d, soil (Lufa 2.1), dressed seeds	Effect on reproduction [%] 11.2	EFSA Scientific Report (2007)
Pardosa spp. (adults)	FS 100	Ext. lab., 14 d, soil (Lufa 2.1), dressed seeds	Corrected mortality [%] -3.1 Effect on feeding rate [%] -18	EFSA Scientific Report (2007)
Folpet				
Predatory mites				
*Typhlodromus pyri** (protonymphs)	Folpan 500SC	Laboratory, residues on glass	Mortality: control: 13%, 0.49 kg/ha: 14% Reproduction: offspring/female control: 8.9 0.49 kg/ha: 9.3	EFSA Scientific Report (2009)
Coccinella septempunctata (larvae)	Folpan 500SC	Laboratory, residues on glass	Mortality: control: 20%, 0.48 kg/ha: 13%. Reproduction, fertile eggs/female: control: 373 0.48 kg/ha: 206	EFSA Scientific Report (2009)
Coccinella septempunctata (larvae)	Folpan 80 WDG	-	Mortality: control: 22%, 0.53 kg/ha: 16%. Reproduction: fertile eggs/female: control: 419 0.53 kg/ha: 188	EFSA Scientific Report (2009)
Chrysoperla carnea (larvae)	Folpan 500SC	Laboratory, residues on glass	Mortality: control: 21.1%, 0.49 g/ha:7.7%. Reproduction, fertile eggs/female: control: 610 0.49 kg/ha: 624	EFSA Scientific Report (2009)
Trichogramma cacoeciae (adult)	Folpan 500SC	Laboratory, residues on glass	Parasitized eggs/wasp: control: 7.7 0.53 kg/ha: 6.3 (18.5% reduction)	EFSA Scientific Report (2009)
Poecilus cupreus (adults)	Folpan 80 WDG	Laboratory, residues on sand	Mortality: 0% No effect on feeding.	EFSA Scientific Report (2009)

Aleochara bilineata (adults)	Folpan 500SC	Laboratory, residues on sand	Parasitism: control: 36% 0.49kg/ha: 29% (19% reduction)		EFSA Scientific Report (2009)
Aphidius rhopalosiphi (adults)	Folpan 500SC	Extended laboratory, residues on apple leaves	Corrected mortality%: 0.1kg/ha:2.5 0.5kg/ha:10 1.2kg/ha:2.5 1.5kg/ha:7.5 2.0kg/ha:32.5	Reduction in patriotization: 0.1kg/ha:32% 0.5kg/ha:33% 1.2kg/ha:23% 1.5kg/ha:68% 2.0kg/ha:75%	EFSA Scientific Report (2009)
*Typhlodromus pyr*i	Folpan 80 WDG	Extended laboratory, bean leaves, whole plants sprayed	Corrected mortality: 1.64kg/ha:0% 3.38kg/ha:0% 5.25kg/ha:0%	Eggs/female: control:4.5 1.64kg/ha:8.1 3.38kg/ha:9.85.25kg/ha:9.2	EFSA Scientific Report (2009)
Aphidius rhopalosiphi	Folpan 80 WDG	Extended laboratory, bean leaves, whole plants sprayed. Fresh residues, and 14 day aged residues	Fresh residues: Corrected mortality%: 1.64kg/ha:2.7 3.38kg/ha:21.6 5.25kg/ha:75.7 mummies/female: control:38.0 1.64kg/ha:27.8 3.38kg/ha:25.6	14 day aged residues: mortality%: 1.64kg/ha:0 3.38kg/ha:0 5.25kg/ha:0 mummies/female: control:31.2 1.64kg/ha:24.6 3.38kg/ha:12.0 5.25kg/ha:28.8	EFSA Scientific Report (2009)
Coccinella septempunctata	Folpan 80 WDG	Extended laboratory, bean leaves, whole plants sprayed.	Corrected mortality%: 0.31kg/ha:0 1.64kg/ha:0 3.38kg/ha:0 5.25kg/ha:11.8	Fertile eggs /female/day: control:4.1 0.31kg/ha:6.8 1.64kg/ha:10.1 3.38kg/ha:8.2 5.25kg/ha:8.4	EFSA Scientific Report (2009)
Chrysoperla carnea	Folpan 80 WDG	Extended laboratory, bean leaves, whole plants sprayed	Corrected mortality%: 1.64kg/ha:20 3.38kg/ha:10 5.25kg/ha:17.5	Eggs/female/day control:36.8 1.64kg/ha:31.8 3.38kg/ha:33.3 5.25kg/ha:34.1	EFSA Scientific Report (2009)
SAP2101F					
Typhlodromus pyri (protonymphs)	SAP2101F	Ext. laboratory test bean leaves, 14 d	LR_{50} > 5100 ml test item/ha		KCP 10.3.2.2/01, Varela S., 2022, S21-05009
			Corrected mortality [%] -7.14 -5.95 -2.38 -1.89 3.57	Effect on reprod. [eggs/female] 6.00 5.42* 4.80* 4.54* 4.52*	
Aphidius rhopalosiphi (adults)	SAP2101F	Ext. laboratory test barley seedlings, 48 hours for mortality and behaviour; 11d after 24h parasitation for reprodcution	LR_{50} > 5100 ml test item/ha		KCP 10.3.2.2/02, Varela S., 2022, S21-05008
			Corrected mortality [%] -7.14 -7.14 -7.14 -7.14 -3.57	Effect on reprod. [mummies/female] 18.00 23.21 18.60 17.20 19.73	
Coccinella septempunctata L. (larvae)	SAP2101F	Ext. laboratory test bean leaves, 14 d	LR_{50} > 5100 ml test item/ha		KCP 10.3.2.2/03, Varela S., 2022, S21-05010
			Corrected mortality [%] -8.57	Effect on reprod. [mean fertile eggs/female/day] 39.1	

			-2.86 8.57 0.00 22.86	30.9 33.5 36.4 36.2	
Chrysoperla carnea (larvae)	SAP2101F	Ext. laboratory test bean leaves, 21 d	LR_{50} > 5100 ml test item/ha		KCP 10.3.2.2/04, Luna F.S., 2022, S21-05012
			Corrected mortality [%] 0.0 10.3 0.0 10.3 -3.4	Effect on reprod. [eggs/female/day] 35.6 35.6 38.3 42.2 33.5	
Field or semi-field tests					
Folpet: No significant population effects on *Typhlodromus pyri* under field conditions, applied at 0.3 to 2.1 kg a.s./ha.					

a) not assessed due to mortality > 50 % at this concentration
* significantly different from control (t-test p <0.05)

zRMS comments:

The studies performed with the formulated product SAP2101F were evaluated and agreed by the zRMS (for details, please refer to respective points in Appendix 2). Endpoints reported in Table 9.7-1 are confirmed to be correct.
EU agreed endpoints for the active substances and representative formulation have not been taken into account, since relevant evaluation was performed with endpoints derived from studies performed with SAP2101F, in line with data requirements.

9.8.1.1 Justification for new endpoints

Not relevant.

9.8.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.8.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment. the risk envelope approach is applied. Here. the assessment for the use group cereals covers the risk for non-target arthropods from all intended uses (see 9.1.2).

Table 9.8-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of SAP2101F in cereals

Intended use	Cereals		
Active substance/product	Prothioconazole + Folpet/ SAP2101F		
Application rate (mL/ha)	2 × 1500		
MAF	1.7		
~~**Test species** **Tier II**~~	~~**LR_{50} (lab.)** **(mL/ha)**~~	~~**$PER_{in\text{-}field}$** **(mL/ha)**~~	~~**$HQ_{in\text{-}field}$** **criterion: HQ ≤ 1**~~
~~*Typhlodromus pyri*~~	~~>5100~~	~~2550~~	~~< 0.5~~
~~*Aphidius rhopalosiphi*~~	~~>5100~~		~~< 0.5~~

~~*Coccinella septempunctata* L.~~	~~>5100~~		~~< 0.5~~
~~*Chrysoperla carnea*~~	~~>5100~~		~~< 0.5~~
Test species Tier II	**Rate with ≤ 50 % effect (ml/ha) a**	**$PER_{in\text{-}field}$ (ml/ha)**	**$PER_{in\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	>5100	2550	Y
Aphidius rhopalosiphi	>5100		Y
Coccinella septempunctata L.	>5100		Y
Chrysoperla carnea	>5100		Y

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment.
Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

zRMS comments:

It should be noted that glass plate-laboratory studies with the formulated product on both sensitive indicator NTA species are not available.
In the same time, two extended laboratory studies on Typhlodromus pyri and Aphidius rhopalosiphi were presented and two additional studies on Coccinella septempunctata and Chrysoperla carnea were conducted.
The calculations provided by the Applicant in Table 9.7-2 have been amended according to recommendation given in ESCORT 2 GD such as: „If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect''.

Due to that $PER_{in\text{-}field}$ is below rate with ≤ 50 % effect for all testes species the risk in-field is considered acceptable from exposure of SAP2101F for all NTA tested species.

9.8.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment. the risk envelope approach is applied. Here. the assessment for the use group cereals covers the risk for non-target arthropods from all intended uses (see 9.1.2).

Table 9.8-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of SAP2101F in cereals

Intended use		Cereals				
Active substance/product		Prothioconazole + Folpet/ SAP2101F				
Application rate (mL/ha)		2 × 1500				
MAF		1.7				
vdf		10 (2D); 1 (3D)				
~~**Test species Tier II**~~	~~**LR_{50} (lab.) (mL/ha)**~~	~~**Drift rate**~~	~~**$PER_{off\text{-}field}$ (mL/ha)**~~	~~**CF**~~		~~**$HQ_{off\text{-}field}$ criterion: HQ ≤ 1**~~
~~*Typhlodromus pyri* (2D)~~	~~>5100~~	~~2.38~~	~~6.069~~	~~10~~		~~0.01~~
~~*Aphidius rhopalosiphi* (3D)~~	~~>5100~~		~~60.69~~			~~0.06~~
~~*Coccinella septempunctata* L. (2D)~~	~~>5100~~		~~6.069~~			~~0.01~~
~~*Chrysoperla carnea* (2D)~~	~~>5100~~					~~0.01~~
Test species Tier II	**Rate with ≤ 50 % effect* (ml/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (mL/ha)**	**CF**	**corr.PERoff -field (ml/ha)**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri (2D)	>5100	2.38	6.069	5	30.34	Y
Aphidius rhopalosiphi (3D)	>5100		60.69 No vdf	5	303.45	Y

Coccinella septempunctata L. (2D)	>5100		6.069	5	30.34	Y
Chrysoperla carnea (2D)	>5100			5	30.34	Y

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
*If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

zRMS comments:

The calculations provided by the Applicant in Table 9.7-3 have been amended according to recommendation given in ESCORT 2 GD such as: „If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect''.
In addition, the correction factor (CF) of 10 used by the Applicant has been replaced by CF of 5, which is relevant for extended laboratory studies according to recommendation given in ESCORT 2.

Overall, acceptable off-field risk to non-target arthropods may be concluded from the intended Central Zone uses of SAP2101F with no need for risk mitigation measures.

9.8.2.3 Additional higher-tier risk assessment

Not relevant.

9.8.2.4 Risk mitigation measures

No risk mitigation needed.

9.8.3 Overall conclusions

No unacceptable risks are expected to the non-target arthropods (*T. pyri, A. rhopalosiphi, C. septempunctata* L. *and C. carnea*) due to exposure to SAP2101F formulation. The assessed risk in- and off-field at extended exposure showed acceptable risk, according to SAP2101F proposed uses, with Hazard Quotients below the trigger value of 1.

9.9 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.9.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with prothioconazole and folpet. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.9-1: Endpoints and effect values relevant of prothioconazole and its metabolites for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
~~*Eisenia foetida*~~	~~Prothioconazole~~	~~acute 10 % peat content~~	~~LC_{50} > 1000 mg/kg wt.s. $LC_{50,corr}$ > 500* mg/kg wt.s.~~	~~EFSA Scientific Report (2007)~~
~~*Eisenia foetida*~~	~~Prothioconazole EC250~~	~~acute 10 % peat content~~	~~LC_{50} > 249.3 mg/kg wt.s. $LC_{50,corr}$ > 124.65* mg/kg wt.s.~~	~~EFSA Scientific Report (2007)~~
Eisenia foetida	Prothioconazole EC250	chronic 10 % peat content	NOEC = 1.33 mg/kg wt.s. $NOEC_{corr.}$ = 0.665* mg/kg wt.s.	EFSA Scientific Report (2007)
Folsomia candida	Prothioconazole	chronic 10 % peat content	NOEC = 64 mg/kg wt.s.	EFSA Scientific Report (2007)
Folsomia candida	Prothioconazole FS 100	chronic 10 % peat content Seed treatment scenario	NOEC = 230 kg seeds/ha (10 g a.s./dt seeds) equivalent to 24.38 g a.s./ha	EFSA Scientific Report (2007)
Folsomia candida	Prothioconazole FS 100	chronic 5 % peat content Seed treatment scenario	NOEC = 1150 kg seeds/ha (10 g a.s./dt seeds) equivalent to 112 g a.s./ha	EFSA Scientific Report (2007)
Hypoaspis aculeifer	Prothioconazole	chronic	NOEC = 100 mg/kg wt.s.	EFSA Scientific Report (2007)
Metabolites (combined spray application and seed treatment scenario)				
~~*Eisenia foetida*~~	~~JAU 6476-desthio~~	~~acute 10 % peat content~~	~~LC_{50} > 1000 mg/kg wt.s. $LC_{50,corr}$ > 500* mg/kg wt.s.~~	~~EFSA Scientific Report (2007)~~
Eisenia foetida	JAU 6476-desthio	chronic 10 % peat content	NOEC = 1 mg/kg wt.s. $NOEC_{corr}$ = 0.5* mg/kg wt.s.	EFSA Scientific Report (2007)
~~*Eisenia foetida*~~	~~JAU 6476-S-methyl~~	~~acute 10 % peat content~~	~~LC_{50} > 1000 mg/kg wt.s. $LC_{50,corr}$ > 500* mg/kg wt.s.~~	~~EFSA Scientific Report (2007)~~
Eisenia foetida	JAU 6476-S-methyl	chronic 10 % peat content	NOEC = 100 mg/kg wt.s. $NOEC_{,corr}$ = 50* mg/kg wt.s.	EFSA Scientific Report (2007)
Folsomia candida	JAU 6476-desthio	chronic 10 % peat content	NOEC = 62.5 mg/kg wt.s.	EFSA Scientific Report (2007)
Folsomia candida	JAU 6476-S-methyl	chronic 10 % peat content	NOEC = 31.6 mg/kg wt.s.	EFSA Scientific Report (2007)
Field studies				

Species	Substance	Exposure System	Results	Reference
Lumbricius terrestris, L. rubellus, L. castanea, Aporrectodea caliginosa, A. terrestris longa	Prothioconazole EC250	Field study (grassland site) 3 × 200 g a.s./ha 5 different species identified and assessed. 46% reduction in the number of A caliginosa juveniles 7 weeks after first application (2 weeks after final application). No adverse effect 5 month after first application. (Maximum measured soil PEC 0.052 mg prothioconazole/kg based on soil sampling depth of 10 cm which is equivalent to a soil PEC of 0.104 mg prothioconazole/kg over the standard 5 cm depth)		EFSA Scientific Report (2007)
Range of species in an arable field study	Prothioconazole FS 100	chronic	NOEC= 1150 kg seeds/ha (10 g a.s./dt seeds) equivalent to 122 g a.s./ha	EFSA Scientific Report (2007)
Litter bag test				
Field Soil Litter Degradation	Prothioconazole FS 100	126 d, (23.2 g a.s./ha) followed by JAU 6476 EC 250 (3 @ 200 g a.s./ha during 26 day period)	Field soil litter degradation [%] after 34 days: test item: 51.7; control: 52.1 after 95 days: test item: 74.3; control: 78.4 after 126 days test item: 92.0; control 91.2	EFSA Scientific Report (2007)

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

Table 9.9-2: Endpoints and effect values relevant of folpet for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
~~*Eisenia foetida*~~	~~Folpet~~	~~Mixed into substrate acute,14-day~~	~~LC_{50} > 1.000 mg folpet/kg $LC_{50\ corr.}$ > 500* mg folpet/kg~~	~~EFSA Scientific Report (2009)~~
~~*Eisenia foetida*~~	~~Folpan 80 WDG~~	~~Mixed into substrate acute,14-day~~	~~LC_{50} >828 mg folpet/kg $LC_{50corr.}$ > 414* mg folpet/kg)~~	~~EFSA Scientific Report (2009)~~
Eisenia foetida	Folpet	Mixed into substrate chronic, 56-day	NOEC = 5.18** mg folpet/kg soil	EFSA Scientific Report (2009)
Metabolites (combined spray application and seed treatment scenario)				
-				
Field studies				
-				
Litter bag test				
-				

*Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
** It was agreed in the EPCO 22 experts´ meeting on ecotoxicology that the lowest endpoint should be used without applying a correction factor.

zRMS comments:

Soil organism toxicity data for prothioconazole, prothioconazole metabolite JAU 6476-desthio and folpet provided in Table 9.8-2 were confirmed that they are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106 and in EFSA Journal (2007) 124, 1-84, respectively.

The acute toxicity data has been struck through in table above as being no longer a data requirement.

Table 9.9-3: Endpoints and effect values relevant of SAP2101F for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Eisenia andrei	SAP2101F	Mixed into substrate chronic, 56-day 5% peat content	$NOEC_{reprod}$ = 23.40 mg t.i./kg sdw $EC_{10,reprod}$ = 24 mg t.i./kg sdw $NOEC_{reprod,corr}$ = 11.70 mg t.i./kg sdw	KCP 10.4.1.1/01, Queralt M., 2022, S21-05013
Folsomia candida	SAP2101F	Mixed into substrate chronic, 28-day 5% peat content	$NOEC_{reprod} \geq$ 1000 mg t.i./kg sdw $EC_{10,reprod}$ > 1000 mg t.i./kg sdw	KCP 10.4.2.1/01, Queralt M., 2023, S23-103641
Hypoaspis aculeifer	SAP2101F	Mixed into substrate chronic, 14-day 5% peat content	$NOEC_{reprod} \geq$ 1000 mg t.i./kg sdw $EC_{10,reprod}$ > 1000 mg t.i./kg sdw	KCP 10.4.2.1/02, Queralt M., 2022, S23-103642
Field studies				
-				
Litter bag test				
-				

*Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.

zRMS comments:

Studies on toxicity of SAP2101F to earthworms and other non-target soil organisms were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. Endpoints reported in Table 9.8-3 are confirmed to be correct.

9.9.1.1 Justification for new endpoints

For the risk assessment of earthworms and other non-target soil organisms (meso- and macrofauna) only the derived values endpoints of the earthworms were corrected (divided by a factor of 2) as recommended, yet not stated, in EPPO 2002. However, according to SANCO/10329/2002 rev. 2 final, and EFSA 2015:EN-924, it is noted that the application of a correction factor of 2 may not be totally appropriate for collembola and mite studies where only 5% of OM is used in the test guideline.

In the EPPO scheme of 2002, it is stated that "For chemicals with log Pow > 2, sorption is expected to be linearly related to soil organic matter content. A factor of 2 (division of all toxicity endpoints) is used to correct the toxicity data from earthworm studies conducted in artificial soil. This derives from the fact that the artificial soil of OECD guideline 207 has a peat content of 10%, whereas atypical agricultural soil has not more than 5% organic matter." This regulation implies the correction of the endpoints obtained in studies conducted with earthworms and only when a peat content of 10% would be used, which is not the case. In 2015, the Outcome on recurring issues in ecotoxicology brought another light on this issue by stating that "it was agreed to retain the factor of 2 for all the first-tier studies, when relevant (i.e. logPow of >2)". This would mean that studies conducted with all different soil organisms with either peat content (5 or 10%) would need to be corrected. In the same document it is also stated that "Some experts considered the factor of 2 as not scientifically underpinned and, in some cases, not even sufficiently protective. It was agreed that there is a clear lack of data regarding the bioavailability of test substances in soils with different amounts of organic matter. It was also noted that this is a complex topic and there could be more influential parameters than just the organic matter used in the test soil affecting the toxicity" and more specifically for col-

lembolan and mites "It was noted that the application of the correction factor of 2 may not be totally appropriate for collembolan and soil mite studies where 5% OM is used in the test guideline. (…)".
On this notice, the correction factor application was not proceeded (since it is overly conservative and quite arbitrary) for both collembolan and predatory mite for prothioconazole, folpet and respective metabolites. Both soil organisms are less dependent on organic matter than the earthworms, which the Applicant considers valid to not address the correction factor of 2 for this specific case. Moreover, during the course of the study, the organisms are not fed with organic matter in opposition to what occurs in the earthworms studies, instead, both collembola and predatory mite are fed with yeast and cheese mites, respectively. Additionally, both organisms are more exposed (in this case to the active substances prothioconazole and folpet) through pore water, while the earthworm continues to be the most exposed of all three organisms to soil.

Considering also the organisms physiology, real scenarios of exposure in-field and based on EFSA 2017;15(2):4690, earthworms are considered "soft-bodies" and their via of exposure through diet and naked skin contributes for higher exposure to PPP present in soil. It must be highlighted that earthworms diet is mainly constituted by soil and its added contents, which increases this exposure rate to chemicals through the soil. On the other hand, both collembola and predatory mites (the so called "hard-bodies") take up chemicals mostly via soil pore water (Gyldenkaerne and Jorgensen, 2000; Fountain and Hopkin, 2005; EFSA, 2009c) and their diet, as briefly mentioned above, is much diverse and less dependent on organic matter, constituted by fungi, yeast and other soil meso- and macro-organisms (in the case of the predatory mite).

Other important factor that must be weighted when considering these organisms is the respective sizes. As mentioned in (EFSA 2017;15(2):4690) "*Surface-area-to-volume ratio is especially important for soft-bodied organisms exposed mostly through the contact of body surface with pore water*" which enhances the importance of correcting the endpoint of earthworms towards the collembola and predatory mite "hard-bodies" group.
Overall, the main reason which the Applicant choose not to apply the correction factor of 2 in collembola and predatory mite endpoints, exposed to prothioconazole, folpet and metabolites are discriminated:

- Hard-body: which gives lower rate exposure to PPP presence in soil.
- Diet: different from earthworms, which diet is constituted mostly by soil and mixed organic matter.
- Size and locomotion: collembola and predatory mites are smaller and faster which allows lower exposure and faster opportunity to escape when in real scenarios of exposure, other than laboratory studies.

Furthermore, since it is a topic that is still open for discussion and is not stated in any guidance, the Applicant considers that, for SAP2101F, both collembola and predatory mite don´t have an additional risk exposure, based on the aforementioned considerations.
Thus, the Applicant suggests that there is no need to apply the correction factor for these two organisms to conduct the risk assessment, and no risk should be expected on these proposed conditions.

9.9.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology". as provided by the Commission Services (SANCO/10329/2002 rev 2 (final). October 17. 2002).

As stated in Commission Regulation EU No 284/2013 of 1 March 2013, "*For plant protection products applied as a foliar spray, data on the relevant two non-target arthropod species might be taken into account for a preliminary risk assessment. If effects do occur on either species, testing on Folsomia candida and Hypoaspis aculeifer shall be required (see point 10.4.2.1). (...) For plant protection products applied as soil treatments directly to soil either as a spray or as a solid formulation, then testing shall be required on both Folsomia candida and Hypoaspis aculeifer (see point 10.4.2.1).*"
The formulated product SAP2101F is not applied as a soil treatment but as a foliar spray one, and it is not a soil directed application. As demonstrated above, no unacceptable risks are expected towards four non-target arthropod species (*T. pyri, A. rhopalosiphi, C. septempunctata* and *C. carnea*). Enough data for both

active substances are available, therefore, performing the risk assessment with the data for the active substance can be used as a surrogate of effects of soil mesofauna exposure to SAP2101F, and is considered conservative.

9.9.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2. Table 8.7-3. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for prothioconazole and folpet.
To achieve a concise risk assessment the risk envelope approach is applied. Here the assessment for the use group cereals covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all intended uses (see 9.1.2).

Table 9.9-4: First-tier assessment of the chronic risk of prothioconazole, folpet and its metabolites and SAP2101F for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of SAP2101F in cereals

Intended use	Cereals		
Acute effects on earthworms: no longer a data requirement**.**			
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Prothioconazole	0.665*	0.050	13.3
Prothioconazole-S-methyl (M01)	50*	0.013	3846.2
Prothioconazole-desthio (M04)	0.5*	0.047	10.6
Folpet	5.18	0.198	26.2
Phthalimide	0.518**	0.069	7.51
Phthalamic acid	0.518**	0.011	47.1
Phthalic acid	0.518**	0.012	43.2
SAP2101F	11.7*	0.456	25.7
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC** (mg/kg dw)	**PEC_{soil}** (mg/kg dw)	**TER_{lt}** (criterion TER ≥ 5)
Prothioconazole *Folsomia candida*	32* ~~64~~	0.050	640 ~~1280.0~~
Prothioconazole *Hypoaspis aculeifer*	50* ~~100~~	0.050	1000 ~~2000.0~~
Prothioconazole-S-methyl (M01) *Folsomia candida*	31.6	0.013	2430.8
Prothioconazole-desthio (M04) *Folsomia candida*	62.5	0.047	1329.8
SAP2101F *Folsomia candida*	≥1000	0.456	2193.0
	≥500*		1096.5
Prothioconazole-S-methyl (M01) *Hypoaspis aculeifer*	10**	0.013	769.2
Prothioconazole-desthio (M04) *Hypoaspis aculeifer*	10**	0.047	212.8
SAP2101F *Hypoaspis aculeifer*	≥1000	0.456	2193.0
	≥500*		1096.5

TER values shown in bold fall below the relevant trigger. *Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002. ** Endpoint derived from the active substance with application of

assessment factor of 10

zRMS comments:

The soil exposure provided in Table 9.8-4 is confirmed to be in line with PEC_{SOIL} values agreed by the zRMS in area of Section 8.

The risk assessment presented in Table 9.8-4 for both active substances and their metabolites is in general agreed by the zRMS. In case of studies for Folsomia and Hypoapsis for prothioconazole the toxicity endpoints have been divided by a factor 2 by zRMS.

Overall, acceptable risk to soil macro- and meso-fauna may be concluded from the intended Central Zone uses of SAP2101F.

9.9.2.2 Higher-tier risk assessment

Not relevant.

9.9.3 Overall conclusions

No unacceptable long-term risks are expected for earthworms and other non-target soil organisms (meso- and macrofauna) due to exposure to either prothioconazole, folpet, relevant metabolites and SAP2101F formulation on its intended uses based on the TER values significantly higher than 5 trigger.

9.10 Effects on soil microbial activity (KCP 10.5)

9.10.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with prothioconazole and folpet and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related.

Effects on soil microorganisms of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
~~C-mineralisation~~	~~Prothioconazole~~	~~28 d, aerobic soil type~~	~~No influence 2.0 Kg a.s./ha (equivalent to 2.67 mg/Kg)*~~	~~EFSA Scientific Report (2007)~~
~~C-mineralisation~~	~~JAU 6476-S-methyl~~	~~28 d~~	~~No influence 2.0 kg p.m./ha (equivalent to 2.67 mg/Kg)*~~	~~EFSA Scientific Report (2007)~~
~~C-mineralisation~~	~~Folpet~~	~~63 d, aerobic soil type~~	~~No influence (< 25% effect compared to untreated control) when tested at 1.593 and 15.93 kg folpet/ha (equivalent to 21.24 mg/kg sdw)*.~~	~~EFSA Scientific Report (2009)~~
N-mineralisation	Prothioconazole	28 d. aerobic soil type	No influence 2.0 Kg a.s./ha (equivalent to 2.67 mg/Kg)*	EFSA Scientific Report (2007)
N-mineralisation	JAU 6476-desthio	28 d. aerobic soil type	No influence 0.2 Kg a.s./ha (equivalent to 0.267 mg/Kg)*	EFSA Scientific Report (2007)
N-mineralisation	JAU 6476-desthio	28 d. aerobic soil type	No influence 1.0 Kg a.s./ha (equivalent to 1.33 mg/Kg)*	EFSA Scientific Report (2007)
N-mineralisation	JAU 6476-S-methyl	28 d. aerobic soil type	No influence 2.0 Kg a.s./ha (equivalent to 2.67 mg/Kg)*	EFSA Scientific Report (2009)
N-mineralisation	Folpet	63 d, aerobic soil type	No influence (< 25% effect compared to untreated control) when tested at 1.593 and 15.93 kg folpet/ha (equivalent to 21.24 mg/kg sdw)*.	EFSA Scientific Report (2009)
N-mineralisation	SAP2101F	28 d. aerobic soil type	No influence < 25% effect compared to untreated control in $conc_{max}$ =23.40 mg/kg sdw	KCP 10.5/01 Queralt M., 2022, S21-05015

*Calculated assuming a soil depth of 5 cm and a soil density of 1.5 g/cm^3.

zRMS comments

Soil micro-organism toxicity data for prothioconazole, prothioconazole metabolite JAU 6476-desthio and folpet provided in Table 9.9-1 were confirmed that they are in line with EU agreed endpoints reported in EFSA Scientific Report (2007) 106 and in EFSA Journal (2007) 124, 1-84, respectively.

The study on effects of SAP2101F on soil nitrogen transformation was evaluated by the zRMS and is considered acceptable.
For details of evaluation, please refer to Appendix 2. The endpoint reported in Table 9.10-1 is confirmed to be correct.
Information regarding effects on carbon mineralization is no longer a data requirement and for that reason is struck

through in Tables 9.10-1.

9.10.1.1 Justification for new endpoints

Not relevant.

9.10.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final) October 17, 2002).
The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.9).
To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals covers the risk for the soil microorganisms from all intended uses (see 9.1.2).

Table 9.10-2: Assessment of the risk for effects on soil micro-organisms due to the use of SAP2101F in crop cereals

Intended use	Cereals		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Prothioconazole	2.67 (at 28 d)	0.050	Yes
JAU 6476-desthio	0.267 (at 28 d)*	0.047	Yes
JAU 6476-S-methyl	2.67 (at 28 d)	0.013	Yes
Folpet	21.24 (at 28 d)	0.198	Yes
Phthalimide	2.124 (at 28 d)*	0.069	Yes
Phthalamic acid	2.124 (at 28 d)*	0.011	Yes
Phthalic acid	2.124 (at 28 d)*	0.012	Yes
SAP2101F	23.40 (at 28 d)	0.456	Yes
C-mineralisation: no longer a data requirement			

***** Endpoint derived from the active substance with application of assessment factor of 10

zRMS comments:

The risk assessment presented in Table 9.9-2 above is in general agreed by the zRMS
The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PECs for the maximum application rate of active substances, relevant soil metabolites and and the product.

Overall, no unacceptable effects on soil microbial activity are expected following application of SAP2101F.

9.10.3 Overall conclusions

The use of prothioconazole, folpet, respective metabolites and SAP2101F according to the proposed use patterns, will not have unacceptable effects on soil micro-organisms. The applied maximum concentration used in RA did not cause any significant effects on soil nitrogen transformation.

9.11 Effects on non-target terrestrial plants (KCP 10.6)

9.11.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with prothioconazole and folpet. Full details of these studies are provided in the respective EU DAR and related documents as well as in Appendix 2 of this document (new studies).

Effects on non-target terrestrial plants of SAP2101F were not evaluated as part of the EU assessment of prothioconazole and folpet. New data submitted with this application are listed in Appendix 1 summarised in Appendix 2.
The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 9.11-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
Amaranthus retroflexus	Prothioconazole	Pre-emergence	Max. phytotoxic effect 5%, at 200 g a.s./ha	EFSA Scientific Report (2007)
Amaranthus retroflexus, *Beta vulgaris*	Prothioconazole	Post-emergence	Max. phytotoxic effect 10%, at 250 g a.s./ha	EFSA Scientific Report (2007)
Amaranthus retroflexus	Prothioconazole EC250	Pre-emergence	Max. phytotoxic effect 5%, at 200 g a.s./ha	EFSA Scientific Report (2007)
-	Prothioconazole EC250	Post-emergence	Max. phytotoxic effect 0%, at 250 g a.s./ha	EFSA Scientific Report (2007)
Other non-target flora	Folpan 80 WDG	Field trial	1st Field trial No phytotoxic effects up to 6.4 Kg folpet/ha 2nd Field trial No phytotoxic effects up to 8.0 kg folpet/ha	EFSA Scientific Report (2009)
Brassica napus[d] *Beta vulgaris*[d] *Glycine max*[d] *Lycopersicon esculentum*[d] *Lolium perenne*[m] *Allium cepa*[m]	SAP2101F	21 d Seedling emergence	[1)] ER_{50} plant weight > 2.850L test item/ha [2)] ER_{50} plant height >2.850L test item/ha	KCP 10.6.2/01 Huerta F., 2022, S21-05016
Brassica napus[d] *Beta vulgaris*[d] *Glycine max*[d] *Lycopersicon esculentum*[d] *Lolium perenne*[m] *Allium cepa*[m]	SAP2101F	21 d Vegetative vigour	[1)] ER_{50} plant weight > 2.850L test item/ha [2)] ER_{50} plant height >2.850L test item/ha	KCP 10.6.2/02 Huerta F., 2022, S21-05017

m: monocotyledonous; d: dicotyledonous

zRMS comments:

Endpoints presented in Table 9.10-1 are in line with the EU agreed endpoints reported in EFSA Scientific Report (2007) 106 and in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.
Studies on toxicity of SAP2101F to non-target terrestrial plants were evaluated by the zRMS and are considered acceptable. For details of evaluation, please refer to Appendix 2. The endpoints reported in Table 9.11-1 for formulation are confirmed to be correct. The EU agreed endpoints for the active substances and representative formulation have not been taken into account, since relevant evaluation was performed with endpoints derived from studies performed with SAP2101F, in line with data requirements.

9.11.1.1 Justification for new endpoints

Not relevant.

9.11.2 Risk assessment

9.11.2.1 Tier-1 risk assessment (based screening data)

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals covers the risk for non-target terrestrial plants from all other intended uses (see 9.1.2). Tests conducted with rates up to 2.850 L test item/ha were conducted with the SAP2101F formulated product and effects were below the critical threshold as defined by the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). A direct comparison of the endpoint with the maximum single application rate is provided in the table below, concluding an acceptable risk for NTTP.

Table 9.11-2: Assessment of the risk for non-target plants due to the use of SAP2101F in crop cereals

Intended use	Cereals			
Active substance/product	Prothioconazole + Folpet / SAP2101F			
Application rate (mL/ha)	1500 mL/ha			
MAF	Not applicable for the tier-1 risk assessment			
Test species	**ER_{50} (mL/ha)**	**Drift rate**	**MAX single application (mL/ha)**	**TER criterion: TER ≥ 1**
Brassica napus *Beta vulgaris* *Glycine max* *Lycopersicon* *Esculentum* *Lolium perenne* *Allium cepa*	>2850	Not applicable for the tier-1 risk assessment	1500	1.9

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

zRMS comments:

The risk assessment for NTTP based on the ER_{50} value> 2850 mL/ha has been amended by zRMS.
According to to "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). the spray drift from one application reaching off-crop habitats from spray-drift predictions of Ganzelmeier & Rautmann (2000) is 2.77% and this value should be considered in the risk assessment.

Therefore, the relevant calculations of Peroff field and TER values is presented in the Table below:

Intended use		Cereals		
Active substance/ product		Prothioconazole + Folpet / SAP2101F		
Application rate (mL/ha)		1500 mL/ha		
MAF		1.0		
Test species	**ER_{50} (mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (mL/ha)**	**TER criterion: TER ≥ 5**
Brassica napus *Beta vulgaris* *Glycine max* *Lycopersicon* *Esculentum* *Lolium perenne* *Allium cepa*	>2850	2.77 %	41.55	68.60

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Based on performed calculations acceptable risk to NTTPs may be concluded for application of SAP2101F at 1.5 L/ha without the need of any specific risk mitigations.

9.11.2.2 Tier-2 risk assessment (based on dose-response data)

Not relevant.

9.11.2.3 Higher-tier risk assessment

Not relevant.

9.11.2.4 Risk mitigation measures

No risk mitigation needed.

9.11.3 Overall conclusions

The worst-case ER_{50} values are greater than the maximum single application dose rate and therefore it is considered that risks to non-target plants after SAP2101F formulation applications are acceptable according to its proposed use.

9.12 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Nor relevant.

9.13 Monitoring data (KCP 10.8)

No data available.

9.14 Classification and Labelling

Classification and Labelling: Prothioconazole – No classification.

Classification and labelling in accordance with Regulation (EC) No 1272/2008

Classification and Labelling: Folpet

Classification and labelling in accordance with Regulation (EC) No 1272/2008

Environmental hazards	Aquatic Acute 1
Hazard pictograms	
Signal word	Warning
Hazard statements	H400: Very toxic to aquatic life
Precautionary statements	P273 P391 P501
For the P phrases, refer to the extant legislation	

For SAP2101F two different methods were used for the classification: 1) summation method and 2) Classification based on the endpoints of required aquatic organisms

No other co-formulant is ecotoxicologically classified.

1) Classification based on summation method

Classification and Labelling: SAP2101F

Classification and labelling in accordance with Regulation (EC) No 1272/2008

Environmental hazards	Aquatic Acute 1
Hazard pictograms	
Signal word	Warning
Hazard statements	H400: Very toxic to aquatic life
Precautionary statements	P273 P391 P501
For the P phrases, refer to the extant legislation	

The classification according to the summation method classifies SAP2101F as category Acute 1 for aquatic organisms.
The follow calculations were used to such classification:
SAP2101F: 12% prothioconazole + 30% folpet.
For the summation method: Acute 1 x M ≥ 25%
M factor for acute toxicity: $0.1 < LC_{50} \leq 1$ is 1
Therefore: 30 x 1 > 25%

2) Classification based on the endpoints of required aquatic organisms

Classification and Labelling: SAP2101F

Classification and labelling in accordance with Regulation (EC) No 1272/2008

Environmental hazards	Aquatic Acute 1, Aquatic Chronic 2
Hazard pictograms	
Signal word	-
Hazard statements	H410: Very toxic to aquatic life with long lasting effects
Precautionary statements	P273 P391 P501
For the P phrases, refer to the extant legislation	

The classification of SAP2101F according to the endpoints of aquatic organisms for prothioconazole, folpet and SAP2101F, classifies the formulation as category Acute 1 and Chronic 2 for aquatic organisms.

The endpoints for SAP2101F:
O. mykiss LC_{50}, 48 h = 0.0492 mg/L
D. magna EC_{50}, 48 h = 0.484 mg/L
P. subcapitata ECr_{50}, 72 h = 0.134 mg/L
Based on:
Category Acute 1:
96 hr LC 50 (for fish) ≤ 1 mg/l and/or
48 hr EC 50 (for crustacea) ≤ 1 mg/l and/or
72 or 96 hr ErC 50 (for algae or other aquatic plants) ≤ 1 mg/l
(a) Short-term (acute) aquatic hazard

Category Chronic 2:
Chronic NOEC or EC x (for fish) ≤ 1 mg/l and/or
Chronic NOEC or EC x (for crustacea) ≤ 1 mg/l and/or
Chronic NOEC or EC x (for algae or other aquatic plants) ≤ 1 mg/l.
(ii) Non-rapidly substances (Note 3) for which there are adequate chronic toxicity data available

zRMS comments:

The classification of the active substances and the product has been summarised by zRMS below:

Classification and Labelling: Prothioconazole (RAC opinion (June 2019))

Classification and labelling in accordance with Regulation (EC) No 1272/2008

Hazard category	Aquatic Acute 1 Aquatic Chronic 1
GHS pictogram	
Signal word	Warning
Hazard statement	H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects Associated hazard statement that could appear on the label: H410: Very toxic to aquatic life with long lasting effects
Precautionary statement response	P391: Collect spillage
Precautionary statement disposal	P501: Dispose of contents/container in accordance with local regulation
Supplemental hazard information	EUH401: To avoid risks to human health and the environment, comply with the instructions for use

Classification and Labelling: Folpet (RAC opinion (June 2023))

Classification and labelling in accordance with Regulation (EC) No 1272/2008

Hazard category	Aquatic Acute 1 Aquatic Chronic 1
GHS pictogram	
Signal word	Warning
Hazard statement	H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects Associated hazard statement that could appear on the label: H410: Very toxic to aquatic life with long lasting effects
Precautionary statement response	P391: Collect spillage
Precautionary statement disposal	P501: Dispose of contents/container in accordance with local regulation

Supplemental hazard information	EUH401: To avoid risks to human health and the environment, comply with the instructions for use

Classification and Labelling: SAP2101F

Classification and labelling in accordance with Regulation (EC) No 1272/2008

Hazard category	Aquatic Acute 1[1] Aquatic Chronic 1[2]
GHS pictogram	
Signal word	Warning
Hazard statement	H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects Associated hazard statement that could appear on the label: H410: Very toxic to aquatic life with long lasting effects
Precautionary statement response	P391: Collect spillage
Precautionary statement disposal	P501: Dispose of contents/container in accordance with local regulation
Supplemental hazard information	EUH401: To avoid risks to human health and the environment, comply with the instructions for use

[1] Acute 1 hazard classification is based on the available endpoints for SAP2101F (*O. mykiss* $EC_{50,}$ 96h = 0.124 mg a.s./L)
[2] No chronic toxicity data on the formulation are available. The summation method applies; both active substances are classified as Chronic 1 (M=10); sum of Chronic 1 components > 25% (considering the M factor).

SAP2101F

The final classification of the product SAP2101F at the label **is H410.**

Appendix 1 Lists of data considered in support of the evaluation

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.2.1/01	Schuler L.	2022	Prothioconazole + Folpet 120 + 300 g/L SC: Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Semi-Static) Eurofins Agroscience Services Study No. S21-05200 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.2.1/02	Schuler L.	2022	Prothioconazole + Folpet 120 + 300 g/L SC: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata* Hindák under Laboratory Conditions Eurofins Agroscience Services Study No. S21- 05199 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.2.1/03	[redacted]	2023	Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions (Acute Toxicity Test – Semi-Static) [redacted] S23-100707 GLP Unpublished	-Y ~~N~~	ASCENZA AGRO S.A.
~~KCP 10.3.1.1/01~~	~~Schmitzer S., Pavic B.~~	~~2007~~	~~Effects of Folpet 80 WG (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the laboratory~~ ~~Ibacon Study No. 33893035~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Belchim Crop Protection~~ ~~ASCENZA AGRO S.A.~~
~~KCP 10.3.1.1/02~~	~~Fauser-Misslin A.~~	~~2015~~	~~Folpet: Acute Oral and Contact Toxicity to Bumble Bee (*Bombus terrestris* L.) under Laboratory Conditions~~ ~~Innovative Environmental Services (IES) Ltd. Study no. 20140156~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Belchim Crop Protection~~ ~~ASCENZA AGRO S.A.~~
~~KCP 10.3.1.1/03~~	~~Amsel, K.~~	~~2015~~	~~Acute toxicity of Folpet 80 WG to the bumblebee *Bombus terrestris* L. under laboratory conditions~~ ~~Biochemagrar Study No. 15 10 48 167 B~~ ~~GLP~~ ~~Unpublished~~	~~N~~	~~Belchim Crop Protection~~ ~~ASCENZA AGRO S.A.~~

~~KCP 10.3.1.1/04~~	~~Schnurr A.~~	~~2015~~	~~Acute toxicity of Folpet 80 WG to the solitary bee *Osmia bicornis* L. under laboratory conditions Biochemagrar Study No. 15-10-48-114-B GLP Unpublished~~	~~N~~	~~Belchim Crop Protection ASCENZA AGRO S.A.~~
KCP 10.3.1.1/05	Ansaloni T.	2022	SAP2101F: Honey Bee (*Apis mellifera* L.) Acute Oral and Contact Toxicity Test under Laboratory Conditions Trialcamp Study No. S21-05005 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.3.1.1/06	Aguilar-Alberola, J.A.	2023	Prothioconazole + Folpet 120+300 g/L SC (SAP2101F): Acute Oral and Contact Toxicity Test to the Bumblebee (*Bombus terrestris* L.) under Laboratory Conditions Eurofins Study No. S22-108802 GLP Unpublished	N	ASCENZA AGRO S.A.
~~KCP 10.3.1.2/01~~	~~Ansaloni T.~~	~~2015~~	~~Chronic toxicity of FOLPET TECHNICAL on honeybees (*Apis mellifera* L.) Trialcamp S.L.L. Study No. TRC14-246BA GLP Unpublished~~	~~N~~	~~Belchim Crop Protection ASCENZA AGRO S.A.~~
KCP 10.3.1.2/02	Ansaloni, T.	2022	SAP2101F: Honey Bee (*Apis mellifera* L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions Eurofins Study No. S21-05006GLP Unpublished (not a new study, but the entry in the table was missing)	N	ASCENZA AGRO S.A.
KCP 10.3.1.3/02	Ansaloni T.	2022	SAP2101F: Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions Eurofins Agroscience Services Study No. S21-05007 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.3.2.1/01	Varela S.	2022	Prothioconazole + Folpet 120+300 g/L - SAP 2101F: Toxicity to the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under Extended Laboratory Conditions Eurofins Agroscience Services Study No. S21-05009 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.3.2.1/02	Varela S.	2022	Prothioconazole + Folpet 120+300 g/L - SAP 2101F: Toxicity to the Aphid Parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) under Extended Laboratory Conditions Eurofins Agroscience Services Study No. S21-05008 GLP	N	ASCENZA AGRO S.A.

			Unpublished		
KCP 10.3.2.1/03	Varela S.	2022	Prothioconazole + Folpet 120+300 g/L - SAP 2101F: Toxicity to the Ladybird, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits Eurofins Agroscience Services Study No. S21-05010 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.3.2.1/04	Luna, F.	2022	Prothioconazole + Folpet 120+300 g/L - SAP 2101F: Toxicity to the Green Lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits Eurofins Agroscience Services Study No. S21-05012 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.4.1.1/01	Queralt M.	2022	Prothioconazole + Folpet 120+300 g/L - SAP2101F: Sublethal Toxicity to the Earthworm *Eisenia andrei* (Oligochaeta, Lumbricidae) in Artificial Soil with 5 % Peat Eurofins Agroscience Services Study No. S21-05013 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.4.2.1/01	Queralt M.	2023	Prothioconazole + Folpet 120 + 300 g/L - SAP2101F: Effects on the Reproductive Output of the Springtail *Folsomia candida* Willem (Collembola, Isotomidae) in Artificial Soil Eurofins Study No. S23-103641 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.4.2.1/02	Queralt M.	2023	Prothioconazole + Folpet 120 + 300 g/L - SAP2101F: Effects on the Reproductive Output of the Predatory Soil Mite *Hypoaspis (Geolaelaps) aculeifer* Canestrini (Acari: Laelapidae) in Artificial Soil Eurofins Study No. S23-103642 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.5/01	Queralt M.	2022	Prothioconazole + Folpet 120+300 g/L - SAP2101F: Effects on the Activity of Soil Microflora under Laboratory Conditions (Nitrogen Transformation) Eurofins Agroscience Services Study No. S21-05015 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP 10.6.2/01	Huerta F.	2022	Prothioconazole + Folpet 120+300 g/L - SAP2101F: Effects on the Seedling Emergence and Growth of Six Non-Target Terrestrial Plant Species under Greenhouse Conditions Eurofins Agroscience Services Study No. S21-05016 GLP Unpublished	N	ASCENZA AGRO S.A.
KCP	Huerta F.	2022	Prothioconazole + Folpet 120+300 g/L - SAP2101F: Effects on the Vegetative Vigour of Six Non-	N	ASCENZA AGRO

10.6.2/02			Target Terrestrial Plant Species under Greenhouse Conditions Eurofins Agroscience Services Study No. S21-05017 GLP Unpublished		S.A.

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
-	-	-	-	-	-

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.3.1.1/01	Schmitzer S., Pavic B.	2007	Effects of Folpet 80 WG (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the laboratory Ibacon Study No. 33893035 GLP Unpublished	N	Belchim Crop Protection ASCENZA AGRO S.A.
KCP 10.3.1.1/02	Fauser-Misslin A.	2015	Folpet: Acute Oral and Contact Toxicity to Bumble Bee (*Bombus terrestris* L.) under Laboratory Conditions Innovative Environmental Services (IES) Ltd. Study no. 20140156 GLP Unpublished	N	Belchim Crop Protection ASCENZA AGRO S.A.
KCP 10.3.1.1/03	Amsel, K.	2015	Acute toxicity of Folpet 80 WG to the bumblebee *Bombus terrestris* L. under laboratory conditions Biochemagrar Study No. 15 10 48 167 B GLP Unpublished	N	Belchim Crop Protection ASCENZA AGRO S.A.
KCP 10.3.1.1/04	Schnurr A.	2015	Acute toxicity of Folpet 80 WG to the solitary bee *Osmia bicornis* L. under laboratory conditions Biochemagrar Study No. 15 10 48 114 B GLP Unpublished	N	Belchim Crop Protection ASCENZA AGRO S.A.

KCP 10.3.1.3/01	Ansaloni T.	2015	Toxicity of FOLPET TECHNICAL on honey bee larvae (*Apis mellifera* L.) after repeated exposure under laboratory conditions Trialcamp S.L.L. Study No. TRC14-245BA GLP Unpublished	N	Belchim Crop Protection ASCENZA AGRO S.A.

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	Owner
-	-	-	-	-	-

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

A 2.2.1.1 KCP 10.2.1/01 Study 1

Comments of zRMS:	The study was conducted in line the "OECD 202" with no minor deviation. The measured initial concentrations of prothioconazole ranged from 94 to 113 % of nominal. In the aged samples, the measured concentrations of prothioconazole were between 88 and 109 % of nominal. The initial concentrations of folpet were between 76 and 110 % of nominal. In the aged samples, the measured concentrations of folpet were between < LOD and 10% of nominal. Therefore, ecotoxicological endpoints were evaluated using the nominal and mean measured concentrations (based on the geometric mean of the analytical recoveries of folpet for each concentration level) of the test item. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 48h EC_{50} = 0.484 mg form./L (0.249-0.862) (mean measured, based on geometric mean of folpet) 48h NOEC = 0.131 mg form./L (mean measured, based on geometric mean of folpet)

Reference:	KCP 10.2.1/01
Report	Prothioconazole + Folpet 120 + 300 g/L SC: Toxicity to the Water Flea *Daphnia magna* Straus under Laboratory Conditions (Acute Immobilisation Test – Semi-Static). Schuler, L., 2022, Report No. S21-05200
Guideline(s):	OECD 202 (2004): OECD Guidelines for Testing of Chemicals No. 202. Daphnia sp., Acute Immobilisation Test. Adopted: 13 April 2004
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	N/A

Objective

The objective of this study was to determine the immobilisation effect of the test item on the water flea *Daphnia magna* under worst-case exposure conditions, the no observed effect concentration (NOEC) and the effect median concentration (EC_{50}), where possible.

Material and methods

Test item:	Prothioconazole + Folpet 120 + 300 g/L SC, batch number: BG-GEA, active ingredients (a.i.): Prothioconazole: content of a.i. analysed: 116.3 g/L; Folpet: content of a.i. analysed: 310.6 g/L
Test organisms:	*Daphnia magna* Straus, Clone V, max. 24 hours old.
Test design:	Semi - static dose-response test with twenty test organisms *per* treatment (4 replicates of 5 test organisms each) were used. The duration of the test was 48 hours.
Endpoints:	Endpoints reported are the EC_{50} and the NOEC after 24 and 48 hours.
Test rates:	Semi-static main test with nominal concentrations of 20.0, 9.09, 4.13, 1.88, 0.854 mg/L and control was performed.
Test conditions:	Temperature, pH-value and oxygen concentration of the test solutions measured after 0, 24 hours aged and fresh and 48 hours are reported. Hardness of the test medium (untreated control) and light intensity were measured on the day of application.
Samples analysed:	Analytical samples taken at 0 hours (initial value) and 24 hours from fresh and aged test solutions and after 48 hours from aged test solution were analysed from control and all test item concentrations.
Statistics:	No statistical evaluation for EC_{50} after 24 hours was performed since the immobilisation was below 50 % at the highest test item concentration. The EC_{50} value after 48 hours was determined by Weibull analysis using linear max. likelihood regression. The NOEC was established based on the highest concentration at which the immobilisation is not higher than the allowed control immobilisation (≤ 10 % immobilisation).
Dates of work:	19 Jan 2022 – 04 Mar 2022

Results and discussions

Validity criteria:	Control immobilisation: The percentage of immobilisation should be ≤ 10 %. In this study the control immobilisation was 0 %. Oxygen concentration: The dissolved oxygen concentration at the end of the test should be ≥ 3 mg/L in all test units. In this test, the dissolved oxygen concentration at the end of the test was ≥ 8.08 mg/L.
Test conditions:	The total hardness (as $CaCO_3$) of the test medium (untreated control) was determined to be 13°dH and 14°dH (232 and 250 mg/L $CaCO_3$); the mean pH-value of the untreated control was determined to be 7.76 ± 0.09 (Std. Dev.), the mean temperature of the control and all test item concentrations was measured to be 19.9 ± 0.20 °C (Std. Dev.) and the mean oxygen concentration was determined to be 8.67 ± 0.25 mg/L (Std. Dev.).
Analytical Results:	The measured initial concentrations of prothioconazole ranged from 94 to 113 % of nominal. In the aged samples, the measured concentrations of prothioconazole were between 88 and 109 % of nominal. The initial concentrations of folpet were between 76 and 110 % of nominal. In the aged samples, the measured concentrations of folpet were between < LOD and 10% of nominal. Therefore, toxicological endpoints were evaluated using the nominal and mean measured concentrations (based on the geometric mean of the analytical recoveries of folpet for each concentration level) of the test item.

Biological results (copied from original study report):

After 48 hours of exposure no immobilisation was observed in the control. No immobilisation higher than the allowed control immobilisation was observed at 0.854 mg/L. 35 % immobilisation was observed at 1.88 mg/L, 50 % immobilisation was observed at 4.13 mg/L and 65 % immobilisation was observed at 9.09 mg/L. At the highest test item concentration of 20.0 mg/L 90 % of the daphnids were immobile. The results are presented in Appendix A: Table 2.

Table 2: Results of the test, 48 h values

	Nominal test item concentration [mg/L]					
	Control	0.854	1.88	4.13	9.09	20.0
	Immobilised daphnids after 48 h					
Replicate 1	0	0	1	2	4	5
Replicate 2	0	1	1	2	4	3
Replicate 3	0	1	3	2	3	5
Replicate 4	0	0	2	4	2	5
Σ	0	2	7	10	13	18
%	0	10	35	50	65	90

Analytical results (copied from original study report):

Table 8: Determined concentration of Prothioconazole

Prothioconazole + Folpet 120 + 300 g/L SC	Prothioconazole	Sampling	Prothioconazole found	
nominal [mg/L]	nominal [mg a.i. /L]	[h]	[mg a.i./L]	% of nominal
Control	0	0 fresh	< LOD	-
		24 aged	< LOD	-
		24 fresh	< LOD	-
		48 aged	< LOD	-
0.854	0.0849	0 fresh	0.0960	113
		24 aged	0.0864	102
		24 fresh	0.0828	98
		48 aged	0.0748	88
1.88	0.187	0 fresh	0.200	107
		24 aged	0.198	106
		24 fresh	0.191	102
		48 aged	0.173	93
4.13	0.411	0 fresh	0.456	111
		24 aged	0.406	99
		24 fresh	0.388	94
		48 aged	0.360	88
9.09	0.904	0 fresh	0.992	110
		24 aged	0.980	108
		24 fresh	0.996	110
		48 aged	0.988	109
20.0	1.99	0 fresh	2.20	111
		24 aged	2.03	102
		24 fresh	2.00	101
		48 aged	2.17	109

- = not calculated; LOD = 0.00200 mg prothioconazole/L; LOQ = 0.00849 mg prothioconazole/L

Statistical Results: EC_{50} and NOEC-values of daphnids exposed to the test item evaluated using nominal and mean measured concentrations (based on folpet analysis) of the test item.

	Prothioconazole + Folpet 120 + 300 g/L SC [mg/L]			
	(nominal)		(mean measured)[3)]	
	24 h	48h	24 h	48h
NOEC	1.88	0.854	0.191	0.131
EC_{50}	> 20.0 [1)]	4.69 [2)]	> 6.52 [1)]	0.484 [2)]

95 % confidence limit of EC_{50}	-	2.89 – 6.81 [2)]	-	0.249 – 0.862 [2)]

- not applicable
[1)] Due to an immobilisation < 50% the EC_{50} was assumed to be higher than the highest test item concentration.
[2)] Weibull analysis using linear max likelihood regression
[3)] Based on geometric mean of the analytical recoveries of folpet for each concentration level

Conclusions

According to the results of the test, the EC50 (48 h) was determined to be 4.69 mg/L (nominal) and 0.484 mg/L (mean measured, based on geometric mean of folpet). The corresponding NOEC (48 h) was 0.854 mg/L (nominal) and 0.131 mg/L (mean measured, based on geometric mean of folpet).

A 2.2.1.2 KCP 10.2.1/02 Study 2

Comments of zRMS:	The study was conducted in line with OECD 201 GD with no minor deviation. The measured initial concentrations of prothioconazole ranged from 79 to 93 % of nominal. In the aged samples, the measured concentrations of prothioconazole were between 75 and 96 % of nominal. The initial concentrations of folpet were between 76 and 120 % of nominal. In the aged samples, the measured concentrations of folpet were between < LOD and 74 % of nominal. Therefore, ecotoxicological endpoints were evaluated using the nominal and mean measured concentrations (based on the geometric mean of the analytical recoveries of folpet for each concentration level) of the test item. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 72h E_rC_{50} = 0.134 mg form./L (0.115 - 0.194) (mean measured, based on geometric mean of folpet) 72h NOE_rC = 0.0879 mg form./L (mean measured, based on geometric mean of folpet)

Reference:	KCP 10.2.1/02
Report	Prothioconazole + Folpet 120 + 300 g/L SC: Toxicity to the Single Cell Green Alga *Pseudokirchneriella subcapitata* Hindák under Laboratory Conditions. Schuler L., 2022. No. S21-05199
Guideline(s):	Yes. OECD Guideline for Testing of Chemicals No. 201, "Freshwater Alga and Cyanobacteria, Growth Inhibition Test" adopted 23 March 2006, Annex 5 corrected: 28 July 2011
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective
The objective of this study was to determine the effects of the test item on the growth of the single cell green alga *Pseudokirchneriella subcapitata*, to determine the no observed effect concentration (NOEC), to determine the lowest observed effect concentration (LOEC) and to determine the effect concentration ($EC_{10, 20, 50}$), where possible.

Material and methods

Test item:	Prothioconazole + Folpet 120 + 300 g/L SC, batch number: BG-GEA, active ingredients (a.i.): Prothioconazole: content of a.i. analysed: 116.3 g/L; Folpet: content of a.i. analysed: 310.6 g/L
Test species:	*Pseudokirchneriella subcapitata*.
Test design:	Initial target cell densities of 0.5×10^4 cells/mL were employed for the individual replicates. The increase of cell numbers was assessed over a test period of 72 hours.
Endpoints:	Where possible inhibition of growth was assessed by the determination of NOEC/LOEC and $EC_{10, 20, 50}$ for growth rate and yield after 72 hours.
Test rates:	A static test with nominal test item concentrations of 100, 31.3, 9.77, 3.05, 0.954 mg/L and control was performed.
Test conditions:	Six replicates were employed for the control and three for each test item concentration. The test was performed in 100 mL Erlenmeyer flasks each containing ~ 50 mL test solution. The pH was recorded at test start and test end. Temperature was measured continuously over the whole test period and recorded daily. Light intensity of the continuous illumination was measured at test start.
Analysis:	Analytical samples were taken and analysed from control and all test item concentrations at 0 hours (initial value) from fresh test solutions and after 24 hours, 48 hours and 72 hours from aged test solutions.
Statistics:	The NOEC and LOEC were determined by using a multiple comparison method (Jonckheere Terpstra test, left sided, for growth rate and yield). The $EC_{10, 20, 50}$-values for growth rate was determined by Weibull analysis using linear maximum likelihood regression. The $EC_{10, 20, 50}$-values for yield was determined by Probit analysis using linear maximum likelihood regression.
Dates of work:	17 Jan 2022 – 08 Mar 2022

Results and discussions

Validity criteria:	Biomass: Cell numbers, measured in the controls between 0 and 72 hours, were found to increase by a factor of 82.51 (corresponding to a specific growth rate of 1.47435 d^{-1}), which exceeds the threshold of 16 (corresponding to a specific growth rate of 0.92 d^{-1}). Coefficient of variation (section by section): The mean coefficient of variation for the section-by-section specific growth rates (hours 0 - 24, 24 - 48 and 48 - 72) in the control cultures was 23 % and did not exceed 35 %. Coefficient of variation (average growth rate): The coefficient of variation of average growth rate in replicate control cultures was 2.2 % and did not exceed 7 % for the whole test period.
Test conditions:	The pH-value of the control ranged from 7.49 to 8.51 during the test period, the temperature was measured to be 22.5 – 23.1 °C during the test and the mean light intensity was 94.7 $\mu Em^{-2}s^{-1}$ at cell culture level.
Analytical Results:	The measured initial concentrations of prothioconazole ranged from 79 to 93 % of nominal. In the aged samples, the measured concentrations of prothioconazole were between 75 and 96 % of nominal. The initial concentrations of folpet were between 76 and 120 % of nominal. In the aged samples, the measured concentrations of folpet were between < LOD and 74 % of nominal. Therefore, toxicological endpoints were evaluated using the nominal and mean measured concentrations (based on the geometric mean of the analytical recoveries of folpet for each concentration level) of the test item.

Biological results:

After 72 h, at termination of the test a concentration response relation was observed for the inhibition of growth rate and yield from nominal test item concentrations of 3.05 to 100 mg/L. The inhibition of growth rate peaked at 167.1 % and the inhibition of yield peaked at 101.2 % at a nominal test item concentration of 100 mg/L (see Appendix A: Table 2 and Table 3).

Analytical results:

Table 12: Determined concentration of Prothioconazole

Test item nominal [mg/L]	Prothioconazole nominal [mg/L]	Sampling	Prothioconazole found	
			[mg/L]	% of nominal
Control	0	0 h fresh	< LOD	-
		24 h aged	< LOD	-
		48 h aged	< LOD	-
		72 h aged	< LOD	-
0.954	0.0948	0 h fresh	0.0752	79
		24 h aged	0.0908	96
		48 h aged	0.0892	94
		72 h aged	0.0712	75
3.05	0.303	0 h fresh	0.281	93
		24 h aged	0.289	95
		48 h aged	0.262	86
		72 h aged	0.246	81
9.77	0.971	0 h fresh	0.772	80
		24 h aged	0.896	92
		48 h aged	0.912	94
		72 h aged	0.876	90
31.3	3.11	0 h fresh	2.50	80
		24 h aged	2.88	93
		48 h aged	2.82	91
		72 h aged	3.06	98
100	9.94	0 h fresh	8.28	83
		24 h aged	9.48	95
		48 h aged	8.76	88
		72 h aged	8.16	82

- = not calculated; LOD = 0.00200 mg prothioconazole /L; LOQ = 0.00948 mg prothioconazole/L

Statistical Results: Toxicological endpoints for the test item

	Test item [mg/L]	
	nominal	mean measured [4]
E_rC_{10} (Growth rate) [1]	5.84	0.0853
95 % confidence limits	5.53 – 6.17	0.0715 – 0.0962
E_rC_{20} [1]	7.76	0.102
95 % confidence limits	7.54 – 7.98	0.0906 – 0.121
E_rC_{50} [1]	11.9	0.134
95 % confidence limits	11.6 – 12.2	0.115 – 0.194
E_yC_{10} (Yield) [2]	3.96	0.0606
95 % confidence limits	3.68 – 4.27	0.0193 – 0.0812
E_yC_{20} [2]	4.80	0.0769
95 % confidence limits	4.53 – 5.10	0.0397 – 0.102
E_yC_{50} [2]	6.94	0.121
95 % confidence limits	6.72 – 7.16	0.0917-0.274
NOEC (Growth rate) [3]	3.05	0.0879
LOEC (Growth rate) [3]	9.77	0.111
NOEC (Yield) [3]	3.05	0.0879
LOEC (Yield) [3]	9.77	0.111

[1] Weibull analysis using linear maximum likelihood regression
[2] Probit analysis using linear maximum likelihood regression

[3] Jonckheere Terpstra test (left-sided, p≤0.05) for growth rate and for yield
[4] Based on geometric mean of the analytical recoveries of folpet for each concentration level

Conclusions

Significant inhibitory effects were determined for growth rate and for yield at test item concentrations of 9.77 mg/L (nominal) and 0.111 mg/L (mean measured) and above. The overall LOEC was therefore determined to be 9.77 mg/L (nominal) and 0.111 mg/L (mean measured), the corresponding NOEC was set at 3.05 mg/L (nominal) and 0.0879 mg/L (mean measured). The EC_{10}-value for growth rate (E_rC_{10}) was determined to be 5.84 mg/L (nominal) and 0.0884 mg/L (mean measured). The EC_{10}-value for yield (E_yC_{10}) was 3.96 mg/L (nominal) and 0.0606 mg/L (mean measured). The EC_{20}-value for growth rate (E_rC_{20}) was determined to be 7.76 mg/L (nominal) and 0.102 mg/L (mean measured). The EC_{20}-value for yield (E_yC_{20}) was 4.80 mg/L (nominal) and 0.0769 mg/L (mean measured). The EC_{50}-value for growth rate (E_rC_{50}) was determined to be 11.9 mg/L (nominal) and 0.134 mg/L (mean measured). The EC_{50}-value for yield (E_yC_{50}) was 6.94 mg/L (nominal) and 0.121 mg/L (mean measured).

A 2.2.1.3 KCP 10.2.1/03 Study 3

Comments of zRMS:	The study was conducted in line with OECD 203 with no major deviations During the study the temperature range (11.5 – 14.1 °C) exceeded the range of 2 °C during the course of the study. This did not influence the integrity of the study, since no adverse effects in control fish mortality were observed. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 96h EC_{50} = 0.124 mg form./L (geomean measured conc., based on mean measured concentrations of prothioconazole and mean measured concentration folpet)
Reference:	KCP 10.2.1/03
Report	SAP2101F: Toxicity to the Rainbow Trout *Oncorhynchus mykiss* under Laboratory Conditions (Acute Toxicity Test – Semi-Static). [redacted] Report No. S23-100707
Guideline(s):	OECD 203 (2019): OECD Guideline for the testing of chemicals, No. 203; Fish, Acute Toxicity Testing. Adopted: 18 June 2019.
Deviations:	Yes, The Temperature range (11.5 – 14.1°C) exceeded the range of 2 °C during the course of the study. Since the validity criteria were met, this aspect is concluded to have a negligible impact on the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Objective

The objectives of the study were to determine the effects of SAP2101F on the mortality of the rainbow trout *Oncorhynchus mykiss* under worst-case exposure conditions and to determine the lethal concentration (LC_{50}), where possible.

Material and methods

Test item: SAP2101F; batch number: X-MFA, active ingredients (a.i.): prothioconazole, content of a.i. (analysed): 122.8 g/L; folpet, content of a.i. (analysed): 309.6 g/L.
Test species: *Oncorhynchus mykiss* Walbaum (Salmoniformes: Salmonidae), size between 3 and 6 cm
Test design: The test item was evaluated in a semi-static test with daily water renewal, 96 h exposure in treated test medium; 7 organisms per test concentration were used in the test.
Assessments on effects and mortality after 0 h, 2 - 3 h, 5 – 6 h, 24 h, 27 – 30 h, 48 h, 51 – 54 h, 72 h, 75 – 78 h and 96 h were conducted.
Endpoints: LC_{50} (lethal concentration for 50 % mortality), if possible.

Test rates: A semi-static test with nominal test item concentrations of 1.00, 0.455, 0.207, 0.0939, 0.0427 mg/L and control was performed.

Test conditions: Temperature, pH-value and % oxygen saturation of the test solutions, measured after 0, 24, 48, 72 and 96 hours from fresh and aged test solutions, are reported. Hardness of the test medium and light intensity was measured at the start of the exposure.

Samples analysed: Analytical samples were taken and anlysed from the control and the test item concentrations 0.455, 0.207, 0.0939 and 0.0427 mg/L at 0 hours (initial value) from fresh test solution and after 24 hours, 48 hours, 72 hours from fresh and aged test solutions and 96 hours from aged test solutions. From test item concentration 1.00 mg/L samples were taken and analysed at 0 hours (initial value) from fresh test solution and at 24 hours from aged solution.

Statistics: The LC_{50}-values after 24, 48, 72 and 96 h were calculated by Weibull analysis using linear max. likelihood regression for nominal concentrations and mean measured concentrations based on recoveries of prothioconazole and by trimmed Spearman-Karber procedure for mean measured concentrations based on recoveries of folpet.

Dates of work: 30 May 2023 – 05 Jul 2023

Results and discussions

Validity criteria: Control mortality: The mortality in the control should not exceed one fish at the end of the test. In this study there was no control mortality.
Oxygen saturation: The dissolved oxygen saturation in control and test vessels should be at least 60 % of the air saturation value throughout the test. In this test, the dissolved oxygen concentration was ≥ 81.7 % throughout the test.
Analytical measurements: Analytical measurements of the test is compulsory. In this test analytical measurements were done.

Test conditions: The total hardness (as CaCO3) of the test water was determined to be 11°dH (196 mg CaCO3/L); the mean pH-value of the untreated control was determined to be 7.89 ≥ 0.14. The mean temperature of the untreated control and test item concentrations was measured to be 12.5 ± 0.6 °C and the oxygen saturation was determined to be 87.9± 3.0 %. The range measured light intensity was between 541 - 798 lux.

Analytical Results: The measured fresh concentrations of prothioconazole ranged from 44 % to 105 % of nominal. In the aged samples the measured concentrations were between 40 % and 114 % of nominal. The measured fresh concentrations of folpet ranged from 94 % to 113 % of nominal. In the aged samples the measured concentrations were between < LOD and 10 % of nominal. Therefore, toxicological endpoints were evaluated using the nominal and the mean measured concentrations (based on the geometric mean of the analytical recoveries of prothioconazole and folpet for each concentration level) of the test item.
Based on the analytical recoveries of prothioconazole, the nominal test item concentrations (1.00, 0.455, 0.207, 0.0939, 0.0427 mg/L) correspond to geometric mean measured test item concentrations of 1.10, 0.367, 0.135, 0.0585, 0.0250 mg/L. Based on the analytical recoveries of folpet, the nominal test item concentrations (1.00, 0.455, 0.207, 0.0939, 0.0427 mg/L) correspond to geometric mean measured test item concentrations of 0.0461, 0.0685, 0.0318, 0.0128, 0.00818 mg/L.

Biological results (copied from original report):

In the control and at the test item concentrations up to and including 0.207 mg/L no mortality was observed within the period of the test. At 0.455 mg/L four fish were found dead after 24 h and five fish after 48 h until test end. At 1.00 mg/L all fish were found dead after 24 h. The results are presented in Appendix A, Table 1.

Table 1: % mortality of fish in the test

Test item conc.[mg/L]	Control	0.0427	0.0939	0.207	0.455	1.00
Time [h]	Mortality [%]					
Day 0 (0)	0	0	0	0	0	0
Day 0 (2-3)	0	0	0	0	0	0
Day 0 (5-6)	0	0	0	0	0	0
Day 1 (24)	0	0	0	0	57	100
Day 1 (27-30)	0	0	0	0	57	100
Day 2 (48)	0	0	0	0	71	100
Day 2 (51-54)	0	0	0	0	71	100
Day 3 (72)	0	0	0	0	71	100
Day 3 (75-78)	0	0	0	0	71	100
Day 4 (96)	0	0	0	0	71	100

Seven fish were used for each replicate in the test

Analytical results

Table 9: Determined concentrations of prothioconazole

Test item nominal [mg/L]	Prothioconazole [mg a.i./L]	Sampling	Prothioconazole found		Mean measured test item conc. [mg/L]
			[mg a.i./L]	% of nominal	
0	0	0 h fresh	< LOD	-	-
		24 h aged	< LOD	-	
		24 h fresh	< LOD	-	
		48 h aged	< LOD	-	
		48 h fresh	< LOD	-	
		72 h aged	< LOD	-	
		72 h fresh	< LOD	-	
		96 h aged	< LOD	-	
0.0427	0.00444	0 h fresh	0.00396	89	0.0250 [1]
		24 h aged	0.00250	56	
		24 h fresh	0.00412	93	
		48 h aged	0.00178	40	
		48 h fresh	0.00197	44	
		72 h aged	0.00222	50	
		72 h fresh	0.00269	61	
		96 h aged	0.00224	50	
0.0939	0.00977	0 h fresh	0.00825	84	0.0585 [1]
		24 h aged	0.00484	50	
		24 h fresh	0.00575	59	
		48 h aged	0.00607	62	
		48 h fresh	0.00688	70	
		72 h aged	0.00514	53	
		72 h fresh	0.00659	67	
		96 h aged	0.00573	59	
0.207	0.0215	0 h fresh	0.0226	105	0.135 [1]
		24 h aged	0.0135	63	
		24 h fresh	0.0167	78	
		48 h aged	0.0127	59	
		48 h fresh	0.0165	77	
		72 h aged	0.0100	47	
		72 h fresh	0.0115	53	
		96 h aged	0.0108	50	

Table 10: Calculation mean measured test item concentration based on recoveries of prothioconazole

Test item nominal [mg/L]	Prothioconazole [mg a.i./L]	Sampling	Prothioconazole used for calculation [mg a.i./L]	Geometric mean of each renewal phase* [mg a.i./L]	Arithmetic mean of the geometric means of the renewal phases* [mg a.i./L]	Mean measured test item conc. [mg/L]
0.0427	0.00444	0 h fresh	0.00396	0.003146427	0.002600116	0.0250
		24 h aged	0.00250			
		24 h fresh	0.00412	0.002708062		
		48 h aged	0.00178			
		48 h fresh	0.00197	0.002091268		
		72 h aged	0.00222			
		72 h fresh	0.00269	0.00245471		
		96 h aged	0.00224			
0.0939	0.00977	0 h fresh	0.00825	0.006319019	0.006079631	0.0585
		24 h aged	0.00484			
		24 h fresh	0.00575	0.005907834		
		48 h aged	0.00607			
		48 h fresh	0.00688	0.005946697		
		72 h aged	0.00514			
		72 h fresh	0.00659	0.006144974		
		96 h aged	0.00573			
0.207	0.0215	0 h fresh	0.0226	0.017467112	0.01400504	0.135
		24 h aged	0.0135			
		24 h fresh	0.0167	0.01456331		
		48 h aged	0.0127			
		48 h fresh	0.0165	0.012845233		
		72 h aged	0.0100			
		72 h fresh	0.0115	0.011144505		
		96 h aged	0.0108			
0.455	0.0473	0 h fresh	0.0440	0.039242834	0.038216777	0.367
		24 h aged	0.0350			
		24 h fresh	0.0420	0.040627577		
		48 h aged	0.0393			
		48 h fresh	0.0279	0.030019494		
		72 h aged	0.0323			
		72 h fresh	0.0444	0.042977203		
		96 h aged	0.0416			
1.00	0.104	0 h fresh	0.109	0.113890298	-	1.10
		24 h aged	0.119			

*unrounded values

Table 11: Determined concentrations of folpet

Test item nominal [mg/L]	Folpet [mg a.i./L]	Sampling	Folpet found		Mean measured test item conc. [mg/L]
			[mg a.i./L]	% of nominal	
0	0	0 h fresh	< LOD	-	-
		24 h aged	< LOD	-	
		24 h fresh	< LOD	-	
		48 h aged	< LOD	-	
		48 h fresh	< LOD	-	
		72 h aged	< LOD	-	
		72 h fresh	< LOD	-	
		96 h aged	< LOD	-	
0.0427	0.0112	0 h fresh	0.0126	113	0.00818 [3]
		24 h aged	< LOD [1]	-	
		24 h fresh	0.0126	113	
		48 h aged	< LOD [1]	-	
		48 h fresh	0.0124	111	
		72 h aged	< LOQ [2]	-	
		72 h fresh	0.0115	103	
		96 h aged	< LOD [1]	-	
0.0939	0.0246	0 h fresh	0.0253	103	0.0128 [3]
		24 h aged	< LOD [1]	-	
		24 h fresh	0.0259	105	
		48 h aged	< LOD [1]	-	
		48 h fresh	0.0267	109	
		72 h aged	< LOQ [2]	-	
		72 h fresh	0.0254	103	
		96 h aged	< LOQ [2]	-	
0.207	0.0542	0 h fresh	0.0552	102	0.0318 [3]
		24 h aged	< LOQ [2]	-	
		24 h fresh	0.0538	99	
		48 h aged	< LOQ [2]	-	
		48 h fresh	0.0580	107	
		72 h aged	0.00208	4	
		72 h fresh	0.0510	94	
		96 h aged	0.00252	5	

Statistical Results: Toxicological endpoints for fish exposed to the test item based on nominal and mean measured concentrations

SAP2101F nominal		
Time [h]	LC50 [mg/L]	95% confidence limit of LC50 [mg/L]
24	0.445 [1)]	0.321-0.639
48	0.401 [1)]	0.301-0.516
72	0.401 [1)]	0.301-0.516
96	0.401 [1)]	0.301-0.516
1) Weibull analysis using linear maximum likelihood regression		

SAP2101F measured [1)]				
	Based on mean measured concentrations of prothioconazole		Based on mean measured concentrations of folpet	
Time [h]	LC50 [mg/L]	95% confidence limit of LC50 [mg/L]	LC50 [mg/L]	95% confidence limit of LC50 [mg/L]
24	0.350 [2)]	0.241-0.601	0.0505 [3)]	0.0472-0.0542
48	0.311 [2)]	0.216-0.458	0.0492 [3)]	0.0462-0.0524
72	0.311 [2)]	0.216-0.458	0.0492 [3)]	0.0462-0.0524
96	0.311 [2)]	0.216-0.458	0.0492 [3)]	0.0462-0.0524
1) Based on geometric mean of the analytical recoveries for each concentration level 2) Weibull analysis using linear maximum likelihood regression 3) Trimmed Spearman-Karber procedure				

Conclusions

According to the results of the test, the LC50 (96 h) of the test item was determined to be 0.401 mg/L (nominal), 0.311 mg/L (measured, based on prothioconazole) and 0.0492 mg/L (measured, based on folpet). Sublethal effects were observed in nominal test item concentrations 0.207 mg/L and 0.455 mg/L during the course of study.

A 2.2.1.4 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.2 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

A 2.3.1.1.3 KCP 10.3.1.1/01 Study 1

Comments of zRMS:	The study was evaluated at EU level in the context of the renewal of the active substance folpet. It is not considered at the current risk assessment for SAP2101F.

~~Reference:~~ ~~KCP 10.3.1.1/01~~

~~Report~~ ~~Effects of Folpet 80 WG: (Acute Contact and Oral) on Honey Bees (*Apis mellifera* L.) in the laboratory. Schmitzer S., 2007. No. 33893035.~~

~~Guideline(s):~~ ~~Yes. OECD Guidelines No. 213 and 214 (1998)~~

~~Deviations:~~ ~~No~~

~~GLP:~~ ~~Yes~~

~~Acceptability:~~ ~~Yes~~

~~Duplication (if vertebrate study)~~ ~~N/A~~

~~Objective:~~

~~This study provides:~~
- ~~the acute toxicity levels of the test item to honey bees;~~
- ~~toxicity information comparable to expected residues from standard rates, for assessment of the potential hazard to honey bees;~~
- ~~information to support precautionary label statements;~~
- ~~information to indicate the need for further testing e.g. semi-field or field studies.~~

~~Material and Methods:~~

~~Teste item:~~ ~~Folpet 80 WG, Batch No.: M-BOA, a.i. content 80.0% (nominalU, 81.5% w/w (analysed) according to certificate of analysis.~~

~~Test species~~ ~~Honey Bee (*Apis mellifera* L.); female worker bees; obtained from a healthy and queen-right colony, bred by IBACON, collected on the morning of use.~~

~~Test design:~~ ~~Limit test; acute oral and contact toxicity test; duration 48h; 5 replicates, each consisting of 10 bees in one cage per test concentration; assessment of mortality after 4, 24 and 48h; reference item: Dimethoate 400g/L (nominal).~~

~~Test concentrations*:~~ ~~Contact test: 100.0 µg a.i./bee,~~
~~Oral test: 104.8 µg a.i./bee~~
~~* in the following, e.g. 100.0 µg a.i./bee as folpet 80 WG will be mentioned as 100.0 µg a.i./bee since throughout the report the test concentrations are expressed in µg active ingredient per bee~~

Test conditions: Temperature: 25 ºC; relative humidity: 26%-46%; photoperiod: 24h darkness.

Results and discussions

At the end of the contact toxicity test (48 hours after application), the was 0.0% mortality at 100.0 µg a.i./bee. No mortality occurred in the control (water+0.5% Adhasit).
In the oral toxicity test the maximum nominal test level of folpet 80 WG (100 µg a.i./bee) corresponded to an actual intake of 104.8 µg a.i./bee. After 48 hours this dose level led to a mortality of 2.0%. A mortality of 2.0% occurred as well in the control (50% sugar solution).
No test item induced behavioural effects were observed at any time.

Toxicity of folpet 80 WG to honey bee (*Apis mellifera* L.) in contact and oral toxicity

	Contact Test [48h]	Oral test [48h]
LD_{50}	> 100.0 µg a.i./bee	> 104.8 µg a.i./bee

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

Conclusions:

The toxicity of folpet 80 WG was tested in both an acute contact and oral toxicity test on honey bees. The LD50 (48h) was > 100.0 µg a.i./bee in the contact toxicity test. The LD50 (48h) was > 104.8 µg a.i./bee in the oral toxicity test.

Validity criteria:

Control Mortality	Contact Test CO_2 /water control: 0.0% Oral Test Water/sugar control: 2.0%
LD_{50} of Reference Item (24h)	Contact Test: 0.30 µg a.i./bee Oral Test: 0.14 µg a.i./bee
Validity of the tests:	The contact and oral test are considered valid as the control mortality in each case was < 10% and the LD50 values obtained with the reference item (dimethoate), were within the required ranges.

A 2.3.1.1.4 KCP 10.3.1.1/02 Study 2

Comments of zRMS:	The study was evaluated at EU level in the context of the renewal of the active substance folpet. The endpoints are not used in current evaluation in the risk assessment.

Reference:	KCP 10.3.1.1/02
Report	Folpet: Acute Oral and Contact Toxicity to Bumble Bee (*Bombus terrestris* L.) under Laboratory Conditions. Fauser-Misslin 2015, Study No. 20140156
Guideline(s):	OECD. 1998. Test no. 213: honey bees, acute oral toxicity. In OECD Guidelines for testing of chemical section 2: effects on biotic systems. OECD publishing. OECD. 1998. Test no. 214: honey bees, acute contact toxicity. In OECD Guidelines for testing of chemical section 2: effects on biotic systems. OECD publishing. Van der Steen, JM. 2001. Review of the methods to determine hazards and toxicity of pesticides to bumblebees (*Bombus terrestris* L.). Apidologie, 32: 399–406. AFPP. 2012. CEB Method no. 230: Méthode d'évaluation des effets des préparations phytopharmaceutiques sur l'abeille domestique *Apis mellifera* L. AFPP publishing. EFSA. 2013. Guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, Bombus spp. and solitary bees)
Deviations:	No

GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	N/A

Objective

The purpose of the study was to determine the acute oral and contact toxicity of Folpet to bumble bees, *Bombus terrestris* L., under laboratory conditions. Mortality and sublethal effects of the bumble bees were assessed. The LD50, NOED and LOED values were calculated.

Materials and Methods

Test item:	Folpet technical
Content of a.i.(analysed):	978.2 g/kg;
Test species:	Worker bumble bees (Bombus terrestris).
Test units oral test:	Plastic vials (for the feeding period) and then well-ventilated plastic boxes (10 × 9 × 5 cm) during the rest of the study.
Test unit contact test:	Well-ventilated plastic boxes (10 × 9 × 5 cm).
Test system:	Adult worker bumble bees
Oral treatments:	Four treatments: a control, a solvent control, one test item and a reference item treatment.
Contact treatments:	Four treatments: a control, a solvent control, one test item and a reference item treatment.
Limit test oral dose:	The target dose was 100 µg a.i./bee. The final dose tested was 100 µg a.i./bee.
Limit test contact dose:	The target dose was 100 µg a.i./bee. The final dose tested was 100 µg a.i./bee.
Reference item oral dose:	The target dose was 10 µg dimethoate/bee. The final dose tested was 10 µg dimethoate/bee.
Reference item contact dose:	The target dose was 10 µg dimethoate/bee. The final dose tested was 10 µg dimethoate/bee.
Replicates:	Three replicate units, each consisting of 10 worker bees, were set up for control, solvent control and reference item. Six replicate units, each consisting of 10 worker bees, were set up for the test item.
Observations:	Mortality and abnormalities were determined after 4, 24, 48, 72 and 96 hours.
Test conditions:	Mean temperature: 25.3 °C (range 24.5 – 25.9 °C); mean relative humidity: 60.2 % (range 51.5 – 64.3 %) and constant darkness.
Statistics:	The solvent control was used for statistical analyses. For the oral and the contact test, Fisher's exact test with Bonferroni Holm Adjustment, $\alpha = 0.05$, one-sided greater was performed at 48 and 96 hours to compare the mortality rates between the solvent control and test item treatment.

Results and Discussion

Results 48h		Treatment			
		Control	Solvent control	Reference item	Folpet 100 µg/bee
Oral test (48h)	Mortality (%)	0.0	6.7	100	4.0[a]
	48h LD50	>100 µg/bee[b]			
	48h NOED	≥ 100 µg/bee			
	48h LOED	>100 µg/bee			
Contact test (48h)	Mortality (%)	0.0	3.0	27	0.0[a]
	48h LD50	>100 µg/bee[b]			
	48h NOED	≥ 100 µg/bee			

	48h LOED	>100 µg/bee

a not statistically significantly different when compared to the solvent control (Fisher's exact test with Bonferroni-Holm Adjustment, α = 0.05, one-sided greater, p = 0.146).
b Based on the low toxicity of the limit test dose of 100 µg a.i./bee in both the oral and contact test the 48h LD50 of Folpet was determined to be > 100 µg a.i./bee.

Results 96h		Treatment			
		Control	Solvent control	Reference item	Folpet 100 µg/bee
Oral test (96h)	Mortality (%)	0.0	10	100	4.0[a]
	96h LD50	>100 µg/bee[b]			
	96h NOED	≥ 100 µg/bee			
	96h LOED	>100 µg/bee			
Contact test (96h)	Mortality (%)	0.0	3.3	70	3.3[c]
	96h LD50	>100 µg/bee[b]			
	96h NOED	≥ 100 µg/bee			
	96h LOED	>100 µg/bee			

c not statistically significantly different when compared to the solvent control (Fisher's exact test with Bonferroni-Holm Adjustment, α = 0.05, one-sided greater, p = 0.270).
d Based on the low toxicity of the limit test dose of 100 µg Folpet/bee in both the oral and contact test the 96h LD50 of Folpet was determined to be > 100 µg Folpet/bee.

Conclusion

Oral Test
After 48 and 96 hours acute oral exposure of adult worker bumble bee to Folpet technical, the cumulative mortality at 100 µg a.i./bee test item was 4 % and not statistically significantly different when compared to the solvent control.
The 48 and 96 hours LD50 for the oral treatment was determined to be > 100 µg Folpet/bee. NOED and LOED for the oral test were determined to be ≥ 100 µg a.i./bee and > 100 µg a.i./bee, respectively.
Contact Test
After 48 and 96 hours acute contact exposure of adult worker bumble bee to Folpet technical, the cumulative mortality at 100 µg a.i./bee test item was 0.0 and 3.3%, respectively and not statistically significantly different when compared to the solvent control.
The 48 and 96 hours LD50 for the contact treatment was determined to be > 100 µg Folpet/bee. NOED and LOED for the contact test were determined to be ≥ 100 µg a.i./bee and > 100 µg a.i./bee, respectively.

Validity criteria

Oral test
At 48 hours, bumble bee worker mortality in the control and solvent control treatment was 0.0 % and 6.7 %, respectively.
At 96 hours, bumble bee worker mortality in the control and solvent control treatment was 0.0 % and 10 %, respectively.
Mortality in the reference item treatment after 48 and 96 hours was 100 % at 10 µg dimethoate/bee.
All validity criteria were met, therefore, the study is valid.
Contact test
At 48 hours, bumble bee worker mortality in the control and solvent control treatment was 0.0 % and 3.0 %, respectively.
At 96 hours, bumble bee worker mortality in the control and solvent control treatment was 0.0 % and 3.3 %, respectively.
Mortality in the reference item treatment after 48 hours was 27 % and after 96 hours 70% at 10 µg dimethoate/bee.
All validity criteria were met, therefore, the study is valid.

A 2.3.1.1.5 KCP 10.3.1.1/03 Study 3

Comments of zRMS:	The study was evaluated at EU level in the context of the renewal of the active substance folpet. This study is not considered in the risk assessment for the current formulation SAP2101F.

Reference:	KCP 10.3.1.1/03
Report	Acute toxicity of Folpet 80 WG to the bumblebee *Bombus terrestris* L. under laboratory conditions. Amsel K., 2015, No. 15-10-48-167-B
Guideline(s):	Adapted from VAN DER STEEN (1996 & 2001), OECD 213 (1998), OECD 214 (1998) and HANEWALD et al. (2013)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	N/A

Objective

The purpose of this study was to determine the acute toxicity of Folpet 80 WG to the bumblebee *Bombus terrestris* L. in a laboratory test after oral and contact exposure.

Materials and Methods

Test item:	Folpet 80 WG Batch code.: A CXN Active substance(s)/Content: Folpet: 798.1 g/kg (analysed)
Test species:	*Bombus terrestris*, L. (bumblebee), young adult worker bumblebees derived from queen right standard hives; source: Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium delivered: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany; collected from the bumblebee micro hive in the morning prior to use
Guideline(s) & puplications:	OECD 213 (1998), OECD 214 (1998), VAN DER STEEN (1996 & 2001), HANEWALD *et al.* (2013)
Test design:	Contact: 96 hours; 1 dose rate of test item, 60 replicates with 1 bumblebees each; Oral: 96 hours; 1 dose rates of test item; 60 replicates with 1 bumblebee each; Reference item: 96 hours; 4 dose rates of test item, 30 replicates with 1 bumblebees each; Dimethoate EC 400 (Dimethoate 420.3 g/L analysed content); Dose rates for contact tets: 10.1, 6.4, 4.0, 2.5 µg a.s./bumblebee Dose rates for oral tets: 1.50, 0.82, 0.45, 0.25 µg a.s./bumblebee

Assessments of mortality and behavioural effects were done after 4, 24, 48, 72 and 96 hours.

Endpoints:	Mortality, behaviour
Dose rates [product/bee]:	Contact test: 250.0 µg/bumblebee Oral test (offered): 500.0 µg/bumblebee Oral test (consumed): 487.8 µg/bumblebee
Dose rates [a.s./bee]:	Contact test: 199.5 µg/bumblebee Oral test (offered): 399.1 µg/bumblebee Oral test (consumed): 389.3 µg/bumblebee

The unit [µg a.s./bumblebee] refers to the CoA analysed content of the active substance in the formulated product.

Test conditions:	Temperature: 24.3 – 25.5 °C Relative humidity: 52 – 70 % Illumination: constant darkness throughout the test (diffuse artificial light of about 100 lx only during handling and assessments) Food: 50 % w/v sucrose solution

Statistics: Statistical program used: ToxRat Professional 3.1 (2015)
Statistical significance of mortality values
- Fisher`s Exact Binomial Test for test item
- Fisher`s Exact Binomial Test with Bonferroni Correction for reference item
LD50 values for reference item
- Logit analysis (linear weight regression) for contact test
- Logit analysis (linear maximum likelihood regression) for oral test

Date of work: 10 – 14 August 2015

Results and discussions

Contact test

Both control groups treated with deionised water or with TritonX solution showed no mortality within 96 hours. In the test item treatment, no mortality occurred after dorsal application of 250.0 µg Folpet 80 WG/bumblebee, after 96 hours.

Therefore, the LD50 (96 h) was estimated to be > 250.0 µg Folpet 80 WG/bumblebee, corresponding to > 199.5 µg a.s./bumblebee.

No effects on behaviour of surviving bumblebees occurred in all tested dose rates in the contact toxicity test when compared to the control.

Oral test

The control group fed with 50 % (w/v) sucrose solution showed no mortality within 96 hours. In the test item treatment, slight mortality of 1.7 % occurred after oral consumption of 487.8 µg Folpet 80 WG/bumblebee, after 96 hours.

Therefore, the LD50 (96 h) was estimated to be > 487.8 µg consumed Folpet 80 WG/bumblebee, corresponding to > 389.3 µg consumed a.s./bumblebee.

No effects on behaviour of surviving bumblebees occurred in all tested dose rates in the oral toxicity test when compared to the control.

The respective LD_{50} values of the contact and oral toxicity test are presented in Table I.

LD50 values of the contact and oral toxicity test

LD50	**Contact toxicity test**	**Oral toxicity test[1]**
	24h, 48 h, 72 h, 96 h	24h, 48 h, 72 h, 96 h
LD_{50} [µg product/bumblebee]	>250.0	>487.8 (180.1 – 516.9)
LD_{50} [µg a.s./bumblebee]	>199.5	>389.3 (179.7 – 515.6)

1 Doses of the oral toxicity test are referring to consumed doses

The contact and oral LD50 (96 h) of the reference item was calculated to be 5.0 µg dimethoate/bumblebee and 0.71 µg dimethoate/bumblebee, respectively. All validity citeria have been met in this study.

Conclusion

The toxicity of Folpet 80 WG was tested in both acute contact and acute oral toxicity tests on bumblebees. Based on the obtained results the LD50 (96 h) in the contact toxicity test was estimated to be > 250.0 µg Folpet 80 WG/bumblebee, which corresponds to > 199.5 µg a.s./bee.

In the oral test the LD50 (96 h) was estimated to be > 487.8 µg consumed Folpet 80 WG/bumblebee, which corresponds to > 389.3 µg consumed a.s./bee. The contact and oral LD_{50} (96 h) of the reference item was calculated to be 5.0 µg dimethoate/bumblebee and 0.71 µg dimethoate/bumblebee, respectively. All validity citeria have been met in this study.

A 2.3.1.1.6 KCP 10.3.1.1/04 Study 4

Comments of zRMS:	The study was evaluated at EU level in the context of the renewal of the active substance folpet. The zRMS-PL agrees with the evaluation and the derived endpoints. This study is not considered in the risk assessment for the current formulation SAP2101F.

Reference: KCP 10.3.1.1/04

Report	Acute toxicity of Folpet 80 WG to the solitary bee *Osmia bicornis* L. under laboratory conditions. Schnurr A., 2015, Study No. 151048114B
Guideline(s):	OECD Guidelines No. 213 and 214 (1998), EFSA (2013)
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	N/A

Objective

The purpose of this study was to determine the acute toxicity of Folpet 80 WG to the solitary bee Osmia bicornis L. in a laboratory test after oral and contact exposure.

Materials and Methods

Test item:	Folpet 80 WG Batch No.: A CXN Analysed content: 798.1 g/kg Folpet
Test species:	Solitary bee *Osmia bicornis L.* (Hymenoptera, Apoidea); females in good health condition; age: newly emerged; weight range: 70–150 mg; source: DSP (Dr. Schubert Pflanzenzucht), An den Linden 34, 06188 Landsberg, Germany.
Guideline(s):	OECD 213 (1998), OECD 214 (1998), EFSA (2013)
Test design:	Contact (LD50 test): 96 h; 5 dose rates of test item, 3 replicates with 10 bees each; Oral (LD50 test): 96 h; 5 dose rates of test item; 30 replicates with 1 bee each; The mortality and the behaviour were assessed 4 h, 24 h, 48 h, 72 h and 96 h after application in the contact and oral toxicity tests. Reference item: Dimethoate EC 400 (analysed dimethoate of 420.3 g/L)
Endpoints:	Mortality, behavioural impairments
Dose rates:	Contact test: 200.0, 100.0, 50.0, 25.0, 12.5 µg a.s./solitary bee Oral test (offered): 400.0, 182.0, 82.8, 37.7, 17.1 µg a.s./solitary bee Oral test (consumed): 361.3, 162.3, 77.2, 34.1, 15.6 µg a.s./solitary bee
The unit [µg a.s./bee] refers to the nominal content of the active ingredients in the formulated product.	
Test conditions:	Temperature: 19.4 – 21.4 °C Relative humidity: 64.7 – 78.1 % Illumination: 12:12 h light-dark-cycle Food: 50 % (w/v) sucrose solution (500 g/L) with anise oil (20 µL/L)
Statistics:	Statistical program used: ToxRat Professional 3.1.0 (2015) LD50 – contact reference item: Probit analysis (linear weighted regression) – oral test item: Probit analysis (linear maximum likelihood regression) – oral reference item: Probit analysis (linear maximum likelihood regression) Statistical significance of mortality values – test item: Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm Correction ($p \leq 0.05$) – reference item: Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm Correction ($p \leq 0.05$)
Dates of work:	August 03 – August 07, 2015

Results and discussions

Contact test

The control group treated with 0.5 % Triton X solution revealed a low mortality of 6.7 % within 96 hours.
In the test item group no statistically, significant mortalities occurred after thoracal application of nominal 200.0, 100.0, 50.0, 25.0 and 12.5 µg Folpet/solitary bee after 96 h, respectively. Only low mortalities of 23.3, 16.7, 20.0, 16.7, and 16.7 % occurred after the application of nominal 200.0, 100.0, 50.0, 25.0 and 12.5 µg Folpet/solitary bee, which were not statistically significant in comparison to the control group. The LD50 (96 h) was >200.0 µg Folpet/solitary bee. No effects on behaviour of surviving bees occurred after thoracal application of nominal 200.0, 100.0, 50.0, 25.0 and 12.5 µg Folpet/solitary bee at any time.

Oral test

The control groups fed with 50 % (w/v) sucrose solution with anise oil (20 µL/L) showed a low mortality of 10.0 % within 96 hours. In the test item group statistically significant mortalities of 90.0, 70.0 and 43.3 % occurred after oral consumption of nominal 361.3, 162.3 and 77.2 µg Folpet/solitary bee after 96 h. The consumption of 34.1 and 15.6 µg Folpet/solitary bee resulted in lower, not statistically significant mortalities of 10.0 % after 96 h, respectively. The LD50 (96 h) based on the corrected mortality was 104.3 µg Folpet/solitary bee. Effects on behaviour of surviving bees were observed in one bee (3.7 %) after the consumption of nominal 361.3 µg Folpet/solitary bee after 24 h. This war characterized by moribund behaviour. All other bees showed no effects on behaviour after the consumption of 361.3, 162.3, 77.2, 34.1 and 15.6 µg Folpet/solitary bee, respectively, at any time. The respective LD50 values of the contact and oral toxicity test are summarised in Table I.

LD_{50} values of the contact and oral toxicity test

LD501 (95% CL)[2]	Contact toxicity test		Oral toxicity test[3]	
	48 h	96 h	48 h	96 h
LD50 [µg a.s./s. bee][4] (95 % CL / lower-upper)	>200.0	>200.0	305.1 (180.1 – 516.9)	104.3 (81.5 – 133.5)
LD50 [µg a.s./s. bee][5] (95 % CL / lower-upper)	>199.5	>199.5	304.4 (179.7 – 515.6)	104.1 (81.3 – 133.1)

[1] based on the corrected mortality; [2] CL: confidence limits; [3] Doses of the oral toxicity test are referring to consumed doses; [4] based on nominal content; [5] based on analysed content

The contact and oral LD50 (96 h) of the reference item was calculated to 0.704 µg dimethoate/solitary bee and 0.454 µg dimethoate/solitary bee, respectively. The control mortality in both tests was ≤10 %. All validity criteria have been met.

Conclusion

The toxicity of Folpet 80 WG was tested in both acute contact and acute oral toxicity tests on solitary bees. The LD50 (96 h) based on corrected mortality in the contact toxicity test was nominal >200.0 µg Folpet/ solitary bee (analysed >199.5 µg Folpet/ solitary bee). In the oral toxicity test the LD50 (96 h) based on corrected mortality was nominal 104.3 µg consumed Folpet/solitary bee (analysed 104.1 µg consumed Folpet/solitary bee).

Validity criteria

Validity criteria of the acute solitary bee study

	Validity criterion	Occurred / calculated	Recommended
Control mortality (96 h)	Contact test: - deionised water - Triton X-solution	10.0 % 6.7 %	≤ 10 %
	Oral test: - 50 % sucrose solution with anise oil (20µL/L)	10.0 %	≤ 10 %

A 2.3.1.1.7 KCP 10.3.1.1/05 Study 5

Comments of zRMS:	The study is conducted in line with OECD Guidelines No. 213 and 214 with no major deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 48 h LD_{50} = 986 µg formulation/bee, oral 48 h LD_{50} > 2340 µg formulation/bee, contact

Reference:	KCP 10.3.1.1/05
Report	SAP2101F: Honey Bee (*Apis mellifera* L.) Acute Oral and Contact Toxicity Test under Laboratory Conditions. Ansaloni T., 2022, Study No. S21-05005
Guideline(s):	OECD Guidelines No. 213 and 214 (1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

To determine the effects of SAP2101F on the Honey bees (Apis mellifera L.), from oral and contact exposure, and to determine the Median Lethal Dose (LD50) and the No Observed Effect Dose (NOED), where possible.

Materials and methods

Test item:	SAP2101F
Batch No.:	BG-GEA
Active ingredient 1 (a.i.1):	prothioconazole
Content (analysed):	116.3 g/L documented in the CoA (120 g/L nominal value)
Active ingredient 2 (a.i.2):	folpet
Content (analysed):	310.6 g/L documented in the CoA (300 g/L nominal value)
Reference item:	BAS 152 65 I (dimethoate, analysed content: 409 g/L; density 1.062 g/mL)

Place of test:	Eurofins Trialcamp S.L.U., Polígon Industrial l'Alter. Avda. Antic Regne de València, 25. 46290, Alcàsser (Valencia), Spain
Test Species:	*Apis mellifera* L.
Age:	Adult worker bees
Source:	Queen-right, healthy colony from our commercial apiary
Collection:	Honey bees were collected from the outer combs of the beehive and distributed into test cages one day before start of exposure.
Acclimatisation:	The collected honey bees were kept under test conditions until test start. During the acclimatisation period they were fed *ad libitum* with untreated 50% (w/v) aqueous sucrose solution.

Test design:	Acute oral and contact toxicity dose response test; 48-hours test duration; one control, five test doses of the test item, 4 doses of the reference item; 5 replicates, with 10 bees each, per control and test item group and 4 replicates of 10 bees each per reference group. Assessment of mortality 4, 24, and 48 hours after exposure start.
Test doses:	Control groups: Oral Toxicity Test: Untreated 50% (w/v) aqueous sucrose solution (C) Contact Toxicity Test: 0.1% Triton X solution in deionsied water (C) Test item groups: Oral Toxicity Test: 993.60, 1490.40, 2235.60, 3353.40, 5030.09 µg test item (hereafter t.i.)/bee, equivalent to 98.77, 148.15, 222.22, 333.33 and 500.00 µg active ingredient 1 (hereafter a.i.1)/bee and 263.77, 395.66, 593.48, 890.23 and 1335.34 µg active ingredient 2 (hereafter a.i.2)/bee (nominal dose). Contact Toxicity Test: 958.46, 1198.08, 1497.60, 1872.00, 2340.00 µg test item (hereafter t.i.)/bee, equivalent to 95.27, 119.09, 148.86, 186.08 and 232.60 µg active ingredient 1 (hereafter a.i.1)/bee and 254.44, 318.05, 397.57, 496.96 and 621.20 µg active ingredient 2 (hereafter a.i.2)/bee. Reference Item groups: Oral Toxicity Test: 0.062, 0.093, 0.140 and 0.210 µg dimethoate/bee (nominal dose) Contact Toxicity Test: 0.080, 0.120, 0.180 and 0.270 µg dimethoate/bee
Test conditions:	Contact Toxicity Test: Temperature: 24.8 – 25.2°C Relative Humidity: 61.7 – 78.9*% * short term deviation Oral Toxicity Test: Temperature: 24.9 – 25.2°C Relative Humidity: 55.4 – 60.0% Exposure to light: 24-h darkness, except during application and assessments

Statistics:	Statistical calculations were made using the statistical program ToxRatPro® Version 3.3.0. The 24-h LD50 contact values with 95% confidence limits of the reference item were calculated by Weibull analysis using linear max. likelihood regression. The 24-h LD50 oral values with 95% confidence limits of the reference item were calculated by Logit analysis using linear max. likelihood regression. The 24-h and 48-h LD50 oral values with 95% confidence limits of the test item were calculated by Moving average computation after Thompson. For the test item groups in the contact test, the 24-h and 48-h LD50 values could not be calculated since no test item dose caused > 50% mortality. Accordingly, these values were empirically estimated from the results. The 24-h and 48-h NOED values for the oral test were determined by a Step-down Rao-Scott-Cochran-Armitage Test Procedure, alpha 0.05, one-sided greater. For the test item groups in the contact test, the 24-h and 48-h NOED values were empirically estimated from the results.

Results and discussions

In the oral toxicity tests, 2.00% mortality was observed at the end of the observation period after 48 hours. In the contact toxicity tests, 4.00% mortality was observed at the end of the observation period after 48 hours. In the oral toxicity test, in the test item nominal doses of 993.60, 1490.40, 2235.60, 3353.40, 5030.09µg t.i./bee, the actual consumed doses were 815.79, 899.26, 941.20, 1166.63and 1001.79µg t.i./bee, and the cumulative mean mortality was 2.00, 14.00, 22.00, 54.00 and 38.00% (corrected: 0.00, 12.24, 24.41, 53.06and 36.73%) respectively,48hoursafter start of exposure. In the contact toxicity test, in the test item nominal doses of 958.46, 1198.08, 1497.60, 1872.00, 2340.00µg t.i./bee, the cumulative mean mortalitywas10.00, 6.00, 2.00, 4.00 and 2.00% (corrected: 6.25, 2.08, -2.08, 0.00and -2.08%), respectively, 48 hours after start of exposure. In the oral toxicity test, behavioural abnormalities (i.e.lack of coordination) were recorded in one bee in the test item treatments T2andT3, in 3 bees in the test item treatment T4andin 5 bees in the test item treatmentT5atthe assessment at 4 hours after exposure. One and two individuals in treatments T3 and T5, respectively, were observed with behavioural abnormalities at the 24-h assessment, while no affected bees were observed a the 48-h assessment. In the contact toxicity test, 2 affected bees (i.e.lack of coordination) were observed in treatment T2 at the 24-h assessment. No other behavioural abnormalities were recorded in any test item group at any other assessment time. The 24-horal median Lethal Dose (LD50) value with 95% confidence limits for the reference item was 0.11[0.10–0.12] µg dimethoate/bee. The 24-hcontact Medial Lethal Dose (LD50) value with 95% confidence limits for the reference item was 0.15[0.13–0.16] µg dimethoate/bee. The 24-horal median Lethal Dose (LD50) value with 95% confidence limits for the test item was 97.86 [95.16 –100.63] µg prothioconazole/bee. The 48-horal median Lethal Dose (LD50) value with 95% confidence limits for the test item was 98.01 [95.28 –100.83] µg prothioconazole/bee. The 24-hand 48-hcontact median Lethal Dose (LD50) values for the test item SAP2101Fcould not be statistically calculated but empirically estimated since no test item dose caused >50% mortality. The 24-h and 48-h NOED values in the oral test were the consumed dose of 81.09 µg prothioconazole/bee**.** Since corrected mortality at the highest test item dose was null and negative (mortality equal or lower than that observed in the control group) at the 24-h and 48-h assessments, respectively, in the contact test, the contact No Observed Effect Dose (NOED) values for the test item SAP2101F were empirically estimated to be greater than or equal to 232.60 µg prothioconazole/bee.

Conclusion

The oral and contact acute toxicity of SAP2101F was tested under laboratory conditions over a period of 48 hours. The resulting endpoints of this oral and contact acute toxicity test to the honey bees (*Apis mellifera* L.) with the test item SAP2101F are presented below:

Endpoints	**[µg t.i./bee] ***	[µg a.i.1/bee]	[µg a.i.2/bee]*
24 h Oral LD50 [95% CI]	984.49 [957.33-1012.36]	97.86 [95.16-100.63]	261.35 [254.14-268.75]
48 h Oral LD50 [95% CI]	986.00 [958.53-1014.37]	98.01 [95.28-100.83]	261.75 [254.46-269.28]
24 h Oral NOED	815.79	81.09	216.57
48 h Oral NOED	815.79	81.09	216.57
24 h Contact LD50	> 2340.00	> 232.60	> 621.20
48 h Contact LD50	> 2340.00	> 232.60	> 621.20
24 h Contact NOED	≥ 2340.00	≥ 232.60	≥ 621.20
48 h Contact NOED	≥ 2340.00	≥ 232.60	≥ 621.20

* Endpoints equivalences based on the actual content of the active ingredients according to the CoA and the test item density.

The 24-h oral median Lethal Dose (LD50) value with 95% confidence limits for the reference item was 0.11[0.10–0.12] µg dimethoate/bee. The 24-h contact Medial Lethal Dose (LD50) value with 95% confidence limits for the reference item was 0.15[0.13–0.16] µg dimethoate/bee. All validity criteria were met and the sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.

A 2.3.1.1.8 KCP 10.3.1.1/06 Study 6

Comments of zRMS:	The study is conducted in line with OECD Guidelines No. 213 and 214 with no major deviations. 48 h LD_{50} >1083.88 µg formulation/bumble bee, oral 48 h LD_{50} >1153.09 µg formulation/bumble bee, contact

Reference:	KCP 10.3.1.1/06
Report	Prothioconazole + Folpet 120+300 g/L SC (SAP2101F): Acute Oral and Contact Toxicity Test to the Bumblebee (*Bombus terrestris* L.) under Laboratory Conditions. Aguilar-Alberola, J.A., 2023, Study No. S22-108802
Guideline(s):	OECD Guideline Document 246 (2017) OECD Guideline Document 247 (2017) SANTE/2020/12830, rev. 1 (2021)
Deviations:	Yes, Behavioural abnormalities in the reference item treatment were not recorded since the reference item is known to be toxic to bumblebees and therefore effects are expected. Moreover, the administered doses achieved a mortality ≥ 50 %.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

To determine the effects of Prothioconazole + Folpet 120+300 g/L SC (SAP2101F) on the bumblebee *Bombus terrestris* L. from oral and contact exposure, the No Observed Effect Dose (NOED) and the dose which caused 50 % mortality (LD50), where possible.

Materials and methods

Test item: Prothioconazole + Folpet 120+300 g/L SC (SAP2101F); Batch code: X-MFA; active ingredient 1: prothioconazole; content of a.i.1: 122.8 g/L; active ingredient 2: folpet; content of a.i.2: 309.6 g/L (content of active ingredients according to Certificate of Analysis); relative density: 1.18 (at 20 ºC); expiry date: Dec 2024.

Reference item: BAS 152 65 l; Batch code: 10248664A; active ingredient: dimethoate; content of a.i. analysed: 42.0 % w/v (420.1 g/L); density: 1.062 g/mL.

Test organisms:

Test species: Bombus terrestris L.

Life stage: Young adult worker bumblebees.

Source: Queen-right, healthy colonies (containing ~60 – 80 bumblebee workers) from a commercial supplier (BioMip Biological Quality S.L.).

Collection: Medium size workers were collected one day before application. Bumblebees were obtained directly from the hives and they were randomly allocated to test cages.

Acclimatisation: From 30 Jan 2023 to 31 Jan 2023.

Test design: Acute oral and contact toxicity dose-response tests. For both tests, one control group, five different doses of Prothioconazole + Folpet 120+300 g/L SC (SAP2101F) and one dose of the reference item were applied to the test organisms. Each treatment group consisted of 30 and 35 replicates of 1 bumblebee each for contact and oral test, respectively. Assessment of mortality 4, 24 and 48 hours (± 30 min) after test start.

Test doses:

Controls:

Oral toxicity test: C: Control group: 50 % (w/v) aqueous sucrose solution.
Contact toxicity test: C: Control group: 0.1 % (v/v) Triton X solution.
Test Item:

Oral toxicity test:

Treatment group	Concentration [mg t.i./L]	Target doses [µg/bee]			Actual consumed doses [µg/bee]		
T1	1801.70	72.07	7.50	18.91	70.91	7.38	18.60
T2	3603.41	144.14	15.00	37.82	140.19	14.59	36.78
T3	7206.81	288.27	30.00	75.63	282.25	29.37	74.06
T4	14413.63	576.55	60.00	151.27	561.35	58.42	147.28
T5	28827.25	1153.09	120.00	302.54	1083.88	112.80	284.38

t.i.: test item; a.i.: active ingredient; a.i.1: prothioconazole; a.i.2: folpet.

Contact toxicity test:

Treatment group	Concentration [mg test item./L]	Doses [µg/bee]		
		Test item	Prothioconazole	Folpet
T1	18017.03	72.07	7.50	18.91
T2	36034.06	144.14	15.00	37.82
T3	72068.13	288.27	30.00	75.63
T4	144136.25	576.55	60.00	151.27
T5	288272.50	1153.09	120.00	302.54

Reference item:
Oral toxicity test: 3.0 µg dimethoate/bumblebee (target dose), 2.9 µg dimethoate/bumblebee (actual intake).
Contact toxicity test: 10.0 µg dimethoate/bumblebee.

Test conditions: Air Temperature: Min: 24.1 / Max: 25.4 °C
Relative humidity: Min: 45.0 / Max: 80.4* % *Short term deviation (<2 hours).
Exposure to light: Constant darkness, except during application and assessments.

Analytical verification: Samples of the dose application solutions from the lowest and highest concentrations were taken directly after its preparation for both, oral and contact solutions. The samples were stored in the freezer at ≤-18 ºC until shipment for analysis. Analytical phase was performed to verify the concentration of the samples taken. A method was validated and samples of 50 % (w/v) aqueous sucrose solution and aqueous solution with 0.1 % (v/v) of Triton X were analysed for concentration determination of prothioconazole and folpet. Quantification was performed by LC-MS/MS detection. The limit of quantification (LOQ) of the analytical method was 47.2 mg folpet/L and 18.7 mg prothioconazole/L in 50 % (w/v) aqueous sucrose solution and 151 mg folpet/L and 59.9 mg prothioconazole/L in 0.1 % (v/v) Triton X solution. The limit of detection (LOD, defined as the lowest calibration standard) was set at 13.5 mg folpet/L and 5.4 mg prothioconazole/L in 50 % (w/v) aqueous sucrose solution, and 44.0 mg folpet/L and 16.8 mg prothioconazole/L in 0.1 % (v/v) Triton X solution.

Statistics: Since no/little mortality was observed in the test item groups across both tests, the NOED and LD50 values were empirically estimated from the results. Statistical calculations were made with MS Excel 2016 v.16.

Dates of work: 31 Jan 2023 (Start of Biological Phase) to 22 Mar 2023 (end of Analytical Phase)

Results and discussions

The measured concentrations in the samples were within 20 % of nominal test concentration used, except for slight exceedances in the samples S22-108802-L2-S1-T1-A2 (131 % recovery of prothioconazole in the oral test) and S22-108802-L2-S1-T5-A2 (123 % recovery of prothioconazole in the oral test). Since the folpet concentrations have been analytically corroborated in the homologous samples, the concentrations of the test item were confirmed and the endpoints are based on nominal concentrations.

In the oral toxicity test, all the test item treated groups had a minimum of 28 feeder individuals. Slight decrease in both the feeding solution consumption and number of feeder individuals was observed as concentration of test item increased (ranging from a minimum consumption of 94.0 % with 28 feeders in treatment T5 to a maximum

of 98.4 % with 35 feeders in treatment T1). Consumption of the control individuals was 98.2 % of the offered feeding solution (34 feeders).
Since only one individual was registered as dead in the control group and in the treatments T1, T2 and T5 of the oral test, no statistical analysis was performed for this parameter. Also, no individuals were recorded as having behavioural abnormalities (i.e. bumblebees affected or moribund) during the whole test. Mortality in the reference item group at the end of the study was 100 %.

The effects of Prothioconazole + Folpet 120+300 g/L SC (SAP2101F) on *Bombus terrestris* L. in the oral toxicity test							
Nominal Dose (Treatment group) [µg test item/bee]		Mortality [%]			Corrected mortality [a] [%]		
		4 h	24 h	48 h	4 h	24 h	48 h
--	(C)	0.0	0.0	2.9	--	--	--
72.07	(T1)	0.0	0.0	2.9	0.0	0.0	-0.1
144.14	(T2)	0.0	0.0	2.9	0.0	0.0	-0.1
288.27	(T3)	0.0	0.0	0.0	0.0	0.0	-3.0
576.55	(T4)	0.0	0.0	0.0	0.0	0.0	-3.0
1153.09	(T5)	0.0	0.0	3.6	0.0	0.0	0.6

[a] Corrected for control group (C) according to Abbott's formula (1925) modified by SchneiderOrelli (1947).

In the contact toxicity test, one individual in treatment T1 and another in treatment T4 were recorded as dead at the end of the study. For this reason, no statistical analysis was performed on mortality data. Also, no individuals were recorded as having behavioural abnormalities (i.e. bumblebees affected or moribund) during the whole test. Mortality in the reference item group at the end of the study was 96.7 %.

The effects of Prothioconazole + Folpet 120+300 g/L SC (SAP2101F) on *Bombus terrestris* L. in the contact toxicity test				
Nominal Dose (Treatment group) [µg test item/bee]		Mortality [%]		
		4 h	24 h	48 h
--	(C)	0.0	0.0	0.0
72.07	(T1)	0.0	0.0	3.3
144.14	(T2)	0.0	0.0	0.0
288.27	(T3)	0.0	0.0	0.0
576.55	(T4)	0.0	3.3	3.3
1153.09	(T5)	0.0	0.0	0.0

Conclusion

All validity criteria were met, and sensitivity of the test organisms could be confirmed.
Accordingly, the study was deemed valid.
The measured concentrations in the samples were within 20 % of nominal test concentration used except for prothioconazole concentrations in the oral test, that were slightly above. Due to the analysed concentrations of folpet were within 20 % of the nominal test concentrations and, as worst case scenario because the analysed concentration of prothioconazole in the oral test samples was above 120 % of the nominal value, the test item concentrations and endpoints are based on nominal concentrations.
According to the results of the study, the NOED value at 24 and 48 hours for the oral test was determined to be 1083.88 µg test item/bumblebee, equivalent to 112.80 µg prothioconazole/bumblebee and 284.38 µg folpet/bumblebee (actual intake values).
Because the mortality did not reach the 50 % at the end of the test in any treatment, the LD50 were empirically estimated from the results as higher than 284.38 µg test item/bumblebee for all the periods.
According to the results of the study, the NOED value at 24 and 48 hours for the contact test was determined to be 1153.09 µg test item/bumblebee, equivalent to 120.00 µg prothioconazole/bumblebee and 302.54 µg folpet/bumblebee. Because the mortality did not reach the 50 % at the end of the test in any treatment, the LD50 were empirically estimated from the results as higher than 1153.09 µg test item/bumblebee for all the periods.

Endpoints:

	Endpoints	[µg t.i./bee]	[µg a.i.1/bee]	[µg a.i.2/bee]
Oral	24 h and 48 h NOED	1083.88	112.80	284.38
	24 h and 48 h LD50 [95% CI]	>1083.88	>112.80	>284.38
	(95 % confidence limits)	Not determined	Not determined	Not determined
Contact	24 h and 48 h NOED	1153.09	120.00	302.54
	24 h and 48 h LD50 [95% CI]	>1153.09	>120.00	>302.54

	(95 % confidence limits)	Not determined	Not determined	Not determined

t.i.: test item; a.i.: active ingredient; a.i.1: prothioconazole; a.i.2: folpet.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 KCP 10.3.1.2/01 Study 1

Comments of zRMS:	The study was evaluated at EU level in the context of the renewal of the active substance folpet. The study is noy used in the risk assessment.

~~Reference:~~ ~~KCP 10.3.1.2/01~~

~~Report~~ ~~Chronic toxicity of FOLPET TECHNICAL on honeybees (*Apis mellifera* L.) Ansaloni T., 2015, Study No. S21-05007~~

~~Guideline(s):~~ ~~Yes. Based on CEB (2012) method, adaptions of OECD Guidelines nº 213 (1998), publications of Decourty et al. (2005) and Suchail et al (2001), recommendations of the german ring test group (2013) and EPPO 170~~

~~Deviations:~~ ~~No~~

~~GLP:~~ ~~Yes~~

~~Acceptability:~~ ~~Yes/No/Supplementary~~

~~Duplication (if vertebrate study)~~ ~~N/A~~

~~Objective~~

~~The objective was to study the chronic oral toxicity of Folpet technical on young worker honeybees.~~

~~Materials and Methods~~

~~Test item:~~

~~FOLPET TECHNICAL, batch XF20111023G, analytical content for Folpet 978.2 g/kg, expiry November 2016.~~

~~Test species:~~

~~*Apis mellifera* L. Var. Iberica; young adult worker honeybees (≤ 24h old) collected from a healthy queen right colony sourced from a commercial apiary. Honeybees were collected shortly after emergence two days prior to the first application and were maintained under test conditions in holding cages throughout the study.~~

~~Test design:~~

~~Application:~~ ~~The selection of test item doses for the definitive test were based on the maximum solubility of the test item in 50% aqueous sucrose solution (0.83 ug folpet/uL) and under suggestion by the sponsor. Five doses in a geometric series (factor of 2) of the test item were assessed: 6.25, 12.5, 25, 50 and 100 µg Folpet/bee/day when considering consumption of all food provided. A stock solution was prepared daily by mixing a defined amount of the test item with acetone. Aliquots of these stock solutions were mixed with sucrose solution (50% w/v) to achieve the required test concentrations. Five replicates per treatment each enclosing ten bees, were group fed with one feeder per cage containing 1200 µl of test solution, thus providing 120 µl of test solution per bee. Feeders were weighed prior to their placement in the test cages and were changed on a daily basis with new feeders containing fresh test solutions. When removed each feeder was re-weighed and the daily mean dose consumption per bee was calculated taking into account the surviving individuals at the moment of replacement. Two control groups (un-treated sucrose solution 50% w/v and sucrose solution sucrose solution 50% w/v + acetone), and the reference product Dimethoate 40% EC at a daily dose of 0.144 µg a.i./bee/day concurrently tested.~~

~~Assessments:~~ ~~Honeybees were observed daily at approximately the same time (when the feeders were changed) for mortality and behaviour assessments. Dead bees were removed from the test units. At the end of the test, surviving individuals of each treatment were frozen at ≤ 10 ºC.~~ ***~~Statistics:~~*** ~~Daily mean consumption of each dose of the test item were compared with the pooled controls by means of pair-wise non parametric test (Mann-Whitney exact test). No statistical analysis was performed on mortality data. Statistics was performed using the software SPSS 19; SPSS©One, 1989-2010.~~

~~Results and discussions~~

~~Diet consumption and mortality:~~ ~~Mean daily consumption was 20.55 µl/bee of the offered diet in the negative control group and was 19.56 µl/bee in the solvent control group. No statistical significant difference in mean consumed diet was observed between the two control groups. Mean cumulative mortality after the ten days exposure was 2.00 ± 2.00% (mean ± SE) in the negative control and it was 0.00 % in the solvent control. Daily mean daily consumption of the bees exposed to the test item treatments ranged between 19.34 µl/bee to 20.23 µl/bee. As a consequence, the recalculated daily consumed doses ranged between 1.04 µg Folpet/bee/day and 16.29 µg~~

~~Folpet/bee/day. Mean cumulative consumtpion (consumption over the ten days dosing period) ranged between 10.40 µg Folpet/bee and 162.86 µg Folpet/bee. No statistically significant difference in the daily mean consumed diet was observed between any of the test item treatments and the pooled control (Mann–Whitney exact test). Mean cumulative mortality of the honeybees dosed orally with the test item for ten consecutive days ranged between 0.00% and 4.00 ± 4.00%. Estimated LDD50 value at 10 days was higher than the highest consumed cumulative dose of 162.86 µg Folpet/bee corresponding to a daily mean consumed dose of 16.29 µg Folpet/bee/day, therefore, the NOED for mortality was determined to be ≥ 16.29 µg Folpet/bee/day and the LDD10 and LDD20 was estimated to be >16.29 µg Folpet/bee/day. Symptoms of intoxication were observed sporadically and on very few individuals starting on the third day of dosing (one individual showing uncoordinated movements in T5). Symptoms were apaty (little response to an external stimulus, i.e. a gentle air blow) and lack of coordination. By the end of the study (day 10) the percentage of **TRC14-246BA Chronic Toxicity / Folpet technical / *Apis mellifera* 7 of 40** affected bees based on the surviving individuals ranged between 2.04% at the lowest dose (T1 = 10.40 µg Folpet/bee, cumulative) and 10.00% at the second highest dose (T4 = 80.59 µg Folpet/bee, cumulative). Hence, the observed sublethal effects can be considered as not significant.~~

~~**Conclusion**~~

~~Food consumption of the treated diet with Folpet technical in all test item concentrations was not statistically significantly different when compared to the pooled control. Sublethal effects (i.e.apaty and lack of coordination) were observed sporadically and in few individuals starting on the third day of exposure ranging from 2.04% (T1 = 10.40 µg Folpet/bee, cumulative) to 10.00% (T4 = 80.59 µg Folpet/bee, cumulative) of the surviving individuals after 240h chronic exposure. The estimated chronic LDD50 value for the Folpet technical was determined to be higher than the highest consumed dose of 162.86 µg Folpet/bee (cumulative), corresponding to a daily treatment LDD50 value of 16.29 µg Folpet/bee. Based on the mortality data, the NOED was determined to be ≥16.29 µg Folpet/bee/day, the highest achievable dose and the LDD10 and LDD20 was estimated to be >16.29 µg Folpet/bee/day. **Mean Daily Cumulative over ten days Folpet technical Folpet* Folpet technical Folpet* LDD50 values (consumed µg/bee)** >16.65 >16.29 >166.49 >162.86 * Analytical content The results obtained with the toxic reference substance (100% cumulative mortality) confirmed the sensitivity of the bees under the conditions of the test.~~

~~**Validity criteria**~~

~~The test was considered valid as the results obtained met the set validity criterion:~~

- ~~Mortality observed in control treatments was equal or less than 15.00% for the duration of the test (final cumulated mortality = 2.00% for the negative control and 0.00% for the solvent control).~~
- ~~Mean mortality in the reference product concentration was ≥ 50% at the end of the test (final cumulated mortality = 100.00%).~~

A 2.3.1.2.2 KCP 10.3.1.2/02 Study 2 (not a new study, but the summary was missing)

Comments of zRMS:	The study is acceptable as the validity criteria of the OECD test No. 245 Guideline were met. Samples of the test item treatments gave recoveries within the 80 - 120 % of the nominal concentrations (actual recoveries 82.4 % and 84.7 % for prothioconazole and 83.0 % and 83.9 % for folpet). Samples of the untreated control gave results below the limit of detection. Therefore, all endpoints are based on nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC = 4024.08 mg formulation./kg feeding solution NOEDD = 67.14 µg formulation/bee/day LC_{10} = 4489.85 mg formulation./kg feeding solution LDD_{10} = 2.85 µg formulation/bee/day LC_{20} = 5134.23 mg formulation./kg feeding solution LDD_{20} = 81.00 µg formulation/bee/day LC_{50} = 6635.88 mg formulation/kg feeding solution LDD_{50} = 99.21 µg formulation/bee/day

Reference:	KCP 10.3.1.2/02
Report	SAP2101F: Honey Bee (*Apis mellifera* L.) Chronic Oral Toxicity Test (10-Day Feeding) under Laboratory Conditions. Ansaloni T., 2022, Study No. S21-05006
Guideline(s):	Yes. Based on OECD test No. 245 Guideline for the Testing of Chemicals: Honey bee (Apis mellifera L.), Chronic Oral Toxicity Test – 10 Day Feeding (9 October 2017). SANTE/2020/12830, rev.1 (2021)
Deviations:	Yes, Behavioural abnormalities in the reference item treatment group were not recorded since the reference item is known to be toxic to honeybees and therefore effects areexpected. Moreover, validity criteria for reference item group were met.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

To determine the effects of SAP2101F material on the honey bee *Apis mellifera* L. from chronic feeding exposure. To determine the $LDD_{10/20/50}$, $LC_{10/20/50}$, NOEDD and NOEC, where possible.

Materials and Methods

Test item: SAP2101F
Batch code: BG-GEA
Active ingredients: prothioconazole and folpet
Density: 1.17 g/cm3
Content of a.i. determined by certificate of analysis: prothioconazole 116.3 g/L, folpet 310.6 g/L
Storage conditions: Stored in a cool, dry well-ventilated location

Reference item: BAS 152 65 I
Batch code: 10248664A
Active ingredient : dimethoate
Content of a.i. determined by certificate of analysis: 40.9 % w/v

Test species: Apis mellifera L. young adult worker bees (newly hatched; 1 to 2 days old).

Place of test: Eurofins Trialcamp S.L.U., Polígon Industrial l'Alter, Avda. Antic Regne de València, 25. 46290 Alcàsser (Valencia), Spain.

Test design: ten days dose response test including one control group, 5 test item concentrations, one concentration of the reference item; 5 replicates with 10 bees each per treatment group. Mortality and behavioural abnormalities were assessed daily over the test duration. Five additional test units without bees with full food syringes containing pure 50 % (w/v) aqueous sucrose solution for evaluation of the evaporation.

Test concentrations: 1 control group (negative control C), 5 test item groups with 4024.08, 6036.11, 9054.17, 13581.26 and 20371.88 mg test item/kg feeding solution, 1 reference item group with 0.90 mg dimethoate/kg feeding solution.

Endpoints: LC10/LDD10, LC20/LDD20, LC50/LDD50 and NOEC/NOEDD on exposure at day 10, where possible.

Test conditions:
Air temperature: Min / Max: 32.9 / 33.8 °C (target 33 ± 2 ºC)
Relative air humidity: Min / Max: 52.6 / 60.9 % (target 50 – 70 %)
Photoperiod: 24 h darkness, except during application and assessments.

Dates of work: 29 Sep to 05 May 2022 (Experimental Biological Phase start to Experimental Analytical Phase end dates).

Statistics: Statistical calculations were made with MS Excel 2016 and the statistical program ToxRatPro® Version 3.3.0. In order to determine the NOEC / NOEDD and the LOEC / LOEDD values, a Step-down Rao-Scott-Cochran-Armitage Test Procedure ($\alpha = 0.05$, one sided greater) was used. The estimation of the LCx /LDDx values was performed by means of Probit analysis using linear max. likelihood regression. Analytical verification: Analytical Phase was performed to verify the concentration of the samples taken. For the analytical dose verification, prothioconazole and folpet residues were determined.

Results and discussions

Samples of the test item treatments gave recoveries within the 80 - 120 % of the nominal concentrations (actual recoveries 82.4 % and 84.7 % for prothioconazole and 83.0 % and 83.9 % for folpet). Samples of the untreated control gave results below the limit of detection. Therefore, all endpoints are based on nominal concentrations.
Validity criterion for the negative control C (untreated 50 % (w/v) aqueous sucrose solution) was fulfilled (mortality < 15% after 10 days of exposure), with 8.0 % mean mortality after 10 days of continuous exposure.
In the reference item group, the validity criterion was fulfilled with 100.00 % mortality after 10 days of continuous exposure. Since validity criteria were fulfilled, the test was considered valid.
The overall mean daily consumption of feeding solution was 19.0 mg/bee/day in the control group C. The overall mean daily consumption of feeding solution for the test item concentrations of 4024.08, 6036.11, 9054.17, 13581.26 and 20371.88 mg t.i./kg feeding solution was 16.7, 15.1, 14.3, 12.3 and 11.8 mg/bee/day, respectively.
The overall mean daily consumption of feeding solution in the reference item treatment group was 15.3 mg/bee/day.
The mean daily uptake for the test item concentrations of 4024.08, 6036.11, 9054.17, 13581.26 and 20371.88 mg t.i./kg feeding solution was 67.14, 91.25, 129.54, 166.50 and 239.71 µg test item/bee/day, equivalent to 6.67, 9.07, 12.88, 16.55 and 23.83 µg prothioconazole/bee/day and 17.82, 24.22, 34.39, 44.20 and 63.64 µg folpet/bee/day, respectively.
In the test item groups of 67.14, 91.25, 129.54, 166.50 and 239.71 consumed µg test item/bee/day cumulative mortalities of 10.0, 48.0, 86.0, 98.0, and 100.0 % were observed, respectively, at the final assessment after 10 days of exposure.
Symptoms of intoxication (affected bees) were observed at the highest test item concentration starting on the fourth assessment day (D4), and progressively in time at the other test item concentrations with the exception of T1, for which no affected bees were observed throughout the study. By the end of the test (D10), 7.7 % and 57.1 % of the surviving bees were affected at the treatment levels T2 and T3, respectively, while the only surviving bee in treatment T4 was not affected at this same assessment day.
No symptoms of intoxication were observed for the control group throughout the study.
Results of the test and main endpoints are resumed in the table below:

Cumulative mortality, overall mean consumption of feeding solution, feeding dose (DD), accumulated mean uptake of test item, NOEDD/NOEC and LDDx/LCx.					
Treatment	10 day cumulative mortality	Abbot's transformed mortality	Overall mean consumption of feeding solution	Feeding dose 1	Accumulated mean uptake of test item
Control(s):					
	[%]	[%]	[mg/bee/day]	-	-
C (0)	8.0	-	19.0	-	-
Reference item: Dimethoate [mg a.i./kg feeding solution]					
	[%]	[%]	[mg/bee/day]	[µg a.i./bee/day]	[µg a.i./bee]
R (0.90)	100.0	100.0	15.3	0.0137	0.1017
Test item: SAP2101F material [mg test item/kg feeding solution]					
	[%]	[%]	[mg/bee/day]	[µg t.i./bee/day]	[µg t.i./bee]
T1 (4024.08)	10.0	2.17	16.7	67.14	671.36
T2 (6036.11)	48.0	43.48	15.1	91.25	912.45
T3 (9054.17)	86.0	84.78	14.3	129.54	1295.45
T4 (13581.26)	98.0	97.83	12.3	166.50	1565.12
T5 (20371.88)	100.0	100.0	11.8	239.71	1630.06
	µg/bee/day				
	test item		prothioconazole2	folpet2	
NOEDD 3	67.14		6.67	17.82	
LDD10 [95% CI] 4	72.85 [65.30 – 78.77]		7.24 [6.49 – 7.83]	19.34 [17.34 – 20.91]	
LDD20 [95% CI] 4	81.00 [74.23 – 86.54]		8.05 [7.38 – 8.60]	21.50 [19.71 – 22.97]	
LDD50 [95% CI] 4	99.21 [93.45 – 105.17]		9.86 [9.29 – 10.45]	26.34 [24.81 – 27.92]	
	mg/kg feeding solution				
	test item		prothioconazole2	folpet2	
NOEC 3	4024.08		400.00	1068.27	
LC10 (95% CI) 4	4489.85 [3893.26 – 4960.49]		446.30 [387.00 – 493.08]	1191.92 [1033.54 – 1316.86]	

LC20 (95% CI) 4	5134.23 [4588.44 – 5583.17]	510.35 [456.10 – 554.98]	1362.98 [1218.09 – 1482.16]
LC50 (95% CI) 4	6635.88 [6153.45 – 7147.89]	659.62 [611.66 – 710.51]	1761.63 [1633.56 – 1897.55]

1 Based on actual measured consumption of feeding solution
2 Calculated on the basis of the content declared in the Certificate of Analysis (prothioconazole 116.3 g/L, folpet 310.6 g/L) and the test item density (1.17 g/cm3)
3 Step-down Rao-Scott-Cochran-Armitage Test Procedure ($\alpha = 0.05$, one sided greater)
4 Probit regression analysis

Conclusion

The chronic toxicity test of SAP2101F material was tested under laboratory conditions over a period of 10 days. The actual concentrations of the active ingredients in the test item feeding solutions were within the 80 - 120 % of the nominal. Samples of the untreated control gave results below the limit of detection. Therefore, all endpoints are based on nominal concentrations.

The resulting endpoints of this Chronic Oral Toxicity Test (10-Day Feeding) to the Honey Bee, Apis mellifera L. with the test item SAP2101F are presented below:

NOEC	4024.08 mg t.i./kg feeding solution
NOEDD	67.14 µg t.i./bee/day
LC10	4489.85 mg t.i./kg feeding solution
LDD10	72.85 µg t.i./bee/day
LC20	5134.23 mg t.i./kg feeding solution
LDD20	81.00 µg t.i./bee/day
LC50	6635.88 mg t.i./kg feeding solution
LDD50	99.21 µg t.i./bee/day

All validity criteria were met and the sensitivity of the test organisms was confirmed.
Accordingly, the study was deemed valid.

Validity criteria

The study is considered valid because:

- Mean mortality in the control group was ≤ 15% at the end of the test (actual 8.0 %).
- Mean mortality in the reference item group was ≥ 50 % at the end of the test (actual 100.0 %).

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.3.1 KCP 10.3.1.3/01 Study 1

Comments of zRMS:	The study was evaluated at EU level in the context of the renewal of the active substance folpet. The study is not evaluated by zRMS in the current Dossier and not used in the risk assessment.

Reference:	KCP 10.3.1.3/01
Report	Toxicity of FOLPET TECHNICAL on honey bee larvae (*Apis mellifera* L.) after repeated exposure under laboratory conditions. Ansaloni T., 2015, Study No. TRC14-245BA
Guideline(s):	Yes. OECD Guideline nº 237 (2013). EPPO 170
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	N/A

Objective

The objective was to study the toxicity of FOLPET TECHNICAL on honey bees' larvae after repeated exposure.

Material and Methods

Test Item:

Folpet technical, batch XF20111023G, purity for Folpet 978.2g/kg, expiry November 2016.

Test species:

Apis mellifera L. Var. Iberica; larvae of honey bees collected from a healthy queen right colony sourced from a commercial apiary. Honey bee larvae at the stage L1 were selected from three different colonies and individually placed into cellular well plates where they were fed with a standardized amount of artificial diet.

Test procedure:

Selection of test larvae: Queens of a minimum of three colonies were confined within an empty comb or a comb with emerging worker bees and empty cells of their own colony with an exclusion cage 3 days before the beginning of the test (D -3). At Day -2 (D -2), and within a maximum of 30 hours after confinement, the queens were released after checking the presence of fresh laid eggs. The comb with the eggs was left in the cage near the brood combs until hatching (D1), when the first instar (L1) larvae were taken from the combs and individually placed in well plates under controlled conditions.

Test Units: Larvae were reared in sterilised crystal polystyrene grafting cells placed individually into a well of a 48 well plate, with the top maintained at the level of the plate by means of a dental roll wetted with approximately 500 µl of the sterilising solution enhanced with 15% w/v glycerol. The plates were placed into a hermetic Plexiglass desiccator with a dish filled with potassium sulphate saturated solution in order to keep a water saturated atmosphere. The desiccator was placed into an incubator with forced ventilation at 34-35 ºC and water saturated atmosphere for the duration of the test.

Diet composition: All larvae were fed once a day with the exception of D2. Three different diets, adapted to the needs of each larval stage, were prepared during the test: Diet A (D1, 20 µl/larva): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose. Diet B (D3, 20 µl/larva): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose. Diet C (D4 to D6): 50% weight of fresh royal jelly + 50% weight of aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose. The following volumes of diet were administered on days D4 to D6: D4 = 30 µl, D5 = 40 µl, D6 = 50 µl.

Application of the test substance: Six doses of the test item with a spacing factor of 2.2 were assessed daily for four consecutive days (D3 to D6). Each test dose was prepared daily from a fresh stock solution obtained by mixing a defined amount of the test item with a defined amount of an organic solvent (i.e. acetone) and then by mixing this solution with a defined amount the corresponding diet. To maintain constant concentrations in terms of mg

a.i./mL diet/day, daily doses increased progressively in accordance to the increasing volume of diet administered each day. The final cumulative doses (total of four applications) were of 0.41, 0.89, 1.97, 4.32, 9.51 and 20.92 µg Folpet/larva. On D3, a minimum of twelve well fed larvae from each of the three colonies (36 larvae per treatment) were selected for each treatment and dosed with 20 µl of the corresponding diet (diet B) containing the test solution with the corresponding concentration. Administration of the selected doses of test item continued on a daily basis until day 6 with the corresponding diets. Mixing of the test solution with the diet was performed just before administration.

Assessments: Mortality was assessed and recorded at feeding time at D4, D5, D6, D7 and D8. An immobile larva or a larva that did not react to the contact with the grafting tool was noted as dead. Dead larvae were removed at each assessment and anomalies in behaviour were recorded. On D8, the presence of uneaten food was qualitatively recorded.

Toxic reference treatment: A toxic standard reference product, Dimethoate (Dimethoate 40% EC) was applied at a constant concentration of 40 mg a.i./Kg diet/day on thirty six larvae on the same days the test item was applied. Procedures followed those described above for the test item.

Statistics: For mortality data of the test item, a standard probit analysis (Finney 1971) was performed for the calculation of the LD_{50} values and a step down test for monotone response (Jonckheere–Terpstra exact test) for the estimation of the No Observed Effect Dose. All statistics were performed using the statistical software SPSS 19; SPSS©Onc, 1989-2010.

Results and discussions

Validity criteria

The test is considered valid as the results obtained met the set validity criteria:

- Mortality observed in control treatments was 11.11% (negative control) and 13.89% (solvent control) 120 hours after dosing.
- Corrected mortality (Schneider-Orelli) observed in the larvae exposed to the reference product was 93.75% 120 hours after dosing.

Mean mortality in the control groups was 11.11% (negative control) and 13.89% (solvent control) 120 hours after the first application (D8). Mean mortality of honey bees' larvae dosed orally with the test item ranged between 0.00% (T1 and T2 = 0.41 and 0.89 µg Folpet/larva/developmental period) and 44.44% (T6 = 20.92 µg Folpet/larva/developmental period) 24 hours after dosing, between 8.33% (T1= 0.41 µg Folpet/larva/developmental period) and 94.44% (T6 = 20.92 µg Folpet/larva/developmental period) 48 hours after dosing, between 11.11% (T1= 0.41 µg Folpet/larva/developmental period) and 100.00% (T6 = 20.92 µg Folpet/larva/developmental period) 72 and 96 hours after dosing and between 19.44% (T1= 0.41 µg Folpet/larva/developmental period) and 100.00% (T6 = 20.92 µg Folpet/larva/developmental period)
120 hours after dosing.

The estimated LD_{50} values are reported in the following table.

Oral Test	LDD*$_{50}$ (µg Folpet/larva/developmental period)
Test item 96h (D3 to D7)	5.004
Test item 120h (D3 to D8)	4.846

* LDD: Lethal Dietary Dose

A significant effect (mortality significantly higher than the control mortality) both at 96 and 120 hours after the first application (D7 and D8, respectively) was observed starting with treatment T3 (1.97 µg Folpet/larva/developmental period). Therefore, cumulative NOED (No Observed Effect Dose over 4 and 5 days after the first application, cumulative dosing) corresponded to a cumulated dose of 0.89 µg Folpet/larva both at 96 and 120 hours after the first application.

Hours after the first application	NOED (µg Folpet/larva/developmental period D3 to D8)
96	0.89
120	0.89

At 120 hours after the first application, one individual of the surviving larvae in treatment T1 (0.41 µg Folpet/larva/developmental period) and two individuals of the surviving larvae of the negative and solvent controls and treatment T3 (1.97 µg Folpet/larva/developmental period) had unconsumed diet. In all other treatment doses, no abnormal symptoms were observed for the surviving individuals at 120 hours after dosing.

~~***Reference treatment:*** Corrected mortality observed in the larvae exposed to the reference product was 93.75% both at 96 and 120 hours after dosing.~~

~~**Conclusion**~~

~~The estimated LDD_{50} value for Folpet technical corresponded to a cumulative (over 4 days of application) dietary dose of 5.004 µg Folpet/larva 96 hours after dosing and 4.846 µg Folpet/larva 120 hours after dosing.~~
~~A cumulative dietary dose of 0.89 µg Folpet/larva resulted in a NOED at the end of the study (No Observed effect Dose over the 4 days of exposure, cumulative dosing, both at 96 and 120 hours after the first application).~~
~~The results obtained with the toxic reference substance confirmed the sensitivity of the test system (bees' larvae) under the test conditions.~~

A 2.3.1.3.2 KCP 10.3.1.3/02 Study 2

Comments of zRMS:	The study is conducted in line OECD 239 with no major deviations. Samples of the test item treatments gave recoveries within the 80 - 120% of the nominal concentrations (actual recoveries between 81 % and 95% for prothioconazole and between 80% and 97% for folpet). Samples of the untreated control gave results below the limit of detection. Therefore, all endpoints are based on nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOED = 9.05 µg formulation SAP2101F /larva

Reference:	KCP 10.3.1.3/02
Report	Honey Bee (*Apis mellifera* L.) Larval Toxicity Test following Repeated Exposure under laboratory conditions. Ansaloni T., 2022, Study No. S21-05007
Guideline(s):	ENV/JM/MONO (2016) 34: Guidance Document on Honey bee (*Apis mellifera*) Larval Toxicity Test, Repeated Exposure (OECD 239). SANTE/2020/12830, rev.1
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

The objective of this study was to determine the effects of SAP2101F on honey bee (Apis mellifera L.) larvae from repeated exposure, specifically to determine the No Observed Effect Dose/Concentration (NOED/NOEC), the Lowest Observed Effect Dose/Concentration (LOED/LOEC), the Median Effect Dose/Concentration (ED50/EC50) and any EDx/ECx (i.e., ED10/EC10 and ED20/EC20) for adult emergence (from D3 to D22), where possible.

Material and Methods

	SAP2101F Batch code: BG-GEA Active ingredients: prothioconazole and folpet Density: 1.17 g/cm^3 Content of a.i. determined by certificate of analysis: prothioconazole 116.3 g/L, folpet 310.6 g/L Storage conditions: Stored in a cool, dry well-ventilated location.
Reference item:	BAS 152 I Batch code: COD-002332 Active substance: Dimethoate Content (analysed): 98.2 % (w/w) Storage conditions: stored refrigerated in the original container at ≤ 25 ºC.
Test organisms:	Honey bee (Apis mellifera L.), synchronized first instar (L1) larvae not older than 30 hours at grafting time.

Source:	Commercial beehives from the in-house Test Facility stock, adequately fed, healthy, queen-right and as far as possible disease-free. The hives from which the larvae were obtained had not been previously exposed to any chemical treatments within four weeks of test initiation.
Preparation of test organisms and larvae collection:	At D-3, to provide known-aged eggs (and subsequent larvae), queens from at least three colonies were confined in a single frame with empty cells of their own hive by using an excluder cage. At D-2, and maximum 30 hours after confinement, queens were released. Frames containing eggs were left in the excluder cages until hatching (D1). Three frames from different hives, containing the highest number of synchronized larvae, were selected for grafting in the laboratory.
Test design:	Dose response test with duration of 21 days from grafting on day 1 to the final assessment on day 22. From day 3 until day 6 of the test, 5 different concentrations of SAP2101F were applied to the larvae of the groups and one single concentration of the reference item was applied to the larvae of the reference item group. Both were supplied in diet B and C. The daily feeding volume increased progressively from 20 µL to 50 µL diet per larva over the application period. The cumulative feeding volume from day 3 until day 6 of 140 µL diet per larva was considered for the calculation of the cumulative doses per larva. One control group was included in the test. The control group was exposed to identical exposure conditions of the treatments and for the same period of time. Each treatment group consisted of 48 larvae: 16 from each of three different colonies (each colony representing one replicate). Larval mortality assessments were done on days 4, 5, 6, 7, and 8. The presence of uneaten food was qualitatively recorded on day 8. Assessments of mortality during pupation phase were done on days 15 and 22. Emergence rate was also recorded on day 22.
Test concentrations and doses:	Control: C: control group (untreated diet). Test Item: Reference item: R: 48.0 mg dimethoate/kg diet (equivalent to 7.39 µg dimethoate/larva, cumulative).
Endpoints:	NOEC/NOED, LOEC/LOED and EC10,20,50/ED10,20,50 for adult emergence, where possible.
Test conditions:	Air Temperature: Min: 34.0 / Max: 35.0 °C Relative humidity: Min: 48.7* / Max: 98.3 % * Short-term deviation (less than 2 hours) Exposure to light: constant darkness except during feeding and assessments.
Dates of work:	04 Oct 2021 to 21 Jun 2021 (Experimental Biological Phase start to Experimental Analytical Phase end dates)
Sampling:	Treated diet samples of the lowest (T1) and highest (T5) test item concentrations were taken daily directly after preparation; all samples were placed in the freezer at < -18 ºC until shipment.
Analytical verification:	An analytical study was performed as a phase of this multisite study. Test item residues were determined to verify the content of the active ingredients in the treated diet of the lowest (T1) and highest (T5) test item concentrations from day 3 to day 6. Results of the Analytical Phase are shown in section and detailed information is included in Annex 2 in the Analytical Phase Report. The measured concentrations (corrected for the procedural recoveries) of the active ingredients in all the analysed samples were within ± 20 % nominal concentrations for the treatment solutions, with the exception of folpet in treatment T1 at D5, for which recovery was 75 %. Nevertheless, since recovery of prothioconazole for the same treatment and on the same day was 104 %, it is considered that the relatively low recovery for folpet does not reflect the real concentration in the analyzed sample. In addition, no effect was observed at this concentration level, and therefore the relatively low recovery of folpet is considered uninfluencial. Consecuantly, the concentrations of the were sufficiently confirmed and the endpoints are based on nominal concentrations.
Statistics:	Statistical calculations were made with MS Excel 2016 and the statistical program ToxRatPro® Version 3.3.0. In order to determine the NOEC and the LOEC values, a Step-down Cochran-Armitage Test Procedure ($\alpha = 0.05$, one sided greater) was used. The corresponding NOED and LOED values were extrapolated.

The EDx/ECx values and the respective 95 % confidence intervals were determined by means of Probit analysis using linear max. likelihood regression.

Results and discussions

Analytical results (copied from original report)

Analytical recoveries for SAP2101F: prothioconazole

Sampling Code	Timing	Treatment ID	Residue of Prothioconazole (mg/kg)	Nominal Concentration of Prothioconazole (mg/kg)	Recovery (% of nominal)
Larval Diet					
S21-05007-D3-T1-R	D3	T1	5.04	5.84	86%
S21-05007-D4-T1-R	D4	T1	4.88		84%
S21-05007-D5-T1-R	D5	T1	4.71		81%
S21-05007-D6-T1-R	D6	T1	4.79		82%
S21-05007-D3-T5-R	D3	T5	58.5	61.35	95%
S21-05007-D4-T5-R	D4	T5	55.0		90%
S21-05007-D5-T5-R	D5	T5	54.7		89%
S21-05007-D6-T5-R	D6	T5	54.6		89%

Analytical recoveries for SAP2101F: folpet

Sampling Code	Timing	Treatment ID	Residue of Folpet (mg/kg)	Nominal Concentration of Folpet (mg/kg)	Recovery (% of nominal)
Larval Diet					
S21-05007-D3-T1-R	D3	T1	13.7	15.61	88%
S21-05007-D4-T1-R	D4	T1	12.6		81%
S21-05007-D5-T1-R	D5	T1	12.5		80%
S21-05007-D6-T1-R	D6	T1	12.5		80%
S21-05007-D3-T5-R	D3	T5	160	163.84	97%
S21-05007-D4-T5-R	D4	T5	146		89%
S21-05007-D5-T5-R	D5	T5	160		97%
S21-05007-D6-T5-R	D6	T5	149		91%

On day 8, the cumulative larval mortality. On day 22, the adult emergence rate was 85.42 %. Therefore, the validity criteria for the control group were met for both test periods (the D8 mortality was lower than 15.00 % and the D22 emergence rate was greater than 70.00 %, across all replicates). Moreover, cumulative mortality in the Reference Item group also met the validity criteria (> 50 % at day 8, actual value 85.42 %).
On day 8, no individuals with presence of uneaten food were observed. At the end of the test, in the final assessment of the emergence on day 22, no emerged bees were recorded as being affected (i.e. malformation).

The Effects of SAP2101F on Honey Bee (Apis mellifera L.) Larvae from Repeated Exposure t.i.: test item (SAP2101F)
Negative values represent lower mortality compared to the control group. t.i.: test item (SAP2101F)

Conclusion

The repeated exposure of SAP2101F to honey bee (*Apis mellifera* L.) was tested under laboratory conditions over a period of 21 days.
All validity criteria were met and sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.1.7 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.1.7.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

A 2.3.1.8 KCP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

A 2.3.1.8.1 KCP 10.3.2.2/01 Study 1

Comments of zRMS:	The study is acceptable as all validity criteria of the relevant IOBC guideline were met. LR_{50}/ER_{50} > 5100 ml formulation SAP 2101F /ha

Reference:	KCP 10.3.2.2/01
Report	Prothioconazole + Folpet 120+300 g/L - SAP 2101F: Toxicity to the Predatory Mite, *Typhlodromus pyri* Scheuten (Acari, Phytoseiidae) under Extended Laboratory Conditions. Varela S., 2022, Study No. S21-05009
Guideline(s):	Yes. IOBC (Blümel et al., 2000) modified; Grimm C. et al. (2001) and Pia Ternes et al. (2001)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

The objective of the study was to determine the effects of Prothioconazole + Folpet 120+300 g/L – SAP 2101F on mortality and reproduction of the predatory mite *Typhlodromus pyri* Scheuten under extended laboratory conditions (exposure on bean leaf fragments); to determine the rate causing 50 % mortality (LR50) and 50 % reduction in reproduction (ER50), and to determine the No Observed Effect Rate (NOER) where possible.
Typhlodromus pyri was selected because it is a recommended sensitive indicator species for testing the side effects of plant protection products on non-target arthropods and it is one of the two standard species required for EU registration.

Materials and methods

Test item:	Prothioconazole + Folpet 120+300 g/L - SAP 2101F Batch No. BG-GEA Content of active ingredient (a.i.) (nominal / analysed): prothioconazole: 120.0 g/L / 116.3 g/L; folpet: 300.0 g/L / 310.6 g/L
Reference item:	Dimethoate 40 % w/v EC (BAS 152 65 I) Batch No. 10248664A Content of a.i. (nominal / analysed): Dimethoate: 400.0 g/L / 409 g /L
Test species:	*Typhlodromus pyri* Scheuten, Life stage at test start: protonymphs (≤ 24 hours old)
Endpoints:	LR_{50} (median Lethal Rate) and ER_{50} (median Effect Rate), where possible. The No Observed Effect Rate (NOER), where possible.
Test design:	Test and reference items were diluted in deionised water and applied with a laboratory track sprayer to bean leaf fragments. A control group applied with deionised water was included in the study. All applications were performed with a spray volume of 200 L/ha. After assembling the test units, twenty protonymphs of *Typhlodromus pyri* were introduced into each test unit (5 replicates per treatment). Direct treatment effects (mortality) and any change in behaviour, with respect to the control, were assessed after 1, 3 and 7 days. Reproduction was assessed on days 9, 11 and 14 for the control group and each test item group where the corrected mortality was equal to or less than 50 %.

Test rates: Prothioconazole + Folpet 120+300 g/L - SAP 2101F: 0.3188, 0.6375, 1.2750, 2.5500 and 5.1000 L of test item /ha.
[Equivalent to 37.07, 74.14, 148.28, 296.57 and 593.13 g of prothioconazole/ha and 99.00, 198.01, 396.02, 792.03 and 1584.06 g of folpet/ha, according to the analytical concentration, and calculated using unrounded values from highest to lowest value according to a geometric series with a factor of 2.0]
Dimethoate 40 % w/v EC: 0.030 L of reference item/ha
[Equivalent to 12.27 g a.i./ha, according to the analytical concentration]

Test conditions:
Temperature: 24.4 – 25.0 ° C [Target: 25 ± 2 °C]
Relative humidity: 76.6 – 87.1 % [Target: 60 – 90 %]
Light regime: 16 h light / 8 h darkness
Light intensity *: 1313 - 1542 lux during exposure
[* Light intensity is not important for the test; e.g.: ≥ 1000 lux]

Statistics: Chi² 2x2 Table Test with Bonferroni Correction with survival individuals at 7 d (one-sided greater, $\alpha = 0.05$) was used to detect significant differences between mortality data of the test item groups and the control in order to determine the NOER for lethal effects.
It was not possible to determine the LR50 by probit analysis since mortality data with the tested rates of the test item were less than 50%.
Reproduction data met normality (Shapiro-Wilk's Test) and variance homogeneity (Levene's Test). The analysis of contrasts revealed a linear trend, thus the Williams Multiple Sequential t-test (one-sided smaller, $\alpha = 0.05$) was performed with cumulative offspring/female at 14 d.
It was not possible to determine the 14-day-ER50 since reductions of reproduction with the tested rates of the test item were less than 50 %.

Results and discussions

Treatment group	**Rates** [a] [L FP/ha]	**Mean mortality** [%]	**Corrected mortality** [b] [%]	**Reproduction** [c] [eggs/female]	**Reduction in reproduction rate** [%]
Control (deionised water)	-	16.00	-	8.18	-
Test item Prothioconazole + Folpet 120+300 g/L - SAP 2101F	0.3188	10.00	-7.14	6.00	26.65
	0.6375	11.00	-5.95	5.42 sd	33.68
	1.2750	14.00	-2.38	4.80 sd	41.31
	2.5500	15.00	-1.19	4.54 sd	44.54
	5.1000	19.00	3.57	4.52 sd	44.73
Reference item Dimethoate 40% w/v EC	0.0300	97.000	96.43	Not studied	

[a] Rate of the test / reference items in L of formulated product (FP)/ha
[b] Negative values mean a decrease in mortality rate relative to the control group
[c] sd: Significantly decreased compared to control (Williams Multiple Sequential t-test, one-sided smaller, $\alpha = 0.05$)

Conclusion

The study was conducted as a rate response test under extended test conditions with seven treatment groups on *Typhlodromus* pyri Scheuten, including the test item Prothioconazole + Folpet 120+300 g/L - SAP 2101F at five application rates, the reference item (Dimethoate 40 % w/v EC) at a single application rate and the control, applied with deionised water.
Mortality ≤ 20% (16.0 %) was achieved 7 days after the application, and an acceptable reproductive capacity (8.18 eggs /female) was assessed over a further 7 days in the control group, meeting the validity criteria. The toxic reference product caused 96.43 % mortality (corrected relative to control) and confirmed the sensitivity of the test species and the test conditions.

A 2.3.1.8.2 KCP 10.3.2.2/02 Study 2

Comments of zRMS:	The study is acceptable as all validity criteria of the relevant IOBC guideline were met. LR_{50}/ER_{50} > 5100 ml formulation SAP 2101F /ha

Reference:	KCP 10.3.2.2/02
Report	Prothioconazole + Folpet 120+300 g/L - SAP 2101F: Toxicity to the Aphid Parasitoid *Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) under Extended Laboratory Conditions. Varela S., 2022, Study No. S21-05008,
Guideline(s):	IOBC (Mead-Briggs et al. 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

The objectives of the study were to determine the effects of Prothioconazole + Folpet 120+300 g/L - SAP2101F on mortality and reproduction of the parasitoid *Aphidius rhopalosiphi* De Stefani Perez under extended laboratory conditions (exposure to barley seedlings); to determine the rate causing 50 % mortality (LR50) and 50 % reduction in reproduction (ER50), and to determine the No Observed Effect Rate (NOER), where possible.

Aphidius rhopalosiphi was selected because it is a recommended sensitive indicator species for testing the side effects of plant protection products on non-target arthropods and it is one of the two standard species required for EU registration.

Materials and Methods

Test item:	Prothioconazole + Folpet 120+300 g/L - SAP 2101F Batch No. BG-GEA Content of active ingredient (a.i.) (nominal / analysed): prothioconazole: 120.0 g/L / 116.3 g/L; folpet: 300.0 g/L / 310.6 g/L
Reference item:	Dimethoate 40 % w/v EC (BAS 152 65 I) Batch No. 10248664A Content of a.i. (nominal / analysed): dimethoate: 400.0 g/L / 409 g/L
Test species:	*Aphidius rhopalosiphi* De Stefani Perez (Hymenoptera, Braconidae) Life stage at start of exposure: adult wasps (less than 48 hours old)
Endpoints:	LR_{50} (median Lethal Rate) and ER_{50} (median Effect Rate), where possible. The No Observed Effect Rate (NOER), where possible.

Test design: Test and reference item were diluted in deionised water and applied with a laboratory track sprayer to barley seedlings. A control group applied with deionised water was included in the study. All applications were performed with a spray volume of 400 L/ha. After assembling of test units five adult female wasps were introduced into each test unit (6 replicates per treatment). The settling behaviour of the wasps was assessed during the initial three hours after their release. Direct treatment effects and any change in behaviour with respect to the control were assessed approximately 2, 24 and 48 hours. Reproduction (mummies/female) was assessed 11 days following a 24-hour parasitation period. Reproduction was assessed for the control group and each test item group, where the corrected mortality was below 50%.

Test rates: Prothioconazole + Folpet 120+300 g/L - SAP 2101F: 0.3188, 0.6375, 1.2750, 2.5500 and 5.1000 L of test item /ha.
[Equivalent to 37.07, 74.14, 148.28, 296.57 and 593.13 g of prothioconazole/ha and 99.00, 198.01, 396.02, 792.03 and 1584.06 g of folpet/ha, according to the analytical concentration, and calculated using unrounded values from highest to lowest value according to a geometric series with a factor of 2.0]
Dimethoate 40 % w/v EC: 0.010 L of reference item/ha
[Equivalent to 4.09 g a.i./ha, according to the analytical concentration]

Test conditions:

Temperature:	19.8 – 20.3° C
Relative humidity:	79.6 – 87.7 %
Light regime:	16 h light / 8 h darkness
Light intensity:	768– 1111 lux during mortality
	2464 – 3397 * lux during parasitation
	7816 – 12461 * lux during development of mummies

* Data above 3000 lux, included in Parasitism and Reproduction phases, are measured under non-GLP conditions.

Statistics: Chi² 2x2 Table Test with Bonferroni Correction with survival at 48 h (one-sided greater, $\alpha = 0.05$) was used to detect significant differences between mortality data of the test item group and the control.
It was not possible to determine the LR_{50} by probit analysis since mortality data with the tested rates of the test item were less than 50%.
Repellency data met normality (Shapiro-Wilk's Test) and homoscedasticity (Levene Test). The analysis of contrasts did not reveal a linear and quadratic trend, thus the Williams Multiple Sequential t-test was performed with % of adults settling on the seedlings during approximately 3.0 h after the exposure (one-sided smaller, $\alpha = 0.05$).
Reproduction data did not met normality (Shapiro-Wilk's Test) but met homogeneity (Levene's Test). The analysis of contrasts did not reveal a linear trend, thus the Multiple sequentially-rejective U-test after Bonferroni-Holm (one-sided smaller, $\alpha=0.05$) was performed with number of offspring at 14 days after application (one-sided smaller, $\alpha=0.05$).
It was not possible to determine the ER_{50} by probit analysis since reductions of reproduction with the tested rates of the test item were less than 50% relative to control.

Dates of work**:** 09 Nov – 23 Nov 2021 [Experimental Phase]

Results and discussions

Treatment group	**Rates** [L product/ha]	**Mean mortality** [%]	**Corrected mortality** [a] [%]	**Reproduction** [mummies/female]	**Reduction in reproduction rate** [%] [b]
Control (deionised water)	--	6.67	---	19.85	---
Test item Prothioconazole + Folpet 120+300 g/L - SAP 2101F	0.3188	0.00	-7.14	18.00	9.30
	0.6375	0.00	-7.14	23.21	-16.97
	1.2750	0.00	-7.14	18.60	6.28
	2.5500	0.00	-7.14	17.20	13.33
	5.1000	3.33	-3.57	19.73	0.57
Reference item Dimethoate 40% w/v EC	0.010	100.00	100.00	--	--

[a] Negative values means a decrease in mortality rate relative to the control group
[b] Negative values means an increase in reproduction rate relative to the control group

Conclusions: The study was conducted as a rate response test under extended test conditions with seven treatment groups on *Aphidius rhopalosiphi* De Stefani Perez, including the test item Prothioconazole + Folpet 120+300 g/L - SAP 2101F at five application rates, the reference item (Dimethoate 40 % w/v EC) at a single application rate and the control, applied with deionised water.
Mortality less than 10 % (6.67 %) and acceptable reproductive capacity (19.85 mummies per female) were observed during the 48-hour exposure period and subsequent fecundity assessment in the control group, with no female producing 0 mummies. Corrected mortality in the reference item group was between 50 % and 100 % (actual 100.00 % compared to the control). Hence, the validity criteria were fulfilled and, accordingly, the study was deemed valid.

Prothioconazole + Folpet 120+300 g/L - SAP 2101F on *Aphidius rhopalosiphi* De Stefani Perez. Extended laboratory conditions

Endpoint	Rate		
	[L test item/ha] [a]	[g prothioconazole/ha] [b]	[g folpet/ha] [b]
LR_{50} [c]	n.d.; [> 5.1000]	n.d.; [> 593.13]	n.d.; [> 1584.06]
ER_{50} [c]	n.d.; [> 5.1000]	n.d.; [> 593.13]	n.d.; [> 1584.06]
NOER (mortality)	≥ 5.1000	≥ 593.13	≥ 1584.06
NOER (reproduction)	≥ 5.1000	≥ 593.13	≥ 1584.06

[a] Rate in L of test item/ha; [b] Active ingredient content according to the certificate of analysis (prothioconazole 116.3 g/L, folpet 310.6 g/L); [c] n.d.: not determined as mortality and reduction on reproduction were below 50% up to and including 5.1000 L of test item/ha (relative to the control).

A 2.3.1.8.3 KCP 10.3.2.2/03 Study 3

Comments of zRMS:	The study is acceptable as all validity criteria of the relevant IOBC guideline were met. LR_{50}/ER_{50} > 5100 ml formulation SAP 2101F /ha

Reference:	KCP 10.3.2.2/03
Report	Prothioconazole + Folpet 120+300 g/L - SAP 2101F: Toxicity to the Ladybird, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits. Varela S., 2022, Study No. S21-05010,
Guideline(s):	Yes. IOBC (Schmuck R., et al., 2000) modified for the use of natural substrate
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

The objective of the study was to determine the effects of freshly applied spray deposits of Prothioconazole + Folpet 120+300 g/L - SAP 2101F on mortality and reproduction of the ladybird *Coccinella septempunctata* L. under extended laboratory conditions on bean leaflets; to determine the rate producing 50 % mortality (LR50) and the No Observed Effect Rate (NOER), where possible.

Materials and methods

Test item:	Prothioconazole + Folpet 120+300 g/L - SAP 2101F Batch No. BG-GEA Content of a.i. (nominal / analysed): Prothioconazole: 120.0 g/L / 116.3 g/L Folpet: 300.0 g/L / 310.6 g/L
Reference item:	Dimethoate 40 % w/v EC (BAS 152 65 I) Batch No. 10248664A Content of a.i. (nominal / analysed): Dimethoate: 400 g/L / 409 g/L
Control:	Deionised water
Test organism:	*Coccinella septempunctata* L. (Coleoptera, Coccinellidae) life stage at start of exposure: larvae (3 - 4 days old)
Endpoints and Objective:	Percentage mortality, the mean number of eggs/female/day, the percentage of fertile eggs (hatching rate) and the mean number of fertile eggs/female/day. The objective of the study was to determine the effects of freshly applied spray deposits of Prothioconazole + Folpet 120+300 g/L - SAP 2101F on mortality and reproduction of the ladybird *Coccinella septempunctata* L. under extended laboratory conditions on bean leaflets; to determine the No Observed Effect Rate (NOER) and the Median Lethal Rate (LR_{50}), where possible. Reproductive results were compared to the threshold values described in the guideline method as validity criteria for the control treatment, where possible.
Test design:	The test item, Prothioconazole + Folpet 120+300 g/L - SAP 2101F, was diluted in water and applied with a laboratory track sprayer to detached bean leaflets at five different rates. One control group treated with deionised water and one reference item group treated with dimethoate were included in the study. All applications were performed with a spray volume of 200 L/ha. After drying of the treated leaflets, the test units were assembled. Each treatment group included 40 replicates containing one larva each. The larvae were exposed to the dried residues on the bean leaflets. The larvae were fed with aphids of the species *Acyrthosiphon pisum ad libitum.* During the reproduction phase adults were provided with aphids (same species as used for the larvae), honey-water solution (1:1 w/w) and a mixture of unspecified pollen types. The mortality was determined from the larval stage until pupation and emergence of the adults at least every working day. Reproduction was evaluated with 8 synchronisations of egg laying (24-h periods) in two weeks, to calculate the eggs *per* female and day (fecundity rate) and the larvae emerging from eggs to calculate the percentage of viable eggs (fertility rate). In this way the mean of fertile eggs *per* female *per* day was obtained *per* treatment.
Test rates:	Test item: 0.3188, 0.6375, 1.2750, 2.5500 and 5.1000 L of formulated product (FP)/ha Reference item: 30 mL FP/ha [Equivalent to 12.27 g of active ingredient (a.i.)/ha, according to the analysed content]
Test conditions:	Temperature: 24.4 – 25.2 °C [Target: 25 ± 2 °C] Relative humidity: 76.6 – 89.5 % [Target: 60 – 90 %] Light regime: 16 h light / 8 h darkness Light intensity: 1166 - 5243 lux [Target: ≥ 1000 lux]
Statistics:	Chi² 2x2 Table Test with Bonferroni Correction with mortality (one-sided greater, $\alpha = 0.05$) was used to detect significant differences between mortality data of the test item groups and the control. It was not possible to determine the LR_{50} by probit analysis since reductions of mortality with the tested rates of the test item were less than 50%. A statistical analysis of the reproduction data was not conducted; the reproduction test was evaluated only qualitatively due to the very high species-inherent variability in egg laying performance (Schmuck R., *et al.*, 2000).
Dates of work:	[Experimental phase]: 10 Nov 2021 – 27 Dec 2021

Results and discussions

Mortality and reproduction of *Coccinella septempunctata*

Treatment group	Rate [L product/ha]	Mortality [a] [%]	Corrected mortality [b] [%]	Mean fertile eggs/female/day
Control (deionised water)	0 (Control)	12.50	--	26.3
Test item Prothioconazole + Folpet 120+300 g/L - SAP 2101F	0.3188	5.00 [ns]	-8.57	39.1
	0.6375	10.00 [ns]	-2.86	30.9
	1.2750	20.00 [ns]	8.57	33.5
	2.5500	12.50 [ns]	0.00	36.4
	5.1000	32.50 [ns]	22.86	36.2
Reference item Dimethoate 40% w/v EC	0.030	100.00	100.00	--

[a] Total *C. septempunctata* mortality up to the completion of adult emergence (pre-imaginal mortality)
"ns": Not significantly different compared to control (Chi² 2x2 Table Test with Bonferroni Correction, 1-sided greater, $\alpha = 0.05$)
[b] Corrected mortality according to Abbott (1925), modified by Schneider-Orelli (1947):
Corrected Mortality [%] = [(Mt - Mc] / (100 - Mc)] x 100
Mt = Mortality [%] in treated, Mc = Mortality [%] in control

Prothioconazole + Folpet 120+300 g/L - SAP 2101F applied to detached bean leaflets did not cause statistically significant effect on mortality of *Coccinella septempunctata* at the tested rates from 0.3188 to 5.1000 L test item/ha (Chi² 2x2 Table Test with Bonferroni Correction, one-sided greater, $\alpha = 0.05$). Therefore, the NOER (No Observed Effect Rate) was estimated to be higher than or equal to maximum tested rate of 5.1000 L test item/ha.
Mortality (corrected to the control) was below 50 % with up to 5.1000 L test item/ha; actual maximum mortality was 32.50 % for the control group. Therefore, the rate producing 50 % mortality (LR_{50}) was estimated to be greater than the maximum tested rate of 5.1000 L test item/ha.
The mean fecundity in the test item groups included in the reproduction test (0.3188 to 5.1000 L test item/ha) was between 30.9 and 39.1 eggs *per* female *per* day compared to 26.3 eggs *per* female *per* day in the control group. The mean hatching rate was 100 %, in the test item treatment groups, as well as 100 % in the control group. The mean fertility in the test item groups was above the control validity criterion of 2 fertile eggs *per* female *per* day; between 30.9 and 39.1 fertile eggs *per* female *per* day compared to 26.3 fertile eggs *per* female *per* day in the control group.

Conclusions:
All validity criteria were met and the sensitivity of the test organisms was confirmed: 12.50 % mortality in the control group and 26.3 fertile eggs per female per day. Mortality in the reference item group was ≥ 50 % (actual value: 100 %). Hence, the validity criteria were fulfilled and accordingly, the study was deemed valid. Prothioconazole + Folpet 120+300 g/L SAP2101F applied to bean leaflets cause a maximum of 22.86 % corrected mortality on the highest concentration tested. However, no statistically significant effects could be determined in the mortality of Coccinella septempunctata when compared to the control at the tested rates of the test item from 0.3188 to 5.1000 L test item/ha. Therefore, the NOER (No Observed Effect Rate) was estimated to be higher than or equal to 5.1000 L test item/ha. Mortality (corrected to the control) was always below 50 % with up to and including the rate of 5.1000 L test item/ha. Therefore, the rate producing 50 % mortality (LR50) was estimated to be greater than the maximum tested rate of 5.1000 L test item/ha. It can be assumed that there are no adverse effects on the reproductive performance of the test organism at the rates of the test item up to and including 5.1000 L test item/ha, since the mean fertility was above the control validity criterion of 2 fertile eggs per female per day.

Endpoints after exposure of *Coccinella septempunctata* L.

Prothioconazole + Folpet 120+300 g/L - SAP 2101F Extended conditions; fresh and dried residues on bean leaflets	
Endpoint	**[L test item/ha] [a]**
NOER Lethal effects	NOER ≥ 5.1000 L FP/ha
LR_{50}	LR_{50} > 5.1000 L FP/ha
Reproduction [b]	No impact on reproduction up to and including 5.1000 L test item/ha in accordance with the validity criteria for the control group: ≥ 2 fertile eggs/female/day

[a] Rate in L of formulated product (FP)/ha
[b] Reproduction was evaluated only qualitatively and no statistical analysis was performed

A 2.3.1.8.4 KCP 10.3.2.2/04 Study 4

Comments of zRMS:	The study is acceptable as all validity criteria of the relevant IOBC guideline were met. LR_{50}/ER_{50} > 5100 ml formulation SAP 2101F /ha
Reference:	KCP 10.3.2.2/04
Report	Toxicity to the Green Lacewing, *Chrysoperla carnea* Steph. (Neuroptera: Chrysopidae) Using an Extended Laboratory Test with Freshly Applied Spray Deposits. Luna F., 2022, Study No. S21-050012
Guideline(s):	Yes. IOBC (Vogt H., et al., 2000) modified for the use of natural substrate ESCORT I Guidance Document (Barrett et al., 1994) and ESCORT II Guidance Document (Candolfi et al., 2001)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

The objective of the study was to determine the effects of freshly applied spray deposits of Prothioconazole + Folpet 120+300 g/L - SAP 2101F on mortality and reproduction of the green lacewing *Chrysoperla carnea* Steph. under extended laboratory conditions on leaves of Phaseolus vulgaris L.
The endpoints were to determine the No Observed Effect Rate (NOER) and the rate producing 50 % mortality (LR50), where possible. Reproductive performance was evaluated comparing the results obtained in the test with the validity criterion for the historical control of the guideline and also with the reproductive data from concurrent control.

Material and Methods

Test item:	Prothioconazole + Folpet 120+300 g/L - SAP 2101F Batch No. BG-GEA Content of active ingredients (a.i.) (nominal/analysed): prothioconazole: 120.0 g/L / 116.3 g/L folpet: 300.0 g/L / 310.6 g/L
Reference item:	Dimethoate 40 % w/v EC (BAS 152 65 I) Batch No. 10248664A Content of a.i. (nominal / analysed): dimethoate: 400 g/L / 409 g /L
Test species:	Chrysoperla carnea Steph. (Neuroptera, Chrysopidae) Life stage at start of exposure: larvae (two-three days old)
Study Objective:	The objective of the study was to determine the effects of freshly applied spray deposits of Prothioconazole + Folpet 120+300 g/L - SAP 2101F on mortality and reproduction of the green lacewing Chrysoperla carnea Steph. under extended laboratory conditions on leaves of Phaseolus vulgaris L. The endpoints were to determine the No Observed Effect Rate (NOER) and the rate producing 50 % mortality (LR50), where possible. Reproductive performance was evaluated comparing the results obtained in the test with the validity criterion for the historical control of the guideline and also with the reproductive data from concurrent control.
Test design:	The test item, Prothioconazole + Folpet 120+300 g/L - SAP 2101F, was diluted in deionised water and applied with a laboratory track sprayer to leaves of Phaseolus vulgaris L. at five different rates. One control group treated with deionised water and one reference item group treated with dimethoate were included in the study. All applications were performed with a spray volume of 200 L/ha. After drying of the treated leaves, the test units were assembled. Each treatment group included 30 replicates containing one larva each. The larvae were exposed to fresh dried spray residues on the leaves. The larvae were fed with small quantities of eggs of Ephestia kuehniella Zeller (Lepidoptera: Pyralidae); the emerged adults with an artificial diet. The mortality was determined from the larval stage until pupation, and also until emergence of the adults, at least 3 times a week. Reproduction (fecundity and fertility expressed as hatching rate) was assessed taking two egg samples (each covering a 24 hour egg laying period) within one week. The eggs from this period were counted and the hatching success of the larvae was recorded. The reproduction performance was assessed for treatment groups with a corrected mortality ≤ 50 % when compared to the control.
Test rates:	Prothioconazole + Folpet 120+300 g/L - SAP 2101F: 0.3188, 0.6375, 1.2750, 2.5500 and 5.1000 L of test item/ha Dimethoate 40 % w/v EC: 0.030 L of reference item /ha [Equivalent to 12.27 g a.i./ha, according to the analytical concentration]
Test conditions:	Temperature: 24.5 – 25.0 °C [Target: 25 ± 2 °C] Relative humidity: 80.3 – 87.7 % [Target: 60 – 90 %] Light regime: 16h light / 8h darkness Light intensity: 1154 – 4743 * lux [Target: ≥ 1000 lux] Pre-adult Mortality and adult stage: 1154 – 1311 Reproduction phase: 4272 – 4743 * * Light intensity above 3000 lux was measured under non-GLP conditions (reproduction phase)

Statistics:

Chi²-2 x 2 Test with Bonferroni Correction (one-sided greater, $\alpha = 0.05$) was used to detect significant differences between mortality data of the test item groups and the control since a previous qualitative trend analysis by contrasts (monotonicity of rate/response) did not reveal a linear trend.
The LR50 was not possible to be determined since mortality with the test item at any tested rate was less than 50 %. Then, the LR50 was estimated according to the corrected mortality when compared to the control.
A quantitative judgement (statistical analysis) of the reproduction data was not conducted.

Results and discussions

Mortality and reproduction of Chryoperla carnea Steph. after exposure to Prothioconazole + Folpet 120+300 g/L - SAP 2101F

Treatment group	Rates [L product/ha]	Mean mortality [a] [%]	Corrected mortality [b] [%]	Fecundity [Eggs per female per day]	Fertility Mean hatching rate [%]
Control (deionised water)	--	3.3	--	42.4	100.0
Prothioconazole + Folpet 120+300 g/L - SAP 2101F	0.3188	3.3	0.0	35.6	100.0
	0.6375	13.3	10.3	35.6	100.0
	1.2750	3.3	0.0	38.3	100.0
	2.5500	13.3	10.3	42.2	100.0
	5.1000	0.0	-3.4	33.5	99.4
Dimethoate 40% w/v EC	0.030	100.0	100.0	Not studied	

a Total Chrysoperla carnea Steph. mortality up to the completion of adult emergence (pre-imaginal mortality)
b Corrected mortality according to Abbott (1925), modified by Schneider-Orelli (1947)
Corrected M (%) = [(Mt-Mc]/(100-Mc)] x 100; Mt = Mortality in treated treatment, Mc = Mortality in control treatment

The study was conducted as a rate response test under extended test conditions with seven treatment groups on *Chrysoperla carnea* Steph., including the test item Prothioconazole + Folpet 120+300 g/L - SAP 2101F at five application rates, the reference item (Dimethoate 40 % w/v EC) at a single application rate and the control, applied with deionised water.In the control group, the cumulative mortality was ≤ 20 % (actual 3.3 %), the green lacewing adults produced ≥ 15 eggs per female per day (actual: 42.4) and the mean hatching rate in the control group was ≥ 70 % (actual: 100 %). Mean mortality in the reference item group was ≥ 50 % (actual 100 %). Hence, the validity criteria were fulfilled and, accordingly, the study was deemed valid. Mortality with the test item applied at rates between 0.3188 and 5.1000 L test item/ha was below 50 %, between 0.0 % and 13.3 % (even below 20 %; threshold value as validity criterion for the control treatment). Prothioconazole + Folpet 120+300 g/L - SAP 2101F applied to leaves of *Phaseolus vulgaris* L. did not cause significant effects on mortality of *Chrysoperla carnea* Steph. when compared to the control at the tested rates up to and including 5.1000 L test item/ha. The mean fecundity was above the critical value of 15 eggs laid per female per day and the mean hatching rate was above 70 % in the test item groups. Reduction of reproductive output with the test item was less than 50 % based on the data of fecundity and fertility when compared to the control group.

Conclusions:

Under these extended laboratory test conditions, LR50 (rate producing 50 % mortality) was estimated to be greater than 5.1000 L test item/ha.
The NOER (No Observed-Lethal Effect Rate when compared to the control) was estimated to be higher than or equal to 5.1000 L test item/ha.
Based on the assessments proposed in the guideline, there were no adverse effects on the reproductive performance of the test organism at the tested rates up to and including 5.1000 L test item/ha, since the mean fecundity was above the critical value of 15 eggs laid per female per day and the mean hatching rate was above 70 %.

Based on the data of fecundity and fertility when compared to the control group, it can be concluded that the ER50 for reproduction (rate causing 50 % reduction of reproductive output) can be estimated to be higher than 5.1000 L test item/ha.

Prothioconazole + Folpet 120+300 g/L - SAP 2101F on *Chrysoperla carnea* Steph. Extended laboratory conditions			
Endpoint	**Rate** [L test item/ha] [a]	[g prothioconazole/ha] [b]	[g folpet/ha] [b]
LR_{50} [c]	> 5.1000	> 593.13	> 1584.06
NOER mortality [d]	≥ 5.1000	≥ 593.13	≥ 1584.06
Reproduction	No impact on reproduction for the tested rates 0.3188, 0.6375, 1.2750, 2.5500 and 5.1000 L test item/ha in accordance with the validity criteria for the control group: Fecundity [eggs/female/day] ≥ 15 Fertility [hatching rate] ≥ 70%		
ER_{50}	> 5.1000	> 593.13	> 1584.06

a Rate in L of formulated product /ha
b Active ingredient content according to the certificate of analysis: prothioconazole: 116.3 g/L and folpet: 310.6 g/L
c LR50 estimated according to the corrected mortality when compared to the control
d According to the Chi²-2 x 2 Test with Bonferroni Correction (one-sided greater, α = 0.05).

A 2.3.1.9 KCP 10.3.2.3 Semi-field studies with non-target arthropods

A 2.3.1.10 KCP 10.3.2.4 Field studies with non-target arthropods

A 2.3.1.11 KCP 10.3.2.5 Other routes of exposure for non-target arthropods

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

A 2.4.1.1.1 KCP 10.4.1.1/01 Study 1

Comments of zRMS:	The study is conducted in line OECD 222 with no major deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC $_{(reproduction)}$ = 23.40 mg formulation SAP 2101F /kg soil dry weight. Since the active substances Folpet and Prothioconazole have a log Kow > 2, the endpoint should be corrected by a factor of 2 for the use in the risk assess-ment: $NOEC_{repr, corr}$ = 11.7 mg SAP 2101F /kg soil dry weight.

Reference:	KCP 10.4.1.1/01
Report	Prothioconazole + Folpet 120+300 g/L SC – SAP2101F: Sublethal Toxicity to the Earthworm *Eisenia andrei* (Oligochaeta, Lumbricidae) in Artificial Soil with 5 % Peat. Queralt M., 2022, Study No. S21-05013,
Guideline(s):	Yes. IOBC (Mead-Briggs et al. 2010), OECD 222
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

Determination of the effects of Prothioconazole + Folpet 120 + 300 g/L SC – SAP2101F on the reproduction of the earthworm Eisenia andrei by dermal and alimentary uptake, using an artificial soil. Determination of the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) for reproduction, body weight change and mortality, where possible. Determination of the median Lethal Concentration (LC50) for mortality and Effect Concentration (EC_{10}, EC_{20} and EC_{50}) for reproduction, where possible.

Material and Methods

Test item:	Prothioconazole + Folpet 120 + 300 g/L SC – SAP2101F; Batch number: BG-GEA; active ingredients content (analysed): prothioconazole = 116.3 g/L – folpet = 310.6 g/L; relative density: 1.17; expiry date: July 2023.
Test species:	Adult earthworms of the species Eisenia andrei between two months and one-year-old, with clitellum and a wet mass between 250 mg and 600 mg were used. The test individuals were selected from a synchronized population and did not differ in age by more than 4 weeks. The earthworms were maintained in artificial substrate under conditions identical to the experimental conditions for 6 days before the start of the study.
Test design:	Dose-response test, 56-days exposure in treated artificial soil. Eight different test item concentrations were mixed into the soil; four replicates per test item concentration as well as a water control (without test item) with eight replicates. Each replicate with ten adult worms. Assessment of worm mortality and body weight change after 28 days, assessment of reproduction after 56 days.

Test item concentrations:	0.00 (control), 4.01, 7.22, 13.00, 23.40, 42.12, 75.82, 136.47 and 245.64 mg test item/kg soil dry weight; corresponding to 0.40, 0.72, 1.29, 2.33, 4.19, 7.54, 13.57 and 24.42 mg of prothioconazole/kg soil dry weight and 1.07, 1.92, 3.45, 6.21, 11.18, 20.13, 36.23 and 65.21 mg of folpet/ kg soil dry weight (based on the analysed content and density).
Endpoints:	Lowest Observed Effect Concentration (LOEC) and No Observed Effect Concentration (NOEC) for reproduction, body weight change and mortality, where possible. Determination of the median Lethal Concentration (LC50) for mortality and the Effect Concentration (EC_{10}, EC_{20} and EC_{50}) of reproduction, where possible.
Test conditions:	Artificial soil according to OECD 222; pH 5.87 to 6.12 at test initiation and pH 6.22 to 6.33 at test termination. Water content 21.42 to 22.01 % at test initiation and 39.47 to 44.26 % at test termination. Temperature: 18.2 ºC to 20.4 ºC; long day conditions (16 h light/8 h darkness), 417.4 to 543.9 lux.
Statistics:	Calculation of treatment means and standard deviations. Analysis with the Shapiro-Wilk's test for normality of data distribution and with the Levene's test for homoscedasticity with a level of significance $\alpha = 0.01$. Level of significance $\alpha = 0.05$ for the comparative tests. Analysis of mortality data using Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm ($\alpha = 0.05$, one-sided). The LC50 was not possible to measure since the mortality was lower than 50%. Analysis of body weight change using Williams Multiple Sequential t-test procedure ($\alpha = 0.05$, two-sided). Analysis of reproduction using the step down Williams multiple sequential t-test ($\alpha = 0.05$, one-sided). The EC10, 20 were calculated by non-linear regression using a 4-parameter normal cumulative distribution function with reliable 95 %-confidence limits. Since there was not observed a reproductive reduction higher than 50% the EC50 was estimated.
Dates of work:	19 Oct 2021 to 16 Dec 2021.
Reference item	The toxic reference item carbendazim (supplier: Sigma-Aldrich, analysed purity: 99.5 %) was tested in a separate study (S21-00372), performed between January - March 2021, see Appendix H for details). A statistically significant reduction in the number of juveniles was determined at 1.03 mg test item/kg soil dry weight. This result is within the range expected from the OECD test guideline No 222 (2016) (1 – 5 mg carbendazim/kg soil dry weight) and hence acceptable sensitivity of the test system is assured.
Results and discussions	No behaviour abnormalities were observed when the worms burrowed into the soil on the application day. At the day assessment, one ulcerated individual was observed in the control group and one individual regenerating lost segment was observed in treatment T2. In the rest of the treatments, no pathological symptoms of the adult earthworms were observed. Food consumption of the adult earthworms was estimated to be similar in all the test item groups compared to the control group during the first four weeks of the study. A 2.5 % mortality of adult earthworms was observed in treatments T3 and T8, but no statistically significant differences were determined compared to the control group. No statistically significant difference in the body weight change of the adult earthworms were determined at any of the test item concentrations, compared to the control group. A statistically significant decrease in the number of juveniles was determined at 42.12 mg test item/kg soil dry weight compared to the control group, and all higher test item concentrations.

Results of mortality, body weight change and reproductive output of *Eisenia andrei* summarised:

Treatment group	Concentration	Mean mortality	Mean body weight change	Mean no. of juveniles	CV	Reduction in reproduction relative to Control[b]

		[mg t.i./kg sdw]	[%]	[mg/worm]	**per replicate**	[%]	[%]
Control	(C)	0.00	0.00	347.13	248.25	13.51	-
Test item	(T1)	4.01	0.00	339.13	262.00	12.61	5.54
	(T2)	7.22	0.00	334.50	273.75	15.40	10.27
	(T3)	13.00	2.50	382.35	260.50	20.28	4.93
	(T4)	23.40	0.00	330.78	253.00	13.90	1.91
	(T5)	42.12	0.00	382.78	189.75[a]	16.23	-23.56
	(T6)	75.82	0.00	406.73	173.00[a]	22.92	-30.31
	(T7)	136.47	0.00	386.10	194.50[a]	20.71	-21.65
	(T8)	245.64	2.50	372.77	194.25[a]	17.60	-21.75

t.i.: test item; sdw: soil dry weight; CV: Coefficient of variation; T: treatment.
[a] statistically significantly different compared to the control (Williams multiple sequential t-test procedure, $\alpha = 0.05$, one-sided).
[b] negative values indicate lower reproduction compared to the control, positive values higher reproduction.

Endpoints	**[mg t.i./kg soil dry weight]**	**[mg prothioconazole/kg sdw] [a]**	**[mg folpet/kg sdw] [b]**	**NW**
LOEC $_{\text{body weight change}}$	>245.64	>24.42	>65.21	--
NOEC $_{\text{body weight change}}$	≥245.64	≥24.42	≥65.21	--
LOEC $_{\text{mortality}}$	>245.64	>24.42	>65.21	--
NOEC $_{\text{mortality}}$	≥245.64	≥24.42	≥65.21	--
$LC_{50\ \text{mortality}}$	>245.64	>24.42	>65.21	--
LOEC $_{\text{reproduction}}$	42.12	4.19	11.18	--
NOEC $_{\text{reproduction}}$	23.40	2.33	6.21	--
EC_{10}	24.00 (19.89 – 28.94)	2.39 (1.98 – 2.88)	6.37 (5.28 – 7.68)	0.38
EC_{20}	26.00 (21.57 – 31.37)	2.58 (2.14 – 3.12)	6.90 (5.73 – 8.33)	0.38
EC_{50}	>245.64	>24.42	>65.21	--

t.i.: test item; sdw: soil dry weight; NW: 95 %-confidence interval normalised width.
[a] based on the test item density and analysed active ingredient content. 1.17 g/cm^3 and 116.3 g prothioconazole/L.
[b] based on the test item density and analysed active ingredient content. 1.17 g/cm^3 and 310.6 g folpet/L.

Conclusions:

All validity criteria were met and sensitivity of the test organisms could be confirmed. Accordingly, the study was deemed valid.

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.1.1 KCP 10.4.2.1/01 Study 1

Comments of zRMS:	The study is conducted in line OECD 23 2 with no major deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC $_{(reproduction)}$ ≥ 1000 mg formulation SAP 2101F /kg soil dry weight. Since the active substances Folpet and Prothioconazole have a log Kow > 2, the endpoint should be corrected by a factor of 2 for the use in the risk assess-ment: $NOEC_{repr, corr}$ ≥ 500 mg SAP 2101F /kg soil dry weight

Reference:	KCP 10.4.2.1/01
Report	Prothioconazole + Folpet 120 + 300 g/L - SAP2101F: Effects on the Reproductive Output of the Springtail *Folsomia candida* Willem (Collembola, Isotomidae) in Artificial Soil. Queralt M., 2023, Study No. S23-103641.
Guideline(s):	OECD Guideline No. 232 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

The objective of the study was to determine the effects of Prothioconazole + Folpet 120 + 300 g/L - SAP2101F on survival and the reproductive output of the springtail *Folsomia candida* Willem (Collembola, Isotomidae) under worst-case exposure conditions. For this purpose, the Lowest Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC) for mortality and reproductive output, the median Lethal Concentration (LC_{50}) for adult mortality, and the Effect Concentration on

reproductive output ($EC_{10,20,50}$) were determined, where possible.

Material and Methods

Test item: Prothioconazole + Folpet 120 + 300 g/L - SAP2101F; batch code: X-MFA; active ingredients: 1: prothioconazole, analysed content: 122.8 g/L, 2: folpet, analysed content: 309.6 g/L; expiry date: Dec 2024.

Test organisms: *Folsomia candida* Willem (Collembola, Isotomidae), from in-house culture, 10-11 day old juveniles.

Test design: 28-day exposure in treated artificial soil; the test item solutions were mixed homogeneously into the soil, deionised water was added for the control group and the treated soil was filled in glass vessels before the springtails were introduced on top of the soil.
8 test item groups with 4 replicates each; 1 control group with 8 replicates; 10 juvenile springtails per replicate.
Assessment of mortality and reproductive output after 28 days of exposure.

Test item concentrations: 0 (control), 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000.00 mg test item/kg soil dry weight. Equivalent to: 0 (control), 1.70, 3.06, 5.51, 9.91, 17.84, 32.12, 57.82 and 104.07 mg prothioconazole/kg soil dry weight and 0 (control), 4.29, 7.71, 13.89, 24.99, 44.99, 80.98, 145.76 and 262.37 mg folpet/kg soil dry weight.

Endpoints: LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) for mortality and reproductive output; LC50 (Lethal Concentration) for adult mortality and EC10, 20, 50 (Effect Concentration of 10, 20, 50 %) for reproductive output, where possible.

Test conditions: Artificial soil with 5 % sphagnum peat content; soil pH between 5.66 and 6.00 at test initiation and pH between 6.24 and 6.32 at test termination; water content at test initiation between 24.48 and 24.84 % (corresponding to 47.58 and 48.29 % of the WHCmax) and 22.26 and 23.77 % (corresponding to 43.26 and 46.20 % of the WHCmax) at test termination, temperature during exposure: 20.33 to 20.80 °C (target: 20 ± 2 °C). 16:8 light-dark cycles with 430.6 to 591.0 lux (target: 400 to 800 lux).

Statistics: Statistical calculations were performed with ToxRat Professional 3.3.0 and Microsoft Office Excel-2016® v.16.0. Calculation of treatment means and standard deviations. Level of significance $\alpha = 0.05$ for the final statistical comparison tests. After pre-testing for trend performing a qualitative trend analysis by contrasts using proportions (monotonicity of concentration/response, $\alpha = 0.05$); mortality data of the control group and the test item groups were compared using the Multiple Sequentiallyrejective Fisher Test After Bonferroni-Holm ($\alpha = 0.05$, one-sided greater). The median Lethal Concentration (LC50) value for adult mortality could not be calculated and it was empirically estimated from the results. The reproductive output data of the control group and the test item groups was pretested for normality of data distribution with Shapiro-Wilk's test ($\alpha = 0.01$) and for homoscedasticity with Levene's test ($\alpha = 0.01$). The reproductive output was analysed using Dunnett's Multiple t-test procedure (one-sided smaller, $\alpha = 0.05$). The effective concentrations EC10, EC20 and EC50 values could not be calculated and they were empirically estimated from the results.

Dates of work: 24 May 2023 (Application) – 22 Jun 2023 (final pH and water content measurements).

Results and discussions

In the control group, the mean mortality of adults was 8.75 %, the mean number of juveniles per replicate was 648.38 and the coefficient of variation of reproductive output was 7.58 %. Therefore, the validity criteria for the control group were met.
The maximum mortality observed was 12.50 % (4.11 % corrected for control), and occurred at the fourth highest concentration tested (171.47 mg test item/kg soil dry weight). No statistically differences were

observed between the control and test item groups for mortality (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, α = 0.05, one-sided greater).
The maximum reduction in reproductive output occurred at the fourth lowest concentration tested (95.26 mg test item/kg soil dry weight) with a 3.64 % reduction compared to the control group; the mean number of juveniles was 624.75. No statistically differences were observed between the control and test item groups for reproduction (Dunnett's Multiple t-test procedure, α = 0.05, one-sided smaller).
No behavioural abnormalities or any pathological symptoms of the test organisms could be observed in the control group and in any of the test item groups at the end of the study.
The toxic reference item Boric acid was tested in a separate study (S22-05316), dates of work: Aug - Sep 2022. The EC50 for reproductive output was determined to be 83.28mg boric acid/kg soil dry weight. This is within the target of about 100 mg/kg soil dry weight given by the OECD guideline 232 (2016) and also within the expected range according to historical facility data. Therefore, acceptable sensitivity of the test system was assured.

Mortality and Reproductive output of *Folsomia candida* after 28 days of exposure to artificial soil treated with Prothioconazole + Folpet 120 + 300 g/L - SAP2101F

Treatment group	Test item concentration	Mean mortality	Corrected mortality a	Mean number of juveniles per replicate	CV	Reduction in reproductive output b
	[mg t.i./kg sdw]	[%]	[%]		[%]	[%]
C	–	8.75	-	648.38	7.58	-
T1	16.33	5.00	-4.11	685.25	11.49	-5.69
T2	29.40	0.00	-9.59	710.25	8.01	-9.54
T3	52.92	2.50	-6.85	637.25	17.91	1.72
T4	95.26	0.00	-9.59	624.75	4.95	3.64
T5	171.47	12.50	4.11	637.25	7.89	1.72
T6	308.64	2.50	-6.85	732.00	3.74	-12.90
T7	555.56	10.00	1.37	648.50	6.85	-0.02
T8	1000.00	0.00	-9.59	647.50	3.90	0.13

C: control group; T: test item group; t.i.: test item Prothioconazole + Folpet 120 + 300 g/L - SAP2101F;
sdw: soil dry weight; CV: Coefficient of Variation.
a Mortality corrected to control group according to Abbott's formula (1925) modified by Schneider-Orelli (1947). Negative values indicate lower mortality compared to control group.
b Negative values indicate higher reproductive output compared to control group.

Conclusions:
In a 28-day Folsomia candida reproduction test in artificial soil for the test item Prothioconazole + Folpet 120 + 300 g/L - SAP2101F, all validity criteria were met and the sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.
No behavioural abnormalities or any pathological symptoms were observed.
Under the conditions of this study, the resulting endpoints are as presented below.
The LOEC value for mortality and reproductive output could not be determined and were estimated to be greater than the highest tested concentration of 1000.00 mg test item/kg soil dry weigh; equivalent to 104.07 mg prothioconazole/kg soil dry weight and 262.37 mg folpet/kg soil dry weight. Accordingly, the NOEC value for mortality and reproductive output were determined to be equal to or greater than the highest tested concentration of 1000.00 mg test item/kg soil dry weight; equivalent to 104.07 mg prothioconazole/kg soil dry weight and 262.37 mg folpet/kg soil dry weight.
Since adult mortality corrected for control was always below 50 %, LC50 value was estimated to be greater than the highest tested concentration of 1000.00 mg test item/kg soil dry weight; equivalent to 104.07 mg prothioconazole/kg soil dry weight and 262.37 mg folpet/kg soil dry weight.
Effects on reproduction were always below 10 %, therefore EC10, EC20 and EC50 values for reproductive output were estimated to be greater than the highest tested concentration of 1000.00 mg test item/kg soil dry weight; equivalent to 104.07 mg prothioconazole/kg soil dry weight and 262.37 mg folpet/kg soil dry weight.

Endpoints

Endpoints	Concentration		
	Test item	Active ingredient	
	[mg t.i./kg sdw]	[mg prothioconazole/kg sdw] a	[mg folpet/kg sdw] b
LOEC mortality c	> 1000.00	> 104.07	> 262.37

NOEC mortality c	≥ 1000.00	≥ 104.07	≥ 262.37
LC50 d	> 1000.00	> 104.07	> 262.37
(95 %-confidence interval)	n.d.	n.d.	n.d.
LOEC reproductive output e	> 1000.00	> 104.07	> 262.37
NOEC reproductive output e	≥ 1000.00	≥ 104.07	≥ 262.37
EC10, 20, 50 d	> 1000.00	> 104.07	> 262.37
(95 %-confidence interval)	n.d.	n.d.	n.d.

t.i.: test item; sdw: soil dry weight; n.d.: not determined.
a Based on the analysed prothioconazole content of the CoA and density: 122.8 g/L and 1.18 g/mL, respectively.
b Based on the analysed folpet content of the CoA and density: 309.6 g/L and 1.18 g/mL, respectively.
c Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm (α = 0.05, one-sided greater).
d Empirically estimated from the results.
e Dunnett's multiple t-test procedure (α = 0.05, one-sided smaller).

A 2.4.2.1.2 KCP 10.4.2.1/02 Study 2

Comments of zRMS:	The study is conducted in line OECD 226 with no major deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: NOEC $_{(reproduction)}$ ≥ 1000 mg formulation SAP 2101F /kg soil dry weight. Since the active substances Folpet and Prothioconazole have a log Kow > 2, the endpoint should be corrected by a factor of 2 for the use in the risk assess-ment: $NOEC_{repr, corr}$ ≥ 500 mg SAP 2101F /kg soil dry weight.
Reference:	KCP 10.4.2.1/02
Report	Prothioconazole + Folpet 120 + 300 g/L - SAP2101F: Effects on the Reproductive Output of the Predatory Soil Mite *Hypoaspis (Geolaelaps) aculeifer* Canestrini (Acari: Laelapidae) in Artificial Soil. Queralt M., 2023, Study No. S23-103642,
Guideline(s):	OECD Guideline No. 226 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective
The objective of the study was to determine the effects of the test item Prothioconazole + Folpet 120 + 300 g/L - SAP2101F in soil on the reproductiveoutput and, additionally, on the mortality of the predatory soil mite species *Hypoaspis (Geolaelaps) aculeifer* Canestrini (Acari: Laelapidae) under worst-case exposure conditions. For this purpose, the Lowest Observed Effect Concentration (LOEC) formortality and reproductive output, the No Observed Effect Concentration (NOEC) for mortality and reproductive output, the median Lethal Concentration (LC_{50}) for adult mortality, and the Effect Concentration ($EC_{10, 20, 50}$) for reproductive output were determined, where possible.

Material and Methods
Test item: Prothioconazole + Folpet 120 + 300 g/L - SAP2101F; batch code: X-MFA; active ingredients: 1: prothioconazole, analysed content: 122.8 g/L, 2: folpet, analysed content: 309.6 g/L; expiry date: Dec 2024.

Test organisms*: Hypoaspis aculeifer* Canestrini (Acari, Laelapidae), from in-house culture, adult mites (30 days after the start of the egg-laying for synchronisation).

Test design: Adult females were exposed to the test item in artificial soil. After 14 days, the surviving individuals were extracted from the test units. The number of juveniles per test unit and, additionally, the number of surviving adult females were determined.
The reproductive output and the mortality in each test item group were compared to that of the control group. A concentration-response test with 8 different test item concentrations and 4 replicates each as well as a deionised water control (without test item) with 8 replicates was performed; 10 adult females were exposed per replicate.

Test item concentrations: 0 (control), 16.33, 29.40, 52.92, 95.26, 171.47, 308.64, 555.56 and 1000.00 mg test item/kg soil dry weight. Equivalent to: 0 (control), 1.70, 3.06, 5.51, 9.91, 17.84, 32.12, 57.82 and 104.07 mg prothioconazole/kg soil dry weight and 0 (control), 4.29, 7.71, 13.89, 24.99, 44.99, 80.98, 145.76 and 262.37 mg folpet/kg soil dry weight.

Endpoints: LOEC (Lowest Observed Effect Concentration) and NOEC (No Observed Effect Concentration) for mortality and reproductive output; LC50 (median Lethal Concentration) for adult mortality and EC10, 20, 50 (Effect Concentration of 10, 20, 50 %) for reproductive output, where possible.

Test conditions: Artificial soil with 5 % sphagnum peat content; soil pH between 5.99 to 6.03 at test initiation and between 5.99 to 6.20 at test termination; water content at test initiation between 27.63 and 28.53 % (corresponding to 47.26 and 48.80 % of the WHCmax) and 26.81 and 27.61 % (corresponding to 45.85 and 47.23 % of the WHCmax) at test termination; temperature during exposure: 20.4 °C to 20.8 °C; 16:8 light:dark cycles (long day conditions), and light intensity 475.9 lux to 657.3 lux.
Statistics: Statistical calculations were performed with ToxRat Professional 3.3.0 and Microsoft Office Excel-2016® v.16.0. Calculation of treatment means and standard deviations. Level of significance α = 0.05 for the final statistical comparison tests. After pre-testing for trend performing a qualitative trend analysis by contrasts using proportions (monotonicity of concentration/response, α = 0.05); mortality data of the control group and the test item groups was compared using the Multiple Sequentiallyrejective Fisher Test After Bonferroni-Holm (α = 0.05, one-sided greater). The median Lethal Concentration (LC50) for adult mortality could not be calculated and it was empirically estimated from the results. The reproductive output data of the control group and the test item groups was pretested for normality of data distribution with Shapiro-Wilk's test (α = 0.01) and for homoscedasticity with Levene's test (α = 0.01). After performing a trend analysis by contrasts (monotonicity of concentration/response, α = 0.05), the reproductive output was analysed using Dunnett's multiple t-test procedure (one-sided smaller, α = 0.05). The EC10, 20, 50 for reproductive output could not be calculated and they were empirically estimated from the results.

Dates of work: 18 May 2023 (Application) – 05 Jun 2023 (counting of adult females and juveniles)

Results and discussions
In the control group, the mean mortality of adult females was 6.25 %, the mean number of juveniles per replicate was 277.00 and the coefficient of variation of reproductive output was 7.50 %. Therefore, the validity criteria for the control group were met.
The maximum mortality observed was 12.50 %, 6.67 % corrected for control, and occurred at the third highest test item concentration (308.64 mg test item/kg soil dry weight). At the highest concentration tested (1000.00 mg test item/kg soil dry weight) a 2.50 % mortality was observed (-4.00 % corrected for control). No statistical significant differences were observed between the control and any of the test item concentrations (Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm, α = 0.05, one-sided greater).
The maximum reduction in reproductive output occurred at the fourth lowest concentration tested (95.26 mg test item/kg soil dry weight) with a 6.50 % reduction compared to the control group; the mean number of juveniles was 259.00. At the highest concentration tested (1000.00 mg test item/kg soil dry

weight) a 4.51 % reduction compared to the control group was observed and the mean number of juveniles was 264.50. No statistical significant differences were observed between the control and any of the test item concentrations (Dunnett's multiple t-test procedure, $\alpha = 0.05$, one-sided smaller).
No behavioural abnormalities or any pathological symptoms of the test organisms could be observed in the control group and in any of the test item groups.
The toxic reference item BAS 152 65 I (Dimethoate 40% EC), a.i. dimethoate, was tested in a separate study (S23-100247, dates of work: Jan - Feb 2023). The EC50 for reproductive output was determined to be 3.08 mg a.i./kg soil dry weight (95 %-confidence interval: 2.76 – 3.44 mg a.i./kg soil dry weight). This is within the target range of 3.0 to 7.0 mg a.i./kg soil dry weight given by the OECD Test Guideline No. 226 (2016) and, hence, acceptable sensitivity of the test system was assured.

Mortality and Reproductive output of *Hypoaspis aculeifer* after 14 days of exposure to artificial soil treated with Prothioconazole + Folpet 120 + 300 g/L - SAP2101F

Treatment group	Test item concentration	Mean mortality	Corrected mortality a	Mean number of juveniles per replicate	CV	Reduction in reproductive output b
	[mg t.i./kg sdw]	[%]	[%]		[%]	[%]
C	–	6.25	-	277.00	7.50	-
T1	16.33	10.00	4.00	275.00	17.06	0.72
T2	29.40	2.50	-4.00	280.25	10.35	-1.17
T3	52.92	2.50	-4.00	284.25	8.79	-2.62
T4	95.26	2.50	-4.00	259.00	17.52	6.50
T5	171.47	2.50	-4.00	272.75	19.18	1.53
T6	308.64	12.50	6.67	260.50	17.33	5.96
T7	555.56	7.50	1.33	263.00	8.63	5.05
T8	1000.00	2.50	- 4.00	264.50	17.26	4.51

C: control group; T: test item group; t.i.: test item Prothioconazole + Folpet 120 + 300 g/L - SAP2101F;
sdw: soil dry weight;
CV: Coefficient of Variation.
a Mortality corrected to control group according to Abbott's formula (1925) modified by Schneider-Orelli (1947). Negative values indicate lower mortality compared to control group.

Conclusions:
In a 14-day *Hypoaspis aculeifer* reproduction test in artificial soil for the test item Prothioconazole + Folpet 120 + 300 g/L - SAP2101F, all validity criteria were met and the sensitivity of the test organisms was confirmed. Accordingly, the study was deemed valid.
No behavioural abnormalities or any pathological symptoms were observed. Survival and reproduction output of *Hypoaspis aculeifer* were not negatively affected by any of the assayed test item concentrations after 14 days of exposure.
Under the conditions of this study, the resulting endpoints are as presented below.
The LOEC for mortality and reproductive output could not be determined and were estimated to be greater than the highest concentration tested of 1000.00 mg test item/kg soil dry weight; equivalent to 104.07 mg prothioconazole/kg soil dry weight and 262.37 mg folpet/kg soil dry weight. Accordingly, the NOEC for mortality and reproductive output were determined to be equal to or greater than the highest concentration tested of 1000.00 mg test item/kg soil dry weight; equivalent to 104.07 mg prothioconazole/kg soil dry weight and 262.37 mg folpet/kg soil dry weight.
Since adult mortality corrected for control was always below 50 %, LC50 was estimated to be greater than the highest concentration tested of 1000.00 mg test item/kg soil dry weight; equivalent to 104.07 mg prothioconazole/kg soil dry weight and 262.37 mg folpet/kg soil dry weight.
Effects on reproduction were always below 10 %, therefore, the EC10, EC20 and EC50 for reproductive output were estimated to be greater than the highest concentration tested of 1000.00 mg test item/kg soil dry weight; equivalent to 104.07 mg prothioconazole/kg soil dry weight and 262.37 mg folpet/kg soil dry weight.

Endpoints

Endpoints	Concentration		
	Test item	Active ingredient	
	[mg t.i./kg sdw]	[mg prothioconazole/kg sdw] a	[mg folpet/kg sdw] b
LOEC mortality c	> 1000.00	> 104.07	> 262.37
NOEC mortality c	≥ 1000.00	≥ 104.07	≥ 262.37
LC50 d	> 1000.00	> 104.07	> 262.37
(95 %-confidence interval)	n.d.	n.d.	n.d.

LOEC reproductive output e	> 1000.00	> 104.07	> 262.37
NOEC reproductive output e	≥ 1000.00	≥ 104.07	≥ 262.37
EC10, 20, 50 d	> 1000.00	> 104.07	> 262.37
(95 %-confidence interval)	n.d.	n.d.	n.d.

t.i.: test item; sdw: soil dry weight; n.d.: not determined.
a Based on the analysed prothioconazole content of the CoA and density: 122.8 g/L and 1.18 g/mL, respectively.
b Based on the analysed folpet content of the CoA and density: 309.6 g/L and 1.18 g/mL, respectively.
c Multiple Sequentially-rejective Fisher Test After Bonferroni-Holm (α = 0.05, one-sided greater).
d Empirically estimated from the results.
e Dunnett's multiple t-test procedure (α = 0.05, one-sided smaller).

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.4.2.2.1 KCP 10.5 Effects on soil nitrogen transformation

A 2.4.2.2.2 KCP 10.5/01 Study 1

Comments of zRMS:	The study has been evaluated according to OECD 216 (2000) and is considered acceptable and reliable for use in the risk assessment. The validity criterion is met: Conclusion from the study results : SAP2101F (Prothioconazole 120 g/l + Folpet 300 g/l, SC') had no effect on the nitrogen transformation of soil microflora at 28 days when applied up to 23.40 mg test item/kg soil dry weight.

Reference:	KCP 10.5/01
Report	'Prothioconazole + Folpet 120+300 g/L SC – SAP2101F' Effects on the Activity of Soil Microflora under Laboratory Conditions (Nitrogen Transformation). Queralt M., 2022, Study No. S21-05013,
Guideline(s):	OECD Guideline for Testing of Chemicals No. 216 (2000): Soil Microorganisms: Nitrogen Transformation Test, 21 January 2000.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	N/A

Objective

The aim of the validation is to assure reliable and repeatable results for nitrate in soil extracts using Segmented Flow Analysis performed on a Seal AA1 system.

Material and Methods

Test item:	Prothioconazole + Folpet 120+300 g/L – SAP 2101F; Batch code: BG-GEA; expiry date: July 2023; analysed content: prothioconazole: 116.3 g/L - folpet: 310.6 g/L; relative density: 1.17.
Test soil:	Sandy loam soil (59.5 % sand), low in organic matter (organic carbon content 0.66 %) was used. Soil pH (in water) was 6.86 and the microbial biomass carbon content was 1.32 % of total soil organic carbon.

Test design:	28-day exposure in treated soil; the test item solutions were mixed homogeneously into the soil, deionised water was added for the control group; the treated soil was distributed in glass test units and incubated in controlled conditions. 2 test item groups and 1 control group with 4 replicates per treatment and control were tested. Soil samples were analysed for nitrate formation rates on days 0, 7, 14, and 28 after application. Effects on NO_3^- production were evaluated.
Endpoint:	Nitrate formation rate (mg NO_3^-/kg soil dry weight/day) and % of deviation from the control. Calculations have been performed considering an accumulative approach and an incremental aproach.
Test item concentrations:	Control (deionised water), 4.68 mg test item/kg soil dry weight, (equivalent to 0.47 mg prothioconazole/kg soil dry weight and 1.24 mg folpet/kg soil dry weight) and 23.40 mg test item/kg soil dry weight, (equivalent to 2.33 mg prothioconazole/kg soil dry weight and 6.21 mg folpet/kg soil dry weight).
Test conditions:	Soil incubation was performed in a controlled environment chamber at a range temperature of 19.79 to 20.16 °C (target: 20 ± 2 °C) and under dark conditions; moisture content was maintained during the test at about 42 % WHC.
Statistics:	Calculation of mean nitrate formation rates (considering an accumulative and an incremental approach), standard deviation and coefficient of variation. Comparison between each concentration of the test item assayed and the control was performed for nitrate formation rates. All data were tested for normality with the Shapiro-Wilk's test and for homoscedasticity with the Levene's test before performing the appropriate statistical test. Student t-test was used for all data, as they showed normal distribution. Level of significance $\alpha = 0.05$ for hypothesis testing.
Dates of work:	Experimental Phase: 26 October 2021 to 24 November 2021

Results and discussions

There were statistically significant differences (Student t-test, $\alpha = 0.05$) to the control in nitrate formation rates on evaluations 0-7 and 0-14 days (accumulative approach) and 0-7, 7-14 and 14-28 days (incremental approach), for concentration of 4.68 mg test item/kg soil dry weight.
In addition, there were significant differences (Student t-test, $\alpha = 0.05$) to the control in nitrate formation rates on evaluations 0-7, 0-14, 0-28 (accumulative approach) and 0-7, 7-14, 14-28 (incremental approach), for concentration of 23.40 mg test item/kg soil dry weight.
The deviation in nitrate formation rate between the treatment T1 and the control exceed 25 % on the evaluation 7-14 days (incremental approach), but it fell below 25 % on the evaluation 14-28 days.
The deviation in nitrate formation rate between the treatment T2 and the control exceed 25 % on the evaluation 0-7 days (both accumulative and incremental approach) and 7-14 days (incremental approach), but it fell below 25 % on the evaluation 0-28 and 14-28 days.
The differences to the control in nitrate formation rates 28 days after the test start were 0.83 % and 11.28 % in the 4.68 and 23.40 mg test item/kg soil dry weight, respectively, considering an accumulative approach.
The differences to the control in nitrate formation rates 28 days after the test start were -10.22 % and -9.00 % in the 4.68 and 23.40 mg test item/kg soil dry weight, respectively, considering an incremental approach.

Results summarised:

NO_3^- content (mg NO_3^-/kg sdw) Mean values									
	Control			T1 4.68 mg test item /kg sdw			T2 23.40 mg test item/kg sdw		
Evaluation	**NO_3^- content**	**SD**	**CV %**	**NO_3^- content**	**SD**	**Deviation %**	**NO_3^- content**	**SD**	**Deviation %**
Day 0	26.37	0.72	**2.73**	25.55	0.51	-3.12	25.57	0.16	-3.02
Day 7	74.61	1.16	**1.55**	65.71	2.05	-11.93	41.68	1.42	-44.14
Day 14	122.61	1.51	**1.23**	127.96	1.84	4.36	142.54	1.20	16.26
Day 28	171.28	1.15	**0.67**	171.66	2.39	0.22	186.83	2.91	9.08

sdw: soil dry weight; SD: standard deviation; CV: Coefficient of variation

NO_3^- formation rate (mg NO_3^-/kg sdw /day). Accumulative approach. Mean values						
	Control		T1 4.68 mg test item /kg sdw		T2 23.40 mg test item/kg sdw	
Evaluation	**NO_3^- formation rate**	**CV %**	**NO^{3-} formation rate**	**Deviation %**	**NO3- formation rate**	**Deviation %**
0-7 days	6.89	3.03	5.74[a]	**-16.75**	2.30[a]	**-66.62**
0-14 days	6.87	1.85	7.31[a]	**6.41**	8.35[a]	**21.54**
0-28 days	5.18	1.15	5.22	**0.83**	5.76[a]	**11.28**

sdw: soil dry weight; CV: Coefficient of variation
[a] Significantly differences from the respective control at the 0.05 probability level (Student t-test, 2-tailed).

NO_3^- formation rate (mg NO_3^-/kg sdw /day). Incremental approach. Mean values						
	Control		T1 4.68 mg test item /kg sdw		T2 23.40 mg test item/kg sdw	
Evaluation	**NO_3^- formation rate**	**CV %**	**NO^{3-} formation rate**	**Deviation %**	**NO3- formation rate**	**Deviation %**
0-7 days	6.89	3.03	5.74[a]	**-16.75**	2.30[a]	**-66.62**
7-14 days	6.86	5.48	8.89[a]	**29.70**	14.41[a]	**110.15**
14-28 days	3.48	2.50	3.12[a]	**-10.22**	3.16[a]	**-9.00**

sdw: soil dry weight; CV: Coefficient of variation
[a] Significantly differences from the respective control at the 0.05 probability level (Student t-test, 2-tailed).

Conclusions:

The coefficient of variation between control replicates remained below 15 % throughout the study period for the soil nitrate content. Accordingly, the study was deemed valid as the results met the validity criteria.

A 2.5 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.5.1 KCP 10.6.1 Summary of screening data

A 2.6 KCP 10.6.2 Testing on non-target plants

A 2.6.1.1.1 KCP 10.6.1/01 Study 1

Comments of zRMS:	The study has been evaluated according to OECD 208 (2006) and is considered acceptable and reliable for use in the risk assessment. All validity criteria are met. The following endpoint is derived from the study: $ER_{50} \geq$ 2.850 L SAP2101F /ha

	Visual phytotoxicity: None of the tested rates of the test item 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' showed phytotoxicity symptoms for any of the tested species.

Reference:	KCP 10.6.1/01
Report	Prothioconazole + Folpet 120+300 g/L SC - SAP2101F': Effects on the Seedling Emergence and Growth of Six Non-Target Terrestrial Plant Species under Greenhouse Conditions. Huerta F.,2022, Study No. S21-05016.
Guideline(s):	OECD 208 (2006) SANTE/2020/12830, rev.1 (2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Objective

The study objective was to determine the effects of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' on the seedling emergence and early growth of non-target terrestrial plant species under greenhouse conditions.
The Lowest Observed Effect Rate (LOER), the No Observed Effect Rate (NOER) and the Effect Rate (ER10, 25, 50) for seedling emergence, shoot height and shoot dry weight and Lethal Rate (LR10, 25, 50) for mortality were determined, where possible.

Materials and Methods

Test item: 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F'

Batch No.: BG-GEA

Active ingredients (a.i.):	Prothioconazole
Content of prothioconazole (analysed):	116.3 [g/L]
Active ingredients (a.i.):	Folpet
Content of prothioconazole (analysed):	310.6 [g/L]

Plant species: Dicotyledonous species: *Brassica napus* (oilseed rape), *Beta vulgaris* (sugar beet), *Glycine max* (soybean), *Lycopersicon esculentum* (tomato).

Monocotyledonous species: *Lolium perenne* (ryegrass), *Allium cepa* (onion).

Place of test: The experimental phase was performed in a controlled greenhouse environment located in E-46900 Torrent, Spain.

Test design: Four dicotyledonous and two monocotyledonous species were cultivated in soil. 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' was applied at five defined application rates ranging from 0.1781 to 2.8500 L test item/ha. In each treatment group a total of 20 seeds were applied. The test observation period was 21 days after 50 % of the seedlings in the control group had emerged. During this period, plants were assessed for mortality and phytotoxicity symptoms on day 7, 14 and 21 after 50 % of the seedlings in the control group had emerged. The effects on plant shoot height and shoot dry weight were determined for day 21. Results were compared to the water treated control.

Exposure time: The test lasted for 21 days after at least 50 % of the seedlings in the control had emerged.

Test rates: 0 (control).
0.1781, 0.3563, 0.7125, 1.4250 and 2.8500 L test item/ha in 200 L spray solution/ha.

Endpoints:	Seedling emergence, mortality, phytotoxicity, growth stage, shoot height and shoot dry weight; NOER (No Observed Effect Rate), LOER (Lowest Observed Effect Rate) and the $ER_{10,\ 25,\ 50}$ (Effect Rate for 10 %, 25 %, 50 % effect) on seedling emergence, shoot height and shoot dry weight for day 21 after at least 50 % of the seedlings in the control had emerged; and $LR_{10,\ 25,\ 50}$ (Lethal Rate for 10 %, 25 %, 50 % effect) for mortality for day 21 after at least 50 % of the seedlings in the control had emerged, where possible.
Soil type:	Sandy-loam composed of 81.28 % sand, 4.00 % silt and 14.72 % clay, with a pH of 7.26, an organic carbon content of 1.26 % and an electric conductivity of 0.561 mS/cm.
Test conditions for:	Air temperature (min/max) [°C]: 12.0 / 30.2 Relative humidity (min/max) [%]: 52 / 91 Photoperiod (light /dark) [h]: 16/8 Light intensity (min) [µE/m²/s]: 406
Statistics:	Mean seedling emergence, mean mortality, mean final heights and mean final shoot dry weight were compared using a suitable statistical test in order to obtain the NOER and LOER values. For quantal data, Fisher's Exact Binomial Test with Bonferroni Correction was performed. Metric data was tested for normality of data with the Shapiro-Wilk's test and for homoscedasticity with the Levene's test before performing the appropriate statistical test. Comparison between each rate of the test item assayed, with at least three replicates with surviving individuals and the relative control, was performed for all the plant species. For metric data, when normal distribution and homogeneity of variance of the data was obtained, a William's test (α=0.05) or Dunnett's test (α = 0.05) were performed. When normal distribution of the data was not obtained, Sequentially-rejective U-test After Bonferroni-Holm were performed. Calculations were performed using unrounded raw data. Numbers are rounded for presentation purposes and therefore manual re-calculation may result in slightly different values.
Dates of work (biological phase):	11th Nov 2021 – 15th Nov 2021
Validity criteria:	The study was considered valid for all species; emergence recorded in the control group was ≥ 70.0 % (actually: 75.0 % to 100.0 %) and mean survival of the control plants was ≥ 90.0 % (actually: 94.1 % to 100.0 %), and moreover, no phytotoxic effects were detected in the control plants and the cultivation conditions for a particular species were identical.
Analytical Rate Verification:	Analytical recoveries of 104 % of prothioconazole and 88 % of folpet were obtained in regard to the target concentration for the stock solution.

Results and discussions

Seedling Emergence: No significant effects on emergence were observed for all the tested species.

NOER, LOER and $ER_{10,\ 25,\ 50}$ of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for seedling emergence

Family	Species	Common Name	'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' [L test item/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Brassica napus*	Oilseed rape	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥2.850	>2.850	>2.850	>2.850	>2.850

Fabaceae	*Glycine max*	Soybean	≥2.850	>2.850	>2.850	>2.850	>2.850
Solanaceae	*Lycopersicon esculentum*	Tomato	≥2.850	>2.850	>2.850	>2.850	>2.850
Monocotyledonous species							
Poaceae	*Lolium perenne*	Ryegrass	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaryllidaceae	*Allium cepa*	Onion	≥2.850	>2.850	>2.850	>2.850	>2.850

Mortality: None of the tested rates of the test item 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' affected the survivorship of the tested species.

NOER, LOER and $LR_{10, 25, 50}$ of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for mortality

Family	Species	Common Name	'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' [L test item/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Brassica napus*	Oilseed rape	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥2.850	>2.850	>2.850	>2.850	>2.850
Fabaceae	*Glycine max*	Soybean	≥2.850	>2.850	>2.850	>2.850	>2.850
Solanaceae	*Lycopersicon esculentum*	Tomato	≥2.850	>2.850	>2.850	>2.850	>2.850
Monocotyledonous species							
Poaceae	*Lolium perenne*	Ryegrass	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaryllidaceae	*Allium cepa*	Onion	≥2.850	>2.850	>2.850	>2.850	>2.850

Phytotoxicity: None of the tested rates of the test item 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' showed phytotoxicity symptoms for any of the tested species.

Growth Stage: No differences in growth stage could be detected between the test item groups and the controls for the six tested species at any of the rates tested.

Shoot Height: No differences in growth stage could be detected between the test item groups and the controls for the six tested species at any of the rates tested

NOER, LOER and $ER_{10, 25, 50}$ of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for Shoot Height

Family	Species	Common Name	'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' [L test item/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Brassica napus*	Oilseed rape	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥2.850	>2.850	>2.850	>2.850	>2.850
Fabaceae	*Glycine max*	Soybean	≥2.850	>2.850	>2.850	>2.850	>2.850

Solanaceae	*Lycopersicon esculentum*	Tomato	≥2.850	>2.850	>2.850	>2.850	>2.850
Monocotyledonous species							
Poaceae	*Lolium perenne*	Ryegrass	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaryllidaceae	*Allium cepa*	Onion	≥2.850	>2.850	>2.850	>2.850	>2.850

Shoot Dry Weight: No statistically significant reductions on shoot dry weight were observed for tested treatment rates of the test item 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for all six tested species.

NOER, LOER and ER $_{10, 25, 50}$ of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for Shoot Dry Weight

Family	Species	Common Name	'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' [L test item/ha]				
			NOER	LOER	ER_{10} (95 % Confidence Limits)	ER_{25} (95 % Confidence Limits)	ER_{50} (95 % Confidence Limits)
Dicotyledonous species							
Brassicaceae	*Brassica napus*	Oilseed rape	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥2.850	>2.850	>2.850	>2.850	>2.850
Fabaceae	*Glycine max*	Soybean	≥2.850	>2.850	>2.850	>2.850	>2.850
Solanaceae	*Lycopersicon esculentum*	Tomato	≥2.850	>2.850	>2.850	>2.850	>2.850
Monocotyledonous species							
Poaceae	*Lolium perenne*	Ryegrass	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaryllidaceae	*Allium cepa*	Onion	≥2.850	>2.850	>2.850	>2.850	>2.850

Conclusion:

It can be concluded that 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' has no significant effects on mortality, on shoot height and on shoot dry weight in any of the tested species.
The overall NOER was estimated to be ≥2.850 L test item/ha (equivalent to 331.46 g prothioconazole/ha and 885.21 g folpet/ha).

A 2.6.1.1.2 KCP 10.6.1/02 Study 2

Comments of zRMS:	The study has been evaluated according to OECD 227 and is considered acceptable and reliable for use in the risk assessment. All validity criteria are met. The following endpoint is derived from the study: $ER_{50} \geq$ 2.850 L SAP2101F /ha <u>Visual phytotoxicity</u>: None of the tested rates of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' showed phytotoxicity symptoms for any of the tested species.

Reference: KCP 10.6.1/02

Report	'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F': Effects on the Vegetative Vigour of Six Non-Target Terrestrial Plant Species under Greenhouse Conditions. Huerta F., 2022, Study No. S21-05017,
Guideline(s):	OECD 227 (2006) SANTE/2020/12830, rev. 1 (2021)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Objective

The study objective was to determine the effects of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' on early growth of six non-target terrestrial plant species under greenhouse conditions.
The Lowest Observed Effect Rate (LOER), the No Observed Effect Rate (NOER) and the Effect Rate ($ER_{10, 25, 50}$) for shoot height and shoot dry weight and Lethal Rate ($LR_{10, 25, 50}$) for mortality were determined, where possible.

Materials and Methods

Test item:	'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F'. Batch No.: BG-GEA Active ingredients (a.i.): Prothioconazole Content of prothioconazole (analysed): 116.3 [g/L] Active ingredients (a.i.): Folpet Content of prothioconazole (analysed): 310.6 [g/L]
Plant species:	Dicotyledonous species: *Brassica napus* (oilseed rape), *Beta* vulgaris (sugar beet), *Glycine max* (soybean), *Lycopersicon esculentum* (tomato). Monocotyledonous species: *Lolium perenne* (ryegrass), *Allium cepa* (onion).
Place of test:	The Experimental Phase was performed in a controlled greenhouse environment located in E-46900 Torrent, Spain.
Test design:	Four dicotyledonous and two monocotyledonous species were cultivated in soil. 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' was applied at five defined application rates ranging from 0.1781 to 2.8500 L test item/ha. In each treatment group a total of 20 plants at BBCH growth stage 12 – 14 were applied. The test observation period was 21 days following application. During this period, plants were assessed for mortality and phytotoxicity symptoms on day 7, 14 and 21. The effects on plant shoot height and shoot dry weight were determined for day 21. Results were compared to the tap water treated control.
Exposure time:	21 days after application.
Test rates:	0 (control). 0.1781, 0.3563, 0.7125, 1.4250 and 2.8500 L test item/ha in 200 L spray solution/ha.
Endpoints:	Mortality, phytotoxicity, growth stage, shoot height and shoot dry weight; NOER (No Observed Effect Rate), LOER (Lowest Observed Effect Rate) and $ER_{10, 25, 50}$ (Effect Rate for 10, 25, 50 %) for effect on shoot height and shoot dry weight on day 21, where possible, and $LR_{10,25,50}$ (Lethal rate for 10, 25, 50 %).
Soil type:	Sandy-loam composed of 81.28 % sand, 4.00 % silt and 14.72 % clay, with a pH of 7.26, an organic carbon content of 1.26 % and an electric conductivity of 0.561 mS/cm.

Test conditions: Air temperature (min/max) [°C]: 13.0 / 27.5
Relative humidity (min/max) [%]: 52 / 100
Photoperiod (light /dark) [h]: 16/8
Light intensity (min) [µE/m^2/s]: 407
Short-term deviations (< 2 hours) from the recommended relative humidity were not considered as deviations as they are unavoidable and do not affect the integrity and outcome of the study.

Statistics: Statistical analysis of data was performed using the ToxRat Solutions program (ToxRat® Professional Version 3.3.0.).
Mean mortality, mean final shoot height and mean final shoot dry weight of the surviving plants were determined for each test rate and the control.
Mean mortality, mean final shoot height and mean final shoot dry weight were compared using a suitable statistical test in order to obtain the NOER and LOER values.
Calculations were performed using unrounded raw data, numbers are rounded for presentation purposes and therefore manual re-calculation may result in slightly different values.
Comparison between each rate of the test item assayed, with at least three replicates with surviving individuals and the relative control was performed for all the plant species.
Metric data was tested for normality of data with the Shapiro-Wilk's test and for homoscedasticity with the Levene's test before performing the appropriate statistical test.
For metric data, when normal distribution and homogeneity of variance of the data was obtained, a William's test (α=0.05) or Dunnett's test (α = 0.05) were performed. When normal distribution of the data was obtained and homogeneity of variance of the data was not obtained, Multiple Sequentially rejective Welch-t-test after Bonferroni-Holm (α=0.05) was performed. When normal distribution of the data was not obtained, Step-down-Jonckeere Terpstra test (α=0.05) or Multiple Sequentially-rejective Median (2 x 2 - Table) Test After Bonferroni-Holm was performed.

Dates of work (Biological Phase): 03rd Dec 2021 – 31st Dec 2021

Validity criteria: The study was considered valid for all species; emergence recorded was ≥ 70 % (actually: 95.96 % to 99.26 %) and mean survival of the control plants was ≥ 90 % (actually: 100 %), and moreover, no phytotoxic effects were detected in the control plants

Analytical Rate Verification: Analytical recoveries of 103 % prothioconazole and 105 % of folpet were obtained in regard to the target concentration for the stock solution.

Mortality: None of the tested rates of the test item 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' affected the survivorship of the tested species.

Results and discussions

NOER, LOER and $LR_{10, 25, 50}$ of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for mortality

Family	Species	Common Name	'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' [L test item/ha]				
			NOER	LOER	LR_{10}	LR_{25}	LR_{50}
Dicotyledonous species							
Brassicaceae	*Brassica napus*	Oilseed rape	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥2.850	>2.850	>2.850	>2.850	>2.850
Fabaceae	*Glycine max*	Soybean	≥2.850	>2.850	>2.850	>2.850	>2.850
Solanaceae	*Lycopersicon*	Tomato	≥2.850	>2.850	>2.850	>2.850	>2.850

	esculentum						
Monocotyledonous species							
Poaceae	*Lolium perenne*	Ryegrass	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaryllidaceae	*Allium cepa*	Onion	≥2.850	>2.850	>2.850	>2.850	>2.850

Phytotoxicity: None of the tested rates of the test item 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' showed phytotoxicity symptoms for any of the tested species.

Growth Stage: No differences in growth stage could be detected between the test item groups and the controls for the six tested species at any of the rates tested.

Shoot Height: No statistically significant reductions on shoot height were observed for tested treatment rates of the test item 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for all the tested species.

NOER, LOER and $ER_{25, 50}$ of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for shoot height

Family	**Species**	**Common Name**	**'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' [L test item/ha]**				
			NOER	**LOER**	**ER_{10}**	**ER_{25}**	**ER_{50}**
Dicotyledonous species							
Brassicaceae	*Brassica napus*	Oilseed rape	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥2.850	>2.850	>2.850	>2.850	>2.850
Fabaceae	*Glycine max*	Soybean	≥2.850	>2.850	>2.850	>2.850	>2.850
Solanaceae	*Lycopersicon esculentum*	Tomato	≥2.850	>2.850	>2.850	>2.850	>2.850
Monocotyledonous species							
Poaceae	*Lolium perenne*	Ryegrass	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaryllidaceae	*Allium cepa*	Onion	≥2.850	>2.850	>2.850	>2.850	>2.850

Shoot Dry Weight: No statistically significant reductions on shoot dry weight were observed for tested treatment rates of the test item 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for all the tested species.

NOER, LOER and $ER_{10,25, 50}$ of 'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' for shoot dry weight

Family	**Species**	**Common Name**	**'Prothioconazole + Folpet 120+300 g/L SC - SAP2101F' [L test item/ha]**				
			NOER	**LOER**	**ER_{10}**	**ER_{25}**	**ER_{50}**
Dicotyledonous species							
Brassicaceae	*Brassica napus*	Oilseed rape	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaranthaceae	*Beta vulgaris*	Sugar beet	≥2.850	>2.850	>2.850	>2.850	>2.850
Fabaceae	*Glycine max*	Soybean	≥2.850	>2.850	>2.850	>2.850	>2.850
Solanaceae	*Lycopersicon esculentum*	Tomato	≥2.850	>2.850	>2.850	>2.850	>2.850
Monocotyledonous species							
Poaceae	*Lolium perenne*	Ryegrass	≥2.850	>2.850	>2.850	>2.850	>2.850
Amaryllidaceae	*Allium cepa*	Onion	≥2.850	>2.850	>2.850	>2.850	>2.850

Conclusion

It can be concluded that ‘Prothioconazole + Folpet 120+300 g/L SC - SAP2101F’ has no significant effects on mortality, on shoot height and on shoot dry weight in any of the tested species.
The overall NOER was estimated to be ≥ 2.850 L test item/ha (equivalent to 331.46 g prothioconazole/ha and 885.21 g folpet/ha).

A 2.6.2 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data

Appendix 3 Calculations considering the minimum proposed application rate

zRMS comments:

The calculations for lower application rate are not verified by zRMS.
The worst-case scenario - the max application rate has been considered in current the Core Dossier.
The calculations can be used at MSs level, if necessary.

Effects on birds (KCP 10.1.1)

Table A3-1: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of SAP2101F in cereals - Prothioconazole

Intended use	**Cereals**				
Active substance/product	Prothioconazole				
Application rate (g/ha)	2 x 120 - Prothioconazole 2 x 108.8 – JAU 6476-desthio				
Acute toxicity (mg/kg bw)	1413 (Prothioconazole) / 297 (JAU 6476-desthio)				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals (Prothioconazole)	Small omnivorous bird	158.8	1.2	22.8672	61.8
Cereals (JAU 6476-desthio)	Small omnivorous bird	158.8	1.2	20.732928	14.3
Reprod. toxicity (mg/kg bw/d)	78 (Prothioconazole) / 14.8 (JAU 6476-desthio)				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals (Prothioconazole)	Small omnivorous bird	64.8	1.4 x 0.53	5.769792	13.5
Cereals (JAU 6476-desthio)	Small omnivorous bird	64.8	1.4 x 0.53	5.23127808	2.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table A3-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of SAP2101F in cereals – JAU 6476-desthio (metabolite)

Intended use	**Cereals**				
Active substance/product	JAU 6476-desthio				
Application rate (g/ha)	2 x 108.8 – JAU 6476-desthio				
Reprod. toxicity (mg/kg bw/d)	14.8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	1.4 x 0.53	0.26640768	55.6
Cereals BBCH 30-39	Small omnivorous bird “lark” Combination (invertebrates with	5.4	1.4 x 0.53	0.43593984	33.9

	interception) 25% crop leaves 25% weed seeds 50% ground arthropods				

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table A3-3: Screening assessment of the acute and long-term/reproductive risk for birds due to the use of SAP2101F in cereals - Folpet

Intended use	**Cereals**				
Active substance/product	Folpet				
Application rate (g/ha)	2 x 300				
Acute toxicity (mg/kg bw)	2150				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	57.168	37.6
Reprod. toxicity (mg/kg bw/d)	74.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small omnivorous bird	64.8	1.4 x 0.53	14.42448	5.2

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table A3-4: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of SAP2101F in cereals

Intended use	**Cereals**				
Active substance/product	SAP2101F				
Application rate (g/ha)	2 x 1140*				
Acute toxicity (mg/kg bw)	1867.5				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	158.8	1.2	217.2	8.6

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Considering a density of the formulation of 1.14 g/mL.

Table A30-5: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of SAP2101F in cereals

Intended use	**Cereals**				
Active substance/product	SAP2101F				
Application rate (g/ha)	2 x 1140*				
Acute toxicity (mg/kg bw)	1867.5				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals BBCH ≥ 40	Small omnivorous bird “lark” Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	7.2	1.2	9.8496	189.6

Cereals BBCH 30-39	Small omnivorous bird "lark" Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	12.0	1.2	16.416	113.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Considering a density of the formulation of 1.14 g/mL.

Table A3-6: Effective application rate

Crop	Substance	DT_{50}	Max application rate (AR) (g as/ha)	No. of applica-tions	$1\text{-}e^{-nki}$	$1\text{-}e^{-ki}$	MAF_{mean}	AR_{eff}
Cereals	Prothioconazole	1.2	120	2	1.000	1.000	1.000	120.04
	Folpet	1.38	300	2	1.000	0.999	1.001	300.26

Table A3-7: Ratio of AReff to acute/long term toxicity endpoint

					No concern if ratio
Prothioconazole: Effective application rate (g/ha) =	120.04				
Prothioconazole					
Koc (l/kg) =	1765				
Acute toxicity (mg/kg bw) =	1413	quotient =	0.08		≤ 3000
Reprod. toxicity (mg/kg bw/d) =	78	quotient =	1.54		≤ 3000
JAU 6476-desthio: Effective application rate (g/ha) =	120.04 (worst-case approach)				
JAU 6476-desthio					
Koc (l/kg) =	375.4				
Acute toxicity (mg/kg bw) =	297	quotient =	0.40		≤ 3000
Reprod. toxicity (mg/kg bw/d) =	14.8	quotient =	8.11		≤ 3000
Folpet: Effective application rate (g/ha) =	300.26				
Folpet					
Koc (l/kg) =	304				
Acute toxicity (mg/kg bw) =	746	quotient =	0.40		≤ 50
Reprod. toxicity (mg/kg bw/d) =	74.6	quotient =	4.02		≤ 50

Table A3-8: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	comments
PEC_{soil} (mg/kg soil)	0.033	Worst-case initial PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	4.05 / 11220	-
Koc	1765	Aged soil column leaching study; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	3.83802	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.12665	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.13299	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	78	-
TER_{lt}	586.5	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-9: Assessment of the risk for earthworm-eating birds due to exposure to JAU 6476-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	comments
PEC_{soil} (mg/kg soil)	0.032	Worst-case PECsoil calculated for multiple applications in cereals

Parameter	JAU 6476-desthio	comments
log P_{ow} / P_{ow}	3.04 / 1096	-
Koc	575.4	Geomean, n=4; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	1.21635	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.00389	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.04087	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	14.8	-
TER_{lt}	362.1	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-10: Assessment of the risk for earthworm-eating birds due to exposure to folpet via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Folpet	comments
PEC_{soil} (mg/kg soil)	0.132	Worst-case initial PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	3.017 / 1040	-
Koc	304	Worst-case assumption; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	2.191	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.289212	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.3036726	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	74.6	-
TER_{lt}	245.7	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-11: Assessment of the risk for fish-eating birds due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	comments
PEC_{sw} (mg/L)	0.01303	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	19.7	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.256691	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.040814	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	78	-
TER_{lt}	1911.1	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-12: Assessment of the risk for fish-eating birds due to exposure to JAU 6476-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	comments
PEC_{sw} (mg/L)	0.04432	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	65	-

Parameter	JAU 6476-desthio	comments
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	2.8808	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.4580	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	14.8	-
TER_{lt}	32.3	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-13: Assessment of the risk for fish-eating birds due to exposure to folpet via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Folpet	comments
PEC_{sw} (mg/L)	0.07392	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	56	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	4.13952	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.65818	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	74.6	-
TER_{lt}	113.3	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

Table A3-14: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of SAP2101F in cereals - Prothioconazole

Intended use	**Cereals**				
Active substance/product	Prothioconazole				
Application rate (g/ha)	2 x 120 - Prothioconazole 2 x 108.8 – JAU 6476-desthio				
Acute toxicity (mg/kg bw)	6200 (prothioconazole) / 2235 (JAU 6476-desthio)				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals (Prothioconazole)	Small herbivorous mammal	118.4	1.2	17.0496	363.6
Cereals (JAU 6476-desthio)	Small herbivorous mammal	118.4	1.2	15.458304	144.6
Reprod. toxicity (mg/kg bw/d)	95.6 (prothioconazole) / 10 (JAU 6476-desthio)				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals (Prothioconazole)	Small herbivorous mammal	48.3	1.4 x 0.53	4.300632	22.2
Cereals (JAU 6476-desthio)	Small herbivorous mammal	48.3	1.4 x 0.53	3.89923968	2.6

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table A3-15: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of SAP2101F in cereals – JAU 6476-desthio

Intended use		**Cereals**			
Active substance/product		JAU 6476-desthio			
Application rate (g/ha)		2 x 108.8 – JAU 6476-desthio			
Reprod. toxicity (mg/kg bw/d)		10			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" ground dwelling invertebrates with interception 100% ground arthropods	1.9	1.4 x 0.53	0.15338624	65.2
Cereals BBCH ≥ 40	Small herbivorous mammal "vole Grass + cereals 100% grass	21.7	1.4 x 0.53	1.75183232	5.7
Cereals BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	1.4 x 0.53	0.18567808	53.9
Cereals BBCH 30-39	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	3.9	1.4 x 0.53	0.31484544	31.8

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table A3-16: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of SAP2101F in cereals - Folpet

Intended use		**Cereals**			
Active substance/product		Folpet			
Application rate (g/ha)		2 x 300			
Acute toxicity (mg/kg bw)		2000			
TER criterion		10			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small herbivorous mammal	118.4	1.2	42.624	46.9
Reprod. toxicity (mg/kg bw/d)		150			
TER criterion		5			
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals	Small herbivorous mammal	48.3	1.4 x 0.53	10.75158	14.0

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table A3-17: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of SAP2101F in cereals – SAP2101F

Intended use	**Cereals**
Active substance/product	SAP2101F
Application rate (g/ha)	2 x 1140*
Acute toxicity (mg/kg bw)	2489

TER criterion	10

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Cereals	Small omnivorous bird	118.4	1.2	161.9712	15.4

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.
*Considering a density of the formulation of 1.14 g/mL.

Table A3-18: Effective application rate

Crop	**Substance**	**DT_{50}**	**Max application rate (AR) (g as/ha)**	**No. of applica-tions**	**$1\text{-}e^{-nki}$**	**$1\text{-}e^{-ki}$**	**MAF_{mean}**	**AR_{eff}**
Cereals	Prothioconazole	1.2	120	2	1.000	1.000	1.000	120.04
	Folpet	1.38	300	2	1.000	0.999	1.001	300.26

Table A3-19: Ratio of AReff to acute/long term toxicity endpoint

					No concern if ratio
Prothioconazole: Effective application rate (g/ha)	=	120.04			
Prothioconazole					
Koc (l/kg)	=	1765			
Acute toxicity (mg/kg bw)	=	6200	quotient =	0.02	≤ 3000
Reprod. toxicity (mg/kg bw/d)	=	95.6	quotient =	1.26	≤ 3000
JAU 6476-desthio: Effective application rate (g/ha) =		120.04 (worst-case approach)			
JAU 6476-desthio					
Koc (l/kg)	=	375.4			
Acute toxicity (mg/kg bw)	=	2235	quotient =	0.05	≤ 3000
Reprod. toxicity (mg/kg bw/d)	=	10	quotient =	12.0	≤ 3000
Folpet: Effective application rate (g/ha)	=	300.26			
Folpet					
Koc (l/kg)	=	304			
Acute toxicity (mg/kg bw)	=	2000	quotient =	0.15	≤ 50
Reprod. toxicity (mg/kg bw/d)	=	150	quotient =	2.00	≤ 50

Table A3-20: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	comments
PECsoil (mg/kg soil)	0.033	Worst-case initial PECsoil calculated for multiple applications in cereals
log Pow / Pow	4.05 / 11220	-
Koc	1765	Aged soil column leaching study; value used for PECgw and PECsw simulations
foc	0.02	Default
BCFworm	3.83802	BCFworm/soil = (PECworm,ww/PECsoil,dw) = (0.84 + 0.012 × Pow) / foc × Koc
PECworm	0.12665	PECworm = PECsoil × BCFworm/soil
Daily dietary dose (mg/kg bw/d)	0.16212	DDD = PECworm × 1.28
NOEL (mg/kg bw/d)	95.6	-
TERlt	589.7	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-21: Assessment of the risk for earthworm-eating mammals due to exposure to JAU 6476-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	comments
PEC_{soil} (mg/kg soil)	0.032	Worst-case PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	3.04 / 1096.5	-
Koc	575.4	Geomean, n=4; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	1.21635	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.03892	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.04982	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	10	-
TER_{lt}	200.7	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-22: Assessment of the risk for earthworm-eating mammals due to exposure to folpet via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals

Parameter	Folpet	comments
PEC_{soil} (mg/kg soil)	0.132	Worst-case PECsoil calculated for multiple applications in cereals
log P_{ow} / P_{ow}	3.017 / 1039.9	-
Koc	304	Worst-case assumption; value used for PECgw and PECsw simulations
foc	0.02	Default
BCF_{worm}	2.191	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.289212	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.370191	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	150	-
TER_{lt}	405.2	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-23: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	**Prothioconazole**	**comments**
PECsw (mg/L)	0.01303	Worst-case Step 1 PECsw calculated for multiple application in winter and spring cereals
BCFfish	19.7	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PECfish	0.256691	PECfish = PECwater × BCFfish
Daily dietary dose (mg/kg bw/d)	0.03645	DDD = PECfish × 0.142
NOEL (mg/kg bw/d)	95.6	-
TERlt	2622.8	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A30-24: Assessment of the risk for fish-eating mammals due to exposure to JAU 6476-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	JAU 6476-desthio	comments
PEC_{sw} (mg/L)	0.04432	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	65	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	2.8808	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.4091	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	10	-
TER_{lt}	24.4	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Table A3-25: Assessment of the risk for fish-eating mammals due to exposure to folpet via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Folpet	comments
PEC_{sw} (mg/L)	0.07392	Worst-case Step 1 PEC_{sw} calculated for multiple application in winter and spring cereals
BCF_{fish}	56	-
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	4.13952	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.58781	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	150	-
TER_{lt}	255.2	>5, no further refinement

TER values shown in bold fall below the relevant trigger.

Effects on aquatic organisms (KCP 10.2)

Table A3 -26: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1, 2 calculations for the use of SAP2101F (minimum dose)

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 1830	NOEC 308	EC_{50} 1300	NOEC 560	E_rC_{50} 2180	NOEC 9140
AF		100	10	100	10	10	10
RAC (µg/L)		18.3	30.8	13	56	218	914
Winter cereals							
FOCUS Scenario	**PEC gl-max (µg/L)**						
Step 1							
	13.03	0.71	0.42	**1.002**	0.23	0.06	0.01
Step 2							

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
N-Europe	1.10	Ok at step 1		0.08	Ok at step 1		
S-Europe	1.10			0.08			
Spring cereals							
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	13.03	0.71	0.42	**1.002**	0.23	0.06	0.0
Step 2							
N-Europe	1.10	Ok at step 1		0.08	Ok at step 1		
S-Europe	1.10			0.08			

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table A3-27: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of SAP2101F (minimum dose)

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudo-kirchn. sub-capitata***	***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 6630	NOEC 3.34	EC_{50} 10000	NOEC 100	E_rC_{50} 550	NOEC 2000
AF		100	10	100	10	10	10
RAC (µg/L)		66.3	0.334	100	10	55	200
Winter cereals							
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	44.32	0.67	**132.7**	0.44	**4.43**	0.81	0.22
Step 2							
N-Europe	7.04	Ok at step 1	**21.08**	Ok at step 1	0.70	Ok at step 1	
S-Europe	5.76		**17.25**		0.58		
Step 3 – Multiple application							
D1/ditch	0.09479	Resolved at previous steps	0.28	Resolved at previous steps			
D1/stream	0.03003		0.09				
D2/ditch	0.1152		0.34				
D2/stream	0.1134		0.34				
D3/ditch	0.04034		0.12				
D4/pond	0.007394		0.02				
D4/stream	0.01937		0.06				
D5/pond	0.008947		0.03				
D5/stream	0.02752		0.08				

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
D6/ditch	0.05258		0.16				
R1/pond	0.06485		0.19				
R1/stream	0.5627		**1.68**				
R3/stream	0.5212		**1.56**				
R4/stream	0.8766		**2.62**				
Step 3 – Single application							
D1/ditch	0.02416	Resolved at previous steps	0.07	Resolved at previous steps			
D1/stream	0.02883		0.09				
D2/ditch	0.0496		0.15				
D2/stream	0.03385		0.10				
D3/ditch	0.02417		0.07				
D4/pond	0.004783		0.01				
D4/stream	0.02164		0.06				
D5/pond	0.005534		0.02				
D5/stream	0.02782		0.08				
D6/ditch	0.01308		0.04				
R1/pond	0.0225		0.07				
R1/stream	0.1953		0.58				
R3/stream	0.2447		0.73				
R4/stream	0.3597		**1.08**				
Spring cereals							
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**						
Step 1							
	44.32	0.67	**132.7**	0.44	**4.43**	0.81	0.22
Step 2							
N-Europe	3.21	Ok at step 1	**9.61**	Ok at step 1	0.32	Ok at step 1	
S-Europe	5.76		**17.25**		0.58		
Step 3 – Multiple application							
D1/ditch	0.2296	Resolved at previous steps	0.69	Resolved at previous steps			
D1/stream	0.05923		0.18				
D3/ditch	0.04191		0.13				
D4/pond	0.009174		0.03				
D4/stream	0.02466		0.07				
D5/pond	0.008809		0.03				
D5/stream	0.02746		0.08				
R4/stream	0.6279		**1.88**				
Step 3 – Single application							
D1/ditch	0.1356	Resolved at previous steps	0.41	Resolved at previous steps			
D1/stream	0.0548		0.16				
D3/ditch	0.04668		0.14				

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged
D4/pond	0.006009		0.02				
D4/stream	0.02532		0.08				
D5/pond	0.00558		0.02				
D5/stream	0.02929		0.09				
R4/stream	0.3274		0.98				

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0 28: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) for prolonged fish group based on FOCUS Steps 4 calculations for the use of SAP2101F in cereals (minimum dose)**

Group		Fish prolonged
Test species		*Oncorhynchus mykiss*
Endpoint (µg/L)		NOEC 3.34
AF		10
RAC (µg/L)		0.334
Winter cereals		
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)	PEC/RAC < 1
Step 4 – Multiple application, 10 meters of VFS		
R1/stream	0.2555	0.76
R3/stream	0.2378	0.71
Step 4 – Single applications, 5 meters of VFS		
R4/stream	0.2347	0.70
Spring cereals		
Step 4 – Multiple application, 10 meters of VFS		
R4/stream	0.2827	0.85

Table A3-29: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-S-methyl (M01) for each organism group based on FOCUS Steps 1, 2 calculations for the use of SAP2101F**

Group		Fish acute	Inverteb. acute	Algae
Test species		*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchn. subcapitata*
Endpoint (µg/L)		LC_{50} 1800	EC_{50} 2800	E_rC_{50} 47400
AF		100	100	10
RAC (µg/L)		18	28	4740
Winter cereals				
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)			
Step 1				
	19.23	**1.1**	0.7	0.0
Step 2				
N-Europe	1.49	0.08	Resolved at Step 1	Resolved at Step 1

Group		Fish acute	Inverteb. acute	Algae
S-Europe	1.29	0.07		
Spring cereals				
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)			
Step 1				
	19.23	**1.1**	0.7	0.0
Step 2				
N-Europe	1.02	0.06	Resolved at Step 1	Resolved at Step 1
S-Europe	1.29	0.07		

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole (M13) for each organism group based on FOCUS Steps 1, 2 calculations for the use of SAP2101F (minimum dose)**

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchn. sub-capitata*
Endpoint (µg/L)		LC_{50} 498000	NOEC 3200	EC_{50} 900000	E_rC_{50} 22500
AF		100	10	100	10
RAC (µg/L)		4980	320	9000	2250
Winter cereals					
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)				
Step 1					
	2.25	0.0	0.0	0.0	0.0
Step 2					
N-Europe	0.10	0.0	0.0	0.0	0.0
S-Europe	0.09	0.0	0.0	0.0	0.0
Spring cereals					
FOCUS Scenario	PEC $_{gl-max}$ (µg/L)				
Step 1					
	2.25	0.0	0.0	0.0	0.0
Step 2					
N-Europe	0.07	0.0	0.0	0.0	0.0
S-Europe	0.09	0.0	0.0	0.0	0.0

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 031: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole- thi-azocine (M12) for each organism group based on FOCUS Steps 1, 2 calculations for the use of SAP2101F**

Group		Fish acute	Fish prolonged	Inverteb. acute	Algae
Test species		*Oncorhynchus mykiss*	*Oncorhynchus mykiss*	*Daphnia magna*	*Pseudokirchn. sub-capitata*
Endpoint		LC_{50}	NOEC	EC_{50}	E_rC_{50}

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**
(µg/L)		49800*	320*	90000*	2250*
AF		100	10	100	10
RAC (µg/L)		498	32	900	225
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**				
Step 1					
	1.64	0.0	0.1	0.0	0.0
Step 2					
S-Europe	0.16	0.0	0.0	0.0	0.0

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold. *Endpoint values estimated from the a.s. with an assessment factor of 10

Table A3-32: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for each organism group based on FOCUS Steps 1, 2 (set 2) and 3 calculations for the use of SAP2101F (minimum dose)**

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***
Endpoint		HC_5	NOEC	EC_{50}	NOEC	E_rC_{50}
(µg/L)		52.7	39	680	320	10000
AF		9*	10	100	10	10
RAC (µg/L)		5.9	3.9	6.8	32	1000
Winter cereals						
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	73.92	**12.5**	**18.9**	**10.9**	**2.31**	0.07
Step 2						
N-Europe	4.99	0.84	**1.3**	0.73	0.16	Resolved at Step 1
S-Europe	4.23	0.72	**1.08**	0.6	0.13	
Step 3 – Multiple application						
D1/ditch	1.678	Resolved at Step 2	0.43	Resolved at step 1 and 2		
D1/stream	1.417		0.36			
D2/ditch	1.682		0.43			
D2/stream	1.471		0.38			
D3/ditch	1.662		0.43			
D4/pond	0.07987		0.02			
D4/stream	1.256		0.32			
D5/pond	0.09247		0.02			
D5/stream	1.449		0.37			
D6/ditch	1.67		0.43			
R1/pond	0.1516		0.04			
R1/stream	2.224		0.57			

R3/stream	2.977		0.76			
R4/stream	1.693		0.43			
Step 3 – Single application						
D1/ditch	1.907	Resolved at Step 2	0.49	Resolved at previous steps		
D1/stream	1.482		0.38			
D2/ditch	1.919		0.49			
D2/stream	1.63		0.42			
D3/ditch	1.9		0.49			
D4/pond	0.06558		0.02			
D4/stream	1.404		0.36			
D5/pond	0.06559		0.02			
D5/stream	1.517		0.39			
D6/ditch	1.879		0.48			
R1/pond	0.06559		0.02			
R1/stream	1.252		0.32			
R3/stream	1.759		0.45			
R4/stream	1.258		0.32			
Spring cereals						
FOCUS Scenario	**PEC $_{gl-max}$ (µg/L)**					
Step 1						
	73.92	**12.5**	**18.9**	**10.9**	**2.31**	0.07
Step 2						
N-Europe	2.76	0.47	0.71	0.41	0.1	Resolved at Step 1
S-Europe	4.23	0.72	**1.08**	0.6	0.13	
Step 3 -Multiple application						
D1/ditch	2.244	Resolved at Step 2	0.58	Resolved at step 2		Resolved at Step 1
D1/stream	1.455		0.37			
D3/ditch	1.663		0.43			
D4/pond	0.0882		0.02			
D4/stream	1.389		0.36			
D5/pond	0.08214		0.02			
D5/stream	1.435		0.37			
R4/stream	5.585		**1.43**			
Step 3 -Single application						
D1/ditch	1.924	Resolved at Step 2	0.49	Resolved at step 2		Resolved at Step 1
D1/stream	1.683		0.43			
D3/ditch	1.902		0.49			
D4/pond	0.06562		0.02			
D4/stream	1.555		0.40			
D5/pond	0.06561		0.02			
D5/stream	1.597		0.41			

R4/stream	3.109		0.80		

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
*According to EFSA Conclusion 2009 "Six species of fish were tested. Brown trout (*Salmo trutta*) was the most sensitive species tested, and this LC_{50} should be used in the higher tier risk assessment. Uncertainty regarding interspecies variation in sensitivity has been reduced. Hence, a TER trigger of 10 should be used."

Table A3-33: **Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for prolonged fish end-point group based on FOCUS Steps 4 calculations for the use of SAP2101F (minimum dose)**

Group		**Fish prolonged**
Test species		***Oncorhynchus mykiss***
Endpoint (µg/L)		NOEC 39
AF		10
RAC (µg/L)		3.9
Spring cereals		
FOCUS Scenario	**PEC gl-max (µg/L)**	**PEC/RAC < 1**
Step 4 – Multiple application, 5 meters of VFS		
R4/stream	3.630	0.93

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold.

Effects on bees (KCP 10.3.1)

Hazard quotients for bees

Honey bees

Risk assessment according to SANCO/10329/2002 rev.2 (final), October 17, 2002)

Table A3-34: First-tier assessment of the risk for bees due to the use of SAP2101F in cereals

Intended use Cereals **Active substance** Prothioconazole **Application rate (g/ha)** 2 x 120			
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}. Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>48.7	120	2.5
Contact toxicity	>200		0.6
Product Folpet **Application rate (g/ha)** 2 x 300			
Test design	**LD_{50} (lab.)** (µg/bee)	**Single application rate** (g/ha)	**Q_{HO}. Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>236	300	1.3
Contact toxicity	>200		1.5
Product SAP2101F **Application rate (g/ha)** 2 x 1140			
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}. Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	986	1140	1.2
Contact toxicity	2340		0.5

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Risk assessment according to EFSA Journal 2013; 11(7):3295

The Applicant would like to highlight that the guidance document used for this risk assessment is not yet noted and is currently under update. Therefore, the risk assessment is only presented for illustrative reasons and no conclusion should be drawn on the basis of these results until the updated and noted document is available.

Table A3-35: Screening step of the risk for bees due to the use of prothioconazole, folpet and SAP2101F in cereals according to EFSA Journal 2013; 11(7):329

Intended use **Active substance** **Application rate (g a.s./ha)**	Cereals Prothioconazole 2 x 120				
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**"Calculation factor"**	**HQ/ETR**	**Trigger**	**Risk indicator**
Acute oral toxicity	>48.7	7.6	0.00	0.2	Ok!
Acute contact toxicity	>200	1	2.5	42	Ok!
Intended use **Active substance** **Application rate (g a.s./ha)**	Cereals Folpet 2 x 300				
Test design	**LD_{50} (lab.) (µg a.s./bee)**	**"Calculation factor"**	**HQ/ETR**	**Trigger**	**Risk indicator**
Acute oral toxicity	>236	7.6	0.01	0.2	Ok!
Acute contact toxicity	>200	1	1.3	42	Ok!
Chronic oral toxicity	16.29	7.6	0.140	0.03	**Not Ok!**
Chronic larvae toxicity	2.16	4.4	0.61	0.2	**Not Ok!**
Intended use **Active substance** **Application rate (g/ha)**	Cereals SAP2101F 2 x 1140				
Test design	**LD_{50} (lab.)/NOED (µg a.s./bee)**	**"Calculation factor"**	**HQ/ETR**	**Trigger**	**Risk indicator**
Acute oral toxicity	986	7.6	0.00	0.2	Ok!
Acute contact toxicity	2340	1	1.2	42	Ok!
Chronic oral toxicity	99.21	7.6	0.077	0.03	**Not Ok!**
Chronic larvae toxicity	9.05	4.4	0.07	0.2	**Not Ok!**

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

Table A3-36: First tier of the risk for honeybees due to the use of SAP2101F in cereals according to EFSA Journal 2013; 11(7):329

Intended use **Active substance** **Application rate (g a.s./ha)**	Cereals Folpet 2 × 300								
Application	**BBCH**	**Category**	**Scenario**	**Ef**	**SV HB**	**TWA HB**	**ETR**	**trigger**	**Risk indicator**
Spray DW	30 - 39	chronic	treated crop	1	0.92	0.72	0.012	0.03	Ok!
	40 - 69	chronic	treated crop	1	0.92	0.72	0.012	0.03	Ok!
	30 - 39	chronic	weeds	0.5	2.9	0.72	0.019	0.03	Ok!
	40 - 69	chronic	weeds	0.3	2.9	0.72	0.012	0.03	Ok!

	30 - 39	chronic	field margin	0.0092	2.9	0.72	0.000	0.03	Ok!
	40 - 69	chronic	field margin	0.0092	2.9	0.72	0.000	0.03	Ok!
	30 - 39	chronic	adjacent crop	0.0033	5.8	0.72	0.000	0.03	Ok!
	40 - 69	chronic	adjacent crop	0.0033	5.8	0.72	0.000	0.03	Ok!
	30 - 39	chronic	next crop	1	0.54	0.72	0.007	0.03	Ok!
	40 - 69	chronic	next crop	1	0.54	0.72	0.007	0.03	Ok!
	30 - 39	larva	treated crop	1	0.15	0.85	0.02	0.2	Ok!
	40 - 69	larva	treated crop	1	0.15	0.85	0.02	0.2	Ok!
	30 - 39	larva	weeds	0.5	2.2	0.85	0.13	0.2	Ok!
	40 - 69	larva	weeds	0.3	2.2	0.85	0.08	0.2	Ok!
	30 - 39	larva	field margin	0.0092	2.2	0.85	0.00	0.2	Ok!
	40 - 69	larva	field margin	0.0092	2.2	0.85	0.00	0.2	Ok!
	30 - 39	larva	adjacent crop	0.0033	4.4	0.85	0.00	0.2	Ok!
	40 - 69	larva	adjacent crop	0.0033	4.4	0.85	0.00	0.2	Ok!
	30 - 39	larva	next crop	1	0.4	0.85	0.05	0.2	Ok!
	40 - 69	larva	next crop	1	0.4	0.85	0.05	0.2	Ok!

Intended use	Cereals
Active substance	SAP2101F
Application rate (g/ha)	2 × 1140

Application	**BBCH**	**Category**	**Scenario**	**Ef**	**SV HB**	**TWA HB**	**ETR**	**trigger**	**Risk indicator**
	30 - 39	chronic	treated crop	1	0.92	0.72	0.008	0.03	Ok!
	40 - 69	chronic	treated crop	1	0.92	0.72	0.008	0.03	Ok!
	30 - 39	chronic	weeds	0.5	2.9	0.72	0.012	0.03	Ok!
	40 - 69	chronic	weeds	0.3	2.9	0.72	0.007	0.03	Ok!
	30 - 39	chronic	field margin	0.0092	2.9	0.72	0.000	0.03	Ok!
	40 - 69	chronic	field margin	0.0092	2.9	0.72	0.000	0.03	Ok!
	30 - 39	chronic	adjacent crop	0.0033	5.8	0.72	0.000	0.03	Ok!
	40 - 69	chronic	adjacent crop	0.0033	5.8	0.72	0.000	0.03	Ok!
	30 - 39	chronic	next crop	1	0.54	0.72	0.004	0.03	Ok!
	40 - 69	chronic	next crop	1	0.54	0.72	0.004	0.03	Ok!
	30 - 39	larva	treated crop	1	0.15	0.85	0.02	0.2	Ok!
	40 - 69	larva	treated crop	1	0.15	0.85	0.02	0.2	Ok!
	30 - 39	larva	weeds	0.5	2.2	0.85	0.12	0.2	Ok!
	40 - 69	larva	weeds	0.3	2.2	0.85	0.07	0.2	Ok!
	30 - 39	larva	field margin	0.0092	2.2	0.85	0.00	0.2	Ok!
	40 - 69	larva	field margin	0.0092	2.2	0.85	0.00	0.2	Ok!
	30 - 39	larva	adjacent crop	0.0033	4.4	0.85	0.00	0.2	Ok!
	40 - 69	larva	adjacent crop	0.0033	4.4	0.85	0.00	0.2	Ok!
	30 - 39	larva	next crop	1	0.4	0.85	0.04	0.2	Ok!
	40 - 69	larva	next crop	1	0.4	0.85	0.04	0.2	Ok!

Bumble bees

- *Risk assessment according to SANCO/10329/2002 rev 2 final*

Table 0-6: First-tier assessment of the risk for bumble bees due to the use of SAP2101F in cereals

Intended use	Cereals
Active substance	SAP2101F
Application rate (g a.s./ha)	2 × 1000

Test design	**LD_{50} (lab.) (µg a.s./bee)**	**Single application rate (g a.s./ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>1083.88	1000	<0.9
Contact toxicity	>1153.09		<0.9

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.

- *Risk assessment according to EFSA Journal 2013; 11(7):3295*

Table 9.6-7: Screening step of the risk for bumble bees due to the use of SAP2101F in cereals according to EFSA Journal 2013; 11(7):329

Intended use	Cereals
Active substance	SAP2101F
Application rate (g a.s./ha)	2 × 1000

Test design	**LD_{50} (lab.) (µg a.s./bee)**	**"Calculation factor"**	**HQ/ETR**	**Trigger**	**Risk indicator**
Acute oral toxicity	>1083.88	11.2	0.01	0.036	OK
Acute contact toxicity	>1153.09	1	0.9	7	OK

Effects on arthropods other than bees (KCP 10.3.2)

Risk assessment for in-field exposure

Table A3-37: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of SAP2101F in cereals

Intended use	Cereals
Active substance/product	Prothioconazole + Folpet/ SAP2101F
Application rate (mL/ha)	2 × 1140
MAF	1.7

Test species Tier I	**LR_{50} (lab.) (mL/ha)**	**$PER_{in\text{-}field}$ (mL/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 1**
Typhlodromus pyri	>5100	1938	< 0.38
Aphidius rhopalosiphi	>5100		< 0.38
Coccinella septempunctata L.	>5100		< 0.38
Chrysoperla carnea	>5100		< 0.38

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment. Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Table A3-38: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of SAP2101F in cereals

Intended use	Cereals
Active substance/product	Prothioconazole + Folpet/ SAP2101F

Application rate (g/ha)	2 × 1140				
MAF	1.7				
vdf	10 (2D); 1 (3D)				
Test species **Tier I**	**LR_{50} (lab.) (mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (mL/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri (2D)	>5100	2.38	4.61	5	<0.01
Aphidius rhopalosiphi (3D)	>5100		46.1		<0.01
Coccinella septempunctata L. (2D)	>5100		4.61		<0.01
Chrysoperla carnea (2D)	>5100				<0.01

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.
*If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

Effects on non-target soil meso- and macrofauna (KCP 10.4)

First-tier risk assessment

Table 039: First-tier assessment of the chronic risk of prothioconazole, folpet, respective metabolites and SAP2101F for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of SAP2101F in cereals (minimum dose)

Intended use	Cereals		
Acute effects on earthworms: no longer a data requirement**.**			
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Prothioconazole	0.665*	0.033	20.2
Prothioconazole-S-methyl (M01)	50*	0.009	5555.6
Prothioconazole-desthio (M04)	0.5*	0.032	15.6
Folpet	5.18	0.132	39.2
Phthalimide	0.518*	0.045	11.5
Phthalamic acid	0.518*	0.007	74
Phthalic acid	0.518*	0.008	64.8
SAP2101F	11.7*	0.304	38.5
Chronic effects on other soil macro- and mesofauna			
Product/active substance	**NOEC** (mg/kg dw)	**PEC_{soil}** (mg/kg dw)	**TER_{lt}** (criterion TER ≥ 5)
Prothioconazole *Folsomia candida*	64	0.033	1939.4
Prothioconazole *Hypoaspis aculeifer*	100	0.033	3030.30
Prothioconazole-S-methyl (M01) *Folsomia candida*	31.6	0.009	3511.1
Prothioconazole-desthio (M04) *Folsomia candida*	62.5	0.032	1953.1
SAP2101F *Folsomia candida*	≥1000	0.304	3289.5
	≥500*		1644.7

Prothioconazole-S-methyl (M01) *Hypoaspis aculeifer*	10**	0.009	1111.1
Prothioconazole-desthio (M04) *Hypoaspis aculeifer*	10**	0.032	312.5
SAP2101F *Hypoaspis aculeifer*	≥1000	0.304	3289.5
	≥500*		1644.7

** Endpoint derived from the active substance with application of assessment factor of 10

Effects on soil microbial activity (KCP 10.5)

Table 040: Assessment of the risk for effects on soil micro-organisms due to the use of SAP2101F in crop cereals

Intended use			
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Prothioconazole	2.67 (at 28 d)	0.033	Yes
JAU 6476-desthio	0.267 (at 28 d)	0.032	Yes
JAU 6476-S-methyl	2.67 (at 28 d)	0.009	Yes
Folpet	21.40 (at 28 d)	0.132	Yes
Phthalimide	2.34 (at 28 d)*	0.045	Yes
Phthalamic acid	2.34 (at 28 d)*	0.007	Yes
Phthalic acid	2.34 (at 28 d)*	0.008	Yes
SAP2101F	23.40 (at 28 d)	0.304	Yes
C-mineralisation: no longer a data requirement			

Effects on non-target terrestrial plants (KCP 10.6)

Table A3-41: Assessment of the risk for non-target plants due to the use of SAP2101F in crop cereals

Intended use	Cereals
Active substance/product	Prothioconazole + Folpet / SAP2101F
Application rate (mL/ha)	1140
MAF	

Test species	**ER_{50} (mL/ha)**	**Drift rate**	**Min single application (mL/ha)**	**TER criterion: TER ≥ 1**
Brassica napus *Beta vulgaris* *Glycine max* *Lycopersicon Esculentum* *Lolium perenne* *Allium cepa*	>2850	Not applicable for the tier-1 risk assessment	1140	<2.5

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Appendix 4 Additional calculations based on soil DT50 of 4.68 days for folpet

zRMS comments:

The calculations are provided for the Applicant have been included in the the risk assessment presented at Point 9.5.2

As indicated in section B8, the following calculations are provided for completeness and the Applicant stands by the risk assessment presented above.

Table A4-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for folpet for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of SAP2101F in cereals

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
Test species		-	*Oncorhynchus mykiss*	*Daphnia magna*	*Daphnia magna*	*Scenedesmus subspicatus*
Endpoint		HC_5	NOEC	EC_{50}	NOEC	E_rC_{50}
(µg/L)		52.7	39	680	320	10000
AF		9	10	100	10	10
RAC (µg/L)		5.9	3.9	6.8	32	1000
FOCUS Scenario	PEC $_{gl\text{-}max}$ (µg/L)					
Winter cereals						
Step 3 – Multiple applications (worst-case value between sets 1 and 2, further details refer to B8)						
D1/ditch	2.522	0.4	0.6	0.4	0.1	0.0
D1/stream	2.129	0.4	0.5	0.3	0.1	0.0
D2/ditch	3.341	0.6	0.9	0.5	0.1	0.0
D2/stream	2.212	0.4	0.6	0.3	0.1	0.0
D3/ditch	2.493	0.4	0.6	0.4	0.1	0.0
D4/pond	0.107	0.0	0.0	0.0	0.0	0.0
D4/stream	1.882	0.3	0.5	0.3	0.1	0.0
D5/pond	0.130	0.0	0.0	0.0	0.0	0.0
D5/stream	2.173	0.4	0.6	0.3	0.1	0.0
D6/ditch	2.504	0.4	0.6	0.4	0.1	0.0
R1/pond	0.443	0.1	0.1	0.1	0.0	0.0
R1/stream	6.840	**1.2**	**1.8**	**1.01**	0.2	0.0
R3/stream	7.645	**1.3**	**2.0**	**1.1**	0.2	0.0
R4/stream	6.974	**1.2**	**1.8**	**1.03**	0.2	0.0
Step 3 – Single application (worst-case value between sets 1 and 2, further details refer to B8)						
D1/ditch	2.864	0.5	0.7	0.4	0.1	0.0
D1/stream	2.227	0.4	0.6	0.3	0.1	0.0
D2/ditch	3.335	0.6	0.9	0.5	0.1	0.0
D2/stream	2.445	0.4	0.6	0.4	0.1	0.0
D3/ditch	2.850	0.5	0.7	0.4	0.1	0.0
D4/pond	0.098	0.0	0.0	0.0	0.0	0.0

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae
D4/stream	2.107	0.4	0.5	0.3	0.1	0.0
D5/pond	0.098	0.0	0.0	0.0	0.0	0.0
D5/stream	2.275	0.4	0.6	0.3	0.1	0.0
D6/ditch	2.818	0.5	0.7	0.4	0.1	0.0
R1/pond	0.131	0.0	0.0	0.0	0.0	0.0
R1/stream	1.878	0.3	0.5	0.3	0.1	0.0
R3/stream	2.638	0.4	0.7	0.4	0.1	0.0
R4/stream	1.886	0.3	0.5	0.3	0.1	0.0
Spring cereals						
Step 3 – Multiple applications (worst-case value between sets 1 and 2, further details refer to B8)						
D1/ditch	3.057	0.5	0.8	0.4	0.1	0.0
D1/stream	2.182	0.4	0.6	0.3	0.1	0.0
D3/ditch	2.494	0.4	0.6	0.4	0.1	0.0
D4/pond	0.125	0.0	0.0	0.0	0.0	0.0
D4/stream	2.082	0.4	0.5	0.3	0.1	0.0
D5/pond	0.113	0.0	0.0	0.0	0.0	0.0
D5/stream	2.152	0.4	0.6	0.3	0.1	0.0
R4/stream	9.871	**1.7**	**2.5**	**1.5**	0.3	0.0
Step 3 – Single application (worst-case value between sets 1 and 2, further details refer to B8)						
D1/ditch	2.888	0.5	0.7	0.4	0.1	0.0
D1/stream	2.523	0.4	0.6	0.4	0.1	0.0
D3/ditch	2.853	0.5	0.7	0.4	0.1	0.0
D4/pond	0.098	0.0	0.0	0.0	0.0	0.0
D4/stream	2.332	0.4	0.6	0.3	0.1	0.0
D5/pond	0.098	0.0	0.0	0.0	0.0	0.0
D5/stream	2.395	0.4	0.6	0.4	0.1	0.0
R4/stream	6.020	**1.02**	**1.5**	0.9	0.2	0.0

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

For the Step 4 calculations, only the comparison of the additional PEC_{SW} values with the lowest RAC of 3.9 µg/L will be presented here.

Table A4-2: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for folpet based on FOCUS Step 4 calculations and toxicity data for fish chronic with mitigation of spray drift and run-off for the use of SAP2101F in cereals

Intended use	Cereals
Active substance	folpet
Application rate (g/ha)	2 x 450
Winter Cereals - Multiple applications –10 meters of vegetated filter strip	
R1/stream	3.107
R3/stream	3.489
R4/stream	3.173

RAC (µg/L) 3.9	**PEC/RAC ratio**
R1/stream	0.8
R3/stream	0.9
R4/stream	0.8
Spring Cereals - Multiple applications –20 m of vegetated filter strip	
R4/strean	2.332
RAC (µg/L) 3.9	**PEC/RAC ratio**
R4/strean	0.6
Spring Cereals - Single application –10 meters of vegetated filter strip	
R4/strean	2.717
RAC (µg/L) 3.9	**PEC/RAC ratio**
R4/strean	0.7

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Maximum dose

Winter cereals:

- a non-spray buffer zone of *10 m of vegetated filter strip*, for multiple application for R1, R3 and R4 scenarios.

Spring cereals:

- a non-spray buffer zone of *20 m of vegetated filter strip*, for multiple application for R4 scenario;
- a non-spray buffer zone of *10 m of vegetated filter strip*, for single application for R4 scenario.