REGISTRATION REPORT

Part B
Section 9
Ecotoxicology

Detailed summary of the risk assessment

Product code: ADM.03503.F.1.A

Product name(s): see Part A

Chemical active substances:

Fluxapyroxad, 75 g/L

Prothioconazole, 150 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Country organisation/representative

as specified in Part A

Submission date: April 2022

MS Finalisation date: June 2023 (initial Core Assessment)

December 2023 (final Core Assessment)

Version history

When	What
2022/04	Version 1 Applicant
June 2023	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.
December 2023	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 cMS and the Applicant are highlighted in yellow. Information no longer relevant is ~~struck through~~ and shaded.

DATA PROTECTION CLAIM

In order to present a dossier fully compliant with today's requirements (Reg. 284/2013), studies have been performed on ADM.03503.F.1.A. Under Article 59, Regulation 1107/2009/EC, on behalf of the Sponsor Company the applicant claims data protection for the studies conducted with ADM.03503.F.1.A. The data protection status and corresponding justification as valid for the respective country will be confirmed in the respective PART A.

STATEMENT FOR OWNERSHIP

The summaries and evaluations contained in this document may be based on unpublished proprietary data submitted for the purpose of the assessment undertaken by the regulatory authority that prepared it. Other registration authorities should not grant, amend, or renew a registration on the basis of the summaries and evaluation of unpublished proprietary data contained in this document unless they have received the data on which the summaries and evaluation are based, either –

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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							
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: devel-opmental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g saf-ener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. num-ber 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 arthro-	Soil organisms	Non-target plants
1	Belgium	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici Drechslera tritici-repentis (DTR) Puccinia striiformis Puccinia recondita, Blumeria graminis f. sp. tritici, Fusarium + microdo-chium*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
2	Belgium	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis Pyrenophora teres Ramularia collo-cygni Puccinia hordei Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
3	Belgium	Rye (SECCW)	F	*Rhyncosporium secalis Puccinia recondita Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
4	Belgium	Triticale (TTLSS)	F	*Zymoseptoria tritici Puccinia recondita Puccinia striiformis Drechslera tritici-repentis (DTR) Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15						
5	Netherlands	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici* *Drechslera tritici-repentis (DTR)* *Puccinia striiformis* *Puccinia recondita,* *Blumeria graminis f. sp. tritici,* *Fusarium + microdochium*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
6	Netherlands	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis* *Pyrenophora teres* *Ramularia collo-cygni* *Puccinia hordei* *Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
7	Netherlands	Rye (SECCW)	F	*Rhyncosporium secalis* *Puccinia recondita* *Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
8	Netherlands	Triticale (TTLSS)	F	*Zymoseptoria tritici* *Puccinia recondita* *Puccinia striiformis* *Drechslera tritici-repentis (DTR)* *Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
9	Czechia	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici* *Drechslera tritici-repentis (DTR)* *Puccinia striiformis* *Puccinia recondita,* *Blumeria graminis f. sp. tritici,* *Fusarium + microdochium*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
10	Czechia	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis* *Pyrenophora teres* *Ramularia collo-cygni* *Puccinia hordei* *Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15						
11	Czechia	Rye (SECCW)	F	*Rhyncosporium secalis* *Puccinia recondita* *Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
12	Czechia	Triticale (TTLSS)	F	*Zymoseptoria tritici* *Puccinia recondita* *Puccinia striiformis* *Drechslera tritici-repentis (DTR)* *Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
13	Germany	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici* *Drechslera tritici-repentis (DTR)* *Puccinia striiformis* *Puccinia recondita,* *Blumeria graminis f. sp. tritici,* *Fusarium + microdo-chium*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
14	Germany	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis* *Pyrenophora teres* *Ramularia collo-cygni* *Puccinia hordei* *Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
15	Germany	Rye (SECCW)	F	*Rhyncosporium secalis* *Puccinia recondita* *Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
16	Germany	Triticale (TTLSS)	F	*Zymoseptoria tritici* *Puccinia recondita* *Puccinia striiformis* *Drechslera tritici-repentis (DTR)* *Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
17	Ireland	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici* *Drechslera tritici-repentis (DTR)* *Puccinia striiformis* *Puccinia recondita,* *Blumeria graminis f. sp. tritici,* *Fusarium + microdo-*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15						
				chium																
18	Ireland	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis* *Pyrenophora teres* *Ramularia collo-cygni* *Puccinia hordei* *Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
19	Ireland	Rye (SECCW)	F	*Rhyncosporium secalis* *Puccinia recondita* *Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
20	Ireland	Triticale (TTLSS)	F	*Zymoseptoria tritici* *Puccinia recondita* *Puccinia striiformis* *Drechslera tritici-repentis (DTR)* *Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400			A	A	R	A	A	A	A
21	Poland	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici* *Drechslera tritici-repentis (DTR)* *Puccinia striiformis* *Puccinia recondita,* *Blumeria graminis f. sp. tritici,* *Fusarium + microdo-chium*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
22	Poland	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis* *Pyrenophora teres* *Ramularia collo-cygni* *Puccinia hordei* *Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
23	Poland	Rye (SECCW)	F	*Rhyncosporium secalis* *Puccinia recondita* *Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
24	Poland	Triticale (TTLSS)	F	*Zymoseptoria tritici* *Puccinia recondita* *Puccinia striiformis* *Drechslera tritici-repentis (DTR)* *Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15						
25	Slovakia	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici Drechslera tritici-repentis (DTR) Puccinia striiformis Puccinia recondita, Blumeria graminis f. sp. tritici, Fusarium + microdo-chium*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
26	Slovakia	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis Pyrenophora teres Ramularia collo-cygni Puccinia hordei Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
27	Slovakia	Rye (SECCW)	F	*Rhyncosporium secalis Puccinia recondita Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
28	Slovakia	Triticale (TTLSS)	F	*Zymoseptoria tritici Puccinia recondita Puccinia striiformis Drechslera tritici-repentis (DTR) Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
29	Hungary	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici Drechslera tritici-repentis (DTR) Puccinia striiformis Puccinia recondita, Blumeria graminis f. sp. tritici, Fusarium + microdo-chium*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
30	Hungary	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis Pyrenophora teres Ramularia collo-cygni Puccinia hordei Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15						
31	Hungary	Rye (SECCW)	F	*Rhyncosporium secalis* *Puccinia recondita* *Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
32	Hungary	Triticale (TTLSS)	F	*Zymoseptoria tritici* *Puccinia recondita* *Puccinia striiformis* *Drechslera tritici-repentis (DTR)* *Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
33	Slovenia	Winter wheat (TRZAW) Spring wheat (TRZAS)	F	*Zymoseptoria tritici* *Drechslera tritici-repentis (DTR)* *Puccinia striiformis* *Puccinia recondita,* *Blumeria graminis f. sp. tritici,* *Fusarium + microdo-chium*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
34	Slovenia	Winter barley (HORVW) Spring barley (HORVS)	F	*Rhyncosporium secalis* *Pyrenophora teres* *Ramularia collo-cygni* *Puccinia hordei* *Blumeria graminis f. sp. hordei*	foliar, spraying, overall	-/ BBCH 30-65 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
35	Slovenia	Rye (SECCW)	F	*Rhyncosporium secalis* *Puccinia recondita* *Puccinia striiformis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	A	A	A	A
36	Slovenia	Triticale (TTLSS)	F	*Zymoseptoria tritici* *Puccinia recondita* *Puccinia striiformis* *Drechslera tritici-repentis (DTR)* *Blumeria graminis*	foliar, spraying, overall	-/ BBCH 30-69 spring	a) 1 (-) b) 1 (-)	-	a) 1.25 L/ha b) 1.25 L/ha	a) 93.75 / 187.5 b) 93.75 / 187.5	125-400		Range of rates 1.0-1.25L	A	A	R	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

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
(15) Overall conclusions - explanation for the column 15 is below *

*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

9.1.1 Overall conclusions

9.1.1.1 Effects on birds (KCP 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 for birds and mammals was carried out according to the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438).

Birds

The acute and reproductive (long-term) risk for birds from dietary exposure to fluxapyroxad, prothioconazole and the prothioconazole-desthio metabolite is indicated to be acceptable based on Screening Step and/or Tier 1 assessments, including considerations of combined exposure.

Likewise, acceptable risk is indicated for the exposure via drinking water and the indirect exposure via secondary poisoning for earthworm- and fish-eating birds.

Overall, the risk for birds exposed following the intended uses of ADM.03503.F.1.A is acceptable.

Terrestrial vertebrates other than birds

The acute and reproductive (long-term) risk for terrestrial vertebrates other than birds from dietary exposure to fluxapyroxad, prothioconazole and the prothioconazole-desthio metabolite is indicated to be acceptable based on Screening Step and/or Tier 1 assessments, including considerations of combined exposure. For the small herbivorous scenario, an acceptable risk is presented based on higher tier assessments accounting for revised crop interception.

Likewise, acceptable risk is indicated for the exposure via drinking water and the indirect exposure via secondary poisoning for earthworm- and fish-eating mammals.

Overall, the risk for terrestrial vertebrates other than birds exposed following the intended uses of ADM.03503.F.1.A is acceptable.

9.1.1.2 Effects on aquatic organisms (KCP 10.2)

The risk assessment for aquatic organisms was carried out according to the Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters (EFSA Journal 2013;11(7):3290).

Based on the available data and risk assessment for aquatic organisms including considerations on potential mixture toxicity, acceptable risk is indicated if a 10 m vegetated buffer distance is taken into account for both uses, i.e. winter and spring cereals.

9.1.1.3 Effects on bees (KCP 10.3.1)

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).

An acceptable acute risk is indicated for exposure of bees towards the formulated product as well as the individual active substances for the intended worst-case use of ADM.03503.F.1.A.

9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

The risk assessment was conducted according to the ESCORT 2 Guidance Document (2000) and the Guidance Document on Terrestrial Ecotoxicology (2002).

An acceptable in-field and off-field risk is indicated for exposure of terrestrial non-target arthropods other than bees towards the formulated product for the intended worst-case use of ADM.03503.F.1.A without the necessity to account for risk mitigations.

9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil microbial activity (KCP 10.5)

The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

Meso- and macrofauna

An acceptable risk is indicated for soil macro- and meso-fauna for the intended worst-case use of ADM.03503.F.1.A in cereals with Toxicity Exposure Ratios greater than five for the active substances, relevant metabolites as well as formulated product, respectively.

Soil microbial functions

An acceptable risk is indicated for soil microflora for the intended worst-case use of ADM.03503.F.1.A in cereals with NOAECs (i.e. the maximum tested concentration with effects < 25% at ≤ 100 days) greater than the maximum predicted environmental concentrations of the active substances, relevant metabolites as well as formulated product, respectively.

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

The risk assessment was conducted according to the Guidance Document on Terrestrial Ecotoxicology (2002).

An acceptable off-field risk is indicated for exposure of terrestrial non-target plants towards the formulated product for the intended worst-case use of ADM.03503.F.1.A without the necessity to account for risk mitigations.

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

No further relevant data available nor considered necessary.

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).

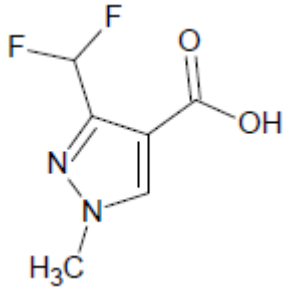
Table 9.1-2: Critical use pattern of ADM.03503.F.1.A grouped according to organism groups

Grouping according to organism groups			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Terrestrial vertebrates (Birds and Mammals; **9.2** and **9.3**)	according to GAP; refer to Document B0, 1x 1.25 L product/ha in winter and spring cereals	Scenarios according to EFSA Birds and Mammals Guidance (2009): **Crop growth stage: BBCH 30-69** **Scenario: ´Cereals´**	**BBCH 30-39**: secondary poisoning (earthworm-eating); minimum crop interception **BBCH 30-69**: cereals (post-emergence): dietary risk
Aquatic organisms (**9.5**)	according to GAP; refer to Document B0, 1x 1.25 L product/ha in winter and spring cereals	Crops according to FOCUS surface water guidance (2015)[1]	**BBCH 30-69**: default window covering post-emergence crop
Bees (**9.6**)	according to GAP; refer to Document B0, 1x 1.25 L product/ha in winter and spring cereals	No distinction required	No distinction required
Terrestrial non-target arthropods other than bees (**9.7**)	according to GAP; refer to Document B0, 1x 1.25 L product/ha in winter and spring cereals	No distinction required	No distinction required
Soil meso- and macrofauna / soil microorganisms (**9.8** and **9.9**)	according to GAP; refer to Document B0, 1x 1.25 L product/ha in winter and spring cereals	**Crop growth stage: BBCH 30-69**	**BBCH 30-39**: minimum crop interception
Non-target terrestrial plants (**9.10**)	according to GAP; refer to Document B0, 1x 1.25 L product/ha in winter and spring cereals	No distinction required	No distinction required

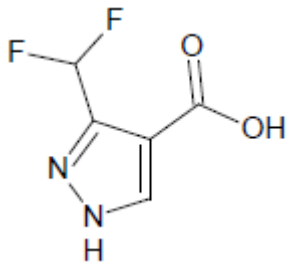
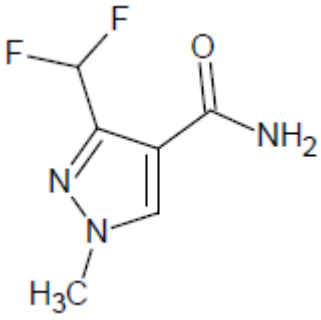
9.1.3 Consideration of metabolites

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 ADM.03503.F.1.A is indicated in the table.

Table 9.1-3 Metabolites of Fluxapyroxad

Metabolite	Chemical structure	Molar mass	Maximum occurrence in com-partments	Risk assessment required?
3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxylic Acid (**M700F001**)		176.12 g/mol	~~Soil: 12.1% (mean)~~ ~~Water: 10.9%~~	~~**Soil** (Terrestrial vertebrates: secondary poisoning, soil organisms)~~ ~~**Water** (Aquatic organisms)~~

[1] FOCUS (2015): Generic guidance for FOCUS surface water Scenarios. Version 1.4.

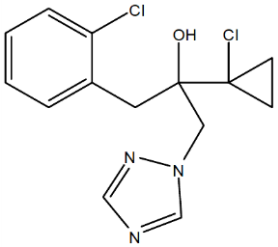
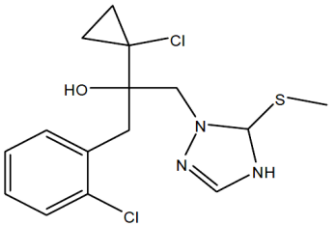
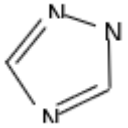
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
3-(difluoromethyl)-1*H*pyrazole-4-carboxylic acid (**M700F002**)		162.1 g/mol	~~Soil: 70.5% (still increasing)~~	~~**Soil** (Terrestrial vertebrates: secondary poisoning, soil organisms) **Water** (Aquatic organisms)~~
3-(difluoromethyl)-1-methyl-1*H*-pyrazole-4-carboxamide (**M700F007**)		175.1 g/mol	~~Water: 17.7%~~	~~**Water** (Aquatic organisms)~~

zRMS comments:

Metabolites relevant for soil and water compartment listed in Table 9.1-3 are the same as indicated in EFSA EFSA Journal 2012; 10(1): 2522.

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 Prothioconazole

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
Prothiocona-zole-desthio (**M04**) (JAU-desthio)		312.2 g/mol	~~Soil: 57.1%~~ ~~Water: 32.3%~~ ~~Sediment: 26.9%~~ ~~Water/sediment: 54.6%~~	**Soil** (Terrestrial vertebrates: secondary poisoning, soil organisms) **Water** (Aquatic organisms)
Prothioconazole-S-methyl (**M01**) (JAU-S-methyl)		358.3 g/mol	~~Soil: 14.6%~~ ~~Water/sediment: 77% (anaerob)~~	**Soil** (Terrestrial vertebrates: secondary poisoning, soil organisms) **Water** (Aquatic organisms)
1,2,4-triazole (**M13**)		69.065 g/mol	~~Water: 37.2%~~ ~~Sediment: 4.6%~~ ~~Water/sediment: 41.8%~~	**Water** (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 (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 9.1-4.

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with the active substances fluxapyroxad and prothioconazole as well as the prothioconazole metabolite M04. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of formulation were not evaluated as part of the EU assessments of the active substances fluxapyroxad and prothioconazole.
However, the provision of further data on the formulation ADM.03503.F.1.A is not considered essential, because the risk for terrestrial vertebrates other than birds is adequately addressed based on the data for the active substances and relevant metabolites.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes. However, in deviation to the agreed acute oral endpoints, extrapolated LD_{50} estimates are considered for fluxapyroxad and prothioconazole. Further justifications are provided below (Refer to Point 9.2.1.1). This approach is considered to be most appropriate to account for potential combined effects not disregarding the actual toxicity data of the individual active substances, rather than the worst-case approach proposed by EFSA for initial assessments which relates exposure of all actives expressed in equivalents of the active with the lowest available endpoint."

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

Species	Substance	Exposure System	Results	Reference
Fluxapyroxad				
Bobwhite quail (*Colinus virginianus*)	Fluxapyroxad (a.s.)	Acute oral toxicity; gavage	LD_{50} > 2000 mg a.s./kg bw $LD_{50;\ extrapolated}$ = 3776 mg a.s./kg bw [a)]	EFSA Journal 2012; 10(1): 2522
Mallard duck (*Anas platyrhynchos*)	Fluxapyroxad (a.s.)	Acute oral toxicity; gavage	**LD_{50} > 2000 mg a.s./kg bw** **$LD_{50;\ extrapolated}$ = 3776 mg a.s./kg bw** [a)]	EFSA Journal 2012; 10(1): 2522
Bobwhite quail (*Colinus virginianus*)	Fluxapyroxad (a.s.)	Short-term (8 day); dietary	LC_{50} > 5000 mg a.s./kg diet LDD_{50} > 912.00 mg a.s./kg bw	EFSA Journal 2012; 10(1): 2522
Mallard duck (*Anas platyrhynchos*)	Fluxapyroxad (a.s.)	Short-term (8 day); dietary	LC_{50} > 5000 mg a.s./kg diet LDD_{50} > 1716 mg a.s./kg bw	EFSA Journal 2012; 10(1): 2522
Bobwhite quail (*Colinus virginianus*)	Fluxapyroxad (a.s.)	Reproductive toxicity; dietary	NOEC = 1000 mg a.s./kg diet NOEL = 74.6 mg a.s./kg bw/day	EFSA Journal 2012; 10(1): 2522
Mallard duck (*Anas platyrhynchos*)	Fluxapyroxad (a.s.)	Reproductive toxicity; dietary	NOEC = 300 mg a.s./kg diet **NOEL = 33.6 mg a.s./kg bw/day**	EFSA Journal 2012; 10(1): 2522
Prothioconazole and relevant metabolites				
Bobwhite quail (*Colinus virginianus*)	Prothioconazole (a.s.)	Acute oral toxicity; gavage	**LD_{50} > 2000 mg a.s./kg bw** **$LD_{50;\ extrapolated}$ = 3776 mg a.s./kg bw** [a)]	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail (*Colinus virginianus*)	Prothiocona-zole-desthio (M04)	Acute oral toxicity; gavage	**LD_{50} > 2000 mg/kg bw** [b)]	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail (*Colinus virginianus*)	Prothioconazole (a.s.)	Short-term (5 day); dietary	LC_{50} > 5000 mg a.s./kg diet LDD_{50} > 1413 mg a.s./kg bw	EFSA Scientific Report (2007) 106, 1-98
Mallard duck (*Anas platyrhynchos*)	Prothioconazole (a.s.)	Short-term (5 day); dietary	LC_{50} > 5000 mg a.s./kg diet LDD_{50} > 2457 mg a.s./kg bw	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail (*Colinus virginianus*)	Prothiocona-zole-desthio (M04)	Short-term (5 day); dietary	LC_{50} = 4090 mg/kg diet LDD_{50} > 297 mg/kg bw	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Exposure System	Results	Reference
Bobwhite quail (*Colinus virginianus*)	Prothioconazole (a.s.)	Reproductive toxicity (22 weeks); dietary	NOEC ≥ 1000 mg a.s./kg diet NOEL ≥ 86 mg a.s./kg bw/d	EFSA Scientific Report (2007) 106, 1-98
Mallard duck (*Anas platyrhynchos*)	Prothioconazole (a.s.)	Reproductive toxicity (21 weeks); dietary	NOEC = 700 mg a.s./kg diet **NOEL = 78 mg a.s./kg bw/d**	EFSA Scientific Report (2007) 106, 1-98
Bobwhite quail (*Colinus virginianus*)	Prothiocona-zole-desthio (M04)	Reproductive toxicity (20 weeks); dietary	NOEC = 173 mg/kg diet **NOEL = 14.8 mg/kg bw/d**	EFSA Scientific Report (2007) 106, 1-98
Mallard duck (*Anas platyrhynchos*)	Prothiocona-zole-desthio (M04)	Reproductive toxicity (20 weeks); dietary	NOEC ≥ 500 mg/kg diet NOEL ≥ 63 mg/kg bw/d	EFSA Scientific Report (2007) 106, 1-98

a.s. technical active substance; **Bold**: Endpoints used for risk assessments

[a)] Extrapolated based on a factor of 1.888 as in accordance with EFSA (2009)[2] for data without mortalities at the limit dose and with 10 birds tested

[b)] 30% mortality observed at the limit dose; no extrapolation applicable

zRMS comments:

Avian toxicity data for fluxapyroxad, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.2-1 above has been confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

Potential mixture toxicity

In order to assess the potential for combined effects on birds, predicted acute mixture toxicity was calculated conservatively assuming dose additivity of the active substances based on the worst-case assumption that the active substances have the same mode of action.

In the following table, a ´toxicity per fraction´ assessment (for an assumed concentration addition) is presented for both, acute as well as reproductive toxicity based on the fractions of active substances as in the formulated product.

The assessment is presented for the pairings of fluxapyroxad with either the parent prothioconazole or the prothioconazole-desthio metabolite assuming 100% generation from parent prothioconazole, i.e. corresponding to an substance content of 170 g prothioconazole-desthio/L accounting for a molecular weight ratio of 1.1026 (344.26 g/mol : 312.2 g/mol), respectively the inverse value of 0.9069.

[2] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. doi: 10.2903/j.efsa.2009.1438.

Table 9.2-2: ´Toxicity per fraction´ assessment – additive mixture toxicity

Organism group	Time scale	Test substance	Fraction in the mixture ($x_{a.s.}$)	LD_{50} / NO(A)EL [mg a.s./kg bw(/d)]	Toxicity per fraction for CA/**Surrogate endpoint**	Contribution to overall toxicity [%]
Birds	acute	Fluxapyroxad	0.33	3776 [b]	11328.0	**33.3**
		Prothioconazole	0.67	3776 [b]	5664.0	**66.7**
		ADM.03503.F.1.A	1.0	n.a.	**3776.0**	n.a.
		Fluxapyroxad	0.36 [a]	3776 [b]	10623.1	**22.6**
		Prothioconazole-desthio	0.64 [a]	> 2000	> 3102.9	**77.4**
		ADM.03503.F.1.A	1.0	n.a.	**> 2401.5**	n.a.
	chronic / reprod.	Fluxapyroxad	0.33	33.6	100.8	**53.7**
		Prothioconazole	0.67	78	117.0	**46.3**
		ADM.03503.F.1.A	1.0	n.a.	**54.1**	n.a.
		Fluxapyroxad	0.36 [a]	33.6	94.5	**19.5**
		Prothioconazole-desthio	0.64 [a]	14.8	23.0	**80.5**
		ADM.03503.F.1.A	1.0	n.a.	**18.5**	n.a.

n.a. not available/not applicable; CA: Concentration Addition.
[a] Calculated from active substance contents for prothioconazole (150 g/L) based on the molar masses of 344.26 and 312.2 g/mol for prothioconazole and prothioconazole-desthio (i.e. corresponding to 136 g/L), respectively.
[b] Extrapolated endpoint based on limit dose endpoint multiplied by a factor of 1.888 as in accordance with EFSA (2009).

The assessment indicates that for both pairings and for acute and chronic/reproductive toxicity, both substances formally contribute significantly to the predicted overall mixture toxicity, i.e. both active substances (including the desthio-metabolite of prothioconazole) are driving overall risk.

Acute mixture toxicity assessments:

As in accordance with EFSA guidance (2009)[2], acute risk assessments are presented for the individual (active) substances as well as for the formulated product accounting for the predicted surrogate endpoint ($LD_{50,\ mix}$) for the combined exposure to fluxapyroxad and prothioconazole (or prothioconazole-desthio, respectively). It is noted, that the estimated $LD_{50,\ mix}$ of > 3776 mg a.s./kg bw for prothioconazole is based on extrapolated endpoints. The surrogate endpoint for the combination with prothioconazole-desthio is based on an extrapolated endpoint for fluxapyroxad and a limit dose endpoint for prothioconazole-desthio. Overall, low avian acute oral toxicity is indicated for all constituents as well as the mixture(s).

Reproductive mixture toxicity assessments:

Principally, combined exposure to the constituents of a formulated product over extended periods as considered to be required to cause sublethal/reproductive effects is not likely due to differing environmental fate behaviours of the different components. The EFSA guidance (2009) requires reproductive mixture toxicity assessments only on a case-by-case basis. Combined reproductive effects are only considered to be likely if the active substances share a common mode of action.

Reported effects defining reproductive and developmental endpoints from mammalian testing in case of fluxapyroxad are restricted to reduced body weight gains of offspring in multi-generation testing in rats at doses toxic for parental animals and post-implantation losses as well as increased incidences of paw hyperflexation observed in rabbits only. No actual reproductive effects nor developmental effects were observed up to the highest doses of fluxapyroxad tested in rats. For prothioconazole, reproductive effects in form of reduced implantations and disruptions of the oestrus cycle were reported. Developmental toxicity studies showed retarded ossification, reduced fetal weights, litter losses, abortions and microphtalmia in rats. No developmental effects were observed in rabbits for this active substance. For prothioconazole-desthio (M04), dystocia was reported as critical effect in the multi-generation test and extranumerary ribs

in the developmental toxicity study in rat.

Chronic bird testing in case of fluxapyroxad (reference is made to the EU DAR; 2011) did not show any treatment-related reproductive effects up to the top feed concentration tested. Likewise, prothioconazole (reference is made to the EU DAR) did not cause reproductive effects in bobwhite quail up to the highest dose tested whereas in mallard, relevant effects (embryo survival, reduced number of 14-day survivors of normal hatchlings) were observed at the top feed concentration of 2000 mg/kg. For prothioconazole-desthio (M04), reduced chick survival rates were reported for bobwhite quail at the top feed concentration of 500 mg/kg, whereas no effects were observed up to and including 500 mg/kg in mallard duck.

Overall, the observed effects, respectively the lack of relevant reproductive effects at critical/top dose levels do not indicate a common mode of action of fluxapyroxad and prothioconacole/prothioconazole-desthio. Accordingly, combined toxic effects based on assumed Concentration Addition (CA) are considered to tend to overestimate the actual risk from combined exposure.

However, in a comprehensive approach, reproductive mixture toxicity assessments are presented by calculating the sum of TER-triggers divided by TER for the individual active substances and in an alternative approach by calculating the sum $(1/TER)^{-1}$. An acceptable reproductive risk from combined toxicity based on assumed Concentration Addition (CA) is indicated by cumulative values below the trigger of 1 or greater than the trigger of 5, respectively. This approach is considered to be most appropriate to account for potential combined effects not disregarding the actual toxicity data of the individual active substances, rather than the worst-case approach proposed by EFSA for initial assessments which relates

zRMS comments:

Combined acute toxicity

The combined acute risk assessment has been amended by zRMS according to recommendation given in EFSA GD for B&M 2009.
It is noted that for the acute risk assessment the Applicant selected higher acute toxicity endpoints for active compound and its metabolite although that lower endpoints from short-term studies are listed in LoEP
In zRMS's opinion as no treatment related mortalities were observed in the short-term toxicity study for the a.s prothioconazole indicating that the dietary exposure has not resulted with increased mortality of tested birds and using by the Applicant the acute LD_{50}>2000 kg a.s./kg bw is sufficiently protective in the risk assessment.

This approach zRMS-PL was accepted for the other products with a.s.-prothioconazole and was agreed by the most of MSs during commenting period process.
However, for completeness, the acute combined risk assessment with consideration of LDD_{50}>1413 mg a.s./kg bw value has been also presented by zRMS.

In case of the acute risk for metabolite JAU 6476-desthio acute LD_{50} >2000 mg pm/kg bw is used by the Applicant, while short-term dietary studies with this compound with lower LD_{50} of 297 mg pm/kg bw/d should be considered as treatment related mortalities were observed in these short-term dietary studies.

zRMS calculations are presented below.

Avian LD_{50} (mix) for JAU 6476-desthio metabolite and Fluxapyroxad when combined in ADM.03503.F.1.A (step 1 in EFSA GD 2009, Appendix B)

	JAU 6476-desthio	Fluxapyroxad
Relative amount of a.s. (%)	15	7.5
Fraction in the a.s. mixture	0.67	0.33
LD_{50} of a.s. or met[mg/kg bw]	>297	>2000
Fraction / LD_{50}	0.002255	0.000165
Sum	0.00242	
1/ sum = predicted LD_{50} (mix)	413.22	

[1)] Relative amount of the parent assuming immediate and complete conversion of prothioconazole to JAU 6476-desthio; this in combination with metabolite endpoint represents worst case and covers also contribution of prothioconazole to the mixture toxicity as it is expected that consideration of prothioconazole endpoint of >2000 mg a.s./kg bw in the LD_{50mix} calculation would give higher combined value.

Avian "tox per fraction" for the JAU 6476-desthio metabolite and Fluxapyroxad when combined in ADM.03502.F.1.A (step 1 in EFSA GD 2009, Appendix B)

	JAU 6476-desthio	**Fluxapyroxad**	"mix"
Content in the formulation	15	7.5	
Fraction in mixture	0.67	0.33	
LD_{50} (mg/kg bw)	>297	>2000	LD_{50mix} =413.22
Tox per fraction	443.30	6060.60	
Contribution to predicted toxicity	**93.2%**	6.8%	

JAU6476-desthio metabolite contributes **93.2%** to mixture toxicity, while the fluxapyroxad has an impact on the predicted risk of 6.8 %, therefore, LD_{50} of 297 mg/kg bw covers the acute combined risk assessment.

Avian "(mix)" for the Prothioconazole and fluxapyroxad when combined in ADM.03503.F.1.A (step 1 in EFSA GD 2009, Appendix B)

	Prothioconazole	**Fluxapyroxad**
Content in the formulation	15	7.5
Fraction in mixture	0.67	0.33
LD_{50} (mg/kg bw)	>2000	>2000
Fraction/LD50	0.000335	0.000165
	0.0005	
1/ sum = predicted LD_{50} (mix)	>2000	

Avian "tox per fraction" for the Prothioconazole and fluxapyroxad when combined in ADM.03502.F.1.A (step 1 in EFSA GD 2009, Appendix B)

	JAU 6476-desthio	**Fluxapyroxad**	"mix"
Content in the formulation	15	7.5	
Fraction in mixture	0.67	0.33	
LD_{50} (mg/kg bw)	>2000	>2000	LD_{50mix} =2000
Tox per fraction	2985.1	6060.60	
Contribution to predicted toxicity	**67%**	33%	

Prothioconazole contributes 67% to mixture toxicity, while the fluxapyroxad has an impact on the predicted risk of 33 %, therefore, LD_{50} of 20000 mg/kg bw should be considered in combined risk assessment.

Avian "tox per fraction" for the Prothioconazole and fluxapyroxad when combined in ADM.03503.F.1.A (step 1 in EFSA GD 2009, Appendix B)

	Prothioconazole	**Fluxapyroxad**
Content in the formulation	15	7.5
Fraction in mixture	0.67	0.33
LD_{50} (mg/kg bw)	>1413	>2000
Fraction / LD_{50}	0.000474	0.000165
Sum	0.000639	
1/ sum = predicted LD_{50} (mix)	1564.94	

Avian "tox per fraction" for prothioconazole and fluxapyroxad when combined in ADM.03503.F.1.A (step 1 in EFSA GD 2009, Appendix B)

	Prothioconazole	**Fluxapyroxad**	"mix"
Content in the formulation	15	7.5	
Fraction in mixture	0.67	0.33	
LD_{50} (mg/kg bw)	>1413	>2000	LD_{50mix} = 1564.94
Tox per fraction	2108.95	6060.60	
Contribution to predicted toxicity	**74.2%**	25.8%	

Prothioconazole contributes 74.2 % to mixture toxicity, while the fluxapyroxad has an impact on the predicted risk of 25.8 %, therefore, LD_{50} of 1564.94 mg/kg bw is considered in combined risk assessment.

Combined long-term toxicity

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD_{50}, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing according to GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B. Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.
The TER_{mix} approach is generally agreed among MSs in Central Zone and this approach was considered by the Applicant.

9.2.1.1 Justification for new endpoints

The formulated product ADM.03503.F.1.A was not tested. The provision of further data on the formulation is not considered to be required as an increased toxicity of the product is not expected as indicated by acute oral testing in mammals (Chande 2021; KCP 7.1.1/01) providing a limit median lethal dose endpoint (LD_{50} > 2000 mg ADM.03503.F.1.A/kg bw) with no reported mortalities in the test.

The EU agreed acute oral toxicity endpoints for fluxapyroxad and prothioconazole are each limit dose endpoints (LD_{50} > 2000 mg a.s./kg bw) which due to no observed mortalities in 10 test birds at the top dose in the respective studies in accordance with EFSA Guidance (2009) can be extrapolated applying a factor of 1.888 to a predicted LD_{50} of 3776 mg a.s./kg bw each. These endpoints are, accordingly, applied for risk assessments.

With LD_{50} values of > 2000 mg/kg bw/d for fluxapyroxad, prothioconazole and prothioconazole-desthio, the surrogate toxicity endpoints for parental reproductive toxicity (LD_{50}/10) is > 200 mg/kg bw/day for all substances. This is greater than the NO(A)ELs of 33.6, 78 and 14.8 mg/kg bw/day, respectively. Accordingly, long-term (reproductive) risk assessments are based on the respective worst-case endpoints from chronic bird testing.

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).

To achieve a concise risk assessment, the risk envelope approach is applied, i.e. for a maximum single use rate of 1.25 L product/ha corresponding to 93.75 g fluxapyroxad/ha and 187.5 prothioconazole/ha, respectively for a maximum BBCH range of 30 to 69 (see 0).

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

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

In the absence of indications for long-term (reproductive) effects due to short-term exposure, the default time-weighted average factor based on a DT_{50} of 10 days and the default averaging window of 21 days is applied.

Risk assessments are presented for the individual active substances fluxapyroxad and prothioconazole, as well as for the relevant metabolite prothioconazole-desthio. In addition to Screening Step assessments, Tier 1 assessments are presented for the active substances even if an acceptable risk is indicated based on Screening Step level assessments if exposure is considered relevant in context of combined risk assessments for the mixture (i.e. for reproductive risk assessments).

Table 9.2-3: Screening step and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) - fluxapyroxad

Intended use		**Cereals (BBCH 30-69) [a)]**			
Active substance		Fluxapyroxad			
Application rate [g/ha]		1 × 93.75			
Acute toxicity [mg/kg bw]		~~3776~~ [b)] >2000			
TER criterion		10			
Crop scenario Growth stage	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [c)] [mg/kg bw/d]**	**TER_a**
Not applicable	Small omnivorous bird	158.8	1.0	14.88	~~253.7~~ 134.40
Reprod. toxicity [mg/kg bw/d]		33.6			
TER criterion		5			
Crop scenario Growth stage	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m [c)] [mg/kg bw/d]**	**TER_{lt}**
Not applicable	Small omnivorous bird	64.8	0.53	3.22	10.4
Crop scenario Growth stage	**Generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [c)] [mg/kg bw/d]**	**TER_{lt}**
BBCH 30-39	Small omnivorous bird ´lark´	5.4	0.53	0.27	126.0
BBCH ≥ 40	Small omnivorous bird ´lark´	3.3	0.53	0.16	206.2
~~Cereals late season seed heads~~	~~Small granivorous~~	~~4.7~~	~~0.53~~	~~0.23~~	~~144.20~~

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.

[a)] Risk envelope.

[b)] Extrapolated from limit dose of > 2000 mg a.s./kg bw in accordance with EFSA (2009) using a factor of 1.888.

[c)] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates.

zRMS comments:

The calculations in the Table 9.2-3 has been amended by zRMS taken into account LD_{50}>2000 mg a.s./kg bw as the worst-case scenario.

Table 9.2-4: Screening step and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) - prothioconazole

Intended use		**Cereals (BBCH 30-69) [a)]**			
Active substance		Prothioconazole			
Application rate [g/ha]		1 × 187.5			
Acute toxicity [mg/kg bw]		~~3776~~ [b)] 2000 1413			
TER criterion		10			
Crop scenario **Growth stage**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [c)] [mg/kg bw/d]**	**TER_a**
Not applicable	Small omnivorous bird	158.8	1.0	29.76	~~126.9~~ 67.20 47.50
Reprod. toxicity [mg/kg bw/d]		78			
TER criterion		5			
Crop scenario **Growth stage**	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m [c)] [mg/kg bw/d]**	**TER_{lt}**
Not applicable	Small omnivorous bird	64.8	0.53	6.44	12.1
Crop scenario **Growth stage**	**Generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [c)] [mg/kg bw/d]**	**TER_{lt}**
BBCH 30-39	Small omnivorous bird ´lark´	5.4	0.53	0.53	146.2
BBCH ≥ 40	Small omnivorous bird ´lark´	3.3	0.53	0.33	239.3
~~Cereals late season seed heads~~	~~Small granivorous~~	~~4.7~~	~~0.53~~	~~0.46~~	~~169.60~~

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.

[a)] Risk envelope.

[b)] Extrapolated from limit dose of > 2000 mg a.s./kg bw in accordance with EFSA (2009) using a factor of 1.888.

[c)] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates.

Table 9.2-5: Screening step and first-tier assessment of the acute and long-term/reproductive risk for birds due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – prothioconazole-desthio

Intended use		**Cereals (BBCH 30-69) [a)]**			
Active substance		Prothioconazole-desthio			
Application rate [g/ha]		1 × 170 (calculated for 100% generation considering molecular weight ratios)			
Acute toxicity [mg/kg bw]		>297 ~~> 2000~~			
TER criterion		10			
Crop scenario Growth stage	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [c)] [mg/kg bw/d]**	**TER_a**
Not applicable	Small omnivorous bird	158.8	1.0	26.99	>11.0 ~~74.1~~
Reprod. toxicity [mg/kg bw/d]		14.8			
TER criterion		5			
Crop scenario Growth stage	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m [c)] [mg/kg bw/d]**	**TER_{lt}**
Not applicable	Small omnivorous bird	64.8	0.53	5.84	**2.5**
Crop scenario Growth stage	**Generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [c)] [mg/kg bw/d]**	**TER_{lt}**
BBCH 30-39	Small omnivorous bird ´lark´	5.4	0.53	0.48	30.6
BBCH ≥ 40	Small omnivorous bird ´lark´	3.3	0.53	0.30	50.1
~~Cereals late season seed heads~~	~~Small granivorous~~	~~4.7~~	~~0.53~~	~~0.42~~	~~35.23~~

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.
[a)] Risk envelope.
[b)] Limit dose endpoint (30% mortality observed).
[c)] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates.

Acceptable acute and reproductive risk is indicated for birds for exposure towards the individual (active) substances for the intended uses of ADM.03503.F.1.A based on Screening Step or Tier 1 assessments.

zRMS comments:

The calculations on the Tables 9.2-4 and 9.2-5 have been amended by zRMS by using differ endpoints than proposed by the Applicant.

It is noted that for the acute risk assessment the Applicant selected higher acute toxicity endpoints for active compound-prothioconazole and its metabolite, although that lower endpoints from short-term studies are listed in LoEP

In zRMS's opinion as no treatment related mortalities were observed in the short-term toxicity study for the a.s prothioconazole indicating that the dietary exposure has not resulted with increased mortality of tested birds and the acute LD_{50}>2000 kg a.s./kg bw is sufficiently protective to use in the risk assessment.

This approach zRMS-PL was accepted for the other products with a.s.-prothioconazole and was agreed by the most of MSs during commenting period process.

However, for completeness, the acute combined risk assessment with consideration of LDD_{50}>1413 mg a.s./kg bw value has been also presented by zRMS.
In case of the acute risk for metabolite JAU 6476-desthio acute LD_{50} >2000 mg pm/kg bw is used by the Applicant, while short-term dietary studies with this compound with lower LD_{50} of 297 mg pm/kg bw/d should be considered as treatment related mortalities were observed in these short-term dietary studies.

Assessments for combined exposure

Acute risk

The acute risk assessments for combined exposure towards the active substances as presented in the tables below is based on the derived surrogate endpoints (′LD_{50}′) accounting for the model of Concentration Addition (CA). Reference is made to Table 9.2-2.

The assessments are presented for the two pairings, fluxapyroxad and prothioconazole as well as fluxapyroxad and prothioconazole-desthio, respectively. This approach is conservatively based on the assumption that prothioconazole is completely transformed to prothioconazole-desthio which formally provides the lowest acute endpoint. The respective exposure is calculated as sum of the Daily Dietary Doses for the individual (active) substances.

Table 9.2-6: Screening step assessment of the acute risk for birds due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole

Intended use		**Cereals (BBCH 30-69) [a)]**			
Product		Fluxapyroxad + Prothioconazole			
Application rate [g/ha]		1 × 93.75 + 1 × 187.5			
Acute toxicity [mg/kg bw]		~~3776~~ [b)] 2000 1564.94			
TER criterion		10			
Crop scenario **Growth stage**	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [c); d)] [mg/kg bw/d]**	**TER_a**
Not applicable	Small omnivorous bird	158.8	1.0	44.65	~~84.6~~ 44.80 35.04

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.
[a)] Risk envelope.
[b)] Surrogate toxicity endpoint for combined exposure based on Concentration Addition.
[c)] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates.
[d)] Sum of Daily Dietary Doses for both (active) substances.

~~**Table 9.2-7: Screening step assessment of the acute risk for birds due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole-desthio**~~

~~**Intended use**~~		~~**Cereals (BBCH 30-69) [a)]**~~			
~~**Product**~~		~~Fluxapyroxad + Prothioconazole-desthio~~			
~~**Application rate [g/ha]**~~		~~1 × 93.75 + 1 × 170 (calculated for 100% generation considering molecular weight ratios)~~			
~~**Acute toxicity [mg/kg bw]**~~		~~2402 [b)]~~			
~~**TER criterion**~~		~~10~~			
~~**Crop scenario**~~ ~~**Growth stage**~~	~~**Indicator species**~~	~~**SV_{90}**~~	~~**MAF_{90}**~~	~~**DDD_{90} [c); d)] [mg/kg bw/d]**~~	~~**TER_a**~~
~~Not applicable~~	~~Small omnivorous bird~~	~~158.8~~	~~1.0~~	~~41.87~~	~~57.4~~

~~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.~~
~~[a)] Risk envelope.~~
~~[b)] Surrogate toxicity endpoint for combined exposure based on Concentration Addition.~~
~~[c)] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates.~~
~~[d)] Sum of Daily Dietary Doses for both (active) substances.~~

~~An acceptable acute risk is indicated for the exposure towards ADM.03503.F.1.A if accounting for combined exposure towards the (active) substances.~~

zRMS comments:

The calculations of combined acute risk assessment for product provided in the Table 9.2-6 for have been amended by zRMS taken into account both calculated surrogate toxicity endpoints for mixture of prothioconazole and fluxapyroxad: LD_{mix}>2000 mg a.s./kg bw (based on acute test) and LD_{50mix}>1564.94 mg a.s./kg bw (based on the dietary endpoint for prothioconazole).

In case of fluxapyroxad and prothioconazole-desthio the combined risk assessment is covered by the risk for prothioconazole-desthio.

Reproductive risk

The below assessment of potential reproductive risk from combined exposure of birds towards fluxapyroxad and prothioconazole or fluxapyroxad and prothioconazole-desthio is based on Tier 1 Toxicity Exposure Ratios for the individual (active) substances.

Table 9.2-8: Tier 1 assessment of the reproductive risk for birds due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole.

Intended use		**Cereals (BBCH 30-69) [a)]**			
Product		Fluxapyroxad + Prothioconazole			
Application rate [g/ha]		1 × 93.75 + 1 × 187.5			
Reproductive toxicity [mg/kg bw/d]		33.6 + 78			
TER criterion		1 or 5			
Crop scenario Growth stage	**Generic focal species**	**TER_{lt}**		**∑ (TER-trigger/TER)**	**∑ $(1/TER)^{-1}$**
		Fluxapyroxad	**Prothioconazole**		
BBCH 30-39	Small omnivorous bird ´lark´	126.0	146.2	0.07	67.7
BBCH ≥ 40	Small omnivorous bird ´lark´	206.2	239.3	0.05	110.8

Sum of (TER-trigger/TER) or sum of (1/TER)-1 shown in **bold** exceed or fall below the relevant trigger of 1 and 5, respectively
[a)] Risk envelope.

Table 9.2-9: Tier 1 assessment of the reproductive risk for birds due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole-desthio.

Intended use		**Cereals (BBCH 30-69) [a)]**			
Product		Fluxapyroxad + Prothioconazole-desthio			
Application rate [g/ha]		1 × 93.75 + 1 × 170			
Reproductive toxicity [mg/kg bw/d]		33.6 + 14.8			
TER criterion		1 or 5			
Crop scenario Growth stage	**Generic focal species**	**TER_{lt}**		**∑ (TER-trigger/TER)**	**∑ $(1/TER)^{-1}$**
		Fluxapyroxad	**Prothioconazole-desthio**		
BBCH 30-39	Small omnivorous bird ´lark´	126.0	30.6	0.20	24.6
BBCH ≥ 40	Small omnivorous bird ´lark´	206.2	50.1	0.12	40.3

Sum of (TER-trigger/TER) or sum of (1/TER)-1 shown in **bold** exceed or fall below the relevant trigger of 1 and 5, respectively
[a)] Risk envelope.

An acceptable reproductive risk is indicated for the exposure towards ADM.03503.F.1.A if accounting for combined exposure towards the (active) substances.

zRMS comments:

An acceptable reproductive risk is indicated for the exposure towards ADM.03503.F.1.A if accounting for combined exposure towards the (active) substances.

9.2.2.2 Higher-tier risk assessment

An acceptable avian acute and reproductive risk is indicated for the dietary exposure towards the individual (active) substances and the formulated product ADM.03503.F.1.A if accounting for combined exposure towards the (active) substances. No higher tier assessments are required.

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 ADM.03503.F.1.A 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 K(f)oc values of 728, 1765 and 575.4 L/kg, fluxapyroxad, prothioconazole and prothioconazole-desthio, respectively all belong to the group of more sorptive substances with the relevant trigger of 3000 for the ratio of effective application rate to endpoint.

To achieve a concise risk assessment, the risk envelope approach is applied, i.e. for a maximum single use rate of 1.25 L product/ha (corresponding to 93.75 g fluxapyroxad and 187.5 prothioconazole/ha (or 170 g prothioconazole-desthio/ha), respectively for a maximum BBCH range of 30 to 69 (see 0).

Effective application rate (g/ha)	=	93.75	**Fluxapyroxad**	
Acute toxicity (mg/kg bw)	=	>2000 ~~3776 a)~~	quotient =	<0.047 ~~0.025~~
Reprod. toxicity (mg/kg bw/d)	=	33.6	quotient =	2.79

a) Extrapolated from limit dose of > 2000 mg a.s./kg bw in accordance with EFSA (2009) using a factor of 1.888.

Effective application rate (g/ha)	=	187.5	**Prothioconazole**	
Acute toxicity (mg/kg bw)	=	>2000 ~~3776 a)~~	quotient =	<0.093 ~~0.050~~
Reprod. toxicity (mg/kg bw/d)	=	78	quotient =	2.40

a) Extrapolated from limit dose of > 2000 mg a.s./kg bw in accordance with EFSA (2009) using a factor of 1.888.

Effective application rate (g/ha)	=	170 a)	**Prothioconazole-desthio**	
Acute toxicity (mg/kg bw)	=	>297 ~~> 2000~~	quotient =	0.572 ~~<0.5785~~
Reprod. toxicity (mg/kg bw/d)	=	14.8	quotient =	11.49

a) Pseudo-application rate calculated based on ratio of molecular weights (molar masses of 344.26 and 312.2 g/mol for prothio-

conazole and prothioconazole-desthio, respectively).

Accordingly, the risk for birds from exposure towards the (active) substances is indicated to be acceptable.

zRMS comments:

The leaf scenario does not have to be considered taking into account the proposed uses (cereals).
The evaluation of the risk resulting from uptake of contaminated water in Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is <3000.

In order to apply consistent approach, the drinking water risk assessment was performed also for metabolite JAU 6476-S-methyl and is presented below. Calculations were performed with assumption of 10 times toxicity of the parent.

JAU 6476-S-methyl effective application rate 1 x 187.5 g/ha

Acute toxicity (mg/kg bw)	>200/141.3	quotient =	0.93/1.32	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	7.8	quotient =	24.03	

Overall, the risk birds from exposure towards the active substances and relevant metabolites is indicated to be acceptable.

9.2.2.4 Effects of secondary poisoning

The (worst-case) log P_{ow} values for fluxapyroxad, prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl are reported to be 3.13, 3.82 at pH=7, and 4.19 at pH=4, 3.04 and 4.19, respectively and thus exceed the trigger value of 3. Risk assessments for effects due to secondary poisoning are required for each (active) substance.

No toxicity data are available for prothioconazole-S-methyl. In a conservative approach, a ten-times increased toxicity as compared to the parent prothioconazole is assumed for secondary poisoning assessments. Likewise, no bioconcentration study in fish is available for this metabolite. This point is addressed based on worst-case QSAR modelling results. It is noted that this approach is conservative. Alternatively, an assumption of a ten-times increased BCF compared to the parent ($BCF_{extrapolated}$ = 197) would be less conservative as the modelled BCF (BCF_{QSAR} = 800.1).

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 dry soil.

For relevant soil matrix concentrations, reference is made to Section B.8 of this submission. 21-day time-weighted average soil concentrations are applied as there are no indications for long-term effects to be caused by short-term exposure. PEC_{soil} corresponds to long-term maxima where applicable (i.e. accounting for plateau soil concentration in case of fluxapyroxad).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals at BBCH 30 to 39 with minimum crop interception (80%) covers the risk for birds from all other intended uses at BBCH > 39 (see 0).

Table 9.2-10: Assessment of the risk for earthworm-eating birds due to exposure to fluxapyroxad via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (BBCH ≥ 30)

Parameter	Fluxapyroxad	comments
PEC_{soil} (twa = 21 d) [mg/kg soil]	0.0308	21-day time-weighted average based on long-term maximum
log P_{ow} / P_{ow}	3.13 / 1349	
$K_{(f)OC}$	728	Mean (n = 7)
f_{OC}	0.02	Default
BCF_{worm}	1.17	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.036	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose [mg/kg bw/d]	0.038	$DDD = PEC_{worm} \times 1.05$
NO(A)EL [mg/kg bw/d]	33.6	
TER_{lt}	888.4	

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 prothioconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (BBCH ≥ 30)

Parameter	Prothioconazole	comments
PEC_{soil} (twa = 21 d) [mg/kg soil]	0.0096	21-day time-weighted average
log P_{ow} / P_{ow}	3.82/6607* 4.16 / 14454	EFSA, 2007
$K_{(f)OC}$	1765	Aged soil column leaching (n = 1)
f_{OC}	0.02	Default
BCF_{worm}	2.27* 4.94	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.022* 0.047	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose [mg/kg bw/d]	0.023	$DDD = PEC_{worm} \times 1.05$
NO(A)EL [mg/kg bw/d]	78	
TER_{lt}	3391.30* 1567	

TER values shown in **bold** fall below the relevant trigger.
*pH=7

Table 9.2-12: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (BBCH ≥ 30)

Parameter	Prothioconazole-desthio	comments
PEC_{soil} (twa = 21 d) [mg/kg soil]	0.0259 ~~0.0239~~	Max PECs ~~21-day time-weighted average~~
log P_{ow} / P_{ow}	3.04 / 1096	
$K_{(f)OC}$	575.4	Mean (n = 4)
f_{OC}	0.02	Default
BCF_{worm}	1.21~~2~~	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.029	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$

Parameter	Prothioconazole-desthio	comments
Daily dietary dose [mg/kg bw/d]	0.031	DDD = PEC_{worm} × 1.05
NO(A)EL [mg/kg bw/d]	14.8	
TER_{lt}	484.9	

TER values shown in **bold** fall below the relevant trigger.

Table 9.2-13: Assessment of the risk for earthworm-eating birds due to exposure to prothioconazole-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (BBCH ≥ 30)

Parameter	Prothioconazole-S-methyl	comments
PEC_{soil} (twa = 21 d) [mg/kg soil]	0.0060	21-day time-weighted average
log P_{ow} / P_{ow}	4.19 / 15488	
$K_{(f)OC}$	2556.3	Mean (n = 4)
f_{OC}	0.02	Default
BCF_{worm}	3.65	$BCF_{worm/soil}$ = ($PEC_{worm,ww}$/$PEC_{soil,dw}$) = (0.84 + 0.012 × P_{ow}) / (foc × Koc)
PEC_{worm}	0.022	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose [mg/kg bw/d]	0.023	DDD = PEC_{worm} × 1.05
NO(A)EL [mg/kg bw/d]	7.8 [a)]	
TER_{lt}	339	

TER values shown in **bold** fall below the relevant trigger.

[a)] As no toxicity data are available for the metabolite of concern, it was assumed that the metabolite is 10 × more toxic than the parent compound (worst-case approach).

Accordingly, the risk for earthworm-eating birds exposed to the active substances or the prothioconazole-desthio and prothioconazole-S-methyl metabolites is indicated to be acceptable for the intended worst-case use of ADM.03503.F.1.A by a large margin of safety.

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. 21-day time-weighted average surface water concentrations (FOCUS Step 1) are applied as there are no indications for long-term effects to be caused by short-term exposure.

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

Parameter	Fluxapyroxad	comments
PEC_{sw} (twa = 21 d) [mg/L]	0.016187	21-day time-weighted average
BCF_{fish}	37	
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.599	PEC_{fish} = PEC_{water} × BCF_{fish}
Daily dietary dose [mg/kg bw/d]	0.095	DDD = PEC_{fish} × 0.159
NO(A)EL [mg/kg bw/d]	33.6	
TER_{lt}	352.8	

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 prothioconazole via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	comments
PEC_{sw} (twa = 21 d) [mg/L]	0.002796	21-day time-weighted average
BCF_{fish}	19.7	
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.055	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose [mg/kg bw/d]	0.0088	$DDD = PEC_{fish} \times 0.159$
NO(A)EL [mg/kg bw/d]	78	
TER_{lt}	8906	

TER values shown in **bold** fall below the relevant trigger.

Table 9.2-16: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole-desthio	comments
PEC_{sw} (twa = 21 d) [mg/L]	0.031501	21-day time-weighted average
BCF_{fish}	65	
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	2.048	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose [mg/kg bw/d]	0.326	$DDD = PEC_{fish} \times 0.159$
NO(A)EL [mg/kg bw/d]	14.8	
TER_{lt}	45.5	

TER values shown in **bold** fall below the relevant trigger.

Table 9.2-17: Assessment of the risk for fish-eating birds due to exposure to prothioconazole-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole-S-methyl	comments
PEC_{sw} (twa = 21 d) [mg/L]	0.011628	21-day time-weighted average
BCF_{fish}	800.1 [a)]	
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	9.304	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose [mg/kg bw/d]	1.479	$DDD = PEC_{fish} \times 0.159$
NO(A)EL [mg/kg bw/d]	7.8 [b)]	
TER_{lt}	5.3	

TER values shown in **bold** fall below the relevant trigger.

[a)] PEC_{fish} was modelled using QSAR data. For maximum conservatism, the worst-case model output (i.e. Arnot-Grobas, mid-trophic, using BCFBAF as part of EPISUITE 4.1 a) was selected.

[b)] As no toxicity data are available for the metabolite of concern, it was assumed that the metabolite is 10× more toxic than the parent compound (worst-case approach).

Accordingly, the risk for fish-eating birds exposed to the active substances or the prothioconazole-desthio and prothioconazole-S-methyl metabolites is indicated to be acceptable for the intended worst-case use of ADM.03503.F.1.A.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009).
Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of both active substances due to their log Pow <3.

Overall, an acceptable risk of secondary exposure from all relevant compounds could be concluded for birds.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant. Reference is made to the conclusions of the EU reviews of the individual active substances (Refer to the EU DAR and EFSA conclusions).

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

Not relevant. The formulated product is intended for use as spray application.

9.2.4 Overall conclusions

The acute and reproductive (long-term) risk for birds from dietary exposure to fluxapyroxad, prothioconazole and the prothioconazole-desthio metabolite is indicated to be acceptable based on Screening Step and/or Tier 1 assessments, including considerations of combined exposure. Likewise, acceptable risk is indicated for the exposure via drinking water and the indirect exposure via secondary poisoning for earthworm- and fish-eating birds.

Overall, the risk for birds exposed following the intended uses of ADM.03503.F.1.A is acceptable.

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 active substances fluxapyroxad and prothioconazole as well as the metabolites of fluxapyroxad (i.e. M700F001, M700F002 and M700F007) and prothioconazole (i.e. M04). Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of ADM.03503.F.1.A were not evaluated as part of the EU assessments of the active substances fluxapyroxad and prothioconazole. An acute oral mammalian formulation study with ADM.03503.F.1.A is made available. The new data submitted with this application are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology) of this report.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review processes. Further justifications are provided below (Refer to Point 9.3.1.1).

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

Species	Substance	Exposure System	Results	Reference
Formulated product ADM.03503.F.1.A				
Rat	ADM.03503.F.1.A	Acute oral toxicity; gavage	**LD_{50} > 2000 mg product/kg bw**	KCP 7.1.1/01 Chande, 2021
Fluxapyroxad, representative formulation and metabolites				
Rat	BAS 700 00 F	Acute oral toxicity; gavage	LD_{50} > 2000 mg/kg bw	EFSA Journal 2012; 10(1): 2522
Rat	Fluxapyroxad	Acute oral toxicity; gavage	**LD_{50} > 2000 mg a.s./kg bw**	EFSA Journal 2012; 10(1): 2522
Rat	M700F001	Acute oral toxicity; gavage	**LD_{50} > 2000 mg/kg bw**	EFSA Journal 2012; 10(1): 2522
Rat	M700F002	Acute oral toxicity; gavage	**LD_{50} > 2000 mg/kg bw**	EFSA Journal 2012; 10(1): 2522
Rat	M700F007	Acute oral toxicity; gavage	**LD_{50} > 500 mg/kg bw**	EFSA Journal 2012; 10(1): 2522
Rat	Fluxapyroxad	Reproductive toxicity; dietary; 2-generation study	**NOAEL = 10 mg a.s./kg bw/day**	EFSA Journal 2012; 10(1): 2522
Rat	Fluxapyroxad	Developmental toxicity; teratology	NOAEL = 1000 mg a.s./kg bw/day	EFSA Journal 2012; 10(1): 2522
Rabbit	Fluxapyroxad	Developmental toxicity; teratology	NOAEL = 25 mg a.s./kg bw/day	EFSA Journal 2012; 10(1): 2522
Rabbit	M700F001	Developmental toxicity; teratology	NOAEL = 250 mg/kg bw/day	EFSA Journal 2012; 10(1): 2522
Rabbit	M700F002	Developmental toxicity; teratology	NOAEL = 300 mg/kg bw/day	EFSA Journal 2012; 10(1): 2522
Prothioconazole and relevant metabolites				
Rat	Prothioconazole EC 250	Acute oral toxicity; gavage	LD_{50} > 2500 mg a.s./kg bw	EFSA Scientific Report (2007) 106, 1-98
Rat	Prothioconazole FS 100	Acute oral toxicity; gavage	LD_{50} > 2500 mg a.s./kg bw	EFSA Scientific Report (2007) 106, 1-98
Rat	Prothioconazole	Acute oral toxicity;	**LD_{50} > 6200 mg a.s./kg bw**	EFSA Scientific

Species	Substance	Exposure System	Results	Reference
	(a.s.)	gavage		Report (2007) 106, 1-98
Rat	Prothiocona-zole-desthio (M04)	Acute oral toxicity; gavage	LD_{50} = 2806 mg/kg bw (males) LD_{50} = 2506 mg/kg bw (females)	EFSA Scientific Report (2007) 106, 1-98
Mouse	Prothiocona-zole-desthio (M04)	Acute oral toxicity; gavage	**LD_{50} = 2235 mg/kg bw** (males) LD_{50} = 3459 mg/kg bw (females)	EFSA Scientific Report (2007) 106, 1-98
Rat	Prothioconazole (a.s.)	Reproductive toxicity; gavage; 2-generation study	**NOAEL = 95.6 mg a.s./kg bw/day**	EFSA Scientific Report (2007) 106, 1-98
Rat	Prothiocona-zole-desthio (M04)	Reproductive toxicity; dietary; 2-generation study	**NOAEL = 10 mg mg/kg bw/day**	EFSA Scientific Report (2007) 106, 1-98
Rat	Prothioconazole (a.s.)	Developmental toxicity; teratology	NOAEL = 20 mg a.s./kg bw/day	EFSA Scientific Report (2007) 106, 1-98
Rat	Prothiocona-zole-desthio (M04)	Developmental toxicity; teratology	NOAEL = 1 mg/kg bw/day	EFSA Scientific Report (2007) 106, 1-98
Rabbit	Prothiocona-zole-desthio (M04)	Developmental toxicity; teratology	NOAEL = 2 mg/kg bw/day	EFSA Scientific Report (2007) 106, 1-98

a.s. technical active substance; **Bold**: Endpoints used for risk assessments.
Grey shading: Data for representative products during EU review of the active substances. Data not considered for the assessments for ADM.03503.F.1.A.

zRMS comments:

Mammalian toxicity data for fluxapyroxad, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.2-1 above has been confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

Potential mixture toxicity

In order to assess the potential for combined effects on terrestrial vertebrates other than birds (mammals), predicted acute mixture toxicity was calculated conservatively assuming dose additivity of the active substances based on the worst-case assumption that the active substances have the same mode of action.

In the following table, a ´toxicity per fraction´ assessment (for an assumed concentration addition) is presented for both, acute as well as reproductive toxicity based on the fractions of active substances as in the formulated product.

The assessment is presented for the pairings of fluxapyroxad with either the parent prothioconazole or the prothioconazole-desthio metabolite assuming 100% generation from parent prothioconazole, i.e. corresponding to an substance content of 136 g prothioconazole-desthio/L.

Table 9.3-2: ´Toxicity per fraction´ assessment – additive mixture toxicity

Organism group	Time scale	Test substance	Fraction in the mixture ($x_{a.s.}$)	LD_{50} / NO(A)EL [mg a.s./kg bw(/d)]	Toxicity per fraction for CA/**Surrogate endpoint**	Contribution to overall toxicity [%]
Mammals	acute	Fluxapyroxad	0.33	> 2000	> 6000.0	**60.8**
		Prothioconazole	0.67	> 6200	> 9300.0	**39.2**
		ADM.03503.F.1.A	1.0	n.a.	**> 3647.1**	n.a.
		Fluxapyroxad	0.36 [a)]	> 2000	> 5626.7	**38.1**
		Prothioconazole-desthio	0.64 [a)]	2235	3467.5	**61.9**
		ADM.03503.F.1.A	1.0	n.a.	**> 2145.4**	n.a.
	chronic / reprod.	Fluxapyroxad	0.33	10	30.0	**82.7**
		Prothioconazole	0.67	95.6	143.4	**17.3**
		ADM.03503.F.1.A	1.0	n.a.	**24.8**	n.a.
		Fluxapyroxad	0.36 [a)]	10	28.1	**35.5**
		Prothioconazole-desthio	0.64 [a)]	10	15.5	**64.5**
		ADM.03503.F.1.A	1.0	n.a.	**10.0**	n.a.

n.a. not available/not applicable; CA: Concentration Addition.
[a)] Calculated from active substance contents for prothioconazole (150 g/L) based on the molar masses of 344.26 and 312.2 g/mol for prothioconazole and prothioconazole-desthio (i.e. corresponding to 136 g/L), respectively.

The assessment indicates that for both pairings and for acute and chronic/reproductive toxicity, both substances formally contribute significantly to the predicted overall mixture toxicity, i.e. both active substances (including the desthio-metabolite of prothioconazole) are driving overall risk.

Acute mixture toxicity assessments:

As in accordance with EFSA guidance (2009)[3], acute risk assessments are presented for the individual (active) substances as well as for the formulated product accounting for the predicted surrogate endpoint ($LD_{50,\ mix}$) for the combined exposure to fluxapyroxad and prothioconazole (or prothioconazole-desthio, respectively). It is noted, however, that the estimated $LD_{50,\ mix}$ is a limit dose endpoint indicating low acute toxicity as are the individual active substance endpoints.

Reproductive mixture toxicity assessments:

Principally, combined exposure to the constituents of a formulated product over extended periods as considered to be required to cause sublethal/reproductive effects is not likely due to differing environmental fate behaviours of the different components. The EFSA guidance (2009) requires reproductive mixture toxicity assessments only on a case-by-case basis. Combined reproductive effects are only considered to be likely if the active substances share a common mode of action.
Reported effects defining reproductive and developmental endpoints from mammalian testing in case of fluxapyroxad are restricted to reduced body weight gains of offspring in multi-generation testing in rats at doses toxic for parental animals and post-implantation losses as well as increased incidences of paw hyperflexation observed in rabbits only. No actual reproductive effects nor developmental effects were observed up to the highest doses of fluxapyroxad tested in rats. For prothioconazole, reproductive effects in form of reduced implantations and disruptions of the oestrus cycle were reported. Developmental toxicity studies showed retarded ossification, reduced fetal weights, litter losses, abortions and microphtalmia in rats. No developmental effects were observed in rabbits for this active substance. For prothioconazole-desthio (M04), dystocia was reported as critical effect in the multi-generation test and extranumerary ribs in the developmental toxicity study in rat.
Chronic bird testing in case of fluxapyroxad (reference is made to the EU DAR; 2011) did not show any

[3] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. doi: 10.2903/j.efsa.2009.1438.

treatment-related reproductive effects up to the top feed concentration tested. Likewise, prothioconazole (reference is made to the EU DAR) did not cause reproductive effects in bobwhite quail up to the highest dose tested whereas in mallard, relevant effects (embryo survival, reduced number of 14-day survivors of normal hatchlings) were observed at the top feed concentration of 2000 mg/kg. For prothioconazole-desthio (M04), reduced chick survival rates were reported for bobwhite quail at the top feed concentration of 500 mg/kg, whereas no effects were observed up to and including 500 mg/kg in mallard duck.

Overall, the observed effects, respectively the lack of relevant reproductive effects at critical/top dose levels do not indicate a common mode of action of fluxapyroxad and prothioconacole/prothioconazole-desthio. Accordingly, combined toxic effects based on assumed Concentration Addition (CA) are considered to tend to overestimate the actual risk from combined exposure.

However, in a comprehensive approach, reproductive mixture toxicity assessments are presented by calculating the sum of TER-triggers divided by TER for the individual active substances and in an alternative approach by calculating the sum $(1/TER)^{-1}$. An acceptable reproductive risk from combined toxicity based on assumed Concentration Addition (CA) is indicated by cumulative values below the trigger of 1 or greater than the trigger of 5, respectively. This approach is considered to be most appropriate to account for potential combined effects not disregarding the actual toxicity data of the individual active substances, rather than the worst-case approach proposed by EFSA for initial assessments which relates exposure of all actives expressed in equivalents of the active with the lowest available endpoint.

zRMS comments:

Acute combined risk assessment:

The calculations of LD_{50mix} of 3647.1 mg a.s/kg bw and 2145.4 mg a.s./kg bw for the combined exposure to fluxapyroxad and prothioconazole or prothioconazole-desthio, respectively are validated by zRMS.

Based on these calculations it can be concluded that both active substances (including the desthio-metabolite of prothioconazole) are driving overall risk. Therefore, the risk assessment is based with consideration of the surrogate LDmix endpoints and for each of the a.s., separately.

Combined long-term toxicity

zRMS agrees that for the approach assuming dose additivity of the active substances, reliable results would only be expected for combinations of effect levels with a defined x, e.g. a LD_{50}, but not for NOELs since the latter effect indicators may represent varying risk or response levels for different compounds depending on dose-spacing (GD B&M, EFSA Journal 2009; 7(12): 1438, Appendix B). Therefore, for the risk assessment based on long-term effects it is not recommended to consider the use of predicted toxicity values.
Therefore, the calculated NOELmix was not considered by zRMS in the current risk assessment.
It should be noted that according to recommendation given in Appendix B of the Guidance Document 2009 for the evaluation of sublethal effects, the use of the lowest NO(A)EL of the actives in the formulation, along with the combined exposure estimate from both active substances provides a conservative representation of long-term risks to mammals.
However, we accepted also a comprehensive approach for reproductive mixture toxicity assessment by calculating the sum of TER-triggers divided by TER for the individual active substances and in an alternative approach by calculating the sum $(1/TER)^{-1}$. An acceptable reproductive risk from combined toxicity based on assumed Concentration Addition (CA) is indicated by cumulative values below the trigger of 1 or greater than the trigger of 5, respectively.

9.3.1.1 Justification for new endpoints

The formulated product ADM.03503.F.1.A was tested in an acute oral test on rats (Chande 2021; Refer to Part B, Section B.7, Point 7.1.1). As an LD_{50} of > 2000 mg product/kg bw/day and no mortalities up to the limit dose tested were reported in this study, there is no indication of an increased toxicity of the formulated product.

Likewise, for the individual active substances fluxapyroxad and prothioconazole (LD_{50} > 2000 and > 6200 mg a.s./kg bw, respectively) as well as the metabolites M700F001, M700F002 (LD_{50} > 2000 mg/kg bw) and M700F007 (LD_{50} > 500 mg/kg bw), the median lethal dose exceeds the limit doses tested, whereas a defined worst-case LD_{50} of 2235 mg/kg bw for males was reported for prothioconazole-desthio.

In accordance with the EU agreed endpoints, for long-term/reproductive risk assessments, the NO(A)ELs from multi-generation testing of 10, 95.6 and 10 mg (a.s.)/kg bw/day for fluxapyroxad, prothioconazole and prothioconazole-desthio, respectively, are applied. The respective endpoints form developmental toxicity testing with the active substances fluxapyroxad and prothioconazole exceed the reproductive endpoints from the two-generation studies. In contrast, formally, the NOAELs for prothioconazole-desthio from the teratology studies on rats and rabbits are lower than the EU agreed NOAEL of 10 mg/kg bw/day. However, the effects (i.e. supernumery ribs; reference is made to the information provided in the EU DAR) observed in the developmental toxicity studies are not relevant in context with reproductive risk and occur as normal variation in control animals. Accordingly, the endpoints from teratology testing are not relevant for the assessments of potential risk on reproductive performance. Assessments are based on the NOAEL from multi-generation testing as in line with the EU agreed endpoint list for wild mammals.

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).

To achieve a concise risk assessment, the risk envelope approach is applied, i.e. for a maximum single use rate of 1.25 L product/ha (corresponding to 93.75 g fluxapyroxad/ha and 187.5 prothioconazole/ha, respectively for a maximum BBCH range of 30 to 69 (see 0).

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

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

In the absence of indications for long-term (reproductive) effects due to short-term exposure, the default time-weighted average factor based on a DT_{50} of 10 days and the default averaging window of 21 days is applied.

Risk assessments are presented for the individual active substances fluxapyroxad and prothioconazole, as well as for the relevant metabolite prothioconazole-desthio. In addition to Screening Step assessments, Tier 1 assessments are presented for the active substances even if an acceptable risk is indicated based on Screening Step level assessments if exposure is considered relevant in context of combined risk assessments for the mixture (i.e. for reproductive risk assessments).

Table 9.3-3: Screening step and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) - fluxapyroxad

Intended use	**Cereals (BBCH 30-69) [a)]**				
Active substance	Fluxapyroxad				
Application rate [g/ha]	1 × 93.75				
Acute toxicity [mg/kg bw]	> 2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [b)] [mg/kg bw/d]**	**TER_a**
Not applicable	Small herbivorous mammal	118.4	1.0	11.10	> 180.2
Reprod. toxicity [mg/kg bw/d]	10.0				
TER criterion	5				
Crop scenario Growth stage	**Indicator species**	**SV_m**	**MAF_m × TWA**	**DDD_m [b)] [mg/kg bw/d]**	**TER_{lt}**
Not applicable	Small herbivorous mammal	48.3	0.53	2.40	**4.2**
Crop scenario Growth stage	**Generic focal species**	**SVm**	**MAF_m × TWA**	**DDD_m [b)] [mg/kg bw/d]**	**TER_{lt}**
BBCH ≥ 20	Small insectivorous mammal ´shrew´	1.9	0.53	0.09	106.6
BBCH ≥ 40	Small herbivorous mammal ´vole´	21.7	0.53	1.07	9.3
BBCH 30-39	Small omnivorous mammal ´mouse´	3.9	0.53	0.19	51.9
BBCH ≥ 40	Small omnivorous mammal ´mouse´	2.3	0.53	0.11	88.0

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.

[a)] Risk envelope.

[b)] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates.

zRMS comments:

The calculations of acute and long-term risk assessment for fluxapyroxad is agreed by the zRMS.

Acceptable acute and long-term risk may be concluded for mammals exposed to in ADM.03503.F.1.A

Table 9.3-4: Screening step and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) - prothioconazole

Intended use	**Cereals (BBCH 30-69) [a)]**				
Active substance	Prothioconazole				
Application rate [g/ha]	1 × 187.5				
Acute toxicity [mg/kg bw]	> 6200				
TER criterion	10				
Crop scenario Growth stage	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [b)] [mg/kg bw/d]**	**TER_a**
Not applicable	Small herbivorous mammal	118.4	1.0	22.19	> 279.4
Reprod. toxicity [mg/kg bw/d]	95.6				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [b)] [mg/kg bw/d]**	**TER_{lt}**
Not applicable	Small herbivorous mammal	48.3	0.53	4.80	19.9
Crop scenario Growth stage	**Generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [b)] [mg/kg bw/d]**	**TER_{lt}**
BBCH ≥ 20	Small insectivorous mammal ´shrew´	1.9	0.53	0.19	509.4
BBCH ≥ 40	Small herbivorous mammal ´vole´	21.7	0.53	2.14	44.6
BBCH 30-39	Small omnivorous mammal ´mouse´	3.9	0.53	0.39	248.2
BBCH ≥ 40	Small omnivorous mammal ´mouse´	2.3	0.53	0.23	420.8

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.

[a)] Risk envelope.

[b)] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates.

zRMS comments:

The calculations of acute and long-term risk assessment for prothioconazole is agreed by the zRMS.

Acceptable acute and long-term risk may be concluded for mammals exposed to in ADM.03503.F.1.A.

Table 9.3-5: Screening step and first-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – prothioconazole-desthio

Intended use	**Cereals (BBCH 30-69) [a)]**				
Active substance	Prothioconazole-desthio				
Application rate [g/ha]	1 × 170 (calculated for 100% generation considering molecular weight ratios)				
Acute toxicity [mg/kg bw]	2235				
TER criterion	10				
Crop scenario Growth stage	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} [b)] [mg/kg bw/d]**	**TER_a**
Not applicable	Small herbivorous mammal	118.4	1.0	20.12	111.1
Reprod. toxicity [mg/kg bw/d]	10.0				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [b)] [mg/kg bw/d]**	**TER_{lt}**
Not applicable	Small herbivorous mammal	48.3	0.53	4.35	**2.3**
Crop scenario Growth stage	**Generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m [b)] [mg/kg bw/d]**	**TER_{lt}**
BBCH ≥ 20	Small insectivorous mammal ´shrew´	1.9	0.53	0.17	58.8
BBCH ≥ 40	Small herbivorous mammal ´vole´	21.7	0.53	1.94	5.1
BBCH 30-39	Small omnivorous mammal ´mouse´	3.9	0.53	0.35	28.6
BBCH ≥ 40	Small omnivorous mammal ´mouse´	2.3	0.53	0.21	48.6

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.

[a)] Risk envelope.

[b)] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates.

Acceptable acute and reproductive risk is indicated for terrestrial vertebrates other than birds for exposure towards the individual (active) substances for the intended uses of ADM.03503.F.1.A based on Screening Step or Tier 1 assessments.

zRMS comments:

The calculations of acute and long-term risk assessment for Prothioconazole-desthio metabolite is agreed by the zRMS.

Acceptable acute and long-term risk may be concluded for mammals exposed to in ADM.03503.F.1.A

Assessments for combined exposure

Acute risk

The acute risk assessments for combined exposure towards the active substances as presented in the tables below is based on the derived surrogate endpoints (′LD_{50}′) accounting for the model of Concentration Addition (CA). Reference is made to Table 9.3-2.

The assessments are presented for the two pairings, fluxapyroxad and prothioconazole as well as fluxapyroxad and prothioconazole-desthio, respectively. This approach is conservatively based on the assumption that prothioconazole is completely transformed to prothioconazole-desthio which provides the lowest acute endpoint. The respective exposure is calculated as sum of the Daily Dietary Doses for the individual (active) substances.

Table 9.3-6: Screening step assessment of the acute risk for mammals due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole

Intended use		**Cereals (BBCH 30-69)** [a]			
Product		Fluxapyroxad + Prothioconazole			
Application rate [g/ha]		1 × 93.75 + 1 × 187.5			
Acute toxicity [mg/kg bw]		> 3647 [b]			
TER criterion		10			
Crop scenario Growth stage	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90}** [c; d] **[mg/kg bw/d]**	**TER_a**
Not applicable	Small herbivorous mammal	118.4	1.0	33.29	> 109.6

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.
[a] Risk envelope
[b] Surrogate toxicity endpoint for combined exposure based on Concentration Addition
[c] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates
[d] Sum of Daily Dietary Doses for both (active) substances

Table 9.3-7: Screening step assessment of the acute risk for mammals due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole-desthio

Intended use		**Cereals (BBCH 30-69)** [a]			
Product		Fluxapyroxad + Prothioconazole-desthio			
Application rate [g/ha]		1 × 93.75 + 1 × 170 (calculated for 100% generation considering molecular weight ratios)			
Acute toxicity [mg/kg bw]		> 2145 [b]			
TER criterion		10			
Crop scenario Growth stage	**Indicator species**	**SV_{90}**	**MAF_{90}**	**DDD_{90}** [c; d] **[mg/kg bw/d]**	**TER_a**
Not applicable	Small herbivorous mammal	118.4	1.0	31.22	> 68.7

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.
[a] Risk envelope
[b] Surrogate toxicity endpoint for combined exposure based on Concentration Addition
[c] Exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the unrounded exposure estimates
[d] Sum of Daily Dietary Doses for both (active) substances

An acceptable acute risk is indicated for the exposure towards ADM.03503.F.1.A if accounting for combined exposure towards the (active) substances.

zRMS comments:

The calculations of acute combined risk assessment from exposure to fluxapyroxad and prothioconazole as well as fluxapyroxad and prothioconazole-desthio, respectively are validated by zRMS.

Reproductive risk

The below assessment of potential reproductive risk from combined exposure of birds towards fluxapyroxad and prothioconazole or fluxapyroxad and prothioconazole-desthio is based on Tier 1 Toxicity Exposure Ratios for the individual (active) substances.

Table 9.3-8: Tier 1 assessment of the reproductive risk for mammals due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole

Intended use		**Cereals (BBCH 30-69) [a)]**			
Product		Fluxapyroxad + Prothioconazole			
Application rate [g/ha]		1 × 93.75 + 1 × 187.5			
Reproductive toxicity [mg/kg bw/d]		10 + 95.6			
TER criterion		1 or 5			
Crop scenario Growth stage	**Generic focal species**	**TER_{lt}**		~~**∑ (TER-trigger/TER)**~~	**$\sum (1/TER)^{-1}$**
		Fluxapyroxad	**Prothioconazole**		
BBCH ≥ 20	Small insectivorous mammal ´shrew´	106.6	509.4	~~0.06~~	88.2
BBCH ≥ 40	Small herbivorous mammal ´vole´	9.3	44.6	~~0.65~~	7.7
BBCH 30-39	Small omnivorous mammal ´mouse´	51.9	248.2	~~0.12~~	42.9
BBCH ≥ 40	Small omnivorous mammal ´mouse´	88.0	420.8	~~0.07~~	72.8

Sum of (TER-trigger/TER) or sum of (1/TER)-1 shown in **bold** exceed or fall below the relevant trigger of 1 and 5, respectively.
[a)] Risk envelope.

Table 9.3-9: Tier 1 assessment of the reproductive risk for mammals due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole-desthio

Intended use		**Cereals (BBCH 30-69) [a)]**			
Active substance/product		Fluxapyroxad + Prothioconazole-desthio			
Application rate [g/ha]		1 × 93.75 + 1 × 170 (calculated for 100% generation considering molecular weight ratios)			
Reproductive toxicity [mg/kg bw/d]		10 + 10			
TER criterion		1 or 5			
Crop scenario Growth stage	**Generic focal species**	**TER_{lt}**		~~**∑ (TER-trigger/TER)**~~	**$\sum (1/TER)^{-1}$** **TER_{mix}**
		Fluxapyroxad	**Prothioconazole-desthio**		
BBCH ≥ 20	Small insectivorous mammal ´shrew´	106.6	58.8	~~0.13~~	37.9
BBCH ≥ 40	Small herbivorous mammal ´vole´	9.3	5.1	~~1.52~~	**3.3**
BBCH 30-39	Small omnivorous mammal ´mouse´	51.9	28.6	~~0.27~~	18.4
BBCH ≥ 40	Small omnivorous mammal ´mouse´	88.0	48.6	~~0.16~~	31.3

Sum of (TER-trigger/TER) or sum of (1/TER)-1 shown in **bold** exceed or fall below the relevant trigger of 1 and 5, respectively.
[a)] Risk envelope.

An acceptable reproductive risk is indicated for the exposure towards ADM.03503.F.1.A if accounting for combined exposure towards the (active) substances with exception of the risk for small herbivorous mammals for combined exposure towards fluxapyroxad and the metabolite prothioconazole-desthio.
However, it is noted that the sum of TER trigger/TER or sum of 1/TER is not much greater or below the trigger of 5, respectively. Besides, the assumption of the co-occurrence of fluxapyroxad and prothioconazole-desthio is conservative. Combined exposure is more relevant for the pairing fluxapyroxad and prothioconazole as constituents of the formulated product ADM.03503.F.1.A. Prothioconazole-desthio evolves from prothioconazole. Finally, and as argued above under Point 9.3.1, reproductive effects observed (or the absence of effects) indicate that a common mode of action is unlikely. For this reason, it is assumed that assessments according to the model of Concentration Addition overestimate the actual risk from combined exposure.

Therefore, the reproductive risk for the exposure towards ADM.03503.F.1.A if accounting for combined exposure towards the (active) substances is considered to be acceptable.

However, in a comprehensive approach, refined risk assessments are conducted for small herbivorous mammals for fluxapyroxad and prothioconazole-desthio as basis for revised mixture toxicity assessments (reference is made to Point 9.3.2.2 below).

zRMS comments:

The zRMS checked the combined long-term risk assessment based TER_{mix} approach (sum of 1/TER) and trigger of 5, accepted in Central Zone by the most of the MSs, and confirmed that it is correct.
Based on the calculations presented in the Table 9.3-8 and Table 9.3-9 an acceptable reproductive risk is indicated for the exposure towards ADM.03503.F.1.A if accounting for combined exposure towards the (active) substances with exception of the risk for small herbivorous mammals at BBCH>40 (vole) for combined exposure towards fluxapyroxad and the metabolite prothioconazole-desthio.

Further refinement for is presented in the Point 9.3.2.2.

9.3.2.2 Higher-tier risk assessment

Whereas an acceptable acute and reproductive risk was presented for the exposure of mammals towards the individual (active) substances fluxapyroxad, prothioconazole and prothioconazole-desthio, formally, risk from combined exposure cannot be excluded for the small herbivorous scenario (´vole´) based on the presented assessments for the pairing of fluxapyroxad with the desthio-metabolite of prothioconazole. The respective combined risk quotients marginally exceed (∑ of TER trigger/TER = 1.52) or fall below ($\sum (1/TER)^{-1} = 3.3$) the acceptability criteria (i.e. ≤ 1 or ≥ 5, respectively).

As argued above, the assumption of significant co-occurrence of both substances is not realistic. Accordingly, the mixture toxicity assessments for combined exposure towards fluxapyroxad and prothioconazole showing acceptable risk are considered to supersede the respective assessments for fluxapyroxad and prothioconazole-desthio.

However, refined risk assessments are presented based on the following refinements.

- Deposition Factor (DF)

Deposition Factor (DF)

The standard deposition factor applied for Tier 1 assessments is 0.3 based on an assumed interception of 70% for BBCH ≥ 40, i.e. the plant growth stage at which the small herbivorous scenario is considered to be relevant in cereals.

The EFSA Guidance Document (2009, reference is made to Appendix E) allows for the refinement crop interception based on the more detailed FOCUS Groundwater Report (refer to recent version of 2014[4]). For a BBCH range of 40 to 69 which covers the intended uses of ADM.03503.F.1.A with a BBCH range of 30 to 69 (with BBCH < 40 not relevant for the ´vole scenario´), crop interception is 90%. Accordingly, a DF of 0.1 can be assumed for the calculation of exposure.

The revised risk assessments based on a DF of 0.1 are presented in the following tables for fluxapyroxad and prothioconazole-desthio, respectively.

Table 9.3-10: Higher tier assessment of the reproductive risk for small herbivorous mammals due to the use of ADM.03503.F.1.A in cereals at BBCH ≥ 40 (1.25 L product/ha) – fluxapyroxad

(Generic) focal species	Relative Food Intake Rate (FIR/bw)	Application rate [kg a.s./ha]	RUD [mg a.s./kg food]	DF	MAF	f_{TWA}	DDD_m [a)] [mg/kg bw/d]	NO(A)EL [mg/kg bw/d]	TER_{lt}
´Vole´	1.33	0.09375	54.2	0.1	1.0	0.53	0.36	10	27.8

RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; f_{TWA}: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.
[a)] exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the actual exposure estimates: DDD = [(Σi (PD_i · $FIR_{total,\ fresh}$ · Application Rate · RUD · DF · ftwa · MAF)) / body weight].
bold: TERs below the trigger and refined parameter.

Table 9.3-11: Higher tier assessment of the reproductive risk for small herbivorous mammals due to the use of ADM.03503.F.1.A in cereals at BBCH ≥ 40 (1.25 L product/ha) – prothioconazole-desthio

(Generic) focal species	Relative Food Intake Rate (FIR/bw)	Application rate [kg a.s./ha]	RUD [mg a.s./kg food]	DF	MAF	f_{TWA}	DDD_m [a)] [mg/kg bw/d]	NO(A)EL [mg/kg bw/d]	TER_{lt}
´Vole´	1.33	0.170	54.2	0.1	1.0	0.53	0.65	10	15.4

RUD: residue unit dose; DF: deposition factor (considering possible interception by the crop); MAF: multiple application factor; f_{TWA}: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio.
[a)] exposure estimates are rounded to 2 decimal places; TERs represent accurate values underlying the actual exposure estimates: DDD = [(Σi (PD_i · $FIR_{total,\ fresh}$ · Application Rate · RUD · DF · ftwa · MAF)) / body weight]
bold: TERs below the trigger and refined parameter.

The revised reproductive mixture toxicity assessments are presented in the following table.

Table 9.3-12: Higher tier assessment of the reproductive risk for small herbivorous mammals due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha) – fluxapyroxad + prothioconazole-desthio

Intended use		**Cereals (BBCH ≥ 40) [a)]**			
Product		Fluxapyroxad + Prothioconazole-desthio			
Application rate [g/ha]		1 × 93.75 + 1 × 170 (calculated for 100% generation considering molecular weight ratios)			
Reproductive toxicity [mg/kg bw/d]		10 + 10			
TER criterion		1 or 5			
Crop scenario Growth stage	**Generic focal species**	**TER_{lt}**		~~**∑ (TER-trigger/ TER)**~~	**$\sum (1/TER)^{-1}$**
		Fluxapyroxad	**Prothioconazole-desthio**		
BBCH ≥ 40	Small herbivorous mammal ´vole´	27.8	15.4	~~0.50~~	9.9

Sum of (TER-trigger/TER) or sum of (1/TER)-1 shown in **bold** exceed or fall below the relevant trigger of 1 and 5, respectively.
[a)] Risk envelope.

Accordingly, refined risk assessments for the small herbivorous scenario applying the revised Deposition Factor indicate an acceptable reproductive risk for exposure towards the individual (active) substances as well as for the combined exposure.
Therefore, the risk for mammals exposed towards fluxapyroxad and prothioconazole as well as the relevant metabolite prothioconazole-desthio via the diet following the intended uses of ADM.03503.F.1.A is

[4] FOCUS (2014): Generic Guidance for Tier 1 – FOCUS Ground Water Assessments. Version 2.2. May 2014.

acceptable.

zRMS comments:

The refinement of combined long-term risk assessment based on calculations of refined TER_{LT} for fluxapyroxad and prothioconazole metabolite JAU 6476-desthio with consideration of fdep of 0.1 (reported in the latest 'Generic Guidance for Tier-1 FOCUS Ground Water Assessment' (vers. 2.2; May 2014)) instead of 0.3 for cereal crop stages at BBCH 40-65 (growth stages relevant for the common vole) has been considered by zRMS.

Overall, based on the performed calculations, acceptable acute and long-term dietary risk to mammals from active substances and metabolite prothioconazole-desthio may be concluded following the intended Central Zone uses of ADM.03503.F.1.A.

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 K(f)oc values of 728, 1765 and 575.4 L/kg, fluxapyroxad, prothioconazole and prothioconazole-desthio, respectively all belong to the group of more sorptive substances with the relevant trigger of 3000 for the ratio of effective application rate to endpoint.

To achieve a concise risk assessment, the risk envelope approach is applied, i.e. for a maximum single use rate of 1.25 L product/ha (corresponding to 93.75 g fluxapyroxad and 187.5 prothioconazole/ha (or 170 g prothioconazole-desthio/ha), respectively for a maximum BBCH range of 30 to 69 (see 0).

Effective application rate (g/ha)	=	93.75	**Fluxapyroxad**	
Acute toxicity (mg/kg bw)	=	> 2000	quotient =	< 0.047
Reprod. toxicity (mg/kg bw/d)	=	10.0	quotient =	9.38

Effective application rate (g/ha)	=	187.5	**Prothioconazole**	
Acute toxicity (mg/kg bw)	=	> 6200	quotient =	< 0.030
Reprod. toxicity (mg/kg bw/d)	=	95.6	quotient =	1.96

Effective application rate (g/ha)	=	170 [a)]	**Prothioconazole-desthio**	
Acute toxicity (mg/kg bw)	=	2235	quotient =	0.076
Reprod. toxicity (mg/kg bw/d)	=	10.0	quotient =	17.00

[a)] Pseudo-application rate calculated based on ratio of molecular weights (molar masses of 344.26 and 312.2 g/mol for prothioconazole and prothioconazole-desthio, respectively).

Accordingly, the risk for terrestrial vertebrates other than birds from exposure towards the (active) substances is indicated to be acceptable.

zRMS comments:

The leaf scenario does not have to be considered taking into account the proposed uses (cereals).
The evaluation of the risk resulting from uptake of contaminated water for Puddle scenario was not required since ratio between effective application rate and endpoint relevant for acute risk and long-term assessment is <3000.
In order to apply consistent approach, the drinking water risk assessment was performed also for metab-olite JAU 6476-S-methyl and is presented below. Calculations were performed with assumption of 10 times toxicity of the parent.

JAU 6476-S-methyl effective application rate 1 x 187.5 g/ha

Acute toxicity (mg/kg bw)	620	quotient =	0.28	Trigger: 3000
Reprod. toxicity (mg/kg bw/d)	9.56	quotient =	18.30	

Overall, the risk for terrestrial vertebrates other than birds from exposure towards the active substances and relevant metabolites is indicated to be acceptable.

9.3.2.4 Effects of secondary poisoning

The (worst-case) log P_{ow} values for fluxapyroxad, prothioconazole, prothioconazole-desthio and prothioconazole-S-methyl are reported to be 3.13, 4.19 at pH=7, 3.04 and 4.19, respectively and thus exceed the trigger value of 3. Risk assessments for effects due to secondary poisoning are required for each (active) substance.

No toxicity data are available for prothioconazole-S-methyl. In a conservative approach, a ten-times increased toxicity as compared to the parent prothioconazole is assumed for secondary poisoning assessments. Likewise, no bioconcentration study in fish is available for this metabolite. This point is addressed based on worst-case QSAR modelling results. It is noted that this approach is conservative. Alternatively, an assumption of a ten-times increased BCF compared to the parent ($BCF_{extrapolated}$ = 197) would be less conservative as the modelled BCF (BCF_{QSAR} = 800.1).

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 dry soil.

For relevant soil matrix concentrations, reference is made to Section B.8 of this submission. 21-day time-weighted average soil concentrations are applied as there are no indications for long-term effects to be caused by short-term exposure. PEC_{soil} corresponds to long-term maxima where applicable (i.e. accounting for plateau soil concentration in case of fluxapyroxad).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group cereals at BBCH 30 to 39 with minimum crop interception (80%) covers the risk for birds from all other intended uses at BBCH > 39 (see 0).

Table 9.3-13: Assessment of the risk for earthworm-eating mammals due to exposure to fluxapyroxad via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (BBCH ≥ 30)

Parameter	Fluxapyroxad	comments
PEC_{soil} (twa = 21 d) [mg/kg soil]	0.0308	21-day time-weighted average based on long-term maximum
log P_{ow} / P_{ow}	3.13 / 1349	
Koc	728	Mean (n = 7)
foc	0.02	Default
BCF_{worm}	1.17	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.036	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose [mg/kg bw/d]	0.046	$DDD = PEC_{worm} \times 1.28$
NO(A)EL [mg/kg bw/d]	10.0	
TER_{lt}	216.9	

TER values shown in **bold** fall below the relevant trigger.

Table 9.3-14: 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 (BBCH ≥ 30)

Parameter	Prothioconazole	comments
PEC_{soil} (twa = 21 d) [mg/kg soil]	0.0096	21-day time-weighted average
log P_{ow} / P_{ow}	4.16 / 14454	
Koc	1765	Aged soil column leaching (n = 1)
foc	0.02	Default
BCF_{worm}	4.94	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.047	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose [mg/kg bw/d]	0.061	$DDD = PEC_{worm} \times 1.28$
NO(A)EL [mg/kg bw/d]	95.6	
TER_{lt}	1576	

TER values shown in **bold** fall below the relevant trigger.

Table 9.3-15: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-desthio via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (BBCH ≥ 30)

Parameter	Prothioconazole-desthio	comments
PEC_{soil} (twa = 21 d) [mg/kg soil]	0.0259 ~~0.0239~~	PECsmax ~~21-day time-weighted average~~
log P_{ow} / P_{ow}	3.04 / 1096	
Koc	575.4	Mean (n = 4)
foc	0.02	Default
BCF_{worm}	1.21~~12~~	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.031~~29~~	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose [mg/kg bw/d]	0.039~~37~~	$DDD = PEC_{worm} \times 1.28$
NO(A)EL [mg/kg bw/d]	10.0	
TER_{lt}	256.41	

Parameter	Prothioconazole-desthio	comments
	~~268.7~~	

TER values shown in **bold** fall below the relevant trigger.

Table 9.3-16: Assessment of the risk for earthworm-eating mammals due to exposure to prothioconazole-S-methyl via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (BBCH ≥ 30)

Parameter	Prothioconazole-S-methyl	comments
PEC_{soil} (twa = 21 d) [mg/kg soil]	0.007~~6~~	21-day time-weighted average,
log P_{ow} / P_{ow}	4.19 / 15488	
Koc	2556.3	Mean (n = 4)
foc	0.02	Default
BCF_{worm}	3.65	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC_{worm}	0.025~~2~~	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose [mg/kg bw/d]	0.032~~28~~	$DDD = PEC_{worm} \times 1.28$
NO(A)EL [mg/kg bw/d]	9.56 [a)]	
TER_{lt}	298.75 ~~340.9~~	

TER values shown in **bold** fall below the relevant trigger.

[a)] As no toxicity data are available for the metabolite of concern, it was assumed that the metabolite is 10 × more toxic than the parent compound (worst-case approach).

Accordingly, the risk for earthworm-eating mammals exposed to the active substances or the prothioconazole-desthio and prothioconazole-S-methyl metabolites is indicated to be acceptable for the intended worst-case use of ADM.03503.F.1.A by a large margin of safety.

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. 21-day time-weighted average surface water concentrations (FOCUS Step 1) are applied as there are no indications for long-term effects to be caused by short-term exposure.

Table 9.3-17: Assessment of the risk for fish-eating mammals due to exposure to fluxapyroxad via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Fluxapyroxad	comments
PEC_{sw} (twa = 21 d) [mg/L]	0.016187	21-day time-weighted average, STEP1
BCF_{fish}	37	
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.599	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose [mg/kg bw/d]	0.085	$DDD = PEC_{fish} \times 0.142$
NO(A)EL [mg/kg bw/d]	10.0	
TER_{lt}	117.6	

TER values shown in **bold** fall below the relevant trigger.

Table 9.3-18: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole via bio-accumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole	comments
PEC_{sw} (twa = 21 d) [mg/L]	0.002796	21-day time-weighted average, STEP1
BCF_{fish}	19.7	
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.055	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose [mg/kg bw/d]	0.0078	$DDD = PEC_{fish} \times 0.142$
NO(A)EL [mg/kg bw/d]	95.6	
TER_{lt}	12223	

TER values shown in **bold** fall below the relevant trigger.

Table 9.3-19: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-desthio via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole-desthio	comments
PEC_{sw} (twa = 21 d) [mg/L]	0.031501	21-day time-weighted average, STEP1
BCF_{fish}	65	
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	2.048	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose [mg/kg bw/d]	0.291	$DDD = PEC_{fish} \times 0.142$
NO(A)EL [mg/kg bw/d]	10.0	
TER_{lt}	34.4	

TER values shown in **bold** fall below the relevant trigger.

Table 9.3-20: Assessment of the risk for fish-eating mammals due to exposure to prothioconazole-S-methyl via bioaccumulation in fish (secondary poisoning) for the intended use in cereals

Parameter	Prothioconazole-S-methyl	comments
PEC_{sw} (twa = 21 d) [mg/L]	0.011628	21-day time-weighted average, STEP1
BCF_{fish}	800.1 [a)]	
BMF	Not relevant	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	9.304	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose [mg/kg bw/d]	1.3211	$DDD = PEC_{fish} \times 0.142$
NO(A)EL [mg/kg bw/d]	9.6 [b)]	
TER_{lt}	7.2	

TER values shown in **bold** fall below the relevant trigger.

[a)] PEC_{fish} was modelled using QSAR data. For maximum conservatism, the worst-case model output (i.e. Arnot-Grobas, mid-trophic, using BCFBAF as part of EPISUITE 4.1 a) was selected.

[b)] As no toxicity data are available for the metabolite of concern, it was assumed that the metabolite is 10× more toxic than the parent compound (worst-case approach).

Accordingly, the risk for fish-eating mammals exposed to the active substances or the prothioconazole-desthio and prothioconazole-S-methyl metabolites is indicated to be acceptable for the intended worst-case use of ADM.03503.F.1.A.

zRMS comments:

The Applicants' approach in evaluation of the risk of secondary poisoning is in line with EFSA (2009). Compounds selected for this assessment are agreed by the zRMS. Evaluation was not triggered for remaining metabolites of active substance due to their log Pow <3.

Some additional corrections were added in tables above in case PEC_s values according to evaluation in area of Section 8.

Despite all corrections of the zRMS, acceptable risk of secondary exposure from all relevant compounds could be concluded for mammals.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant. Reference is made to the conclusions of the EU reviews of the individual active substances (Refer to the EU DAR and EFSA conclusions).

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

Not relevant. The formulated product is intended for use as spray application.

9.3.4 Overall conclusions

The acute and reproductive (long-term) risk for terrestrial vertebrates other than birds from dietary exposure to fluxapyroxad, prothioconazole and the prothioconazole-desthio metabolite is indicated to be acceptable based on Screening Step and/or Tier 1 assessments, including considerations of combined exposure. For the small herbivorous scenario, an acceptable risk is presented based on higher tier assessments accounting for revised crop interception. Likewise, acceptable risk is indicated for the exposure via drinking water and the indirect exposure via secondary poisoning for earthworm- and fish-eating mammals.

Overall, the risk for terrestrial vertebrates other than birds exposed following the intended uses of ADM.03503.F.1.A is acceptable.

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

According to the new data requirements set forth in the Annex to Reg. (EU) no 283/2013 and 284/2013, at present toxicity tests might be requested for birds and mammals but not for amphibians and reptiles. Nevertheless, it is stated that relevant data, including data from the open literature for the active substance of concern, regarding the potential effects to amphibians and reptiles shall be presented and taken into account in the risk assessment, if available.

However, it should be noted that no official risk assessment guideline has been developed so far that could be used to estimate the extent of different exposure routes for amphibians and reptiles under natural conditions. Further, almost no validated standard protocols are yet available for amphibian and reptile testing. The only official test guidelines are the amphibian metamorphosis assay (AMA; not developed to generate endpoints for risk assessment other than endocrine disruption) (OECD 231, September 2009) and the larval amphibian growth and development assay (LAGDA) (OECD 241, July 2015).

In the absence of appropriate test and risk assessment guidelines, only information from the open literature on potential side effects on reptiles and amphibians could be taken into account to estimate a theoretical risk to amphibians and reptiles following the intended uses of ADM.03503.F.1.A. This approach is in line with the recommendations of the guidance document SANCO/10181/2013, Section 4, where it is stated that waivers are acceptable for data requirements for which no agreed test methods or guidance documents are available.

Aquatic life stages of amphibians
According to the new ‘Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters’ (EFSA Journal 2013; 11 (7): 3290), aquatic life stages of amphibians should be included in the risk assessment for aquatic organisms. In the review article from Weltje *et al*. (2013)[5] pairwise comparisons of acute and chronic toxicity data obtained from laboratory tests with different fish and amphibian species were done to determine whether sensitivity systematically differs between these two groups of organisms. As a result, the authors could demonstrate that fish and amphibian toxicity data are highly correlated and fish are more sensitive than amphibians in almost all cases. They concluded that acute and chronic risk to the aquatic life stages of amphibians could be considered as covered by the currently requested risk assessment for aquatic organisms (in particular fish). Similar conclusions can be found also from other authors (e.g. Fryday & Thompson, 2012)[6] and are in line with the EFSA Journal 2013; 11 (7): 3290.

In summary, no adverse effects on aquatic life stages of amphibians need to be expected for the intended uses of ADM.03503.F.1.A, since acceptable effects on fish and other aquatic organisms were identified in the corresponding risk assessment (for details please refer to point 9.5 (*Effects on aquatic organisms*) of this section).

[5] Weltje L, Simpson P, Gross M, Crane M & Wheeler J, 2013. Environmental Toxicology and Chemistry, 32, 984–994
[6] Fryday S & Thompson H, 2012. Supporting Publications 2012: EN-343, 348 pp.

Reptiles and terrestrial life stages of amphibians
Reptiles and terrestrial life stages of amphibians will be addressed in future in a revised guidance document on terrestrial ecotoxicology. At present, a separate risk assessment for reptiles and terrestrial life stages of amphibians is not possible.

While a relatively large number of toxicity data were found for aquatic life stages of amphibians suitable for comparisons with fish data, a far smaller number of studies of variable quality are available on effects of pesticides on terrestrial stages of amphibians or reptiles. This makes a comparison with other terrestrial vertebrate data, i.e. for birds and mammals, more difficult.

However, for reptiles the risk from dietary exposure can be assumed much lower than for birds and mammals, since reptiles are poikilothermic and thus unlike birds and mammals they do not have to feed regularly (e.g. to maintain body temperature). As a result, feeding activity may be restricted to warm days and will be negligible during hibernation or at cold days (Fryday & Thompson, 2009[7]).

In addition, Fryday & Thompson (2012) found several examples where adult amphibians were tested in the same study under the same conditions as birds and mammals. In almost all cases, amphibians were less sensitive than birds and/or mammals, indicating that the currently requested and conducted risk assessments for terrestrial vertebrates exposed to fluxapyroxad and prothioconazole are sufficiently conservative for the terrestrial phase of amphibians and reptiles.

In conclusion, based on the uses intended for ADM.03503.F.1.A, an acceptable risk for terrestrial vertebrates (including amphibians and reptiles) can be reasonably expected for acute or long-term exposure to food burdened with residues of fluxapyroxad and prothioconazole (and metabolites), as indicated by TER values that are above the corresponding trigger values. For details, please refer to data points 9.2 (*Effects on birds*) and 9.3 (*Effects on terrestrial vertebrates other than birds*) of this section.

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.

[7] Fryday S and Thompson H, 2009. Literature reviews on ecotoxicology of chemicals with a special focus on plant protection products. Lot 1. Exposure of reptiles to plant protection products. EFSA (CFT/EFSA/PPR/2008/01).

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 active substances fluxapyroxad and prothioconazole as well as with their relevant transformation/degradation products. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of ADM.03503.F.1.A were not evaluated as part of the EU assessment of the active substances fluxapyroxad or prothioconazole. 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 partly deviates from the results of the EU review process. Justifications are provided below.

Table 9.5-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – active substances fluxapyroxad and prothioconazole as well as relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish - acute toxicity				
Fluxapyroxad, relevant metabolites and representative formulation				
Oncorhynchus mykiss	Fluxapyroxad	96 hours, static	LC_{50} = 0.546 mg a.s./L $_{nom}$	EFSA Journal 2012; 10(1): 2522
Lepomis macrochirus	Fluxapyroxad	96 hours, static	LC_{50} = 1.15 mg a.s./L $_{mm}$	EFSA Journal 2012; 10(1): 2522
Pimephales promelas	Fluxapyroxad	96 hours, static	LC_{50} = 0.466 mg a.s./L $_{mm}$	EFSA Journal 2012; 10(1): 2522
Cyprinus carpio	Fluxapyroxad	96 hours, semi-static	**LC_{50} = 0.29 mg a.s./L $_{mm}$**	EFSA Journal 2012; 10(1): 2522
Cyprinodon variegatus	Fluxapyroxad	96 hours, static	LC_{50} = 1.3 mg a.s./L $_{mm}$	EFSA Journal 2012; 10(1): 2522
Oncorhynchus mykiss	M700F001	96 hours, static	**LC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2012; 10(1): 2522
Oncorhynchus mykiss	M700F002	96 hours, static	**LC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2012; 10(1): 2522
Oncorhynchus mykiss	M700F007	96 hours, static	**LC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2012; 10(1): 2522
Oncorhynchus mykiss	BAS 700 00 F	96 hours, static	LC_{50} = 7.1 mg product/L $_{nom}$ i.e. 0.44 mg a.s./L $_{nom}$	EFSA Journal 2012; 10(1): 2522
Prothioconazole, relevant metabolites and representative formulation				
Oncorhynchus mykiss	Prothioconazole	96 hours, static	**LC_{50} = 1.83 mg a.s./L $_{mm}$**	EFSA Scientific Report (2007) 106, 1-98
Lepomis macrochirus	Prothioconazole	96 hours, static	LC_{50} = 4.59 mg a.s./L $_{mm}$	EFSA Scientific Report (2007) 106, 1-98
Cyprinus carpio	Prothioconazole	96 hours, static	LC_{50} = 6.91 mg a.s./L $_{mm}$	EFSA Scientific Report (2007) 106, 1-98
Oncorhynchus mykiss	Prothioconazole-desthio (M04)	96 hours, static	**LC_{50} = 6.63 mg/L $_{nom}$**	EFSA Scientific Report (2007) 106, 1-98
Leuciscus idus melanotus	Prothioconazole-desthio (M04)	96 hours, static	LC_{50} = 13.2 mg/L $_{mm}$	EFSA Scientific Report (2007) 106, 1-98
Oncorhynchus mykiss	Prothioconazole- S-methyl	96 hours, semi-static	**LC_{50} = 1.8 mg/L $_{mm}$**	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	1,2,4-triazole	96 hours, static	**LC_{50} = 498 mg/L $_{mm}$**	EFSA Scientific Report (2007) 106, 1-98
Oncorhynchus mykiss	Prothioconazole 250 EC	96 hours, static	LC_{50} = 4.02 mg product/L $_{nom}$ i.e. 1.0 mg a.s./L $_{nom}$	EFSA Scientific Report (2007) 106, 1-98
Cyprinus carpio	Prothioconazole 250 EC	96 hours, static	LC_{50} = 10.6 mg product/L $_{nom}$ i.e. 3.72 mg a.s./L $_{nom}$	EFSA Scientific Report (2007) 106, 1-98
Fish - chronic toxicity				
Fluxapyroxad				
Pimephales promelas	Fluxapyroxad	33 days, ELS, flow-through	**NOEC = 0.0359 mg a.s./L $_{mm}$** (growth)	EFSA Journal 2012; 10(1): 2522
Prothioconazole and relevant metabolites				
Oncorhynchus mykiss	Prothioconazole	97 days, ELS, flow-through	**NOEC = 0.308 mg a.s./L $_{mm}$** (reduced swim-up)	EFSA Scientific Report (2007) 106, 1-98
Oncorhynchus mykiss	Prothioconazole-desthio (M04)	97 days, ELS, flow-through	**NOEC = 0.00334 mg/L $_{mm}$** (treatment-related deformities)	EFSA Scientific Report (2007) 106, 1-98
Oncorhynchus mykiss	1,2,4-triazole	28 days, semi-static	**NOEC = 3.2 mg/L $_{nom}$** (transient effect on respiration and behaviour)	EFSA Scientific Report (2007) 106, 1-98 (DAR 2005)
Aquatic invertebrates – acute toxicity				
Fluxapyroxad, relevant metabolites and representative formulation				
Daphnia magna	Fluxapyroxad	48 hours, static	EC_{50} = 6.78 mg a.s./L $_{mm}$	EFSA Journal 2012; 10(1): 2522
Americamysis bahia	Fluxapyroxad	96 hours, static	EC_{50} = 3.6 mg a.s./L $_{mm}$	EFSA Journal 2012; 10(1): 2522
Crassostrea virginica	Fluxapyroxad	96 hours, flow-through	**EC_{50} = 1.1 mg a.s./L $_{mm}$**	EFSA Journal 2012; 10(1): 2522
Daphnia magna	M700F001	48 hours, static	**EC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2012; 10(1): 2522
Daphnia magna	M700F002	48 hours, static	**EC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2012; 10(1): 2522
Daphnia magna	M700F007	48 hours, static	**EC_{50} > 100 mg/L $_{nom}$**	EFSA Journal 2012; 10(1): 2522
Daphnia magna	BAS 700 00 F	48 hours, static	EC_{50} = 19.8 mg product/L $_{nom}$ i.e. 1.24 mg a.s./L $_{nom}$	EFSA Journal 2012; 10(1): 2522
Prothioconazole, relevant metabolites and representative formulation				
Daphnia magna	Prothioconazole	48 hours, static	**EC_{50} = 1.3 mg a.s./L $_{nom}$**	EFSA Scientific Report (2007) 106, 1-98
Daphnia magna	Prothioconazole-desthio (M04)	48 hours, static	**EC_{50} > 10 mg/L $_{nom}$** (extrapolated)	EFSA Scientific Report (2007) 106, 1-98
Daphnia magna	Prothioconazole- S-methyl	48 hours, static	**EC_{50} = 2.8 mg/L $_{nom}$**	EFSA Scientific Report (2007) 106, 1-98
Daphnia magna	1,2,4-triazole	24 hours, static	**EC_{50} = 900 mg/L $_{nom}$**	EFSA Scientific Report (2007) 106, 1-98
Daphnia magna	Prothioconazole 250 EC	48 hours, static	EC_{50} = 2.9 mg product/L $_{nom}$ i.e. 0.71 mg a.s./L $_{nom}$	EFSA Scientific Report (2007) 106, 1-98
Aquatic invertebrates – chronic toxicity				
Fluxapyroxad				
Daphnia magna	Fluxapyroxad	21 days, static	**NOEC = 0.5 mg a.s./L $_{nom}$**	EFSA Journal 2012;

Species	Substance	Exposure System	Results	Reference
			(reproduction)	10(1): 2522
Prothioconazole and relevant metabolites				
Daphnia magna	Prothioconazole	21 days, semi-static	**NOEC = 0.56 mg a.s./L $_{nom}$** (reproduction)	EFSA Scientific Report (2007) 106, 1-98
Daphnia magna	Prothioconazole-desthio (M04)	21 days, semi-static	**NOEC = 0.10 mg/L $_{nom}$** (reproduction)	EFSA Scientific Report (2007) 106, 1-98
Sediment dwelling organisms – chronic toxicity				
Fluxapyroxad				
Chironomus riparius	Fluxapyroxad	28 days, static, spiked water	**NOEC = 75.9 mg a.s./L $_{imm}$**	EFSA Journal 2012; 10(1): 2522
Prothioconazole				
Chironomus riparius	Prothioconazole	28 days, static, spiked water	**NOEC = 9.14 mg a.s./L $_{im}$**	EFSA Scientific Report (2007) 106, 1-98
Chironomus riparius	Prothioconazole-desthio (M04)	28 days, static, spiked water	**NOEC = 2.0 mg/L $_{nom}$**	EFSA Scientific Report (2007) 106, 1-98
Algae				
Fluxapyroxad, relevant metabolites and representative formulation				
Pseudokirchneriella subcapitata	Fluxapyroxad	72 hours, static	**E_rC_{50} = 0.7 mg a.s./L $_{nom}$** E_yC_{50} = 0.4 mg a.s./L $_{nom}$	EFSA Journal 2012; 10(1): 2522
Anabaena flos-aquae	Fluxapyroxad	72 hours, static	E_rC_{50} = 2.61 mg a.s./L $_{mm}$ E_yC_{50} = 1.38 mg a.s./L $_{mm}$	EFSA Journal 2012; 10(1): 2522
Navicula pelliculosa	Fluxapyroxad	72 hours, static	E_rC_{50} > 3.42 mg a.s./L $_{mm}$ E_yC_{50} = 2.31 mg a.s./L $_{mm}$	EFSA Journal 2012; 10(1): 2522
Pseudokirchneriella subcapitata	M700F001	72 hours, static	**E_rC_{50} = 36.31 mg/L $_{nom}$** E_yC_{50} = 26.42 mg/L $_{nom}$	EFSA Journal 2012; 10(1): 2522
Pseudokirchneriella subcapitata	M700F002	72 hours, static	**E_rC_{50} = 26.52 mg/L $_{nom}$** E_yC_{50} = 22.44 mg/L $_{nom}$	EFSA Journal 2012; 10(1): 2522
Pseudokirchneriella subcapitata	M700F007	72 hours, static	**E_rC_{50} > 100 mg/L $_{nom}$** E_yC_{50} > 100 mg/L $_{nom}$	EFSA Journal 2012; 10(1): 2522
Pseudokirchneriella subcapitata	BAS 700 00 F	72 hours, static	E_rC_{50} = 42.4 mg product/L $_{nom}$ i.e. 2.65 mg a.s./L $_{nom}$ E_yC_{50} = 5.4 mg product/L $_{nom}$ i.e. 0.34 mg a.s./L $_{nom}$	EFSA Journal 2012; 10(1): 2522
Prothioconazole, relevant metabolites and representative formulation				
Pseudokirchneriella subcapitata	Prothioconazole	96 (72) hours, static	**72 h E_rC_{50} = 2.18 mg a.s./L $_{im}$** 72 h E_bC_{50} = 1.10 mg a.s./L $_{im}$	EFSA Scientific Report (2007) 106, 1-98
Scenedesmus subspicatus	Prothioconazole-desthio (M04)	96 hours, static	**E_rC_{50} = 0.55 mg/L $_{nom}$** E_bC_{50} = 0.073 mg/L $_{nom}$	EFSA Scientific Report (2007) 106, 1-98
Pseudokirchneriella subcapitata	Prothioconazole- S-methyl	72 hours, static	**E_rC_{50} = 47.4 mg/L $_{im}$** E_bC_{50} = 3.77 mg/L $_{im}$	EFSA Scientific Report (2007) 106, 1-98
Pseudokirchneriella subcapitata	1,2,4-triazole	96 hours, static	**E_rC_{50} = 22.5 mg/L $_{mm}$** E_bC_{50} = 8.2 mg/L $_{mm}$	EFSA Scientific Report (2007) 106, 1-98
Pseudokirchneriella subcapitata	Prothioconazole 250 EC	72 hours, static	72 h E_rC_{50} = 12.7 mg product/L $_{nom}$ i.e. 2.92 mg a.s./L $_{nom}$ 72 h E_bC_{50} = 5.2 mg poroduct/L $_{nom}$ i.e. 1.11 mg a.s./L $_{nom}$	EFSA Scientific Report (2007) 106, 1-98
Aquatic macrophytes				

Species	Substance	Exposure System	Results	Reference
Fluxapyroxad				
Lemna gibba	Fluxapyroxad	7 days, static	**E_rC_{50} > 3.43 mg a.s./L $_{mm}$** E_yC_{50} = 2.19 mg a.s./L $_{mm}$	EFSA Journal 2012; 10(1): 2522
Prothioconazole				
Not tested nor required				
Higher-tier studies (micro- or mesocosm studies)				
Not tested nor required				

Bold: Endpoints used for risk assessments; nom: based on nominal concentrations; mm: based on mean measured concentrations; imm: based on initial mean measured concentrations; im: initial measured concentration; ELS: Fish Early Life Stage test
Grey shading: Data for representative products during EU review of the active substances. Data not considered for the assessments for ADM.03503.F.1.A.

zRMS comments:

Aquatic toxicity data for fluxapyroxad, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.5-1 above has been confirmed by zRMS that they are in line with EU agreed end-points reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

No studies on effects of prothioconazole and metabolite JAU 6476-desthio to Lemna gibba were available during the first EU review. It is noted that testing of aquatic macrophytes was not required for prothioconazole being a fungicide.

Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – ADM.03503.F.1.A

Species	Substance	Exposure System	Results	Reference
Fish - acute toxicity				
Oncorhynchus mykiss	ADM.03503.F.1.A	96 hours, semi-static	**LC_{50} = 3.49 mg product/L $_{mm}$ correspond to 0.7276 mg sum of a.s./L***	KCP 10.2.1/01 [redacted]
Aquatic invertebrates – acute toxicity				
Daphnia magna	ADM.03503.F.1.A	48 hours, static	**EC_{50} = 6.58 mg product/L $_{nom}$ correspond to 1.3718 mg sum of a.s./L***	KCP 10.2.1/02 Juckeland, 2021b
Algae				
Pseudokirchneriella subcapitata	ADM.03503.F.1.A	72 hours, static	**E_rC_{50} = 16.9 mg/L $_{nom}$ correspond to 3.5234 mg sum of a.s./L*** E_yC_{50} = 8.70 mg/L $_{nom}$	KCP 10.2.1/03 Juckeland, 2021c
Higher-tier studies (micro- or mesocosm studies)				
Not tested nor required				

Bold: Endpoints used for risk assessments; nom: based on nominal concentrations; nom: based on nominal concentrations; mm: based on mean measured concentrations.
* Product endpoints corrected for active substance content (sum: 225 g/L) and product density (1.0792 g/mL).

zRMS comments:

Studies on effects of the formulated product on aquatic organisms listed in Table 9.5-2 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

In agreement with EFSA guidance (2013)[8], risk assessments for primary producers, i.e. algae and higher aquatic plants are most adequately based on growth rate endpoints (E_rC_{50}). Accordingly, and in deviation to some endpoints from previous EU reviews, assessments are conducted based on E_rC_{50}.

A summary of relevant endpoints and the derivation of Regulatory Acceptable Concentrations (RACs) is presented in the following table.

Table 9.5-3: Regulatory Acceptable Concentrations for aquatic organisms

Organism group	Test species	Time scale	Test item	Assess-ment tier	Endpoint type	Endpoint [µg/L]	AF	RAC [µg/L]
Fish	*O. mykiss*	Acute	ADM.03503.F.1.A	1	96 h LC_{50}	3490 (prod.)	100	**34.9**
	C. carpio	Acute	Fluxapyroxad	1	96 h LC_{50}	290 (a.s.)	100	**2.9**
	O. mykiss	Acute	M700F001	1	96 h LC_{50}	> 100000 (met.)	100	**> 1000**
	O. mykiss	Acute	M700F002	1	96 h LC_{50}	> 100000 (met.)	100	**> 1000**
	O. mykiss	Acute	M700F007	1	96 h LC_{50}	> 100000 (met.)	100	**> 1000**
	O. mykiss	Acute	Prothioconazole	1	96 h LC_{50}	1830 (a.s.)	100	**18.3**
	O. mykiss	Acute	Prothioconazole-desthio	1	96 h LC_{50}	6630 (met.)	100	**66.3**
	O. mykiss	Acute	Prothioconazole- S-methyl	1	96 h LC_{50}	1800 (met.)	100	**18**
	O. mykiss	Acute	1,2,4-triazole	1	96 h LC_{50}	498000 (met.)	100	**4980**
	P. promelas	Chronic	Fluxapyroxad	1	33 d NOEC	35.9 (a.s.)	10	**3.59**
	O. mykiss	Chronic	Prothioconazole	1	97 d NOEC	308	10	**30.8**
	O. mykiss	Chronic	Prothioconazole-desthio	1	97 d NOEC	3.34	10	**0.334**
	O. mykiss	Chronic	1,2,4-triazole	1	28 d NOEC	3200	10	**320**
Aquatic in-vertebrates	*D. magna*	Acute	ADM.03503.F.1.A	1	48 h EC_{50}	6580 (prod.)	100	**65.8**
	C. virginica	Acute	Fluxapyroxad	1	96 h EC_{50}	1100 (a.s.)	100	**11**
	D. magna	Acute	M700F001	1	48 h EC_{50}	> 100000 (met.)	100	**> 1000**
	D. magna	Acute	M700F002	1	48 h EC_{50}	> 100000 (met.)	100	**> 1000**
	D. magna	Acute	M700F007	1	48 h EC_{50}	> 100000 (met.)	100	**> 1000**
	D. magna	Acute	Prothioconazole	1	48 h EC_{50}	1300 (a.s.)	100	**13**
	D. magna	Acute	Prothioconazole-desthio	1	48 h EC_{50}	> 10000 (met.)	100	**> 100**
	D. magna	Acute	Prothioconazole- S-methyl	1	48 h EC_{50}	2800 (met.)	100	**28**
	D. magna	Acute	1,2,4-triazole	1	48 h EC_{50}	900000 (met.)	100	**9000**
	D. magna	Chronic	Fluxapyroxad	1	21 d NOEC	500 (a.s.)	10	**50**
	D. magna	Chronic	Prothioconazole	1	21 d NOEC	560 (a.s.)	10	**56**
	D. magna	Chronic	Prothioconazole-desthio	1	21 d NOEC	100 (met.)	10	**10**
Sediment dweller	*C. riparius*	Chronic	Fluxapyroxad	1	28 d NOEC	75900 (a.s.)	10	**7590**
	C. riparius	Chronic	Prothioconazole	1	28 d NOEC	9140 (a.s.)	10	**914**
	C. riparius	Chronic	Prothioconazole-desthio	1	28 d NOEC	2000 (met.)	10	**200**
Algae	*P. subcapitata*	Chronic	ADM.03503.F.1.A	1	72 h E_rC_{50}	16900 (prod.)	10	**1690**
	P. subcapitata	Chronic	Fluxapyroxad	1	72 h E_rC_{50}	700 (a.s.)	10	**70**
	P. subcapitata	Chronic	M700F001	1	72 h E_rC_{50}	36310 (met.)	10	**3631**
	P. subcapitata	Chronic	M700F002	1	72 h E_rC_{50}	26520 (met.)	10	**2652**
	P. subcapitata	Chronic	M700F007	1	72 h E_rC_{50}	> 100000 (met.)	10	**> 10000**
	P. subcapitata	Chronic	Prothioconazole	1	72 h E_rC_{50}	2180	10	**218**
	S. subspicatus	Chronic	Prothioconazole-desthio	1	96 h E_rC_{50}	550	10	**55**
	P. subcapitata	Chronic	Prothioconazole- S-methyl	1	72 h E_rC_{50}	47400	10	**4740**
	P. subcapitata	Chronic	1,2,4-triazole	1	96 h E_rC_{50}	22500	10	**2250**
Aquatic my-crophytes	*L. gibba*	Chronic	Fluxapyroxad	1	7 d E_rC_{50}	> 3430	10	**> 343**

AF: Assessment Factor; RAC: Regulatory Acceptable Concentration; prod.: product; a.s.: active substance; met.: metabolite

[8] European Food Safety Authority (2013): Scientific Opinion - Guidance Document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. European Food Safety Authority (EFSA), Parma, Italy; EFSA Journal 11(7): 3290.

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-of-field surface waters in the context of Regulation (EC) No 1107/2009" (EFSA 2013[8]), as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below. Assessments based on FOCUS Step 2 are presented for an average crop cover (worst-case) and alternatively for full canopy, both for Northern and Southern Europe.

For refined risk assessments and in order to account for potential risk from combined exposure towards the active substances and the prothioconazole-desthio metabolite based on comparable modelling tiers, FOCUS Step 4 modelling is provided in addition accounting for a 10 m vegetated (i.e. combined drift and run-off) buffer distance.

Risk assessments are presented for the worst-case uses in winter and spring cereals as in accordance with the relevant risk envelope (see 0).

In the following tables, the ratios between Predicted Environmental Concentrations in surface water bodies (PEC_{SW}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group

The following text is added due to agreements during the Central Zone harmonisation meetings. It should be noted that this text has no impact on the outcome of zonal evaluation of formulation ADM.03502.F.1.A which was performed in line with the EU agreed methodology.

"The endpoint ErC50 is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: "... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future". Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone."

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Fluxapyroxad:

Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fluxapyroxad for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.03503.F.1.A in winter cereals – spring treatment (1.25 L product/ha)

Group		**Fish acute**	**Fish chronic**	**Inverteb. acute**	**Inverteb. chronic**	**Sed. dwell. chronic**	**Algae**	**Aquatic macro-phytes**
Test species		***Cyprinus car-pio***	***Pimephales promelas***	***Crassostrea virginica***	***Daphnia magna***	***Chironomus riparius***	***Pseudokircheriella subcapitata***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}	E_rC_{50}
[µg/L]		290	35.9	1100	500	75900	700	> 3430
AF		100	10	100	10	10	10	10
RAC [µg/L]		2.9	3.59	11	50	7590	70	> 343
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**							
Step 1								
	16.720	**5.77**	**4.66**	**1.52**	0.33	0.0022	0.24	< 0.049
Step 2 (Average crop cover)								
N-Europe	3.013	**1.04**	0.84	0.27	-	-	-	-
S-Europe	5.504	**1.90**	**1.53**	0.50	-	-	-	-
Step 2 (Full canopy)								
N-Europe	1.456	0.50	0.41	0.13	-	-	-	-
S-Europe	2.391	0.82	0.67	0.22	-	-	-	-
Step 3								
D1/ditch	1.840	0.63	0.51	-	-	-	-	-
D1/stream	1.152	0.40	0.32	-	-	-	-	-
D3/ditch	0.593	0.20	0.17	-	-	-	-	-
D4/pond	0.246	0.085	0.069	-	-	-	-	-
D4/stream	0.439	0.15	0.12	-	-	-	-	-
D5/pond	0.136	0.047	0.038	-	-	-	-	-
D5/stream	0.478	0.16	0.13	-	-	-	-	-

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Sed. dwell. chronic	Algae	Aquatic macro-phytes
D6/ditch	0.626	0.22	0.17	-	-	-	-	-
R1/pond	0.067	0.023	0.019	-	-	-	-	-
R1/stream	0.463	0.16	0.13	-	-	-	-	-
R3/stream	0.630	0.22	0.18	-	-	-	-	-
R4/stream	0.874	0.30	0.24	-	-	-	-	-

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 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for fluxapyroxad for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.03503.F.1.A in spring cereals – spring treatment (1.25 L product/ha)

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Sed. dwell. chronic	Algae	Aquatic macro-phytes
Test species		***Cyprinus carpio***	***Pimephales promelas***	***Crassostrea virginica***	***Daphnia magna***	***Chironomus riparius***	***Pseudokircheriella subcapitata***	***Lemna gibba***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}	E_rC_{50}
[µg/L]		290	35.9	1100	500	75900	700	> 3430
AF		100	10	100	10	10	10	10
RAC [µg/L]		2.9	3.59	11	50	7590	70	> 343
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**							
Step 1								
	16.720	**5.77**	**4.66**	**1.52**	0.33	0.0022	0.24	< 0.049
Step 2 (Average crop cover)								
N-Europe	3.013	**1.04**	0.84	0.27	-	-	-	-
S-Europe	5.504	**1.90**	**1.53**	0.50	-	-	-	-
Step 2 (Full canopy)								
N-Europe	1.456	0.50	0.41	0.13	-	-	-	-
S-Europe	2.391	0.82	0.67	0.22	-	-	-	-

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Sed. dwell. chronic	Algae	Aquatic macro-phytes
Step 3								
D1/ditch	1.591	0.55	0.44	-	-	-	-	-
D1/stream	0.998	0.34	0.28	-	-	-	-	-
D3/ditch	0.594	0.20	0.17	-	-	-	-	-
D4/pond	0.233	0.080	0.065	-	-	-	-	-
D4/stream	0.486	0.17	0.14	-	-	-	-	-
D5/pond	0.138	0.048	0.038	-	-	-	-	-
D5/stream	0.501	0.17	0.14	-	-	-	-	-
R4/stream	0.803	0.28	0.22	-	-	-	-	-

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

Conclusion on fluxapyroxad

An acceptable risk for aquatic organisms exposed towards fluxapyroxad following the intended worst-case uses of ADM.03503.F.1.A is indicated based on FOCUS Step 1 to Step 3 modelling, i.e. without the necessity to consider risk mitigation measures.

zRMS comments:

Based on calculations provided in the Tables 9.5-4 and 9.5-5 no unacceptable risk to aquatic organisms from exposure to fluxapyroxad has been identified on FOCUS step 3 for spring and winter cereals.

Metabolites of fluxapyroxad:

Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for M700F001 for each organism group based on FOCUS Steps 1 calculations for the use of ADM.03503.F.1.A in winter/spring cereals – spring treatment (1.25 L product/ha)

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
[µg/L]		> 100000	> 100000	36310
AF		100	100	10
RAC [µg/L]		> 1000	> 1000	3631
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**			
Step 1	3.351	< 0.0034	< 0.0034	0.00092

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 M700F002 for each organism group based on FOCUS Steps 1 calculations for the use of ADM.03503.F.1.A in winter/spring cereals – spring treatment (1.25 L product/ha)

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
[µg/L]		> 100000	> 100000	26520
AF		100	100	10
RAC [µg/L]		> 1000	> 1000	2652
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**			
Step 1	9.268	< 0.0093	< 0.0093	0.0035

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 M700F007 for each organism group based on FOCUS Steps 1 calculations for the use of ADM.03503.F.1.A in winter/spring cereals – spring treatment (1.25 L product/ha)

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
[µg/L]		> 100000	> 100000	> 100000
AF		100	100	10
RAC [µg/L]		> 1000	> 1000	> 10000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**			
Step 1	2.607	< 0.0026	< 0.0026	< 0.00026

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

Conclusion on metabolites of fluxapyroxad

An acceptable risk for aquatic organisms exposed towards metabolites/metabolites of fluxapyroxad, i.e. M700F001, M700F002 and M700F007 following the intended worst-case uses of ADM.03503.F.1.A is indicated based on conservative FOCUS Step 1 modelling, i.e. without the necessity to consider risk mitigation measures.

zRMS comments:

Based on calculations provided in the Tables from 9.5-6 to 9.5-8 no unacceptable risk to aquatic organisms from exposure to fluxapyroxad metabolites has been identified on FOCUS step 1.

Prothioconazole:

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.03503.F.1.A in winter cereals – spring treatment (1.25 L product/ha)

Group		**Fish acute**	**Fish chronic**	**Inverteb. acute**	**Inverteb. chronic**	**Sed. dwell. chronic**	**Algae**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Chironomus riparius***	***Pseudokircheriella. subcapitata***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}
[µg/L]		1830	308	1300	560	9140	2180
AF		100	10	100	10	10	10
RAC [µg/L]		18.3	30.8	13	56	914	218
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**						
Step 1							
	20.363	**1.11**	0.66	**1.57**	0.36	0.022	0.093
Step 2 (Average crop cover)							
N-Europe	1.724	0.094	0.056	0.13	-	-	-
S-Europe	1.724	0.094	0.056	0.13	-	-	-
Step 2 (Full canopy)							
N-Europe	1.724	0.094	0.056	0.13	-	-	-
S-Europe	1.724	0.094	0.056	0.13	-	-	-
Step 3							
D1/ditch	1.189	-	-	-	-	-	-
D1/stream	0.925	-	-	-	-	-	-
D3/ditch	1.185	-	-	-	-	-	-
D4/pond	0.041	-	-	-	-	-	-
D4/stream	0.876	-	-	-	-	-	-
D5/pond	0.041	-	-	-	-	-	-
D5/stream	0.946	-	-	-	-	-	-
D6/ditch	1.171	-	-	-	-	-	-

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Sed. dwell. chronic	Algae
R1/pond	0.041	-	-	-	-	-	-
R1/stream	0.781	-	-	-	-	-	-
R3/stream	1.097	-	-	-	-	-	-
R4/stream	0.784	-	-	-	-	-	-

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 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.03503.F.1.A in spring cereals – spring treatment (1.25 L product/ha)

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Sed. dwell. chronic	Algae
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Chironomus riparius***	***Pseudokircheriella. subcapitata***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}
[µg/L]		1830	308	1300	560	9140	2180
AF		100	10	100	10	10	10
RAC [µg/L]		18.3	30.8	13	56	914	218
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**						
Step 1							
	20.363	**1.11**	0.66	**1.57**	0.36	0.022	0.093
Step 2 (Average crop cover)							
N-Europe	1.724	0.094	0.056	0.13	-	-	-
S-Europe	1.724	0.094	0.056	0.13	-	-	-
Step 2 (Full canopy)							
N-Europe	1.724	0.094	0.056	0.13	-	-	-
S-Europe	1.724	0.094	0.056	0.13	-	-	-

Group		Fish acute	Fish chronic	Inverteb. acute	Inverteb. chronic	Sed. dwell. chronic	Algae
Step 3							
D1/ditch	1.199	-	-	-	-	-	-
D1/stream	1.049	-	-	-	-	-	-
D3/ditch	1.186	-	-	-	-	-	-
D4/pond	0.041	-	-	-	-	-	-
D4/stream	0.970	-	-	-	-	-	-
D5/pond	0.041	-	-	-	-	-	-
D5/stream	0.996	-	-	-	-	-	-
R4/stream	0.784	-	-	-	-	-	-

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

Conclusion on prothioconazole

An acceptable risk for aquatic organisms exposed towards prothioconazole following the intended worst-case uses of ADM.03503.F.1.A is indicated based on FOCUS Step 1 to Step 3 modelling, i.e. without the necessity to consider risk mitigation measures.

zRMS comments:

Based on calculations provided in the Tables 9.5-9 and 9.5-10 no unacceptable risk to aquatic organisms from exposure to prothioconazole has been identified on FOCUS step 3 for spring and winter cereals.

Metabolites of prothioconazole:

Table 9.5-11: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.03503.F.1.A in winter cereals – spring treatment (1.25 L product/ha)

Group		**Fish acute**	**Fish chronic**	**Invertebrates acute**	**Invertebrates chron-ic**	**Sediment dwellers chron-ic**	**Algae**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***D. magna***	***C. riparius***	***S. subcapitatus***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}
[µg/L]		6630	3.34	> 10000	100	2000	550
AF		100	10	100	10	10	10
RAC [µg/L]		66.3	0.334	> 100	10	200	55
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**						
Step 1							
	36.680	0.55	**109.8**	< 0.37	**3.67**	0.18	0.67
Step 2 (Average crop cover)							
N-Europe	3.406	-	**10.2**	-	0.34	-	-
S-Europe	6.277	-	**18.8**	-	0.63	-	-
Step 2 (Full canopy)							
N-Europe	1.611	-	**4.82**	-	0.16	-	-
S-Europe	2.688	-	**8.05**	-	0.27	-	-

Group		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chron-ic	Sediment dwellers chron-ic	Algae
Step 3							
D1/ditch	0.019	-	0.057	-	-	-	-
D1/stream	0.039	-	0.12	-	-	-	-
D3/ditch	0.019	-	0.057	-	-	-	-
D4/pond	0.006	-	0.018	-	-	-	-
D4/stream	0.022	-	0.066	-	-	-	-
D5/pond	0.007	-	0.021	-	-	-	-
D5/stream	0.033	-	0.10	-	-	-	-
D6/ditch	0.010	-	0.030	-	-	-	-
R1/pond	0.035	-	0.10	-	-	-	-
R1/stream	0.332	-	0.99	-	-	-	-
R3/stream	0.408	-	**1.22**	-	-	-	-
R4/stream	0.603	-	**1.81**	-	-	-	-

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 9.5-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of ADM.03503.F.1.A in spring cereals – spring treatment (1.25 L product/ha)

Group		**Fish acute**	**Fish chronic**	**Invertebrates acute**	**Invertebrates chronic**	**Sediment dwellers chronic**	**Algae**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***D. magna***	***C. riparius***	***S. subcapitatus***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	NOEC	E_rC_{50}
[µg/L]		6630	3.34	> 10000	100	2000	550
AF		100	10	100	10	10	10
RAC [µg/L]		66.3	0.334	> 100	10	200	55
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**						
Step 1							
	36.680	0.55	**109.8**	< 0.37	**3.67**	0.18	0.67
Step 2 (Average crop cover)							
N-Europe	3.406	-	**10.2**	-	0.34	-	-
S-Europe	6.277	-	**18.8**	-	0.63	-	-
Step 2 (Full canopy)							
N-Europe	1.611	-	**4.82**	-	0.16	-	-
S-Europe	2.688	-	**8.05**	-	0.27	-	-

Group		Fish acute	Fish chronic	Invertebrates acute	Invertebrates chronic	Sediment dwellers chronic	Algae
Step 3							
D1/ditch	0.155	-	0.46	-	-	-	-
D1/stream	0.059	-	0.18	-	-	-	-
D3/ditch	0.038	-	0.11	-	-	-	-
D4/pond	0.008	-	0.024	-	-	-	-
D4/stream	0.025	-	0.075	-	-	-	-
D5/pond	0.007	-	0.021	-	-	-	-
D5/stream	0.035	-	0.10	-	-	-	-
R4/stream	0.521	-	**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.**

For the intended uses in winter cereals (spring application) ~~cereals,~~ for prothioconazole-desthio (M04) metabolite the calculated PEC/RAC ratio did not indicate an acceptable risk for the most sensitive group of aquatic organisms (chronic risk for fish as characterised by a NOEC for rainbow trout of 3.34 µg a.s./L in connection with an assessment factor of 10) for FOCUS R3 and R4 stream at FOCUS Step 3. In addition, the intended uses in spring cereals (spring application), the calculated PEC/RAC ratio did not indicate an acceptable risk for the most sensitive group of aquatic organisms (chronic risk for fish as characterised by a NOEC for rainbow trout of 3.34 µg a.s./L in connection with an assessment factor of 10) for FOCUS R4 stream at FOCUS Step 3.

Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies by means of a 10 m vegetated buffer distance (combined drift/run-off buffer).

Table 9.5-13: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) based on FOCUS Step 4 calculations with mitigation of spray drift and run-off for the use of ADM.03503.F.1.A in winter cereals – spring treatment (1.25 L product/ha)

Intended use		Winter cereals
Active substance		Prothioconazole-desthio
Application rate [g/ha]		1 × 187.5 (a.s.)
Nozzle reduction	**No-spray buffer [m]**	10
	Vegetated filter strip [m]	10
	FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**
None	R3 stream	0.186
None	R4 stream	0.274
RAC [µg/L]		Fish (chronic)
0.334		**PEC/RAC ratio**
None	R3 stream	0.56
None	R4 stream	0.82

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

Table 9.5-14: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for prothioconazole-desthio (M04) based on FOCUS Step 4 calculations with mitigation of spray drift and run-off for the use of ADM.03503.F.1.A in spring cereals – spring treatment (1.25 L product/ha)

Intended use		Spring cereals
Active substance		Prothioconazole-desthio
Application rate [g/ha]		1 × 187.5 (a.s.)
Nozzle reduction	**No-spray buffer [m]**	10
	Vegetated filter strip [m]	10
	FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**
None	R4 stream	0.237
RAC [µg/L]		Fish (chronic)
0.334		**PEC/RAC ratio**
None	R4 stream	0.71

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

Table 9.5-15: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for prothioconazole-S-methyl (M01) for each organism group based on FOCUS Steps 1 calculations for the use of ADM.03503.F.1.A in winter/spring cereals – spring treatment (1.25 product/ha)

Group		**Fish acute**	**Invertebrates acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
[µg/L]		1800	2800	47400
AF		100	100	10
RAC [µg/L]		18	28	4740
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**			
Step 1	14.898	0.83	0.53	0.0031

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 9.5-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole (M13) for each organism group based on FOCUS Steps 1 calculations for the use of ADM.03503.F.1.A in winter/spring cereals – spring treatment (1.25 product/ha)

Group		**Fish acute**	**Fish chronic**	**Invertebrates acute**	**Algae**
Test species		***O. mykiss***	***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	NOEC	EC_{50}	E_rC_{50}
[µg/L]		498000	3200	900000	22500
AF		100	10	100	10
RAC [µg/L]		4980	320	9000	2250
FOCUS Scenario	**PEC $_{gl\text{-}max}$ [µg/L]**				
Step 1	4.832	0.00097	0.015	0.00054	0.0021

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

Conclusion on metabolites of prothioconazole

An acceptable risk for aquatic organisms exposed towards metabolites/metabolites of prothioconazole, i.e. prothioconazole-S-methyl (M01) and 1,2,4-triazole (M13) following the intended worst-case uses of ADM.03503.F.1.A is indicated based on conservative FOCUS Step 1 modelling.

However, for the metabolite prothioconazole-desthio (M04), Step 3 modelling is required in order to indicate acceptable risk for aquatic invertebrates in winter cereals.

In case of winter cereals, for FOCUS R3 and R4 stream, and in case of spring cereals for R4 stream potential risk is indicated at the default distance to surface water bodies. A 10 m vegetated buffer distance is required in order to indicate acceptable risk for these scenarios.

zRMS comments:

No unacceptable risk to aquatic organisms of the metabolites prothioconazole-S-methyl (M01) and 1,2,4-triazole (M13) is identified on FOCUS step 1.

For the metabolite prothioconazole-desthio, an unacceptable risk is identified with FOCUS step 3 calculations for the scenarios relevant for the Central Zone countries (R3 and R4 scenarios) applied for in this application.
It should be noted that for R1 scenario PEC/RAC ratio is in borderline (0.99).
Further refinement for these scenarios was presented with FOCUS STEP 4 PECsw calculations with 10 m VFS to surface water bodies.

Assessments based on combined exposure

Assessments for combined exposure of the (relevant) constituents of the formulated product ADM.03503.F.1.A are presented based on data for the formulated product (whole-mixture approach) as well as based on assumed Concentration Addition (CA) using the available data for the individual active substances.

Risk assessment based on product data

Risk assessments based on the data for ADM.03503.F.1.A are most adequately related to Predicted Environmental Concentrations in surface water via drift entry. Deposition following volatilisation does not significantly impact the overall exposure concentrations from direct product entry into surface water. Both active substances have low vapour pressures.

Initial risk assessments for an application rate of 1.25 L product/ha (corresponding to 1349 g product/ha based on a product density of 1.0792 g/mL) are conducted for the default distance to surface water bodies (i.e. 1 m). In addition, the risk assessment expressed in sum of a.s was added.

Table 9.5-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ADM.03503.F.1.A for each organism group based on FOCUS drift entry for the use in winter cereals (1.25 L product/ha)

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
[µg/L]		3490	6580	16900
AF		100	100	10
RAC [µg/L]		34.9	65.8	1690
	PEC $_{gl\text{-}max}$ [µg/L]			
Drift (default distance)				
FOCUS ditch/stream	8.6668	0.25	0.13	0.0051
Rautmann drift	12.456	0.36	0.19	0.0074

Table 9.5 17-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for ADM.03503.F.1.A for each organism group based on FOCUS drift entry for the use in winter cereals (1.25 L product/ha expressed in a.s. unit)

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***
Endpoint		LC_{50}	EC_{50}	E_rC_{50}
[µg sum of a.s./L]		7260	1370	
AF		100	100	10
RAC [µg sum of a.s./L]		7.26	1.37	352.34
	PEC $_{gl\text{-}max}$ [µg sum of a.s./L]			
Drift (default distance)				
FOCUS ditch/stream	1.95	0.26	**1.42**	0.005
5 meter buffer zone	0.52	0.071	0.37	0.0014

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

Accordingly, the assessments based on measured product toxicity indicate and acceptable risk for aquatic organisms, i.e., fish (acute), aquatic invertebrates (acute) and algae, from exposure via drift entry by a great margin of safety without the necessity to account for risk mitigation measures.

zRMS comments:

Calculations with the formulation endpoint and spray-drift for the formulation are only indicative of the risk from one input source (spray-drift).

Component-based mixture toxicity assessments

ADM.03503.F.1.A contains two active substances (i.e. fluxapyroxad and prothioconazole). However, prothioconazole-desthio is a metabolite of prothioconazole for which the available toxicity package indicates significant toxicity, exceeding that of the active substance. For this reason, prothioconazole-desthio is also taken into consideration for the assessment of potential risk from combined exposure. However, the combined consideration of active substances and metabolite are only meaningful in context of an assessment of the predicted time-course of exposure for all three constituents. Mixture considerations are based on the combined toxicity of the pairing of fluxapyroxad and prothioconazole (active substances in the formulated product) and (due to the low persistence of prothioconazole) alternatively for the pairing of fluxapyroxad with prothioconazole-desthio.

Mixture toxicity considerations based on active substance data under consideration of all entry pathways are presented under consideration of the aquatic EFSA guidance document (2013).

A ´toxicity per fraction´ assessment is performed providing information on the relative contribution of the active substances to the overall toxicity of the mixture based on the fractions of active substances as in the formulated product by assuming concentration addition (CA). For detailed explanation of the calculations reference is made to the EFSA birds and mammals guidance (2009[9]) and the Aquatic guidance document (EFSA 2013[10]). A surrogate endpoint for CA is calculated using the following equation.

$$EC_{X\ mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{EC_{Xi}} \right)^{-1}$$

With:

$EC_{X\ mix-CA}$	surrogate endpoint for additive mixture toxicity
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 ($\Sigma\ p_i = 1$)
EC_{Xi}	concentration of component I provoking X % effect (or $NOEC_i$)

Fractions in the mixture are calculated according to the following equation with the sum of fractions being 1.

$$p_1 = c_1/c_1 + \cdots + c_n$$

Based on active substance concentrations of 75 g fluxapyroxad/L and 150 g prothioconazole/L, fractions (p_i) of 0.33 and 0.67, respectively are calculated for the product composition. Alternatively, based on

[9] European Food Safety Authority; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA. EFSA Journal 2009; 7(12): 1438. doi: 10.2903/j.efsa.2009.1438.

[10] European Food Safety Authority (2013): Scientific Opinion - Guidance Document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. European Food Safety Authority (EFSA), Parma, Italy; EFSA Journal 11(7): 3290.

active substance concentrations of 75 g fluxapyroxad/L and 136 g prothioconazole-desthio/L fractions (p_i) of 0.36 and 0.64, respectively are calculated.

The surrogate endpoint is related to the measured EC_X or NOEC ($EC_{X\ PPP}$) from the product studies, where available, building the Model Deviation Ratio (MDR).

$$MDR = \frac{EC_{X\ mix-CA}}{EC_{X\ PPP}}$$

With an MDR in the range of 0.2 to 5 the predicted endpoint for CA is interpreted as to be in line with the measured toxicity. Values below 0.2 indicate a potential antagonism (i.e. CA overestimates mixture toxicity), whereas values greater than 5 might indicate a potential synergism (i.e. CA potentially underestimates mixture toxicity).

In the following table, the acute and chronic mixture toxicity assessments and MDR calculations are presented for combined exposure towards fluxapyroxad and prothioconazole for all relevant aquatic organisms. In addition, the ratios of surrogate toxicity estimates are shown.

Table 9.5-18: Toxicity per fraction assessment and MDR calculation for additive mixture toxicity for aquatic organisms – fluxapyroxad and prothioconazole

Organism	Time scale	Test substance	Fraction of a.s. in the mixture (X_n)	Toxicity endpoint [µg a.s./L]	Toxicity per fraction for PPP/ Surrogate endpoint $EC_{X\ mix-CA\ PPP}$ [µg/L]	Contribution to overall toxicity [%]	MDR
Fish	acute	Fluxapyroxad	0.33	290	870.0	**75.9**	0.91
		Prothioconazole	0.67	1830	2745.0	**24.1**	
		ADM.03503.F.1.A	1.0	3490 / 727.6 [a]	**660.6**	n.a.	
	chronic	Fluxapyroxad	0.33	35.9	107.7	**81.1**	n.a.
		Prothioconazole	0.67	308	462.0	**18.9**	
		ADM.03503.F.1.A	1.0	n.a.	**87.3**	n.a.	
Aquatic invertebrates	acute	Fluxapyroxad	0.33	6780 [b]	20340.0	8.7	1.30
		Prothioconazole	0.67	1300	1950.0	**91.3**	
		ADM.03503.F.1.A	1.0	6580 / 1371.8 [a]	**1779.4**	n.a.	
	chronic	Fluxapyroxad	0.33	500	1500.0	**35.9**	n.a.
		Prothioconazole	0.67	560	840.0	**64.1**	
		ADM.03503.F.1.A	1.0	n.a.	**538.5**	n.a.	
Sediment dweller	chronic	Fluxapyroxad	0.33	75900	227700.0	5.7	n.a.
		Prothioconazole	0.67	9140	13710.0	**94.3**	
		ADM.03503.F.1.A	1.0	n.a.	12931.4	n.a.	
Algae	chronic	Fluxapyroxad	0.33	700	2100.0	**60.9**	0.36
		Prothioconazole	0.67	2180	3270.0	**39.1**	
		ADM.03503.F.1.A	1.0	16900 / 3523.4 [a]	**1278.8**	n.a.	
Aquatic macrophytes (*Lemna*)	chronic	Fluxapyroxad	0.33	> 3430	10290.0	n.a.	n.a.
		Prothioconazole	0.67	n.a.	n.a.	n.a.	
		ADM.03503.F.1.A	1.0	n.a.	**n.a.**	n.a.	

n.a. not available/applicable; PPP Plant Protection Product; MDR: Model Deviation Ratio.
[a] Product endpoint corrected for active substance content (sum: 225 g/L) and product density (1.0792 g/mL).
[b] For comparison, the higher endpoint for *Daphnia magna* was considered instead of the overall lowest endpoint for *C. virginica.*

The ´toxicity per fraction´ assessment based on the composition as in the formulated product indicates that for all organism groups, with exception of aquatic invertebrates (acute toxicity) and sediment dwellers, the theoretical combined toxicity based on the model of Concentration Addition (CA) is driven by both active substances. In case of acute toxicity to invertebrates (based on both daphnid endpoints) as well as for sediment dwellers, the overall toxicity is determined by the active substance prothioconazole, whereas fluxapyroxad does not significantly influence the toxicity (i.e. contribution to overall toxicity is < 10%). Accordingly, no mixture toxicity assessments are deemed necessary for acute invertebrates and

sediment dwellers. In contrast, assessments of risk from combined exposure are presented for fish (acute and chronic), aquatic invertebrates (chronic) and algae. No data on aquatic macrophytes are available nor triggered.

The Model Deviation Ratios (MDRs) with values below 1 for fish (acute) and algae suggest that the measured toxicity from testing of the formulated product ADM.03503.F.1.A is lower than the predicted toxicity based on CA. However, following the criteria as set in the EFSA aquatic guidance (2013) for MDR values between 0.2 and 5, observed and calculated mixture toxicity is in agreement (also for invertebrates acute where MDR slightly exceed 1). Overall, there is no indication of an increased toxicity of the active substances when in formulation (i.e. no synergism), whereas the comparison would rather suggest potential antagonism for fish and algae.

zRMS comments:

Based on the calculations provided in the Table 9.5-18 for fluxapyroxad and prothioconazole with the exception of aquatic invertebrates (acute toxicity) and sediment dwellers, both active substances contribute to the toxicity, and mixture toxicity thus needs to be presented for these organisms.

Alternatively, and in a worst-case approach, additional mixture toxicity considerations are done for the pairing of fluxapyroxad with the prothioconazole-desthio metabolite. The respective ´toxicity per fraction´ assessment is presented in the following table. It is noted that this contemplation is conservative as it cannot be assumed that the desthio metabolite co-occurs with fluxapyroxad in ratios as assumed based on the pseudo-content of prothioconazole-desthio (136 g/L) assuming 100% generation from parent prothioconazole as determined based on their molar mass ratios.

Table 9.5-19: Toxicity per fraction assessment and MDR calculation for additive mixture toxicity for aquatic organisms – fluxapyroxad and prothioconazole-desthio

Organism	Time scale	Test substance	Fraction of a.s. in the mixture (X_n)	Toxicity endpoint [µg a.s./L]	Toxicity per fraction for PPP/ Surrogate endpoint $EC_{X\ mix\text{-}CA}$ PPP [µg/L]	Contribution to overall toxicity [%]	MDR
Fish	acute	Fluxapyroxad	0.36	290	815.9	**92.7**	1.11
		Prothioconazole-desthio	0.64	6630	10286.3	7.3	
		ADM.03503.F.1.A	1.0	3490 / 682.3 [b)]	**755.9**	n.a.	
	chronic	Fluxapyroxad	0.36	35.9	101.0	4.9	n.a.
		Prothioconazole-desthio	0.64	3.34	5.2	**95.1**	
		ADM.03503.F.1.A	1.0	n.a.	**4.9**	n.a.	
Aquatic invertebrates	acute	Fluxapyroxad	0.36	6780 [c)]	19074.4	**44.9**	**6.65**
		Prothioconazole-desthio	0.64	> 10000	15514.7	**55.1**	
		ADM.03503.F.1.A	1.0	6580 / 1286.5 [b)]	**8555.7**	n.a.	
	chronic	Fluxapyroxad	0.36	500	1406.7	9.9	n.a.
		Prothioconazole-desthio	0.64	100	155.1	**90.1**	
		ADM.03503.F.1.A	1.0	n.a.	**139.7**	n.a.	
Sediment dweller	chronic	Fluxapyroxad	0.36	75900	213532.0	1.4	n.a.
		Prothioconazole-desthio	0.64	2000	3102.9	**98.6**	
		ADM.03503.F.1.A	1.0	n.a.	**3058.5**	n.a.	
Algae	chronic	Fluxapyroxad	0.36	700	1969.3	**30.2**	0.18
		Prothioconazole-desthio	0.64	550	853.3	**69.8**	
		ADM.03503.F.1.A	1.0	16900 / 3304.2 [b)]	**595.3**	n.a.	
Aquatic macrophytes (*Lemna*)	chronic	Fluxapyroxad	0.36	> 3430	9649.7	n.a.	n.a.
		Prothioconazole-desthio	0.64	n.a.	n.a.	n.a.	
		ADM.03503.F.1.A	1.0	n.a.	**n.a.**	n.a.	

n.a. not available/applicable; PPP Plant Protection Product; MDR: Model Deviation Ratio.

[a)] Calculated from active substance contents for prothioconazole (150 g/L) based on the molar masses of 344.26 and 312.2 g/mol for prothioconazole and prothioconazole-desthio (i.e. corresponding to 136 g/L), respectively.

[b)] Product endpoint corrected for active substance content (sum: 225 g/L) and product density (1.0792 g/mL).

[c)] For comparison, the higher endpoint for *Daphnia magna* was considered instead of the overall lowest endpoint for *C. virginica.*

For the pairing of fluxapyroxad with the desthio-metabolite of prothioconazole, only for aquatic invertebrates acute and algae, both actives significantly contribute to overall toxicity, whereas for fish acute, overall toxicity is indicated to be driven by fluxapyroxad, whereas prothioconazole-desthio is the driver of overall toxicity in case of the chronic toxicity to fish, aquatic invertebrates and sediment dwellers.

MDRs indicate that the product toxicity would be in agreement with predicted toxicity based on CA assuming immediate conversion to the desthio-metabolite of prothioconazole with the exception of acute toxicity to *Daphnia*, where the MDR exceeds 5 which would fulfil the criterion for potential synergism (i.e. an increased toxicity of the formulated product). For algae, the resulting MDR is below 0.2 indicative of potential antagonism according to EFSA criteria. However, as explained, this theoretical evaluation based on the assumption that prothioconazole-desthio co-occurs with fluxapyroxad in the ratio assuming immediate and complete transformation from prothioconazole is not realistically reflecting the exposure and substance ratios expected in the field.

zRMS comments:

Based on the calculations provided in the Table 9.5-19 for fluxapyroxad and prothioconazole-desthio with the exception of aquatic invertebrates (acute toxicity) and algae, both active substances contribute to the toxicity, and mixture toxicity thus needs to be presented for these organisms.
zRMS also agrees with argument regarding MDR for acute toxicity to Daphnia, and synergism is not assumed.

Based on the initial evaluations for the ratio of fluxapyroxad and prothioconazole in the formulated product, mixture toxicity assessments for fish (acute and chronic), aquatic invertebrates (chronic) and algae are presented by consideration of combined toxicity following the Risk Quotient Approach using the following equation.

$$RQ_{mix} = \sum_{n} \frac{PEC_{SW}}{RAC}$$

Based on this approach, a time-resolved risk assessment over the entire modelling simulation period is feasible. Overlay of the risk quotient curves for all active substances are performed based on relevant RACs and modelling output.

It is noted that this CA approach is more meaningful as the standard approach as proposed in the EFSA guidance document which requests assessments of mixture toxicity based on surrogate endpoints for the mixture. However, those surrogate toxicity estimates are dependent on the fractions of the individual active substances in the mixture. These ratios are time-variable dependent on the different active substance properties. The RQ_{mix} approach pursued here is fully compatible with the time-resolved FOCUS output as well as the principal assessment scheme as in accordance with EFSA guidance, i.e. the assessments based on ratios of PEC_{SW} and RAC (i.e. RQ), whereas EFSA guidance proposes the calculation of ETRs. It is noted that the RQ approach is also presented in the guidance.

In the component-based assessments presented, the maximum RQ (PEC_{SW}/RAC) based on global maximum PEC_{SW} for each active substance is presented along with the RQ for each active substance at the maximum RQ_{mix}, which might deviate from maximum RQ if peak maxima of the individual active substances occur at different times in the simulation period of the different FOCUS scenarios. RQ_{mix} is the sum of RQs where this value reaches the maximum over the entire simulation period. In a conservative approach, RQ_{mix} is also presented based on individual peak maxima (i.e. assessment ignoring the time of peak events).

In addition to the active substances fluxapyroxad and prothioconazole, prothioconazole-desthio (M04) is

considered in a conservative approach as this substance exhibits significant toxicity. Exposure towards M04 conservatively is conservatively considered in combination with the parent prothioconazole (and fluxapyroxad).

The component-based mixture toxicity assessments are presented in the following tables for the intended uses in winter and spring cereals, respectively. The assessments are based on PEC_{SW} for the maximum risk mitigation measures as required for the individual active substances, i.e. a 10 m vegetated (combined drift and run-off) buffer distance as required for R3 and R4 stream in winter cereals and R4 stream in spring cereals, respectively.

Table 9.5-20: Maximum RQ_{mix} for aquatic organisms based on risk assessments for individual active substances – winter cereals (1.25 L product/ha) with 10 m vegetated/drift buffer distance

FOCUS scenario		Max. PEC_{SW} Fluxapyroxad [µg a.s./L]	Max. PEC_{SW} Prothioconazole [µg a.s./L]	Max. PEC_{SW} Prothioconazole-desthio [µg a.s./L]	Max. RQ Fluxapyroxad (PEC/RAC)	Max. RQ Prothioconazole (PEC/RAC)	Max. RQ Prothioconazole-desthio (PEC/RAC)	RQ_{mix} based on max. RQ (sum of RQ)	RQ at max. RQ_{mix} Fluxapyroxad	RQ at max. RQ_{mix} Prothioconazole	RQ at max. RQ_{mix} Prothioconazole-desthio	Max. RQ_{mix} [b)]
Fish acute												
				RAC [µg a.s./L]:	**2.9**	**18.3**	**66.3**		**Time-resolved assessment**			
D1	ditch	1.840	0.171	0.004	0.63	0.0093	0.00006	0.64	0.6345	0.0000	0.0000	0.63
	stream	1.152	0.179	0.008	0.40	0.0098	0.00012	0.41	0.3973	0.0000	0.0000	0.40
D3	ditch	0.085	0.170	0.003	0.029	0.0093	0.000045	0.039	0.0294	0.0093	0.0000	0.039
D4	pond	0.245	0.025	0.003	0.084	0.0014	0.000045	0.09	0.0846	0.0000	0.0000	0.085
	stream	0.350	0.170	0.004	0.12	0.0093	0.000060	0.13	0.1205	0.0000	0.0000	0.12
D5	pond	0.134	0.025	0.004	0.046	0.0014	0.000060	0.048	0.0463	0.0000	0.0000	0.046
	stream	0.226	0.183	0.006	0.078	0.010	0.000090	0.088	0.0779	0.0000	0.0000	0.078
D6	ditch	0.569	0.168	0.001	0.20	0.0092	0.000015	0.21	0.1961	0.0000	0.0000	0.20
R1	pond	0.029	0.025	0.015	0.010	0.0014	0.00023	0.012	0.0101	0.0000	0.0002	0.010
	stream	0.211	0.151	0.151	0.073	0.0083	0.0023	0.083	0.0726	0.0003	0.0023	0.075
R3	stream	0.287	0.212	0.186	0.10	0.012	0.0028	0.11	0.0991	0.0005	0.0028	0.10
R4	stream	0.397	0.152	0.274	0.14	0.0083	0.0041	0.15	0.1370	0.0002	0.0041	0.14
Fish chronic												
				RAC [µg a.s./L]:	**3.59**	**30.8**	**0.334**		**Time-resolved assessment**			
D1	ditch	1.840	0.171	0.004	0.51	0.0056	0.012	0.53	0.5126	0.0000	0.0027	0.52
	stream	1.152	0.179	0.008	0.32	0.0058	0.024	0.35	0.3209	0.0000	0.0018	0.32
D3	ditch	0.085	0.170	0.003	0.024	0.0055	0.0090	0.038	0.0238	0.0055	0.0000	0.029
D4	pond	0.245	0.025	0.003	0.068	0.00081	0.009	0.078	0.0683	0.0000	0.0018	0.070
	stream	0.350	0.170	0.004	0.10	0.0055	0.012	0.11	0.0974	0.0000	0.0090	0.11
D5	pond	0.134	0.025	0.004	0.037	0.00081	0.012	0.050	0.0374	0.0000	0.0009	0.038
	stream	0.226	0.183	0.006	0.063	0.0059	0.018	0.087	0.0629	0.0000	0.0009	0.064
D6	ditch	0.569	0.168	0.001	0.16	0.0055	0.0030	0.17	0.1584	0.0000	0.0003	0.16
R1	pond	0.029	0.025	0.015	0.008	0.00081	0.045	0.054	0.0082	0.0000	0.0416	0.050
	stream	0.211	0.151	0.151	0.059	0.0049	0.45	0.52	0.0586	0.0002	0.4509	0.51
R3	stream	0.287	0.212	0.186	0.080	0.0069	0.56	0.64	0.0801	0.0003	0.5566	0.64
R4	stream	0.397	0.152	0.274	0.11	0.0049	0.82	0.94	0.1107	0.0001	0.8207	0.93

FOCUS scenario		Max. PEC_{SW} Fluxapyroxad [µg a.s./L]	Max. PEC_{SW} Prothioconazole [µg a.s./L]	Max. PEC_{SW} Prothioconazole-desthio [µg a.s./L]	Max. RQ Fluxapyroxad (PEC/RAC)	Max. RQ Prothioconazole (PEC/RAC)	Max. RQ Prothioconazole-desthio (PEC/RAC)	RQ_{mix} based on max. RQ (sum of RQ)	RQ at max. RQ_{mix} Fluxapyroxad	RQ at max. RQ_{mix} Prothioconazole	RQ at max. RQ_{mix} Prothioconazole-desthio	Max. RQ_{mix} [b]
Invertebrates acute												
				RAC [µg a.s./L]:	**11** [a]	**13**	**> 100**		**Time-resolved assessment**			
D1	ditch	1.840	0.171	0.004	0.17	0.013	0.000040	0.18	0.1673	0.0000	0.0000	0.17
	stream	1.152	0.179	0.008	0.10	0.014	0.00008	0.12	0.1047	0.0000	0.0000	0.10
D3	ditch	0.085	0.170	0.003	0.0077	0.013	0.000030	0.021	0.0078	0.0131	0.0000	0.021
D4	pond	0.245	0.025	0.003	0.022	0.0019	0.000030	0.024	0.0223	0.0000	0.0000	0.022
	stream	0.350	0.170	0.004	0.032	0.013	0.000040	0.045	0.0318	0.0000	0.0000	0.032
D5	pond	0.134	0.025	0.004	0.012	0.0019	0.000040	0.014	0.0122	0.0000	0.0000	0.012
	stream	0.226	0.183	0.006	0.021	0.014	0.000060	0.035	0.0205	0.0000	0.0000	0.021
D6	ditch	0.569	0.168	0.001	0.052	0.013	0.000010	0.065	0.0517	0.0000	0.0000	0.052
R1	pond	0.029	0.025	0.015	0.0026	0.0019	0.00015	0.0047	0.0027	0.0000	0.0001	0.003
	stream	0.211	0.151	0.151	0.019	0.012	0.0015	0.032	0.0191	0.0004	0.0015	0.021
R3	stream	0.287	0.212	0.186	0.026	0.016	0.0019	0.044	0.0261	0.0007	0.0019	0.029
R4	stream	0.397	0.152	0.274	0.036	0.012	0.0027	0.051	0.0361	0.0002	0.0027	0.039
Invertebrates chronic												
				RAC [µg a.s./L]:	**50**	**56**	**10**		**Time-resolved assessment**			
D1	ditch	1.840	0.171	0.004	0.037	0.0031	0.00040	0.040	0.0368	0.0000	0.0001	0.037
	stream	1.152	0.179	0.008	0.023	0.0032	0.00080	0.027	0.0230	0.0000	0.0001	0.023
D3	ditch	0.085	0.170	0.003	0.0017	0.0030	0.00030	0.0050	0.0017	0.0030	0.0000	0.0047
D4	pond	0.245	0.025	0.003	0.0049	0.00045	0.00030	0.0056	0.0049	0.0000	0.0001	0.0050
	stream	0.350	0.170	0.004	0.0070	0.0030	0.00040	0.010	0.0070	0.0000	0.0003	0.0073
D5	pond	0.134	0.025	0.004	0.0027	0.00045	0.00040	0.0035	0.0027	0.0000	0.0000	0.0027
	stream	0.226	0.183	0.006	0.0045	0.0033	0.00060	0.0084	0.0045	0.0000	0.0000	0.0045
D6	ditch	0.569	0.168	0.001	0.011	0.0030	0.00010	0.014	0.0114	0.0000	0.0000	0.011
R1	pond	0.029	0.025	0.015	0.0006	0.00045	0.0015	0.0025	0.0006	0.0000	0.0014	0.0020
	stream	0.211	0.151	0.151	0.0042	0.0027	0.015	0.022	0.0042	0.0001	0.0151	0.019
R3	stream	0.287	0.212	0.186	0.0057	0.0038	0.019	0.028	0.0057	0.0002	0.0186	0.025
R4	stream	0.397	0.152	0.274	0.0079	0.0027	0.027	0.038	0.0079	0.0001	0.0274	0.035

FOCUS scenario		Max. PEC_{SW} Fluxapyroxad [µg a.s./L]	Max. PEC_{SW} Prothioconazole [µg a.s./L]	Max. PEC_{SW} Prothioconazole-desthio [µg a.s./L]	Max. RQ Fluxapyroxad (PEC/RAC)	Max. RQ Prothioconazole (PEC/RAC)	Max. RQ Prothioconazole-desthio (PEC/RAC)	RQ_{mix} based on max. RQ (sum of RQ)	RQ at max. RQ_{mix} Fluxapyroxad	RQ at max. RQ_{mix} Prothioconazole	RQ at max. RQ_{mix} Prothioconazole-desthio	Max. RQ_{mix} [b]
Algae												
				RAC [µg a.s./L]:	**70**	**218**	**55**		**Time-resolved assessment**			
D1	ditch	1.840	0.171	0.004	0.026	0.00078	0.00007	0.027	0.0263	0.0000	0.0000	0.026
	stream	1.152	0.179	0.008	0.016	0.00082	0.00015	0.017	0.0165	0.0000	0.0000	0.016
D3	ditch	0.085	0.170	0.003	0.0012	0.00078	0.000055	0.0020	0.0012	0.0008	0.0000	0.0020
D4	pond	0.245	0.025	0.003	0.0035	0.00011	0.000055	0.0037	0.0035	0.0000	0.0000	0.0035
	stream	0.350	0.170	0.004	0.0050	0.00078	0.000073	0.0059	0.0050	0.0000	0.0001	0.0050
D5	pond	0.134	0.025	0.004	0.0019	0.00011	0.000073	0.0021	0.0019	0.0000	0.0000	0.0019
	stream	0.226	0.183	0.006	0.0032	0.00084	0.00011	0.0042	0.0032	0.0000	0.0000	0.0032
D6	ditch	0.569	0.168	0.001	0.0081	0.00077	0.000018	0.0089	0.0081	0.0000	0.0000	0.0081
R1	pond	0.029	0.025	0.015	0.00041	0.00011	0.00027	0.0008	0.0004	0.0000	0.0003	0.0007
	stream	0.211	0.151	0.151	0.0030	0.00069	0.0027	0.0065	0.0030	0.0000	0.0027	0.0058
R3	stream	0.287	0.212	0.186	0.0041	0.00097	0.0034	0.0085	0.0041	0.0000	0.0034	0.0075
R4	stream	0.397	0.152	0.274	0.0057	0.00070	0.0050	0.011	0.0057	0.0000	0.0050	0.011

bold: PEC_{SW}/RAC (RQ_{mix}) exceeding the trigger.

[a] Worst-case endpoint for *C. virginica* conservatively considered.

[b] Maximum RQ_{mix} over the entire FOCUS simulation period; i.e. for time-resolved mixture toxicity assessment.

zRMS comments:

The Mix-tox including PEC_{sw} from FOCUS step 4 for each of a.s. and metabolite Prothioconazole desthio (M04) has been presented in the Table above for all scenarios with consideration of 10 m VFS to surface water bodies for application at rate 1.25 L/ha for winter cereals. The max RQmix is below 1 indicating an acceptable risk.

Based on the calculations for all scenarios 10 m VFS is required from mixture toxicity assessment.

Table 9.5-21: Maximum RQ_{mix} for aquatic organisms based on risk assessments for individual active substances – spring cereals (1.25 L product/ha) with 10 m vegetated/drift buffer distance

FOCUS scenario		Max. PEC_{SW} Fluxapyroxad [µg a.s./L]	Max. PEC_{SW} Prothioconazole [µg a.s./L]	Max. PEC_{SW} Prothioconazole-desthio [µg a.s./L]	Max. RQ Fluxapyroxad (PEC/RAC)	Max. RQ Prothioconazole (PEC/RAC)	Max. RQ Prothioconazole-desthio (PEC/RAC)	RQ_{mix} based on max. RQ (sum of RQ)	RQ at max. RQ_{mix} Fluxapyroxad	RQ at max. RQ_{mix} Prothioconazole	RQ at max. RQ_{mix} Prothioconazole-desthio	Max. RQ_{mix} [b)]
Fish acute												
				RAC [µg a.s./L]:	**2.9**	**18.3**	**66.3**		**Time-resolved assessment**			
D1	ditch	1.591	0.172	0.022	0.55	0.0094	0.00033	0.56	0.5487	0.0000	0.0000	0.55
	stream	0.998	0.203	0.011	0.34	0.0111	0.00017	0.36	0.3442	0.0000	0.0000	0.34
D3	ditch	0.085	0.170	0.005	0.029	0.0093	0.000075	0.039	0.0232	0.0064	0.0001	0.030
D4	pond	0.232	0.025	0.005	0.080	0.0014	0.000075	0.081	0.0799	0.0000	0.0000	0.080
	stream	0.333	0.188	0.005	0.11	0.010	0.000075	0.13	0.1150	0.0000	0.0001	0.12
D5	pond	0.137	0.025	0.004	0.047	0.0014	0.000060	0.049	0.0472	0.0000	0.0000	0.047
	stream	0.234	0.193	0.007	0.081	0.011	0.00011	0.091	0.0808	0.0000	0.0000	0.081
R4	stream	0.365	0.152	0.237	0.13	0.0083	0.0036	0.14	0.1259	0.0050	0.0036	0.13
Fish chronic												
				RAC [µg a.s./L]:	**3.59**	**30.8**	**0.334**		**Time-resolved assessment**			
D1	ditch	1.591	0.172	0.022	0.44	0.0056	0.066	0.51	0.4432	0.0000	0.0039	0.45
	stream	0.998	0.203	0.011	0.28	0.0066	0.033	0.32	0.2780	0.0000	0.0024	0.28
D3	ditch	0.085	0.170	0.005	0.024	0.0055	0.015	0.044	0.0187	0.0038	0.0144	0.037
D4	pond	0.232	0.025	0.005	0.065	0.00081	0.015	0.080	0.0645	0.0000	0.0027	0.067
	stream	0.333	0.188	0.005	0.093	0.0061	0.015	0.11	0.0929	0.0000	0.0126	0.11
D5	pond	0.137	0.025	0.004	0.038	0.00081	0.012	0.051	0.0382	0.0000	0.0009	0.039
	stream	0.234	0.193	0.007	0.065	0.0063	0.021	0.092	0.0653	0.0000	0.0009	0.066
R4	stream	0.365	0.152	0.237	0.10	0.0049	0.71	0.82	0.1017	0.0030	0.7096	0.81
Invertebrates acute												
				RAC [µg a.s./L]:	**11** [a)]	**13**	**> 100**		**Time-resolved assessment**			
D1	ditch	1.591	0.172	0.022	0.14	0.013	0.00022	0.16	0.1446	0.0000	0.0000	0.14
	stream	0.998	0.203	0.011	0.091	0.016	0.00011	0.11	0.0907	0.0000	0.0000	0.091
D3	ditch	0.085	0.170	0.005	0.0077	0.013	0.000050	0.021	0.0061	0.0090	0.0000	0.015
D4	pond	0.232	0.025	0.005	0.021	0.0019	0.000050	0.023	0.0211	0.0000	0.0000	0.021
	stream	0.333	0.188	0.005	0.030	0.014	0.000050	0.045	0.0303	0.0000	0.0000	0.030
D5	pond	0.137	0.025	0.004	0.012	0.0019	0.000040	0.014	0.0125	0.0000	0.0000	0.012
	stream	0.234	0.193	0.007	0.021	0.015	0.000070	0.036	0.0213	0.0000	0.0000	0.021
R4	stream	0.365	0.152	0.237	0.033	0.012	0.0024	0.047	0.0332	0.0071	0.0024	0.043

FOCUS scenario		Max. PEC_{SW} Fluxapyroxad [µg a.s./L]	Max. PEC_{SW} Prothioconazole [µg a.s./L]	Max. PEC_{SW} Prothioconazole-desthio [µg a.s./L]	Max. RQ Fluxapyroxad (PEC/RAC)	Max. RQ Prothioconazole (PEC/RAC)	Max. RQ Prothioconazole-desthio (PEC/RAC)	RQ_{mix} based on max. RQ (sum of RQ)	RQ at max. RQ_{mix} Fluxapyroxad	RQ at max. RQ_{mix} Prothioconazole	RQ at max. RQ_{mix} Prothioconazole-desthio	Max. RQ_{mix} [b]
Invertebrates chronic												
				RAC [µg a.s./L]:	**50**	**56**	**10**		**Time-resolved assessment**			
D1	ditch	1.591	0.172	0.022	0.032	0.0031	0.0022	0.037	0.0318	0.0000	0.0001	0.032
	stream	0.998	0.203	0.011	0.020	0.0036	0.0011	0.025	0.0200	0.0000	0.0001	0.020
D3	ditch	0.085	0.170	0.005	0.0017	0.0030	0.00050	0.0052	0.0013	0.0021	0.0005	0.0039
D4	pond	0.232	0.025	0.005	0.0046	0.00045	0.00050	0.0056	0.0046	0.0000	0.0001	0.0047
	stream	0.333	0.188	0.005	0.0067	0.0034	0.00050	0.011	0.0067	0.0000	0.0004	0.0071
D5	pond	0.137	0.025	0.004	0.0027	0.00045	0.00040	0.0036	0.0027	0.0000	0.0000	0.0028
	stream	0.234	0.193	0.007	0.0047	0.0034	0.00070	0.0088	0.0047	0.0000	0.0000	0.0047
R4	stream	0.365	0.152	0.237	0.0073	0.0027	0.024	0.034	0.0073	0.0017	0.0237	0.033
Algae												
				RAC [µg a.s./L]:	**70**	**218**	**55**		**Time-resolved assessment**			
D1	ditch	1.591	0.172	0.022	0.023	0.00079	0.00040	0.024	0.0227	0.0000	0.0000	0.023
	stream	0.998	0.203	0.011	0.014	0.00093	0.00020	0.015	0.0143	0.0000	0.0000	0.014
D3	ditch	0.085	0.170	0.005	0.0012	0.00078	0.000091	0.0021	0.0010	0.0005	0.0001	0.0016
D4	pond	0.232	0.025	0.005	0.0033	0.00011	0.000091	0.0035	0.0033	0.0000	0.0000	0.0033
	stream	0.333	0.188	0.005	0.0048	0.00086	0.000091	0.0057	0.0048	0.0000	0.0001	0.0048
D5	pond	0.137	0.025	0.004	0.0020	0.00011	0.000073	0.0021	0.0020	0.0000	0.0000	0.0020
	stream	0.234	0.193	0.007	0.0033	0.00089	0.00013	0.0044	0.0033	0.0000	0.0000	0.0034
R4	stream	0.365	0.152	0.237	0.0052	0.00070	0.0043	0.010	0.0052	0.0004	0.0043	0.010

bold: PEC_{SW}/RAC (RQ_{mix}) exceeding the trigger.
[a] Worst-case endpoint for *C. virginica* conservatively considered.
[b] Maximum RQ_{mix} over the entire FOCUS simulation period; i.e. for time-resolved mixture toxicity assessment.

zRMS comments:

The Mix-tox including PECsw from FOCUS step 4 for each of a.s. and metabolite Prothioconazole desthio (M04) has been presented in the Table above for all scenarios with consideration of 10 m VFS to surface water bodies for application at rate 1.25 L/ha for winter cereals. The RQmix is below 1 indicated an acceptable risk.

Based on the calculations for all scenarios 10 m VFS is required from mixture toxicity assessment.

Accordingly, RQ_{mix} values below 1 for all FOCUS scenarios indicate an acceptable risk from combined exposure of fluxapyroxad, prothioconazole and even prothioconazole-desthio if accounting for the 10 m vegetated buffer as is required for the desthio-metabolite of prothioconazole.

9.5.3 Overall conclusions

An acceptable risk for aquatic organisms from exposure towards the active substances and their relevant metabolites in water as well as from combined exposure towards the active substances and the critical metabolite prothioconazole-desthio (M04) was indicated by Risk Quotients below 1 provided a 10 m vegetated (i.e. combined drift and run-off) buffer is taken into account for the uses in winter as well as spring cereals.

zRMS comments:

Conclusions above were amended accordingly with consideration of the outcome of the performed risk assessment.

Please note that Additional calculations may be required by cMS that do not accept surface water exposure derived using FOCUS models.

The acceptability and applicability of the indicated risk mitigation measures has to be confirmed at the cMS level.

The following text is added due to agreements during the Central Zone harmonisation meetings.
It should be noted that this text has no impact on the outcome of zonal evaluation of formulation ADM.03503.F.1.A, which was performed in line with the EU agreed methodology.

"The endpoint E_rC_{50} is selected in this Core Assessment but there are some uncertainties regarding the level of protection reached for primary producers. This is indicated for macrophytes in the aquatic Guidance Document (EFSA Journal 2013;11(7):3290) that recommends: "... a proper calibration between different tiers (higher and lower tier data) for macrophytes should be performed in the future". Such calibration should be extended to algae. Until available relevant information on the level of protection reached is considered at EU level, it is recommended to address this uncertainty at each Member State level in the National Addendum if considered necessary, although it would be highly appreciated to have a harmonised approach in the Central zone."

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the acute toxicity to bees have been carried out with the active substances fluxapyroxad and prothioconazole. Full details of these studies are provided in the respective EU DARs and related documents.

Effects on bees of ADM.03503.F.1.A were not evaluated as part of the EU assessment of the active substances fluxapyroxad and prothioconazole. 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 partly deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System / Design	Results	Reference
ADM.03503.F.1.A				
Apis mellifera	ADM.03503.F.1.A	96 h, oral	48 h LD_{50} = 788 µg product/bee **96 h LD_{50} = 721 µg product/bee**	KCP 10.3.1.1/01 Franke, 2020
Apis mellifera	ADM.03503.F.1.A	96 h, contact	48 h LD_{50} > 1000 µg product/bee **96 h LD_{50} = 974 µg product/bee**	KCP 10.3.1.1/01 Franke, 2020
Apis mellifera	ADM.03503.F.1.A	10 d, chronic oral	LC_{50} = 5.534 g product/kg food **LDD_{50} = 107 µg product/bee/day**	KCP 10.3.1.2/01 Dreßler, 2021
Apis mellifera	ADM.03503.F.1.A	22 d, larval development	NOEC = 3.207 mg product/kg food **NOED = 0.507 µg product/bee** **ED_{10}=0.179 µg product/bee**	KCP 10.3.1.3/01 Hänsel, 2021
Fluxapyroxad and representative formulated product				
Apis mellifera	Fluxapyroxad	48 h, oral	**LD_{50} > 110.9 µg a.s./bee**	EFSA Journal 2012; 10(1): 2522
Apis mellifera	Fluxapyroxad	48 h, contact	**LD_{50} > 100 µg a.s./bee**	EFSA Journal 2012; 10(1): 2522
Apis mellifera	BAS 700 00 F (62.1 g a.s./L)	48 h, oral	LD_{50} > 2721 µg product/bee	EFSA Journal 2012; 10(1): 2522
Apis mellifera	BAS 700 00 F (62.1 g a.s./L)	48 h, contact	LD_{50} = 448 µg product/bee	EFSA Journal 2012; 10(1): 2522
Prothioconazole and representative formulated product				
Apis mellifera	Prothioconazole	48 h, oral	**LD_{50} > 71 µg a.s./bee**	EFSA Scientific Report (2007) 106, 1-98
Apis mellifera	Prothioconazole	48 h, contact	**LD_{50} > 200 µg a.s./bee**	EFSA Scientific Report (2007) 106, 1-98
Apis mellifera	Prothioconazole EC 250	48 h, oral	LD_{50} > 48.7 µg a.s./bee	EFSA Scientific Report (2007) 106, 1-98
Apis mellifera	Prothioconazole EC 250	48 h, contact	LD_{50} > 200 µg a.s./bee	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Exposure System / Design	Results	Reference
Higher-tier studies (tunnel test, field studies)				
Apis mellifera	ADM.03503.F.1.A	Semi-field / Tunnel test in Germany (Central zone) (OECD 75) with *Phacelia tanacetifolia*; 7 days exposure (post-exposure from days 7 to 28); 1 application during bee flight	NOAER = 1.25 L product/ha (in 400 L/ha) No effects ~~on mortality and~~ colony development.	KCP 10.3.1.5/01 Persigehl *et al.*, 2022a
Apis mellifera	ADM.03503.F.1.A	Tunnel test in Spain (Southern zone) (OECD 75) with *Phacelia tanacetifolia*; 10 days exposure (post-exposure from days 10 to 28); 1 application during bee flight	NOAER = 1.25 L product/ha (in 400 L/ha) ~~No effects on mortality and~~ Colony development.	KCP 10.3.1.5/02 Persigehl *et al.*, 2022b
Apis mellifera	BAS 700 00 F (61.6 g a.s./L)	Bee tunnel test acc. to OECD 75/EPPO 170 on *Phacelia tanacetifolia*	No effect on foraging activity, mean brood termination or brood compensation index at 2.0 L product/ha	EFSA Journal 2012; 10(1): 2522

Grey shading: Data for representative products during EU review of the active substances. Data not considered for the assessments for ADM.03503.F.1.A.

zRMS comments:

Bee toxicity data for fluxapyroxad, prothioconazole and prothioconazole metabolite JAU 6476-desthio provided in Table 9.5-1 above has been confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

Studies on effects of the formulated product on bees listed in Table 9.6-1 were evaluated by the zRMS and considered acceptable.

The data requirements for bees according to Commission Regulation (EU) No 284/2013 are fulfilled.

Summaries of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.6.1.1 Justification for new endpoints

Acute risk assessments are provided for the two active substances fluxapyroxad and prothioconazole. Additionally, acute risk assessments are presented for the relevant formulated product which are considered to be most relevant as these data additionally account for potential effects due to the combined exposure towards the active substances fluxapyroxad and prothioconazole.
No chronic data (10-day bee adult and 22-day bee larval exposure) are available for the active substances fluxapyroxad and prothioconazole.
Adult bee chronic and bee larval testing is made available for the formulated product ADM.03503.F.1.A fulfilling the data requirements as in accordance with Commission Regulations (EC) 283/2013 and 284/2013.

In addition, two semi-field tests are provided with exposure of honeybees foraging on *Phacelia* in tunnels for the maximum in-field exposure of 1.25 L product/ha covering bee mortality as well as colony development.

9.6.2 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[11]). The recently developed "EFSA Guidance Document on the risk of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)" (EFSA Journal 2013; 11(7): 3295; updated version published on 4 July 2014) is not yet voted and therefore not taken into account.

To achieve a concise risk assessment, the risk envelope approach is applied, i.e. for a maximum single use rate of 1.25 L product/ha (corresponding to 93.75 g fluxapyroxad and 187.5 prothioconazole/ha, respectively for a maximum BBCH range of 30 to 69 (see 0).

No agreed assessment scheme is available for the chronic risk for adult bees as well as the risk for bee larval development. Therefore, no risk assessments are provided based on the available chronic data. However, it is noted that the semi-field studies made available, for the maximum intended in-field exposure under worst-case tunnel exposure conditions indicate an acceptable risk for bees covering adult survival during bee flight as well as bee colony development.

9.6.2.1 Hazard quotients for bees

The acute risk assessments for the formulated product ADM.03503.F.1.A as well as the active substances is presented in the following table.

[11] European Commission Health & Consumer Protection Directorate-General. Directorate E – Food Safety: plant health, animal health and welfare, international questions (2002): DRAFT Working Document. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev final. 17 October 2002.

Table 9.6-2: First-tier assessment of the risk for bees due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha)

Intended use	**Cereals (BBCH 30-69) [a)]**		
Product	ADM.03503.F.1.A		
Application rate [g/ha]	1 × 1349 [b)]		
Test design	**LD_{50} (lab.) [µg/bee]**	**Single application rate [g/ha]**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	721	1349	1.87
Contact toxicity	974		1.39
Active substance	Fluxapyroxad		
Application rate [g/ha]	1 × 93.75		
Test design	**LD_{50} (lab.) [µg/bee]**	**Single application rate [g/ha]**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 110.9	93.75	< 0.85
Contact toxicity	> 100		< 0.94
Active substance	Prothioconazole		
Application rate [g/ha]	1 × 187.5		
Test design	**LD_{50} (lab.) [µg/bee]**	**Single application rate [g/ha]**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 71	187.5	< 2.64
Contact toxicity	> 200		< 0.94

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in **bold** breach the relevant trigger.
[a)] Risk envelope.
[b)] Based on a product density of 1.0792 g/cm^2 (refer to CoA for the ecotoxicological test batch) and an application rate of 1.25 L product/ha.

The HQ values are well below the trigger indicating an acceptable acute risk for bees for the exposure towards the formulated product as well as the individual active substances for the intended worst-case use of ADM.03503.F.1.A in cereals.

zRMS comments:

Acute risk assessment to bees**:**

The risk assessment presented in Tables 9.6-2 is validated by the zRMS.
HQoral, contact values for the active substances and the formulated product 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, as according to conclusions of the Central Zone Steering Committee (CZSC), recommendations of EFSA (2013) should not be considered for the zonal evaluations until the guidance is noted at the EU level.

The chronic and larvae risk assessment is not required according to SANCO/10329/2002 rev 2 final.
Due to the fact that the chronic tests are available for adult bee and larvae, the screening step and Tier 1 risk assessment in line with EFSA (2013) for request of some cMS in Central Zone has been performed by the zRMS below, using endpoints from submitted studies.

Chronic risk assessment to bees:

All steps for acute and the chronic risk assessment, i.e. the screening step, 1st and 2 nd oral tier calculations were performed using the corresponding EFSA Bee calculator Tool (Bee-Tool v.3) provided by EFSA.

Screening step risk assessment

The acute and chronic risks to adult honey bees and honey bee larvae bees from the use of ADM.03503.F.1.A were assessed using the maximum single application rates and the respective 'hazard quotients' (HQs) and 'exposure toxicity ratios' (ETRs).

Test	Endpoint µg prod./bee	Calculation factor	HQ/ETR	Trigger	Risk acceptable?
Cereals, BBCH 30-69, maximum application dose 0.1349 kg product/ha					
Acute Oral route of exposure					
Honey Bee	721	7.6	0.00	0.2	Y
Acute contact route of exposure					
Honey Bee	974	1	0.1	42	
Chronic Oral route of exposure					
Honey bee, chronic	107	7.6	0.010	0.03	Y
Honey bee, larvae	0.179	4.4	**3.32**	0.2	**N**

HQ/ETR values in bold are above the trigger value

Considering the proposed uses of ADM.03503.F.1.A at a maximum application rate of 0.1349 kg product/ha a potential risk of formulation is indicated following the chronic exposure of honey bee larvae at this stage of testing. Therefore, 1st tier oral risk assessments were carried out (see Table below).

1st tier, oral risk assessment

In the screening step, potential risk was indicated for honey bee larvae. In the following, a crop and life stage-specific (larvae) risk assessment is carried out, which is a first step of refinement. On the one hand, this takes into account crop dependent exposure factors (Ef), and on the other hand it considers SV values, which depend on default values for pollen and nectar consumption, sugar content in nectar, residues (RUDs) in pollen and nectar as well as crop attractiveness (see table below). It is noted that 1st tier risk assessment scheme in EFSA (2013) allows for distinguishing between particular BBCH stages of the crop in question. Therefore, it was decided by the zRMS to perform separate risk assessment for particular stages at which ADM.03503.F.1.A. will be applied to cereals.

tier oral risk assessment for honey bees (chronic and larvae)

Crop (Crop group according to EFSA tool)	Endpoint	ETR (oral exposure scenario)					Trigger
		Treated crop	Weeds	Field margin	Adjacent crop	Next crop	
Maximum single application rate: kg product/ha, BBCH 30-39							
Cereals	larvae	0.10	**0.7**	0.01	0.01	**0.26**	0.2
Maximum single application rate: kg product/ha, BBCH 40-69							
Cereals	larvae	0.10	**0.42**	0.01	0.01	**0.26**	0.2

An unacceptable chronic risk for bee larvae was identified for scenarios: weeds and next crop.
In order to resolve the chronic risk for ADM.03503.F.1.A the Applicant submitted two separate tunnel studies in Germany by Persigehl et al., 2022a and Spain by Persigehl et al., 2022b.
Based on the results of these studies it was noted that there are no effects on larval development at the worst-case application rate of 1.25 L product/ha in semi-field conditions in bee attractive crop (Phacelia tanacetifolia).
Therefore, in zRMS's opinion the chronic risk can be resolved bases on that studies results.

In the same time, it should be noted that the risk assessment based on EFSA (2013) is provided above for informative purposes only and is not the basis for derivation of conclusion regarding the risk to bees at the zonal level.

This issue should be further resolved at the product authorisation in Member States considering indications of the not yet noted EFSA guidance in their national assessments.

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

Not relevant. An acceptable acute risk is presented based on Tier 1 data for the formulated product ADM.03503.F.1.A as well as the individual active substances fluxapyroxad and prothioconazole.

zRMS comments:

Potential chronic risk for honeybee larvae cannot be excluded based on data for the formulated product applying the EFSA GD 2013 risk assessment scheme.

Two separate tunnel studies provided in Germany and Spain by Persigehl et al., 2022a and Persigehl et al., 2022b respectively show that there are no effects on larval development at the worst-case application rate of 1.25 L product/ha in semi-field conditions.

9.6.3 Effects on bumble bees

No data on bumble bees are provided or considered necessary. There is no agreed assessment scheme for bumble bees and the assessments of acute risk for honeybees indicate an acceptable risk by a great margin of safety.

9.6.4 Effects on solitary bees

No data on solitary bees are provided or considered necessary. There is no agreed study protocols and assessment scheme for solitary bees and the assessments of acute risk for honeybees indicate an acceptable risk by a great margin of safety.

9.6.5 Overall conclusions

An acceptable acute risk is indicated for exposure of bees towards the formulated product as well as the individual active substances for the intended worst-case use of ADM.03503.F.1.A.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Studies on the toxicity to non-target arthropods have been carried out with the representative products for EU reviews of the active substances fluxapyroxad and prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of ADM.03503.F.1.A were not evaluated as part of the EU assessment of the active substances fluxapyroxad or prothioconazole. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

Table 9.7-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
ADM.03503.F.1.A				
Aphidius rhopalosiphi (adults)	ADM.03503.F.1.A	Laboratory test glass plates (2D)	LR_{50} = 0.954 L product/ha **ER_{50} > 0.843 L product/ha**	KCP 10.3.2.1/01 Röhlig, 2020a
Typhlodromus pyri (protonymphs)	ADM.03503.F.1.A	Laboratory test glass plates (2D)	LR_{50} > 1.193 L product/ha **ER_{50} > 1.193 L product/ha**	KCP 10.3.2.1/02 Röhlig, 2020b
Representative product for fluxapyroxad				
Aphidius rhopalosiphi (adults)	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	Laboratory test glass plates (2D)	LR_{50} = 4.70 L product/ha	EFSA Journal 2012; 10(1): 2522
Aphidius rhopalosiphi (adults)	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	Extended laboratory test (3D), barley seedlings	LR_{50} > 6.0 L product/ha ER_{50} > 6.0 L product/ha (29.4% reduced fecundity at 6.0 L product/ha)	EFSA Journal 2012; 10(1): 2522
Typhlodromus pyri (protonymphs)	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	Laboratory test glass plates (2D)	LR_{50} = 0.128 L product/ha	EFSA Journal 2012; 10(1): 2522
Typhlodromus pyri (protonymphs)	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	Extended laboratory test (2D), bean leaves	LR_{50} = 1.62 L product/ha ER_{50} < 2.0 L product/ha (53.9% reduced fecundity at 2.0 L product/ha)	EFSA Journal 2012; 10(1): 2522
Typhlodromus pyri (protonymphs)	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	Aged residue test (2D), bean leaves	LR_{50} > 4.0 L product/ha ER_{50} > 4.0 L product/ha (fresh residues, no effects on fecundity at 4.0 L product/ha)	EFSA Journal 2012; 10(1): 2522
Chrysoperla carnea (larvae)	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	Extended laboratory test (2D), bean leaves	LR_{50} > 6.0 L product/ha ER_{50} > 6.0 L product/ha (No effects on fecundity at 6.0 L product/ha)	EFSA Journal 2012; 10(1): 2522
Representative product for prothioconazole				
Aphidius rhopalosiphi (adults)	Prothioconazole 250 g/L EC [a)]	Laboratory test glass plates (2D)	LR_{50} = 139.9 g a.s./ha ER_{50} < 150 g a.s./ha ER_{50} > 112 g a.s./ha	EFSA Scientific Report (2007) 106, 1-98
Aphidius rhopalosiphi (adults)	Prothioconazole 250 g/L EC [a)]	Extended laboratory test (3D), wheat plants	LR_{50} > 600 g a.s./ha ER_{50} > 600 g a.s./ha (No significant effect on reproducton in any treatment)	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	Prothioconazole 250 g/L EC [a)]	Laboratory test glass plates (2D), coffin cells	LR_{50} = 18.7 g a.s./ha ER_{50} > 11 g a.s./ha (19% effect on reproduction at 11 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98
Typhlodromus pyri (protonymphs)	Prothioconazole 250 g/L EC [a)]	Extended laboratory test (2D), bean leaves	LR_{50} = 445.5 g a.s./ha ER_{50} > 380 g a.s./ha (40% effect on reproduction at 380 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98
Typhlodromus pyri (protonymphs)	Prothioconazole 250 g/L EC [a)]	Aged residues (2D), bean leaves	LR_{50} > 300 g a.s./ha ER_{50} > 300 g a.s./ha (7.5% effect on reproduction at 300 g a.s./ha, fresh residues)	EFSA Scientific Report (2007) 106, 1-98
Coccinella septempunctata (larvae)	Prothioconazole 250 g/L EC [a)]	Laboratory test glass plates (2D)	LR_{50} = 229.8 g a.s./ha ER_{50} > 180 g a.s./ha (No treatment-related adverse effects at up to 180 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98
Chrysoperla carnea (larvae)	Prothioconazole 250 g/L EC [a)]	Laboratory test glass plates (2D)	LR_{50} > 600 g a.s./ha ER_{50} > 600 g a.s./ha (No adverse effects on reproduction)	EFSA Scientific Report (2007) 106, 1-98
Poecilus cupreus (adults)	Prothioconazole 250 g/L EC [a)]	Laboratory test, Quartz sand	LR_{50} > 600 g a.s./ha ER_{50} > 600 g a.s./ha (No adverse effects on feeding)	EFSA Scientific Report (2007) 106, 1-98
Poecilus cupreus (adults)	Prothioconazole 100 g/L FS [a)]	Extended laboratory test, LUFA 2.1 soil	LR_{50} > 22.47 g a.s./ha ER_{50} > 22.47 g a.s./ha (5.6 – 9.6% effect on feeding at 22.47 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98
Aleochara bilineata (adults/larvae)	Prothioconazole 250 g/L EC [a)]	Laboratory test, Quartz sand	ER_{50} > 400 g a.s./ha (24.6% effect on reproduction at 400 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98
Aleochara bilineata (adults/larvae)	Prothioconazole 100 g/L FS [a)]	Extended laboratory test, LUFA 2.1 soil	ER_{50} > 19.34 g a.s./ha (11.2% effect on freproduction at 19.34 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98
Pardosa spp. (adults)	Prothioconazole 100 g/L FS [a)]	Extended laboratory test, LUFA 2.1 soil	LR_{50} > 22.3 g a.s./ha ER_{50} > 22.3 g a.s./ha (-18% effect on feeding at 22.3 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Exposure System	Results	Reference
Field or semi-field tests				
Not available nor required				

2D/3D: two- or three-dimensional test design.
Grey shading: Data for representative products during EU review of the active substances. Data not considered for the assessments for ADM.03503.F.1.A.
[a)] Nominal content.

zRMS comments:

The studies performed with the formulated product 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.

9.7.1.1 Justification for new endpoints

New standard laboratory testing on *Aphidius* and *Typhlodromus* is provided with the formulated product ADM.03503.F.1.A. Risk assessments are conducted based on the most relevant data for ADM.03503.F.1.A, whereas the data for the representative products for EU review of the active substances are not considered relevant.

9.7.2 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[12]), and in consideration of the recommendations of the guidance document ESCORT 2[13].

In a conservative approach, Tier 1 risk assessments in contrast to the recommendations of ESCORT 2 guidance are based on the median effective rate (ER_{50}) for sublethal effects.

To achieve a concise risk assessment, the risk envelope approach is applied, i.e. for a maximum single use rate of 1.25 L product/ha (corresponding to 93.75 g fluxapyroxad and 187.5 g prothioconazole/ha, respectively (see 0).

[12] European Commission Health & Consumer Protection Directorate-General. Directorate E – Food Safety: plant health, animal health and welfare, international questions (2002): DRAFT Working Document. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev final. 17 October 2002.

[13] Candolfi M.P., Barrett K.L., Campbell P.J., Forster R., Grandy N., Huet M.-C., Lewis G., Oomen P.A., Schmuck R., Vogt H. (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods. From the workshop: European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha)

Intended use	**Cereals (BBCH 30-69) [a)]**		
Product	ADM.03503.F.1.A		
Application rate [L/ha]	1 × 1.25 (MAF = 1.0)		
MAF	1.0		
Test species Tier I	**LR_{50}/ER_{50} (lab.) [L/ha]**	**$PER_{in\text{-}field}$ [L/ha]**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Aphidius rhopalosiphi	> 0.843	1.25	< 1.48
Typhlodromus pyri	> 1.193		< 1.05

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.
[a)] Risk envelope.

Accordingly, the risk quotients indicate an acceptable in-field risk for terrestrial non-target arthropods other than bees for exposure towards ADM.03503.F.1.A for the intended worst-case use.

zRMS comments:

The risk assessment presented in Table 9.7-2 is validated by the zRMS.
Based on calculations performed with consideration of the Tier I laboratory data acceptable in-field risk to non-target arthropods from all intended uses of ADM.03503.F.1.A may be concluded.

9.7.2.2 Risk assessment for off-field exposure

The off-field risk is assessed based on predicted exposure for field crops at the default distance of 1 m relating to 90th percentile drift values (2.77% at 1 m) as provided by BBA (2000[14]). In deviation to ESCORT 2 guidance, a Vegetation Distribution Factor (VDF) of 5 is applied in a conservative approach. This is in agreement with the majority of EU experts as detailed in EFSA (2019[15]).

Table 9.7-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of ADM.03503.F.1.A in cereals (1.25 L product/ha)

Intended use	**Cereals (BBCH 30-69) [a)]**					
Product	ADM.03503.F.1.A					
Application rate [L/ha]	1 × 1.25 (MAF = 1.0)					
MAF	1.0					
VDF	5 [b)] (Tier 1) 10 (Tier 1)*					
Test species Tier I	**ER_{50} (lab.) [L/ha]**	**90th percentile drift value [%]**	**VDF**	**$PER_{off\text{-}field}$ [L/ha]**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Aphidius rhopalosiphi	> 0.843	2.77 (1 m)	5	0.0069 0.00345	10	< 0.082 <0.0241
Typhlodromus pyri	> 1.193					< 0.058 <<0.0029

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.
[a)] Risk envelope; [b)] Conservative approach in agreement with EFSA (2019)[15].
*According to Escort 2 VDF=10

Accordingly, the risk quotients indicate an acceptable off-field risk for terrestrial non-target arthropods other than bees for exposure towards ADM.03503.F.1.A for the intended worst-case use without the necessity to consider risk mitigations.

zRMS comments:

The risk assessment presented in Table 9.7-3 is validated by the zRMS.

As a worst case the VDF of 5 has been considered by the Applicant, since available investigations indicate that VDF of 10 recommended by ESCORT 2 guidance document is not appropriate and may lead to underestimation of the exposure.
It should be, however, noted that according to EFSA Supporting publication 2019:EN-1673, VDF of 5 should be considered as the interim solution that will be reflected in the SANCO/10329/2002 rev 2 final with its implementation considered further.

Since use of VDF of 5 was not reflected in the current SANCO terrestrial guidance, its use is not yet mandatory. Nevertheless, the risk assessment performed with VDF of 5 is more protective and is thus agreed by the zRMS and generally by the most of MSs during Harmonisation Meeting in CZ.

It should be also noted that in line with Bullet Points: Ecotoxicology (CZSC November 2021) as long as adjustment to the guidance document has not been made, a VDF of 10 should be applied in core risk assessment.

We are aware that VDF of 10 should be used until the update of the guidance document.
However, despite these agreements, we constantly receive comments from several Central Zone Member States to

[14] 90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000). Bekanntmachung über die Abdrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.
[15] EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673.

present the off-field risk assessment performed with consideration of VDF of 5. Taking this into account, it was decided to present such calculation to avoid these potential comments. Instead, we receive comment that we should not use VDF of 5.

Nevertheless, calculations for both VDF values are presented in Table 9.7-3 and the concerned Member States may decide which calculation is relevant at the national level.

Based on calculations performed with consideration of the Tier I laboratory data acceptable off-field risk to non-target arthropods from all intended uses of ADM.03503.F.1.A may be concluded with no need for risk mitigation measures.

9.7.2.3 Additional higher-tier risk assessment

An acceptable risk for terrestrial non-target arthropods other than bees is indicated based on Tier 1 data.

9.7.2.4 Risk mitigation measures

An acceptable off-field risk is presented based on Tier 1 data without the necessity to account for risk mitigations.

9.7.3 Overall conclusions

An acceptable in-field and off-field risk is indicated for exposure of terrestrial non-target arthropods other than bees towards the formulated product for the intended worst-case use of ADM.03503.F.1.A without the necessity to account for risk mitigations.

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

9.8.1 Toxicity data

Acute and/or chronic studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with the active substances fluxapyroxad and prothioconazole as well as their relevant soil metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of ADM.03503.F.1.A were not evaluated as part of the EU assessment of the active substances fluxapyroxad and prothioconazole. 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 partly deviates from the results of the EU review processes. Further justifications are provided below (Refer to Point 9.3.1.1).

Table 9.8-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Earthworms				
ADM.03503.F.1.A				
Eisenia fetida	ADM.03503.F.1.A	Mixed into substrate 56 days, chronic, artificial soil, 10% peat content	$NOEC_{reprod.}$ = 30.9 mg product/kg soil dw. **$NOEC_{reprod., corr.}$ = 15.45 mg product/kg soil dw. b)** **$NOEC_{reprod.}$ = 3.22 mg sum of the a.s./kg soil dw. b)** **$NOEC_{reprod.corr}$ = 1.51 mg sum of the a.s./kg soil dw. b)** $EC_{10; reprod.}$ = 32.7 mg product/kg dw.	KCP 10.4.1.1/01 Friedrich, 2020a
Fluxapyroxad, metabolites and representative formulated product				
Eisenia fetida	Fluxapyroxad	Mixed into substrate 14 days, acute, artificial soil 5% peat content	LC_{50} > 1000 mg a.s./kg soil dw. $LC_{50, corr.}$ > 500 mg a.s./kg soil dw. b)	EFSA Journal 2012; 10(1): 2522
Eisenia fetida	M700F001	Mixed into substrate 14 days, acute, artificial soil 10% peat content	LC_{50} > 1000 mg/kg soil dw. c)	EFSA Journal 2012; 10(1): 2522
Eisenia fetida	M700F001	Mixed into substrate 56 days, chronic, artificial soil, 5% peat content	**NOEC = 5.33 mg/kg soil dw. c)**	EFSA Journal 2012; 10(1): 2522
Eisenia fetida	M700F002	Mixed into substrate 14 days, acute, artificial soil 10% peat content	LC_{50} > 1000 mg/kg soil dw. c)	EFSA Journal 2012; 10(1): 2522
Eisenia fetida	M700F002	Mixed into substrate 56 days, chronic, artificial soil, 5% peat content	**NOEC = 2.56 mg/kg soil dw. c)**	EFSA Journal 2012; 10(1): 2522
Eisenia fetida	BAS 700 00 F (62.5 g fluxapyroxad/L EC) a)	Mixed into substrate 14 days, acute, artificial soil 5% peat content	LC_{50} = 290.5 mg product/kg soil dw. LC_{50} = 17.22 mg a.s./kg soil dw. $LC_{50, corr.}$ = 8.61 mg a.s./kg soil dw. b)	EFSA Journal 2012; 10(1): 2522
Eisenia fetida	BAS 700 00 F (62.5 g fluxapyroxad/L EC) a)	Mixed into substrate 56 days, chronic, artificial soil, 5% peat content	NOEC = 356.2 mg product/kg soil dw. NOEC = 21.3 mg a.s./kg soil dw. $NOEC_{corr.}$ = 10.65 mg a.s./kg soil dw. b)	EFSA Journal 2012; 10(1): 2522
Prothioconazole, metabolites and representative formulated product				
Eisenia foetida	Prothioconazole	Mixed into substrate 14	LC_{50} > 1000 mg a.s./kg soil dw.	EFSA

Species	Substance	Exposure System	Results	Reference
		days, acute, artificial soil 10% peat content	$LC_{50,\ corr.}$ > 500 mg a.s./kg soil dw. [b)]	Scientific Report (2007) 106, 1-98
Eisenia fetida	Prothioconazole-desthio (M04)	Mixed into substrate 14 days, acute, artificial soil 10% peat content	LC_{50} > 1000 mg/kg soil dw. $LC_{50,\ corr.}$ > 500 mg/kg soil dw. [b)]	EFSA Scientific Report (2007) 106, 1-98
Eisenia fetida	Prothioconazole-desthio (M04)	Mixed into substrate 56 days, acute, artificial soil 10% peat content	NOEC = 1.0 mg/kg soil dw. **$NOEC_{corr}$ = 0.5 mg/kg soil dw. [b)]**	EFSA Scientific Report (2007) 106, 1-98
Eisenia fetida	S-methyl-prothioconazole (M01)	Mixed into substrate 14 days, acute, artificial soil 10% peat content	LC_{50} > 1000 mg/kg soil dw. $LC_{50,\ corr.}$ > 500 mg/kg soil dw. [b)]	EFSA Scientific Report (2007) 106, 1-98
Eisenia fetida	S-methyl-prothioconazole (M01)	Mixed into substrate 56 days, acute, artificial soil 10% peat content	NOEC = 100 mg/kg soil dw. **$NOEC_{corr}$ = 50 mg/kg soil dw. [b)]**	EFSA Scientific Report (2007) 106, 1-98
Eisenia foetida	Prothioconazole 250 g/L EC [a)]	Mixed into substrate 14 days, acute, artificial soil 10% peat content	LC_{50} > 249.3 mg a.s./kg soil dw. $LC_{50,\ corr.}$ > 124.7 mg a.s./kg soil dw. [b)]	EFSA Scientific Report (2007) 106, 1-98
Eisenia foetida	Prothioconazole 250 g/L EC [a)]	Mixed into substrate 56 days, acute, artificial soil 10% peat content	NOEC = 1.33 mg a.s./kg soil dw. $NOEC_{corr.}$ = 0.665 mg a.s./kg soil dw. [b)]	EFSA Scientific Report (2007) 106, 1-98
Eisenia fetida	Prothioconazole 100 g/L FS [a)]	Mixed into substrate, 56 days, exposure via treated seed in artificial soil; 0.5 cm/5 cm soil 10% peat content	NOER ≥ 1150 kg seeds/ha (i.e. 10 g a.s./100 kg seeds) NOER ≥ 122 g a.s./ha $NOER_{corr.}$ ≥ 61 g a.s./ha [b)]	EFSA Scientific Report (2007) 106, 1-98
Field studies				
Representative formulated product for fluxapyroxad				
Earthworm populations	BAS 701 00 F (62.5 g/L fluxapyroxad + 62.5 g/L epoxiconazole) [a)]	1-year field study, winter barley	NOAER = 10 L product/ha $NOAEC_{measured;\ 5\ cm}$ = 0.104 mg a.s./kg soil dw. (No significant effect on earthworm total number or total biomass at 10 L product/ha)	EFSA Journal 2012; 10(1): 2522
Representative formulated product for prothioconazole				
Earthworm populations	Prothioconazole 250 g/L EC [a)]	1-year field study, grassland	NOAER = 3 × 200 g a.s./ha (No adverse effect 5 month after first application)	EFSA Scientific Report (2007) 106, 1-98
Soil macro- and mesofauna other than earthworms				
ADM.03503.F.1.A				
Folsomia candida	ADM.03503.F.1.A	Mixed into substrate 28 days, chronic, artificial soil 5% peat content	NOEC ≥ 100 mg product/kg soil dw. $EC_{10;\ reprod.}$ > 100 mg product/kg soil dw. **$EC_{10;\ reprod.;\ corr.}$ > 50 mg product/kg soil dw. [b)]** **$EC_{10;\ reprod.}$ > 10.42 mg sum of the a.s./kg soil dw. [b)]** **$EC_{10;\ reprod.corr.}$ > 5.21 mg sum of the a.s./kg soil dw. [b)]**	KCP 10.4.2.1/01 Friedrich, 2020b

Species	Substance	Exposure System	Results	Reference
Hypoaspis aculeifer	ADM.03503.F.1.A	Mixed into substrate 14 days, chronic, artificial soil 5% peat content	NOEC ≥ 40.0 mg product/kg soil dw. $EC_{10;\ reprod.}$ > 40.0 mg product/kg soil dw. **$EC_{10;\ reprod.;\ corr.}$ > 20 mg product/kg soil dw.** [b] **$EC_{10;\ reprod.;}$> 4.17 mg sum of the a.s.t/kg soil dw.** [b] **$EC_{10;\ reprod.;corr}$> 2.1 mg sum of the a.s.t/kg soil dw.** [b]	KCP 10.4.2.1/02 Schulz, 2020a
Fluxapyroxad, metabolites and representative formulated product				
Folsomia candida	M700F002	Mixed into substrate 28 days, chronic, artificial soil 5% peat content	**NOEC = 1000 mg/kg soil dw.** [c]	EFSA Journal 2012; 10(1): 2522
Folsomia candida	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a]	Mixed into substrate 28 days, chronic, artificial soil 5% peat content	NOEC = 50.0 mg product/kg soil dw. NOEC = 2.99 mg a.s./kg soil dw. $NOEC_{corr.}$ = 1.50 mg a.s./kg soil dw. [b]	EFSA Journal 2012; 10(1): 2522
Hypoaspis aculeifer	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a]	Mixed into substrate 14 days, chronic, artificial soil 5% peat content	NOEC = 500 mg product/kg soil dw. NOEC = 29.64 mg a.s./kg soil dw. $NOEC_{corr.}$ = 14.82 mg a.s./kg soil dw. [b]	EFSA Journal 2012; 10(1): 2522
Prothioconazole, metabolites and representative formulated product				
Folsomia candida	Prothioconazole	Mixed into substrate 28 days, chronic, artificial soil 10% peat content	NOEC = 64 mg a.s./kg soil dw. **$NOEC_{corr.}$ = 32 mg a.s./kg soil dw.** [b]	EFSA Scientific Report (2007) 106, 1-98
Hypoaspis aculeifer	Prothioconazole	Mixed into substrate 28 days, chronic, natural LUFA 2.1 soil, ca. 0.9% organic carbon	**NOEC = 100 mg a.s./kg soil dw.** [d] **$NOEC_{corr}$=50 mg a.s./kg dws** [d]	EFSA Scientific Report (2007) 106, 1-98
Folsomia candida	Prothioconazole-desthio (M04)	Mixed into substrate 28 days, chronic, artificial soil 10% peat content	NOEC ≥ 62.5 mg/kg soil dw. **$NOEC_{corr}$ ≥ 31.25 mg/kg soil dw.** [b]	EFSA Scientific Report (2007) 106, 1-98
Folsomia candida	S-methyl-prothioconazole (M01)	Mixed into substrate 28 days, chronic, artificial soil 10% peat content	NOEC ≥ 31.6 mg/kg soil dw. **$NOEC_{corr}$ ≥ 15.8 mg/kg soil dw.** [b]	EFSA Scientific Report (2007) 106, 1-98
Folsomia candida	Prothioconazole 100 g/L FS [a]	Mixed into substrate, 56 days, exposure via treated seed in artificial soil; 0.5 cm/1.3 cm soil 10% peat content	NOER ≥ 230 kg seeds/ha (i.e. 10 g a.s./100 kg seeds) NOER ≥ 24.38 g a.s./ha $NOER_{corr.}$ ≥ 12.19 g a.s./ha [b]	EFSA Scientific Report (2007) 106, 1-98
Folsomia candida	Prothioconazole 100 g/L FS [a]	Mixed into substrate, 56 days, exposure via treated seed in artificial soil; 2.5 cm/5 cm soil 5% peat content	NOER ≥ 1150 kg seeds/ha (i.e. 10 g a.s./100 kg seeds) NOER ≥ 112 g a.s./ha $NOER_{corr.}$ ≥ 56 g a.s./ha [b]	EFSA Scientific Report (2007) 106, 1-98
Litter bag test / Organic matter breakdown				
Representative formulated product for fluxapyroxad				
Litter bag test	BAS 701 00 F (62.5 g/L fluxapyroxad + 62.5 g/L epoxiconazole	1-year litter bag test	Effects below 10 % after 12 months exposure to total application rate of 5 L product/ha. Effects between 10-25 % after 12 months exposure to total application rates of 8 and 10 L product/ha.	EFSA Journal 2012; 10(1): 2522

Species	Substance	Exposure System	Results	Reference
Representative formulated product for prothioconazole				
Litter bag test	Prothioconazole 100 g/L FS [a] followed by Prothioconazole 250 g/L EC [a]	126 day litter bag test	Subsequent treatment corresponding to (NOAEC) 23.2 g a.s./ha + 3x 200 g a.s./ha 92.0 vs. 91.2% litter degradation in treatment and control, respectively.	EFSA Scientific Report (2007) 106, 1-98

dw: dry weight.
Endpoints in **bold** are relevant for risk assessments.
Grey shading: Data for representative products during EU review of the active substances. Data not considered for the assessments for ADM.03503.F.1.A.
[a] Nominal content.
[b] Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002. Endpoint correction in agreement with EFSA (2015)[16] was performed regardless of organic carbon content in the test matrix.
[c] Endpoint correction not required due to log P_{OW} < 2.
[d] Endpoint correction not is required due to low soil organic carbon content of natural soil according to-EFSA Supporting publication 2015:EN-924.

zRMS comments:

Soil meso and macro fauna data for fluxapyroxad, prothioconazole and prothioconazole metabolite JAU 6476-desthio and Fluxapyroxad metabolites (M700F001, M700F002) provided in Table 9.8-1 above has been confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

The representative products considered for EU review of the active substances fluxapyroxad and prothioconazole, respectively are not considered relevant and endpoints are listed in the Table above only for completeness.
However, during commenting period some of MSs requested for the risk assessment based on these endpoints.
The relevant calculations were added by zRMS in the Table 9.8-2.
Studies on effects of the formulated product ADM.03503.F.1.A on soil organism (meso- and macrofauna) listed in Table 9.8-1 were evaluated by the zRMS and considered acceptable.

Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.8.1.1 Justification for new endpoints

Most relevant data are provided for the formulated product ADM.03503.F.1.A from chronic testing with earthworms, spring tails and soil mites, covering also potential effects from combined exposure towards the two active substances fluxapyroxad and prothioconazole.

The data on the formulated product ADM.03503.F.1.A are most relevant for the characterisation of potential risk and in line with Commission Regulations (EC) 283/2013 and 284/2013 cover data requirements for the individual active substances.

The representative products considered for EU review of the active substances fluxapyroxad and prothioconazole, respectively are not considered relevant and endpoints are listed here only for completeness.

In line with the EFSA Technical Report (2015)[16], and in deviation to the EU agreed endpoints, an endpoint correction is performed for the substances with log P_{OW} of >2, regardless of the organic (peat) content in the artificial test matrix, except for the study on soil mites with prothioconazole conducted in natural soil at a low organic carbon content.

As in accordance with recent data requirements (reference is made to Commission Regulations (EC) 283/2013 and 284/2013), acute studies with earthworms and respective risk assessments are no longer required. Reference is made to chronic testing with earthworms.

[16] EFSA (European Food Safety Authority) (2015): Technical report on the outcome of the pesticides peer review meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2015:EN-924. 62 pp.

Conservatively, the lowest endpoint, i.e. either NOEC or EC_{10} is selected for risk assessments as in agreement with EFSA Technical Report (2019)[17].

9.8.2 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)[18].

9.8.2.1 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. According to the assessment of environmental fate data, multi-annual accumulation in soil is considered for active substances and relevant metabolites in soil as applicable.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for cereals at BBCH 30-39 with minimum crop interception (80%) also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses cereals at BBCH ≥ 40 (see 0). Where applicable, the plateau concentrations were taken into consideration (i.e. assessments were based on long-term maximum PEC_{soil}).

Table 9.8-2: First-tier assessment of the chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of ADM.03503.F.1.A in cereals (BBCH 30-39)

Intended use	**Cereals (BBCH 30-69) [a)]**			
Chronic effects on earthworms				
Product / active substance / metabolite	**Species**	**$NOEC_{(corr.)}$ / $EC_{10;(corr.)}$ [mg/kg dw]**	**PEC_{soil} [mg/kg dw]**	**TER_{lt} (criterion TER ≥ 5)**
ADM.03503.F.1.A	*Eisenia fetida*	15.45 (product) 1.61 (sum of the a.s.)	0.360 0.0813	42.9 19.4
Prothioconazole		0.665	0.0500	13.3
Fluxapyroxad		10.65	0.0382	278.8
M700F001	*Eisenia fetida*	5.33 (met.)	0.0021 ~~0.0014 04~~ [b)]	2538.1 ~~9817.14~~ ~~13325~~
M700F002	*Eisenia fetida*	2.56 (met.)	0.0144 ~~0.0078~~ [b)]	177.7 ~~326.8~~
Prothioconazole-desthio (M04)	*Eisenia fetida*	0.5 (met.)	0.0259 ~~00.0249~~	19.30 ~~20.1~~
S-methyl-prothioconazole (M01)	*Eisenia fetida*	50 (met.)	0.0076 ~~0.0064~~	6578.94 ~~7813~~
Chronic effects on other soil macro- and mesofauna				
Product / active substance / metabolite	**Species**	**$NOEC_{(corr.)}$ / $EC_{10;(corr.)}$ [mg/kg dw]**	**PEC_{soil} [mg/kg dw]**	**TER_{lt} (criterion TER ≥ 5)**

[17] EFSA (European Food Safety Authority), 2019. Technical report on the outcome of the Pesticides Peer Review Meeting on general recurring issues in ecotoxicology. EFSA supporting publication 2019:EN-1673. 117 pp. doi:10.2903/sp.efsa.2019.EN-1673.

[18] European Commission Health & Consumer Protection Directorate-General. Directorate E – Food Safety: plant health, animal health and welfare, international questions (2002): DRAFT Working Document. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev final. 17 October 2002.

ADM.03503.F.1.A	*Folsomia candida*	> 50 (product) > 5.21 (sum of a.s.)	0.360 0.0813	> 138.9 >64.1
	Hypoaspis aculeifer	> 20 (product) >2.1 (sum of a.s.)	0.360 0.0813	> 55.6 >25.83
M700F002	*Folsomia candida*	1000 (met.)	0.0076 [b)]	131579
Prothioconazole	*Folsomia candida*	32 (a.s.)	0.0500	640.0
	Hypoaspis aculeifer	50 ~~100~~ (a.s.)	0.0500	1 ~~000~~
Fluxapyroxad	*Folsomia candida*	1.5	0.0382	39.3
	Hypoaspis aculeifer	14.82	0.0382	387.6
Prothioconazole-desthio (M04) [1]	*Hypoaspis aculeifer*	10	0.0259	386.1
JAU-S-methyl (M1) (metabolite of prothioconazole) [1]	*Hypoaspis aculeifer*	10	0.0076	1315.8
Prothioconazole-desthio (M04)	*Folsomia candida*	31.25 (met.)	0.0259 ~~0.0249~~	1206.60 ~~1255~~
S-methyl-prothioconazole (M01)	*Folsomia candida*	>15.8 (met.)	0.0076 ~~0.0064~~	>2078.94 ~~2469~~

TER values shown in **bold** fall below the relevant trigger.; a.s. active substance; met. metabolite.
[a)] Risk envelope: BBCH 30-39 with minimum crop interception.
[b)] Long-term maximum PEC_{soil} (accounting for $PEC_{plateau}$).
[1] Since no measured toxicity data are available, it was assumed that the metabolite is 10 x more toxic than the parent compounds prothioconazole.

Accordingly, an acceptable risk is indicated for earthworms and soil macro- and mesofauna other than earthworms for the intended worst-case use of ADM.03503.F.1.A in cereals.

zRMS comments:

Soil microorganism data for fluxapyroxad, prothioconazole and prothioconazole metabolite JAU 6476-desthio and Fluxapyroxad metabolites (M700F001, M700F002) provided in Table 9.8-1 above has been confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

The risk assessment assessment provided in the Table 9.8-2 has been amended by zRMS with consideration of PEC_{sw} values agreed in Section 8.

The calculations of the risk assessment for both of a.s. and their metabolites has been checked by zRMS.
The assumption that prothioconazole metabolites are 10 x more toxic than the parent compounds prothioconazole was done by zRMS in case of H.aculeifer since no measured toxicity data are available for them.

In case of a.s. fluxapyroxad technical for H.aculeifer and Folsomia candidia species no toxicity data was available from the EU review.

Nevertheless, study on toxicity of ADM.03503.F.1.A to Hypoaspis aculeifer cover effects of fluxapyroxad and its metabolites in the product and are considered sufficient.

All TER_{LT} values for soil meso- and macrofauna (other than earthworms) are greater than the trigger of 5, indicating an overall acceptable risk.

TER_{mix} approach was requested by some of Ms during commenting period process:
The relevant calculations are presented below:

TER_{mix} values based on TER_{LT} Tier 1 values for each active substance for earthworm

Active substances				Σ1/TER	Σ1/TER^{-1}	Trigger
Fluxapyroxad		Prothioconazole				
278.8	0.0035	13.3	0.075	0.078	12.74	5

[1)] the lowest TER_{LT} at Tier 1

TERmix values based on TER_{LT} Tier 1) values for each active substance for Folsomia candida

Active substances				Σ1/TER	Σ1/TER^{-1}	Trigger
Fluxapyroxad		Prothioconazole				
39.3	0.025	640	0.0015	0.0265	37.74	5

[1)] the lowest TER_{LT} at Tier 1

TERmix values based on TER_{LT} Tier 1) values for each active substance for Hypoaspis aculeifer

Active substances				Σ1/TER	Σ1/TER^{-1}	Trigger
Fluxapyroxad		Prothioconazole				
387.6	0.0026	1000	0.001	0.0036	277.7	5

[1)] the lowest TER_{LT} at Tier 1

Based on TERmix approach the risk for soil organism is considered acceptable.

9.8.2.2 Higher-tier risk assessment

An acceptable risk for earthworms and soil macro- and mesofauna other than earthworms is indicated based on Tier 1 data. No higher tier risk assessments are required.

9.8.3 Overall conclusions

An acceptable risk is indicated for soil macro- and meso-fauna for the intended worst-case use of ADM.03503.F.1.A in cereals with Toxicity Exposure Ratios greater than five for the active substances, relevant metabolites as well as formulated product, respectively.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with the active substances fluxapyroxad and prothioconazole as well as their relevant soil metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of ADM.03503.F.1.A were not evaluated as part of the EU assessment of active substance 1. 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. Justifications are provided below.

Table 9.9-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
ADM.03503.F.1.A				
N-mineralisation	ADM.03503.F.1.A	28 d, aerobic soil type	**NOAEC = 3.5 mg product/kg soil dw.**	KCP 10.5/01 Schulz, 2020b
Fluxapyroxad, metabolites and representative formulated product				
N-mineralisation	Fluxapyroxad	28 d, aerobic soil type	**NOAEC = 2.01 mg a.s./kg soil dw.**	EFSA Journal 2012; 10(1): 2522
~~C-mineralisation~~	~~Fluxapyroxad~~	~~28 d, aerobic soil type~~	~~NOAEC = 2.01 mg a.s./kg soil dw.~~	~~EFSA Journal 2012; 10(1): 2522~~
N-mineralisation	M700F001	28 d, aerobic soil type	**NOAEC = 0.37 mg/kg soil dw.**	EFSA Journal 2012; 10(1): 2522
~~C-mineralisation~~	~~M700F001~~	~~28 d, aerobic soil type~~	~~NOAEC = 0.37 mg/kg soil dw.~~	~~EFSA Journal 2012; 10(1): 2522~~
N-mineralisation	M700F002	28 d, aerobic soil type	**NOAEC = 1.0 mg/kg soil dw.**	EFSA Journal 2012; 10(1): 2522
~~C-mineralisation~~	~~M700F002~~	~~28 d, aerobic soil type~~	~~NOAEC = 1.0 mg/kg soil dw.~~	~~EFSA Journal 2012; 10(1): 2522~~
N-mineralisation	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	28 d, aerobic soil type	NOAEC = 27.71 mg product/kg soil dw.	EFSA Journal 2012; 10(1): 2522
~~C-mineralisation~~	~~BAS 700 00 F (62.5 g fluxapyroxad/L EC)~~ [a)]	~~28 d, aerobic soil type~~	~~NOAEC = 27.71 mg product/kg soil dw.~~	~~EFSA Journal 2012; 10(1): 2522~~
Prothioconazole and metabolites				
N-mineralisation	Prothioconazole	28 d, aerobic soil type	NOAER = 2.0 kg a.s./ha **NOAEC = 2.67 mg a.s./kg soil dw.**	EFSA Scientific Report (2007) 106, 1-98
~~C-mineralisation~~	~~Prothioconazole~~	~~28 d, aerobic soil type~~	~~NOAER = 2.0 kg a.s./ha~~ ~~NOAEC = 2.67 mg a.s./kg soil dw.~~	~~EFSA Scientific Report (2007) 106, 1-98~~
N-mineralisation	Prothioconazole-desthio (M04)	28 d, aerobic soil type	NOAER = 1.0 kg/ha **NOAEC = 1.33 mg/kg soil dw.**	EFSA Scientific Report (2007) 106, 1-98
N-mineralisation	S-methyl-prothioconazole (M01)	28 d, aerobic soil type	NOAER = 2.0 kg/ha **NOAEC = 2.67 mg/kg soil dw.**	EFSA Scientific Report (2007) 106, 1-98

Endpoint	Substance	Exposure System	Results	Reference
~~C-mineralisation~~	~~S-methyl-prothioconazole (M01)~~	~~28 d, aerobic soil type~~	~~NOAER = 2.0 kg/ha NOAEC = 2.67 mg/kg soil dw.~~	~~EFSA Scientific Report (2007) 106, 1-98~~

NOAER/C: No Observed Adverse Effect Rate/Concentration; i.e. the maximum rate or concentration at which ≤ 25% effects on soil microflora functions were observed in the test at ≤ 100 days.
Soil concenrations/rates are transformed based on 5 cm soil penetration depth and soil bulk density of 1.5 g/cm^3.
Grey shading: Data for representative products during EU review of the active substances. Data not considered for the assessments for ADM.03503.F.1.A.
Endpoints in **bold** are relevant for risk assessment; dw. dry weight.
[a)] Nominal content.

zRMS comments:

Soil microorganism data for fluxapyroxad, prothioconazole and prothioconazole metabolite JAU 6476-desthio and Fluxapyroxad metabolites (M700F001, M700F002) provided in Table 9.9-1 above has been confirmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason is struck through in tables above

Studies on effects of the formulated product ADM.03503.F.1.A on soil micro-organism were evaluated by the zRMS and considered acceptable.

Summary of the performed studies together with zRMS evaluation may be found in Appendix 2.

9.9.1.1 Justification for new endpoints

Most relevant data are provided for the formulated product ADM.03503.F.1.A covering also potential effects from combined exposure towards the two active substances fluxapyroxad and prothioconazole. However, assessments are also presented based on available data for the active substances as well as relevant soil metabolites.

In line with recent data requirements provided in Commission Regulations (EC) 283/2013 and 284/2013, only effects on nitrogen transformation have to be assessed. In line with this, no risk assessments are required for soil respiration (carbon mineralisation) endpoints. However, as the respective endpoints correspond to the concentrations relevant for nitrogen mineralisation, the respective assessments are also protective for the data on soil respiration.

9.9.2 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)[19].

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 0).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for cereals at BBCH 30-39 with minimum crop interception (80%) also covers the risk for soil microflora

[19] European Commission Health & Consumer Protection Directorate-General. Directorate E – Food Safety: plant health, animal health and welfare, international questions (2002): DRAFT Working Document. Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev final. 17 October 2002

functions from all other intended uses cereals at BBCH ≥ 40 (see 0). Where applicable, the plateau concentrations were taken into consideration (i.e. assessments were based on long-term maximum PEC_{soil}).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of ADM.03503.F.1.A in cereals (BBCH 30-39)

Intended use	**Cereals (BBCH 30-69) [a)]**			
N / (C)-mineralisation				
Product / active substance / metabolite	**NOAEC [mg/kg dw]**	**PEC_{soil} [mg/kg dw]**	**Risk acceptable?**	**Margin of Safety**
ADM.03503.F.1.A	3.5 (product)* 0.792 (sum of a.s.)	0.360 (product) 0.013 (sum of the a.s.)	Yes	9.7 56.13
Fluxapyroxad	2.01 (a.s.)	0.0382 [a)] ~~0.0313~~ [a)]	Yes	52.62 ~~64~~
M700F001	0.37 (met.)	0.0021 [a)] ~~0.0004~~ [a)]	Yes	176.2 ~~925~~
M700F002	1.0 (met.)	0.0114 [a)] ~~0.0014~~ ~~0.0076~~ [a)]	Yes	87.72 ~~714.30~~ ~~132~~
Prothioconazole	2.67 (a.s.)	0.0500	Yes	53
Prothioconazole-desthio (M04)	1.33 (met.)	0.0259 ~~0.0249~~	Yes	51.35 ~~53~~
S-methyl-prothioconazole (M01)	2.67 (met.)	0.0076 ~~0.0064~~	Yes	351.31 ~~417~~

NOAEC: No Observed Adverse Effect Concentration; i.e. the maximum concentration at which ≤ 25% effects on soil microflora. functions were observed in the test at ≤ 100 days.
a.s. active substance; met. metabolite.
[a)] Long-term maximum PEC_{soil} (accounting for $PEC_{plateau}$).
*study should be considered with caution

Accordingly, an acceptable risk is indicated for soil microflora for the intended worst-case use of ADM.03503.F.1.A in cereals.

zRMS comments:

The risk assessment presented in Table 9.9-2 above is in general agreed by the zRMS with some minor correction of PECsoil values agreed in the course of evaluation in area of Section 8.
During the interval 7-14 days an effect > 25 % was observed (+ 31.1 %) but after 28 days no deviation were noted. Based on the study results no adverse effects (i.e. deviation from control < 25%) were seen at the end of the 28-day incubation period at 0.350 and 3.50 mg product/kg soil d.w.
~~The effects on the nitrogen transformations are acceptable (<25%) after 28 days at concentration which is higher than the maximum relevant PECs for the maximum application rate of active substances and the product ADM.03503.F.1.A.~~
Based on the deviation from the control the results 3.50 mg product/kg dws should be treated with caution. However, in the same time it should be noted the high margin of safety is concluded for both active substances contained in the product. For this reason , in zRMS opinion the risk can be considered acceptable.

~~Overall, no unacceptable effects on soil microbial activity are expected following application of ADM.03503.F.1.A.~~

9.9.3 Overall conclusions

An acceptable risk is indicated for soil microflora for the intended worst-case use of ADM.03503.F.1.A in cereals with NOAECs (i.e. the maximum tested concentration with effects < 25% at ≤ 100 days) greater than the maximum predicted environmental concentrations of the active substances, relevant metabolites as well as formulated product, respectively.

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

9.10.1 Toxicity data

Studies on the toxicity to non-target terrestrial plants have been carried out with representative products of the active substances fluxapyroxad and prothioconazole during EU review. In addition, data are available for the technical active substance prothioconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of ADM.03503.F.1.A were not evaluated as part of the EU assessments of the active substances fluxapyroxad and prothioconazole. 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 deviates from the results of the EU review process. Justifications are provided below.

Table 9.10-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
ADM.03503.F.1.A				
Allium cepa (m) *Triticum aestivum* (m) *Lactuca sativa* (d) *Helianthus annuus* (d) *Solanum lycopersicum* (d) *Glycine max* (d)	ADM.03503.F.1.A	21 d Seedling emergence	**ER_{50} > 1.193 L product/ha** (No phytotoxic symptoms or effects on seedling emergence, plant survival, height and shoot dry weight)	KCP 10.6.2/01 Friedemann, 2021a
Allium cepa (m) *Triticum aestivum* (m) *Lactuca sativa* (d) *Helianthus annuus* (d) *Solanum lycopersicum* (d) *Glycine max* (d)	ADM.03503.F.1.A	21 d Vegetative vigour	**ER_{50} > 1.193 L product/ha** (No effects on plant survival, height and shoot dry weight; phytotoxic effects in sunflower, tomato and soybean ≤ 10%)	KCP 10.6.2/02 Friedemann, 2021b
Representative product for fluxapyroxad				
Allium cepa (m) *Avena sativa* (m) *Lolium multiflorum* (m) *Zea mays* (m) *Daucus carota* (d) *Helianthus annuus* (d) *Brassica napus* (d) *Beta vulgaris* (d) *Pisum sativum* (d) *Vicia faba* (d)	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	21 d Seedling emergence	ER_{50} > 2.0 L product/ha	EFSA Journal 2012; 10(1): 2522
Allium cepa (m) *Avena sativa* (m) *Lolium multiflorum* (m) *Zea mays* (m) *Daucus carota* (d) *Helianthus annuus* (d) *Brassica napus* (d) *Beta vulgaris* (d) *Pisum sativum* (d) *Vicia faba* (d)	BAS 700 00 F (62.5 g fluxapyroxad/L EC) [a)]	21 d Vegetative vigour	ER_{50} > 2.0 L product/ha	EFSA Journal 2012; 10(1): 2522
Prothioconazole and representative formulated product				
5 mono- and 6 dicotyledoneous species	Prothioconazole	Pre- and postemergence application	Pre-emergence: max. 5% phytotoxicity at 200 g a.s./ha Post-emergence: max. 10% phytotoxicity at 250 g a.s./ha (i.e. ER_{50} > 200 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98

Species	Substance	Exposure System	Results	Reference
5 mono- and 6 dicotyledoneous species	Prothioconazole 250 g/L EC [a)]	Pre- and postemergence application	Pre-emergence: max. 5% phytotoxicity at 200 g a.s./ha Post-emergence: max. 0% phytotoxicity at 250 g a.s./ha (i.e. ER_{50} > 200 g a.s./ha)	EFSA Scientific Report (2007) 106, 1-98

m: monocotyledonous; d: dicotyledonous; Endpoints in **bold** are relevant for risk assessments.
Grey shading: Data for representative products during EU review of the active substances. Data not considered for the assessments for ADM.03503.F.1.A.

34) Nominal content.

zRMS comments:

NTTP data for fluxapyroxad, prothioconazole and prothioconazole metabolite JAU 6476-desthio and Fluxapyroxad metabolites (M700F001, M700F002) provided in Table 9.9-1 above has been con-firmed by zRMS that they are in line with EU agreed endpoints reported in EFSA Journal (2007) 124, 1-84 and EFSA Scientific Report (2007) 106, respectively.

Data for technical active substance (referred to in the EU review for prothioconazole) or representative products for both active substances, are not further considered for risk assessment.

Risk assessments based on the data for the formulated product ADM.03503.F.1.A which also cover potential effects from combined exposure towards the active substances fluxapyroxad and prothioconazole.

9.10.1.1 Justification for new endpoints

The intended use of ADM.03503.F.1.A is as a fungicide in cereals. There is no indication for herbicidal activity of either of the active substances. Accordingly, no Tier 2 testing would be required. However, the Applicant provides rate-response data for seedling emergence and growth as well as vegetative vigour for six plant species each.

Risk assessments are most adequately based on the data for the actual formulated product ADM.03503.F.1.A which also cover potential effects from combined exposure towards the active substances fluxapyroxad and prothioconazole. Data for technical active substance (referred to in the EU review for prothioconazole) or representative products for both active substances, all indicating low toxicity towards terrestrial non-target plants, are not further considered for risk assessment.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

No screening data are required. Reference is made to available Tier 2 rate-response (seedling emergence and growth as well as vegetative vigour) data on six plant species.

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

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002)[20]. It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

To achieve a concise risk assessment, the risk envelope approach is applied, i.e. for a maximum single use rate of 1.25 L product/ha corresponding to 93.75 g fluxapyroxad/ha and 187.5 prothioconazole/ha, respec-

[20] European Commission Health & Consumer Protection Directorate-General. Directorate E – Food Safety: plant health, animal health and welfare, international questions (2002): DRAFT Working Document. Guidance Docu-ment on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC. SANCO/10329/2002 rev final. 17 Oc-tober 2002.

tively for a maximum BBCH range of 30 to 69 (see 0).

The off-field risk is assessed based on predicted exposure for field crops at the default distance of 1 m relating to 90th percentile drift values (2.77% at 1 m) as provided by BBA (2000[21]).

Table 9.10-2: Assessment of the risk for non-target plants due to the use of ADM.03503.F.1.A in cereals

Intended use	**Cereals (BBCH 30-69) [a)]**			
Product	ADM.03503.F.1.A			
Application rate [L/ha]	1 × 1.25			
Test species	**ER_{50} [L/ha]**	**90th percentile drift value [%]**	**$PER_{off\text{-}field}$ [L/ha]**	**TER criterion: TER ≥ 5**
Seedling emergence and growth				
6 species	> 1.193	2.77 (1 m)	0.035	> 34.5
Vegetative vigour				
6 species	> 1.193	2.77 (1 m)	0.035	> 34.5

MAF: Multiple application factor; PER: Predicted environmental rate; TER: Toxicity to Exposure Ratio. TER values shown in **bold** fall below the relevant trigger.

Accordingly, the TERs indicate an acceptable off-field risk for terrestrial non-target plants for exposure towards ADM.03503.F.1.A for the intended worst-case use without the necessity to consider risk mitigations.

Besides, less than 50% effect levels observed at the application rate of 1.193 L product/ha in the studies which is close to the maximum in-field rate of 1.25 L product/ha suggests that there additionally is an acceptable risk for terrestrial non-target plants in the treated field.

zRMS comments:

The calculations of the risk assessment based on ER_{50}>1.193 L product/ha and with consideration PER-off field exposure 0.03 L/ha has been accepted by zRMS.

Overall, an acceptable off-field risk for terrestrial non-target plants for exposure towards ADM.03503.F.1.A for the intended worst-case use without the necessity to consider risk mitigations.

9.10.2.3 Higher-tier risk assessment

No higher tier considerations are required for terrestrial non-target plants. An acceptable risk is indicated based on Tier 2 data.

9.10.2.4 Risk mitigation measures

No risk mitigation is needed. An acceptable risk is indicated based on Tier 2 data without the necessity to account for risk mitigations.

9.10.3 Overall conclusions

An acceptable off-field risk is indicated for exposure of terrestrial non-target plants towards the formulated product for the intended worst-case use of ADM.03503.F.1.A without the necessity to account for risk mitigations.

[21] 90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000). Bekanntmachung über die Abdrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden. Public domain.

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

No additional relevant effect data are available or considered necessary. The data available are considered to fully address the requirements as detailed in Commission Regulations (EC) 283/2013 and 284/2013.

9.12 Monitoring data (KCP 10.8)

No monitoring data are available or considered to be necessary.

9.13 Classification and Labelling

Classification:

Studies testing the toxicity of ADM.03503.F.1.A towards fish, *Daphnia magna* and algae resulted in end-points > 1 mg/L (*O. mykiss*: LC_{50} = 3.72 mg product/L, *Daphnia magna*: EC_{50} = 6.58 mg product/L, *Pseudokirchneriella subcapitata* E_rC_{50} = 16.9 mg product). NOEC and EC_{10} for algae based on growth rate is 3.33 and 7.01 mg product/L, respectively. The active substances both are not readily biodegradable. However, the BCF of fluxapyroxad and prothioconazole in fish of 37 and 19.7, respectively does not indicate a high potential for bioaccumulation. Therefore, under Regulation 1272/2008, ADM.03503.F.1.A is not classified for acute and long-term aquatic hazard based on measured data.

Based on the chronic toxicity of fluxapyroxad (at 7.5% in the mixture) with an NOEC of 0.0359 mg a.s./L (i.e. ≤ 0.1 mg/L) for *P. promelas* (Chronic 1; M-factor: 1) and prothioconazole (at 15% in the mixture) with an NOEC of 0.308 mg a.s./L for *O. mykiss* (Chronic 2), the overall classification for the mixture is Chronic 2 (7.5% x 10 + 15% = 90%; i.e. > 25%).

Labelling:

GHS pictogram:

Hazard Statement: **H411** ´Toxic to aquatic life with long lasting effects´.

Precautionary statements: P391, P501

zRMS comments:

zRMS agrees with the classification of the product.

The product should be classified as Aquatic Chronic 2, and be labelled with GHS09 and H411 “Toxic to aquatic life with long lasting effects”.

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	[redacted]	2021a	Acute toxicity of ADM.03503.F.1.A to *Oncorhynchus mykiss* in a 96-hour semi-static test [redacted] GLP Unpublished	Y	ADM
KCP 10.2.1/02	Juckeland, D.	2021b	Acute toxicity of ADM.03503.F.1.A to *Daphnia magna* in a 48-hour static test Test facility report No. 20 48 ADL 0005 (incl. Amendment No. 1), Sponsor report no. 000105070 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.2.1/03	Juckeland, D.	2021c	Effects of ADM.03503.F.1.A on *Pseudokirchneriella subcapitata* in an algal growth inhibition test Test facility report No. 20 48 AAL 0007, Sponsor report no. 000105071 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.3.1.1./01	Franke, M.	2020	Acute toxicity of ADM.03503.F.1.A to the honeybee *Apis mellifera* L. under laboratory conditions Test facility report No. 20 48 BAA 0026, Sponsor report no. 000105072 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.3.1.2./01	Dreßler, K.	2021	Chronic toxicity of ADM.03503.F.1.A to the honeybee *Apis mellifera* L. under laboratory conditions Test facility report No. 20 48 BAC 0010, Sponsor report no. 000105073 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.3.1.3./01	Hänsel, M.	2021	ADM.03503.F.1.A – Repeated exposure of honeybee larvae (*Apis mellifera* L.) under laboratory conditions Test facility report No. 20 48 BLC 0012 (incl. Amendment No. 1), Sponsor report no. 000105074 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.3.1.5/01	Persigehl, M.; Beinert, M.;	2022a	Study on the Effect of ADM.03503.F.1.A on Honey Bee Colonies (*Apis mellifera* L.) under Semi-Field Conditions in Germany	N	ADM

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
	Hotopp, I.		Test facility report No. B20F026, Sponsor report no. 000107305 tier3 solutions GmbH, Leverkusen, Germany GLP Unpublished		
KCP 10.3.1.5/02	Persigehl, M.; Beinert, M.; Hotopp, I.	2022b	Study on the Effect of ADM.03503.F.1.A on Honey Bee Colonies (*Apis mellifera* L.) under Semi-Field Conditions in Spain Test facility report No. B20F027, Sponsor report no. 000107306 tier3 solutions GmbH, Leverkusen, Germany GLP Unpublished	N	ADM
KCP 10.3.2.1./01	Röhlig, U.	2020a	Effects of ADM.03503.F.1.A on the parasitic wasp *Aphidius rhopalosiphi* (DESTAFANI-PEREZ) in a laboratory test Test facility report No. 20 48 NAL 0004, Sponsor report no. 000105076 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.3.2.1./02	Röhlig, U.	2020b	Effects of ADM.03503.F.1.A on the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test Test facility report No. 20 48 NTL 0004, Sponsor report no. 000105075 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.4.1.1./01	Friedrich, S.	2020a	Effects of ADM.03503.F.1.A on the reproduction of the earthworm *Eisenia fetida* in artificial soil Test facility report No. 20 48 TEC 0033, Sponsor report no. 000105077 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.4.2.1./01	Friedrich, S.	2020b	Effects of ADM.03503.F.1.A on the reproduction of the collembolan *Folsomia candida* Test facility report No. 20 48 TCC 0023, Sponsor report no. 000105078 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.4.2.1./02	Schulz, L.	2020a	Effects of ADM.03503.F.1.A on the reproduction of the predatory mite *Hypoaspis aculeifer* Test facility report No. 20 48 THC 0019, Sponsor report no. 000105079 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP	N	ADM

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
			Unpublished		
KCP 10.5./01	Schulz, L.	2020b	Effects of ADM.03503.F.1.A on the activity of soil microflora (Nitrogen transformation test) Test facility report No. 20 48 SMN 0020, Sponsor report no. 000105080 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.6.2./01	Friedemann, A.	2021a	Effects of ADM.03503.F.1.A on seedling emergence and seedling growth of six non-target terrestrial plant species under greenhouse conditions Test facility report No. 20 46 PSE 0004, Sponsor report no. 000105081 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM
KCP 10.6.2./02	Friedemann, A.	2021a	Effects of ADM.03503.F.1.A on vegetative vigour of six non-target terrestrial plant species under greenhouse conditions Test facility report No. 20 46 PVV 0006, Sponsor report no. 000105082 BioChem agrar, Labor für biologische und chemische Analytik GmbH, Gerichshain, Germany GLP Unpublished	N	ADM

ADM = Property of ADAMA Agricultural Solutions and all affiliates.
Under Article 59 of Regulation 1107/2009/EC, the Sponsor Company claims data protection for all ADM studies.

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

For all data referred to and used for the assessments of risk for terrestrial and aquatic non-target organisms other than the data for the formulated product ADM.03503.F.1.A are provided in the FESA List of Endpoints for fluxapyroxad and prothioconazole, respectively.

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
-	-	-	-	-	-

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

No additional data submitted.

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

No additional data submitted.

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

No additional data submitted.

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

No additional data submitted.

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

No additional data submitted.

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 Study 1: Acute toxicity to *Oncorhynchus mykiss* of ADM.03503.F.1.A

Justification for vertebrate study: The conduction of the acute study in fish was considered justified because this is a formulation with a new combination of active substances and no bridging is possible to any existing product.

Comments of zRMS:	The study was conducted in line with OECD 203 with no deviations. The test concentration of both active substances was verified. The measured concentrations of prothioconazole remained within a range of 91 to 98% of nominal concentrations in the freshly prepared test solutions at the start of the test and at the renewal at 48 hours after test start in the freshly prepared test solutions. In the aged test solutions concentrations were determined to be 74 to 93% of nominal concentrations at the renewal at 48 hours after test start and at the end of the test (96 hours). Since one measured concentration of prothioconazole was below 80% of nominal in an aged test solution, geometric mean measured concentrations have been calculated for prothioconazole considering the exposure periods 0 – 48 h and 0 – 96 h. Therefore, 96-hour LC_{50} adjusted to the sum of a.s. contents based on geometric mean measured concentrations for prothioconazole and nominal concentrations for fluxapyroxad was determined. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 96 h LC_{50} = 3.49 mg product /L (based on geomean test concentration)

Reference:	KCP 10.2.1/01
Report	Acute toxicity of ADM.03503.F.1.A to *Oncorhynchus mykiss* in a 96-hour semi-static test [redacted] (Including Amendment No. 1)
Guideline(s):	OECD 203 (2019)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No, representative product study.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)

Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021

2. Vehicle and/or positive control Vehicle control: test water; no positive control required

3. Test organism

Species	Rainbow trout (*Oncorhynchus mykiss*)
Strain	Not applicable
Source	Forellenzucht Trostadt GbR, Reurieth, Germany; held in the test facility under standardised laboratory conditions
Age	Not reported; Mean length: 4.8 ± 0.2 cm; Mean weight: 0.88 ± 0.2 g
Acclimation period	11 days (mortality during acclimatisation was 0%)
Feeding	Daily feeding with fish food until 24 hours prior to test start, no feeding during the test
Test units	13 L stainless steel container with approximately 10 L test solution. Mean fish loading rate: 0.613 g/L test medium.

4. Environmental conditions

Test water	Reconstituted water according to ISO 6341
Conductivity	1.9 µS/cm (measured)
Hardness	2 mmol/L $CaCO_3$, corresponding to 200 mg/L (measured: 230 mg/L)
Alkalinity	0.8 mmol/L
Oxygen saturation	≥ 80% of air saturation value (Oxygen concentration: 8.25 and 8.80 mg/L at 0 and 48 hours, respectively) Measured oxygen concentrations during the test: 8.19 – 9.07 mg/L (fresh media) and 8.58 – 9.76 mg/L (aged media)
pH	7.81 and 7.70 at 0 and 48 hours, respectively Measured pH during the test: 7.61 – 7.91 (fresh media) and 7.56 – 7.80 (aged media)
Water temperature	10 – 14 °C (measured: 11.5 – 12.3 °C)
Lighting	16 : 8 hours light : dark cycle light intensity on average 20 µE m^{-2} s^{-1}
Aeration	The test medium was aerated until oxygen saturation was achieved

B. STUDY DESIGN AND METHODS

1. In-life dates 20th July 2020 to 24th July 2020 (biological phase)

2. Experimental conditions

Test design

Rainbow trout (*Oncorhynchus mykiss*) were exposed in a semi-static test to the test item at five concentrations and an untreated test water control with test water renewal at 48 hours after test start. Mortalities and sublethal effects were recorded effects after 3, 6, 24, 48, 72 and 96.

Number of animals per treatment

Seven fish were used per test item treatment and control.

Test concentrations

Nominal test substance concentrations were 1.82, 2.55, 3.57, 5.00 and 7.00 mg test item/L. In addition, a control group with untreated test water was used.

Treatment/Application

Stock solutions (A) were prepared by weighing 281.2 and 276.5 mg test item at 0 and 48 hours, respectively, filling up to 1000 mL and stirring for 1 minute on a magnetic stirrer. Corresponding amounts of the stock solutions (64.80, 90.72, 127.01, 177.81 and 248.93 mL at 0 hours and 65.90, 92.26 and 129.16 mL at 48 hours, respectively; the highest two concentrations were not prepared due to 100% mortality) were filled up to 1000 mL to prepare stock solutions B to F. Each of these stock solutions was mixed with 9 L test medium in the test unit immediately before transfer of fish into the test vessels.

The control vessels contained test medium (dilution water) only.

Test medium was prepared one day before test start and aerated for 2 hours.

Analytics

Concentrations of Fluxapyroxad and Prothioconazole in test solutions were determined and analysed using HPLC with MS/MS detection at test start (0 hours), and at the test end (96 hours) and after the test solution renewal at 48 hours after test start in the fresh and aged solutions. Details to the analytical method are summarized in Part B, Section 5.

3. Sampling and measurements

The test fish were observed for mortality and sublethal effects at 3, 6, 24, 48, 72 and 96 hours after test start. Weights and lengths of fish were determined at the end of the test.

At the start (0 hours), after 24, 48, 72 hours before and after the renewal as well as and at the end of the test (after 96 hours) the pH and the content of dissolved oxygen were measured. Temperature was recorded continuously in the water bath.

Test item concentrations were measured in test water samples taken at test start, at 48 hours (aged and fresh medium) and at 96 hours after start of exposure (test termination).

4. Calculation of toxicity

Percentage mortality for each exposure period (cumulative) was calculated.

5. Statistics

The determination of NOEC and LOEC was carried out by hypothesis testing using Fisher`s Exact Binomial Test and Step-down Cochran-Armitage test (alpha = 0.05, one-sided greater) for binomial distributed data.

Lethal concentrations (LCx) were determined by concentration-response modelling (Logit analysis, linear weighted regression). Confidence intervals were determined by Fieller’s theorem.

Statistical evaluation was carried out using ToxRat Professional (3.3.0, RATTE, 2018).

II. RESULTS AND DISCUSSION

Analytical results

The measured concentrations of fluxapyroxad remained within a range of 96 to 107% of nominal concentrations in the freshly prepared test solutions at the start of the test and at the renewal at 48 hours after test start in the freshly prepared test solutions. In the aged test solutions concentrations were determined to be 100 to 106% of nominal concentrations at the renewal at 48 hours after test start and at the end of the test (96 hours).

The measured concentrations of prothioconazole remained within a range of 91 to 98% of nominal concentrations in the freshly prepared test solutions at the start of the test and at the renewal at 48 hours after test start in the freshly prepared test solutions. In the aged test solutions concentrations were determined to be 74 to 93% of nominal concentrations at the renewal at 48 hours after test start and at the end of the test (96 hours). Since one measured concentration of prothioconazole was below 80% of nominal in an aged test solution, geometric mean measured concentrations have been calculated for prothioconazole considering the exposure periods 0 – 48 h and 0 – 96 h.

In addition to the nominal formulation endpoints, the formulation endpoints were adjusted to the sum of a.s. contents over the respective exposure periods using the geometric mean measured concentrations of Prothioconazole and nominal concentrations of Fluxapyroxad.

Detailed analytical results are presented in the following table.

Table A 2.2.1.1-1: Concentrations of fluxapyroxad and prothioconazole in the test media during the exposure period

Nominal test concentration		Measured concentration of active substance					Actual concentration [mg a.s./L] [a)]	
[mg product/L]	[mg a.s./L]	Unit	0 h fresh	48 h aged	48 h fresh	96 h aged	48 h	96 h
Control		[mg a.s./L]	< LOQ	< LOQ	< LOQ	< LOQ	0	
		[%] of nominal	n.a.	n.a.	n.a.	n.a.		
Active substance: fluxapyroxad								
1.82	0.131	[mg a.s./L]	0.1259	0.1345	0.1393	0.1365	based on nominal concentrations	
		[%] of nominal	96.3	102.9	106.6	104.5		
2.55	0.183	[mg a.s./L]	0.1833	0.1828	0.1937	0.1939		
		[%] of nominal	100.2	99.9	105.9	106.0		
3.57	0.256	[mg a.s./L]	0.2591	0.2584	0.2746	0.2721		
		[%] of nominal	101.2	100.9	107.2	106.2		
5.00	0.359	[mg a.s./L]	0.3676	0.3732	[b)]	[b)]		
		[%] of nominal	102.5	104.1	n.a.	n.a.		
7.00	0.502	[mg a.s./L]	0.5034	0.5133	[b)]	[b)]		
		[%] of nominal	100.3	102.2	n.a.	n.a.		
Active substance: prothioconazole								
1.82	0.250	[mg a.s./L]	0.2361	0.2141	0.2458	0.2123	0.225	0.227
		[%] of nominal	94.5	85.7	98.4	85.0		
2.55	0.350	[mg a.s./L]	0.3167	0.2890	0.3300	0.3247	0.303	0.315
		[%] of nominal	90.5	82.6	94.3	92.8		
3.57	0.490	[mg a.s./L]	0.4737	0.3613	0.4813	0.4115	0.414	0.429
		[%] of nominal	96.7	73.8	98.3	84.0		
5.00	0.686	[mg a.s./L]	0.6633	0.6043	[b)]	[b)]	0.633	0.633
		[%] of nominal	96.7	88.1	n.a.	n.a.		
7.00	0.960	[mg a.s./L]	0.9212	0.8621	[b)]	[b)]	0.891	0.891
		[%] of nominal	96.0	89.8	n.a.	n.a.		

n.a.: not applicable; LOQ of 0.06515 mg fluxapyroxad/L and 0.03737 mg prothioconazole/L, respectively.

[a)] based on geometric mean measured active substance concentrations.
[b)] not analysed due to 100% mortality 24/48 hours after test start

Mortality

The percental mortalities of the 7 fish per replicate are presented in the following table.

Table A 2.2.1.1-2: Acute mortality of ADM.03503.F.1.A in rainbow trout

Test concentration		Cumulative mortality at time point [%]				
Nominal [mg product/L]	3 h	6 h	24 h	48 h	72 h	96 h
Control	0.0	0.0	0.0	0.0	0.0	0.0
1.82	0.0	0.0	0.0	0.0	0.0	0.0
2.55	0.0	0.0	0.0	0.0	0.0	0.0
3.57	0.0	0.0	0.0	0.0	28.6 [a)]	28.6 [a)]
5.00	0.0	0.0	0.0	100 [a)]	100 [a)]	100 [a)]
7.00	0.0	0.0	100 [a)]	100 [a)]	100 [a)]	100 [a)]

[a)] significantly different from the control (Fisher`s Exact Binomial Test with Bonferroni Correction for 24 and 48 hours, Step-down Cochran-Armitage Test for 72 and 96 hours, alpha = 0.05, one-sided greater)

The endpoints based on nominal and mean measured concentrations are summarised in the following table.

Table A 2.2.1.1-3: Acute mortality of ADM.03503.F.1.A in rainbow trout

Effect concentration (95% confidence intervals)		ADM.03503.F.1.A [mg/L]			
		24 h	48 h	72 h	96 h
NOEC	Nominal	5.00	3.57	2.55	2.55
	Mean measured	4.75 [a)]	3.21 [a)]	2.38 [b)]	2.38 [b)]
LOEC	Nominal	7.00	5.00	3.57	3.57
	Mean measured	6.67 [a)]	4.75 [a)]	3.28 [b)]	3.28 [b)]
LC_{10}	Nominal	n.d.	n.d.	2.12 (1.46 – 2.62)	2.12 (1.46 – 2.62)
	Mean measured	n.d.	n.d.	1.99 [b)] (1.37 – 2.45)	1.99 [b)] (1.37 – 2.45)
LC_{20}	Nominal	n.d.	n.d.	2.61 (1.97 – 3.14)	2.61 (1.97 – 3.14)
	Mean measured	n.d.	n.d.	2.45 [b)] (1.85 – 2.94)	2.45 [b)] (1.85 – 2.94)
LC_{50}	Nominal	n.d.	n.d.	3.72 (3.09 – 4.57)	3.72 (3.09 – 4.57)
	Mean measured	n.d.	n.d.	3.49 [b)] (2.90 – 4.29)	3.49 [b)] (2.90 – 4.29)

n.d. not determined due to mathematical reasons
[a)] calculations based on geometric mean of test item concentrations, adjusted using the measured concentrations for prothioconazole and nominal concentrations of fluxapyroxad over 0-48 hours
[b)] calculations based on geometric mean of test item concentrations, adjusted using the measured concentrations for prothioconazole and nominal concentrations of fluxapyroxad over 0-96 hours

Sublethal effects

The fish in the control group and in the treated test concentrations showed no abnormalities during the test or before they died at the nominal test concentration of 3.57 mg/L test item. At the test concentration of 5.00 mg/L test item the fish showed untypical vertical orientation and irregular breathing before they died. At the test concentration of 7.00 mg/L test item the fish showed untypical turns before they died.

Validity criteria

- The mortality in the control was 0% at test end (required according to test guideline OECD 203 ≤ 10% or ≤ 1 fish in the controls).

- Dissolved oxygen concentrations in the test solutions were ≥ 83% of air saturation (minimum: 8.19 mg O_2/L) (required according to the test guideline ≥ 60%).

The study did fulfil all validity criteria of OECD test guideline 203.

III. CONCLUSION

The 96-hour LC_{50} and NOEC of ADM.03503.F.1.A for rainbow trout were determined to be 3.72 and 2.55 mg test item/L, respectively, based on nominal concentrations. All validity criteria were met in the study.

The 96-hour LC_{50} and NOEC adjusted to the sum of a.s. contents based on geometric mean measured concentrations for prothioconazole and nominal concentrations for fluxapyroxad were determined to be 3.49 and 2.38 mg test item/L, respectively.

A 2.2.1.2 Study 2: Acute toxicity to *Daphnia magna* of ADM.03503.F.1.A

Comments of zRMS:	The study was conducted in line with OECD 202 with no minor deviations. The test concentration of both active substances was verified. The measured concentrations of fluxapyroxad in the test item solutions ranged from 101 to 104% of nominal in freshly prepared test solutions. The measured concentration in the aged solutions was in a range of 108 to 112% of nominal. The measured concentrations of prothioconazole ranged from 98 and 101% of nominal in freshly pre-pared solutions. In aged solutions, measured concentration was in the range of 80 to 105% of nominal. Therefore, 48-hour EC_{50} of ADM.03503.F.1.A in Daphnia magna was determined to be 6.58 mg test item/L based on nominal concentration. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 48 h EC_{50} = 6.58 mg product /L (based on nominal test concentration).

Reference:	KCP 10.2.1/02
Report	Acute toxicity of ADM.03503.F.1.A to *Daphnia magna* in a 48-hour static test, Juckeland, D., 2021b, 20 48 ADL 0005 (report number), 000105070 (sponsor report number) (Including Amendment No. 1)
Guideline(s):	Yes, OECD 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011

Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle control: test water Positive control/Reference item: Potassium dichromate. The 24-hour EC_{50} of the most recent reference item test of July 2020 with 2.08 mg/L was in the expected range of 0.6 – 2.1 mg/L for tests at the test facility.
3. Test organism	
Species	*Daphnia magna* (Straus)
Source	RWTH Aachen University, Institute for Environmental Research (Biology V), Aachen, Germany. Held and bred at the test facility under standardised laboratory conditions.
Age	24 hours; not first brood progeny
Acclimation period	Breeding of daphnids was performed under the same environmental conditions as in the test.
Feeding	Daphnids were daily fed with defined suspensions (dependent on age) of *Desmodesmus subspicatus* algae during breeding. Daphnids were not fed during the test.
Test units	25 mL glass beakers filled with 10 mLtest solution
4. Environmental conditions	
Test water	Elendt M4 medium
Conductivity	1.9 µS/cm (measured)
Hardness	2 mmol/L $CaCO_3$, corresponding to 200 mg/L (measured: 230 mg/L)
Alkalinity	0.8 mmol/L
pH	7.66 before test start (0 hours) Measured pH during the test: 7.66 – 7.71 (0 hours) and 7.48 – 7.69 (48 hours)
Water temperature	nominal: 18 – 22°C, actual: 20.6 – 20.8 °C
Lighting	16 : 8 hours light : dark cycle light intensity on average 20 µE m^{-2} s^{-1}
Aeration	The test medium was aerated before the test until oxygen saturation was achieved

B. STUDY DESIGN AND METHODS

1. In-life dates	21st July 2020 to 23rd July 2020 (biological phase)

2. Experimental conditions

Test design

Daphnia magna were exposed in a static 48-hour test to the test substance at five test concentrations and a test water control. A toxic reference was tested in a separate test. The recorded effect was mortality and immobility of the daphnids after 24 and 48 hours.

Number of animals per treatment

Twenty daphnids per treatment, five daphnids/replicate, four replicates/test substance treatment, reference treatment and test water control. Three additional test vessels were set up per concentration for measurements of water parameters, test item analysis and 1 retain specimen, respectively.

Test concentrations

Nominal test substance concentrations were 0.390, 5.47, 7.65, 10.7 and 15.0 mg test item/L. In addition, a control group with untreated test water was used.

Treatment/Application

Stock solutions (A) were prepared by weighing 20.5 mg test item, filling up to 1000 mL and stirring on a magnetic stirrer. Corresponding amounts of the stock solutions of 1.90, 2.67, 3.73, 5.23 and 7.32 mL were added to 7.60, 6.83, 5.77, 4.27 and 2.18 mL test medium, respectively. Further 0.5 mL test medium with daphnids were added using a Transferpettor to provide a total amount of solution of 10 mL corresponding with the final concentrations of 3.90 , 5.47, 7.65, 10.7 and 15.0 mg test item/L, respectively.

The control vessels contained test medium (dilution water) only.

Test medium was prepared four days before test start and aerated overnight.

Analytics

Concentrations of Fluxapyroxad and Prothioconazole in test solutions were determined and analysed using HPLC with MS/MS detection at test start (0 hours), and at the test end (48 hours). Details to the analytical method are summarized in Part B, Section 5.

3. Sampling and measurements

Observations for *Daphnia* immobilisation and mortality were made after 3, 24 and 48 hours including any abnormal behaviour or appearance.

The test temperature was continuously measured and the pH value, the oxygen concentration and test item concentrations were measured in the fresh medium (0 hours) as well as after 48 hours in an additional replicate.

4. Calculation of toxicity

Percentage immobility was calculated at 24 and 48 hours after application.

5. Statistics

The determination of NOEC and LOEC was carried out using Step-down Cochran-Armitage Test. The EC_{10}, EC_{20} and EC_{50} values were calculated by Probit analysis according to the maximum likelihood method.

Statistical analysis was performed using the software ToxRat Professional (Version 3.3).

II. RESULTS AND DISCUSSION

Analytical results

The measured concentrations of fluxapyroxad in the test item solutions ranged from 101 to 104% of nominal in freshly prepared test solutions. The measured concentration in the aged solutions was in a range of 108 to 112% of nominal.

The measured concentrations of prothioconazole ranged from 98 and 101% of nominal in freshly prepared solutions. In aged solutions, measured concentration was in the range of 80 to 105% of nominal.

Calculated endpoints were based on nominal concentrations of the test item.

Table A 2.2.1.2-1: Concentrations of fluxapyroxad and prothioconazole in the test media during the exposure period

Nominal test concentration		Sampling Measured concentration of active substance		
[mg product/L]	**[mg a.s./L]**	**Unit**	**0 h fresh**	**48 h aged**
Control		[mg a.s./L]	< LOQ	< LOQ
		[%] of nominal	n.a.	n.a.
Active substance: fluxapyroxad				
3.90	0.279	[mg a.s./L]	0.2859	0.3003
		[%] of nominal	102	108
5.47	0.393	[mg a.s./L]	0.4081	0.4284
		[%] of nominal	104	109
7.65	0.548	[mg a.s./L]	0.5708	0.6116
		[%] of nominal	104	112
10.7	0.769	[mg a.s./L]	0.7926	0.8334
		[%] of nominal	103	108
15.0	1.08	[mg a.s./L]	1.087	1.169
		[%] of nominal	101	109
Active substance: prothioconazole				
3.90	0.534	[mg a.s./L]	0.5252	0.4255
		[%] of nominal	98	80
5.47	0.751	[mg a.s./L]	0.7530	0.6439
		[%] of nominal	100	86
7.65	1.05	[mg a.s./L]	1.031	0.9207
		[%] of nominal	98	88
10.7	1.47	[mg a.s./L]	1.473	1.4069
		[%] of nominal	100	96
15.0	2.06	[mg a.s./L]	2.072	2.157
		[%] of nominal	101	105

n.a.: not applicable; LOQ of 0.1394 mg fluxapyroxad/L and 0.2665 mg prothioconazole/L, respectively.

Immobilisation

The results for immobilisation are presented in the following table.

No abnormal behaviour or appearance was observed in the *Daphnia magna*.

Table A 2.2.1.2-2: Acute toxicity of ADM.03503.F.1.A to *Daphnia magna*

Nominal test concentration [mg product/L]	Immobilised test animals					
	3 hours		24 hours		48 hours	
	No.	[%]	No.	[%]	No.	[%]
Control	0	0.0	0	0.0	0	0.0
3.90	0	0.0	0	0.0	0	0.0
5.47	0	0.0	0	0.0	2	10.0 [a)]
7.65	0	0.0	4	20.0 [a)]	17	85.0 [a)]
10.7	0	0.0	10	50.0 [a)]	20	100.0 [a)]
15.0	0	0.0	15	75.0 [a)]	20	100.0 [a)]

[a)] significantly different from the control (Step-down Cochran-Armitage Test; $\alpha = 0.05$, one-sided greater)

The endpoints based on nominal concentrations are summarised in the following table.

Table A 2.2.1.2-3: Acute effects on mobility of ADM.03503.F.1.A in *Daphnia magna*

Effect concentration (95% confidence intervals)	ADM.03503.F.1.A [mg/L]	
	24 h	48 h
NOEC	5.47	3.90
LOEC	7.65	5.47
EC_{10}	6.92 (5.28 – 8.04)	5.48 (4.66 – 5.95)
EC_{20}	8.15 (6.70 – 9.25)	5.83 (5.14 – 6.29)
EC_{50}	11.1 (9.83 – 13.0)	6.58 (6.08 – 7.12)

Validity criteria

In the control the immobility was 0% at test end (required according to test guideline OECD 202 ≤ 10%) and dissolved oxygen concentration was ≥ 7.97 mg/L (required ≥ 3 mg/L). No daphnids were trapped at the water surface.

The toxic reference indicated the sensitivity of the test organisms. Therefore, the validity criteria were fulfilled.

III. CONCLUSION

The 48-hour EC_{50} of ADM.03503.F.1.A in *Daphnia magna* was determined to be 6.58 mg test item/L and the 48-hour NOEC was determined to be 3.90 mg test item/L, respectively. All validity criteria were fulfilled.

A 2.2.1.3 Study 3: Toxicity to algae – *Pseudokirchneriella subcapitata* of ADM.03503.F.1.A

Comments of zRMS:	The study was conducted in line with OECD 201 with no minor deviations. The test concentration of both active substances was verified. The measured content of fluxapyroxad was between 96 and 104% of nominal in fresh samples. In aged samples, measured concentrations were between 98 and 104 % of nominal. The measured content of prothioconazole was between 89% and 103% of nominal in fresh samples. In aged samples, measured concentrations were between 91% and 100% of nominal. Since the content of fluxapyroxad and prothioconazole in the samples were between 80 and 120% of nominal, biological results were based on the nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The 72-hour E_rC_{50} = 16.9 mg product/L (based on nominal concentrations)

Reference:	KCP 10.2.1/03
Report	Effects of ADM.03503.F.1.A on *Pseudokirchneriella subcapitata* in an algal growth inhibition test, Juckeland, D., 2021c, 20 48 AAL 0007 (report number), 000105071 (sponsor report number)
Guideline(s):	Yes, OECD 201 (2011)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material — ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)

Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021

2. Vehicle and/or positive control — Vehicle control: test water
Toxic reference: In the most recent valid positive control test with *Pseudokirchneriella subcapitata* in October 2020, the 72-hour E_rC_{50} and E_yC_{50} of potassium dichromate were 1.41 and 0.57 mg/L, respectively.

3. Test organism

Species	*Pseudokirchneriella subcapitata* Korshikov
Strain	SAG 61.81
Source	SAG Culture Collection of Algae, Goettingen, Germany
Age	Algae cells were taken from an axenic stock culture
Acclimation period	Stock cultures grew in culturing vessels (glass flasks) for 4 days prior to test initiation in the same medium and temperature and light conditions as in the test
Test units	250 mL Erlenmeyer flasks with air-permeable stoppers filled with 100 mL test volume

4. Environmental conditions

Test water — Algae were cultivated and tested in AAP-medium (according to OECD 201) with the following concentrations:

$NaHCO_3$	50.0 mg/L

KH_2PO_4	1.6 mg/L
$MgSO_4 \cdot 7H_2O$	15.0 mg/L
NH_4Cl	15.0 mg/L
$CaCl_2 \cdot 2H_2O$	18.0 mg/L
$MgCl_2 \cdot 6H_2O$	12.0 mg/L
H_3BO_3	0.185 mg/L
$MnCl_2 \cdot 4H_2O$	0.415 mg/L
$ZnCl_2$	0.003 mg/L
$CoCl_2 \cdot 6H_2O$	0.0015 mg/L
$CuCl_2 \cdot 2H_2O$	$1 \cdot 10^{-5}$ mg/L
$Na_2MoO_4 \cdot 2H_2O$	0.007 mg/L
$FeCl_3 \cdot 6H_2O$	0.064 mg/L
$Na_2EDTA \cdot 2H_2O$	0.100 mg/L

Water temperature	actual: 22.7 – 23.0 °C
Lighting	Continuous illumination; actual mean: 64 $\mu Em^{-2}s^{-1}$
Shaking / CO_2 supply:	Continuously agitated; test vessels were placed in a controlled shaker in a climatic test room

B. STUDY DESIGN AND METHODS

1. In-life dates 21st July 2020 to 24th July 2020 (biological phase)

2. Experimental conditions

Test design

The single cell green alga *Pseudokirchneriella subcapitata* was exposed in a static 72-hour test to the test item at five concentrations and to a control, each test concentration with three replicates and the control with six replicates. The inhibition of algal growth was quantified based on yield and growth rates of the algae.

Inoculum at test start

The cell density was adjusted to 0.5×10^4 cells/mL in all treatments and in the control at start of the exposure period.

Test concentrations

Nominal test substance concentrations were 3.33, 6.00, 10.8, 19.4 and 35.0 mg product/L (spacing factor: 1.8). In addition, a control group with untreated test medium was tested. A toxic reference was tested in a separate test.

Treatment/Application

A stock solution (A) was prepared by weighing 69.8 mg test item, bringing up to a volume of 500 mL corresponding to 139.6 mg test item/L. The stock solution was homogenised by shaking. The solution was clear and transparent. Test concentration solutions were prepared by dilution of the stock solution (i.e. 11.96, 21.49, 38.69, 69.64 and 125.36 mL) with test medium to a final volume of 500 mL for final test concentrations of 3.33, 6.00, 10.8, 19.4 and 35.0 mg test item/L, respectively (nominal). Approximately 100 mL of the prepared solutions were transferred to each test vessel. 2.2 mL algal inoculum was added to 500 mL to result in the initial biomass of 0.5×10^4 cells/mL.

Analytics

An additional replicate per treatment was incubated for analytical sampling. The concentrations of fluxapyroxad and prothioconazole were analysed in the test solutions of all concentration levels and the control at the start and after 72 hours of the exposure period by HPLC-MS/MS. Details to the analytical method are summarized in Part B, Section 5.

3. Sampling and measurements

At 24, 48 and 72 hours, the number of cells in each replicate was determined by direct counting (actual microscopic cell count using a Neubauer counting chamber). Additional observations, such as sedimentation of test solution, cell aggregation or colour differences of algae cells were recorded at 24, 48 and 72 hours after test start.

The pH was measured at the beginning and at the end of the test. The temperature in the test was recorded continuously. The light intensity was measured in different positions over the test area once before test start.

4. Calculation of toxicity

The average specific growth rate for a specific period was calculated as the logarithmic increase of the cell numbers for each single vessel of controls and treatments. For each test concentration and control, a mean value for growth rate along with variance estimates was calculated. The percentage inhibition of growth rates (% I_μ) was calculated as the difference between the growth rates of the control (μ_c) and the growth rates in the treatment (μ_t).

Yield was calculated as the cell numbers at the end of the test minus the starting cell numbers for each single vessel of controls and treatments. The percent inhibition in yield (% I_y) was calculated for each treatment replicate.

5. Statistics

A test for normality of the data was performed by calculating the Shapiro-Wilk's statistic and the homogeneity of variance of the data was evaluated by using the Levene's Test. The NOEC and LOEC for effects on growth rate were determined using ANOVA techniques (Williams's t-test for homogeneous variances and Bonferroni-Holms corrected Welch test for non-homogeneous variances). The EC_{50}, EC_{20} and EC_{10} values for growth rate and yield were determined by Probit analysis.

The statistical evaluation for the 72 hours period was performed for growth rate and yield using ToxRat Professional Version 3.3 (2018).

II. RESULTS AND DISCUSSION

Analytical results
Analytically measured concentrations of fluxapyroxad and prothioconazole were determined in the test solution samples from all test concentrations and the control.

The measured content of fluxapyroxad was between 96 and 104% of nominal in fresh samples. In aged samples, measured concentrations were between 98 and 104 % of nominal.

The measured content of prothioconazole was between 89% and 103% of nominal in fresh samples. In aged samples, measured concentrations were between 91% and 100% of nominal.

Since the content of fluxapyroxad and prothioconazole in the samples were between 80 and 120% of nominal, biological results were based on the nominal concentrations.

Detailed analytical results are presented in the following table.

Table A 2.2.1.3-1: Concentrations of fluxapyroxad and prothioconazole in the test media during the exposure period

Nominal test concentration		Sampling	Measured concentration of active substance	
[mg product/L]	[mg a.s./L]	[h]	[mg a.s./L]	[% nominal]
Control		0	< LOQ	n.a.
		72	< LOQ	n.a.
Active substance: fluxapyroxad				
3.33	0.239	0	0.2285	96
		72	0.2347	98
6.00	0.430	0	0.4146	96
		72	0.4243	99
10.8	0.775	0	0.7596	98
		72	0.7771	100
19.4	1.39	0	1.403	101
		72	1.427	102
35.0	2.51	0	2.606	104
		72	2.618	104
Active substance: prothioconazole				
3.33	0.457	0	0.4060	89
		72	0.4141	91
6.00	0.823	0	0.7715	94
		72	0.7882	96
10.8	1.48	0	1.419	96
		72	1.486	100
19.4	2.67	0	2.741	103
		72	2.459	92
35.0	4.80	0	4.861	101
		72	4.719	98

n.a.: not applicable; LOQ of 0.06831 mg fluxapyroxad/L and 0.2277 mg prothioconazole/L, respectively.

Biological results

After 72 hours (at termination), a concentration response relationship was observed for the inhibition of growth rate and yield from nominal test item concentrations of 3.33 mg/L to 35.0 mg/L. The inhibition of growth rate peaked at 80.9% and the inhibition of yield peaked at 98.4% at a nominal test item concentration of 35.0 mg/L.

Additional observations were recorded at 24, 48 and 72 hours after test start. Destroyed cells were observed at concentrations of 19.4 and 35.0 mg/L at 48 and 72 hours after test start.

Results and relevant endpoints are summarized in the following tables.

Table A 2.2.1.3-2: Biomass of algae exposed to ADM.03503.F.1.A during the exposure period

Nominal concentration [mg product/L]	Average cell numbers [a] [× 10^4/mL]			
	0 hours	24 hours	48 hours	72 hours
Control	0.50	2.0	11.3	41.3
3.33	0.50	1.8	11.0	41.7
6.00	0.50	1.5	8.5	30.2
10.8	0.50	1.1	4.7	15.6
19.4	0.50	0.6	1.9	2.6
35.0	0.50	0.4	0.9	1.2
72-hour endpoints				
	Growth rate (µ)		Yield (y)	
	Nominal concentration [mg product/L]		Nominal concentration [mg product/L]	
EC_{50} (95% Cl)	16.9 (15.7 – 18.2)		8.70 (8.34 – 9.08)	
EC_{20} (95% Cl)	9.49 (8.31 – 10.5)		5.46 (5.08 – 5.81)	
EC_{10} (95% Cl)	7.01 (5.85 – 8.03)		4.28 (3.89 – 4.64)	
NOEC	3.33		3.33	
LOEC	6.00		6.00	

[a] mean of three replicates for the treatments and of six replicates in the control.
Cl: confidence limits.

Table A 2.2.1.3-3: Inhibition of average growth rate and yield of algae exposed to ADM.03503.F.1.A

Nominal test concentration [mg product/L]	Percent inhibition of growth rate [a]			Percent inhibition of yield [a]		
	0 – 24 h	0 – 48 h	0 – 72 h	0 – 24 h	0 – 48 h	0 – 72 h
Control	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.
3.33	4.7	0.8	-0.2 [c]	8.6	3.1	-0.8 [c]
6.00	20.0	9.1 *	7.1 *	31.4 *	26.2 *	27.3 *
10.8	45.2	28.4 *	22.2 *	60.0 *	61.5 *	63.1 *
19.4	90.1 *	57.1 *	63.5 *	94.3 *	86.9 *	94.9 *
35.0	100.0 [b] *	81.5 *	80.9 *	100.0 [b] *	96.2 *	98.4 *

[a] mean of three replicates for the treatments and of six replicates in the control; negative values indicate increase compared to the control.
[b] inhibition higher than 100 % (lower cell counts compared to test start).
[c] negative values indicate a higher cell growth relative to that of the control.
* statistically significant difference to controls.
n.r.: not relevant.

Validity criteria

In the control, the biomass had increased by a factor of 82.7 after 72 hours (required factor ≥ 16 according to test guideline OECD 201) corresponding to a growth rate of 1.471 d^{-1}. The mean coefficient of variation of the daily growth rates in the control (section-by-section growth rates) over 72 hours was 18.1% (required according to test guideline ≤ 35%). The coefficient of variation of the average specific growth rates in the replicates of the control was 1.2% after 72 hours (required according to test guideline ≤ 7%). Thus, the study did fulfil all validity criteria of OECD test guideline 201.

III. CONCLUSION

The effects of ADM.03503.F.1.A on the growth and biomass of the single cell green alga *Pseudokirchneriella subcapitata* were assessed. The 72-hour E_rC_{50}, E_rC_{20} and E_rC_{10} were 16.9, 9.49 and 7.01 mg product/L, respectively based on nominal concentrations. All validity criteria were met in the study.

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

No additional data submitted.

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

No additional data submitted.

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 2.3.1.1.1.1 Study 1: Acute oral and contact toxicity to the honeybee

Comments of zRMS:	The study was conducted in line with OECD 213 and 214 with no minor deviations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The 96-hour LD_{50} = 974 µg product/bee The 96-hour LD_{50} = 721 µg product/bee

Reference:	KCP 10.3.1.1/01
Report	Acute toxicity of ADM.03503.F.1.A to the honeybee *Apis mellifera* L. under laboratory conditions, Franke, M., 2020, 20 48 BAA 0026 (report number), 000105072 (sponsor report number)
Guideline(s):	Yes, OECD 213 and 214 (1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)

Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle oral toxicity test: aqueous sucrose solution (50%, w/v) Vehicle contact toxicity test: deionised water with wetting agent Tween® 80 (1.0% v/v) Positive control: reference item
Reference item	Dimethoate EC 400
Description	Apricot liquid
Lot/Batch #	10214034
Purity	400.0 g/L dimethoate (nominal content) 411.20 g/L dimethoate (analysed content) Density: 1.069 g/mL
Stability of reference item	Stable under storage conditions (at room temperature) Expiry date: 6th September 2021
3. Test organism	
Species	Honeybee, *Apis mellifera* L. Buckfast (Hymenoptera, Apoidea); female adult worker bees
Source	Apiary: BioChem agrar GmbH, Machern, Germany
Acclimatisation	Bees were collected on the morning of use. Groups of ten bees were transferred to each test cage without anaesthesia. Test bees were collected from an entrance located on the top of the bee hive using an automatic trapping device (carousel with glass tubes). For collecting the bees, the carousel with the glass tubes was placed on the top of the bee hive. The glass tubes were fixed in the carousel. Then, the entrance on the top of the bee hive was opened. After bees had walked into the glass tube, the carousel was turned so that the next empty glass tube was located over the entrance. The tubes containing the bees were removed and placed at the test cage entrance. The bees were introduced into the test cage by gentle blowing in the end of the glass tube. After transferring the bees into the test cages, they had time for acclimatisation to the test room conditions for about 1 hour (corresponding to the starvation period in the oral toxicity test) before application of the treatments.
Diet	Oral toxicity test: The bees were starved 1 hour before test start. During the exposure phase, the bees were provided with an aqueous sucrose (50% (w/v)) solution containing either no test substance (control) or the test substance or the reference substance. Contact toxicity test: During the test phase, the bees were supplied *ad libitum* with 50% (w/v) aqueous sucrose solution.
Test units	In both tests, the bees were kept in cages made of cardboard (base: 95 mm × 50 mm; height: 65 mm) with holes in the bottom for ventilation and a glass plate in front.
4. Environmental conditions	
Temperature	Nominal: 25 ± 2°C; actual: 24 – 25 °C
Relative humidity	Nominal: 50 – 70%; actual: 50 – 68%
Photoperiod	Throughout the test, bees were kept in constant darkness, except during handling and assessments at diffuse artificial light

B. STUDY DESIGN AND METHODS

1. In life dates 6th August 2020 to 10th August 2020

2. Experimental conditions

Test design

Lethal effects of the test substance on the honeybee, *Apis mellifera* L., after oral and contact exposure were assessed at five doses of ADM.03503.F.1.A under laboratory conditions. In addition, one water control, one wetting agent control (contact test only) and a reference item (four doses) were tested. For the oral treatment, the test substance was provided via feeding solution. For the contact treatment, the test substance was applied to the bee thorax. Bee mortality and sublethal effects were assessed.

Number of animals/treatment

Ten bees/replicate; three replicates/test and reference substance treatment and controls.

Doses tested

Oral toxicity test
ADM.03503.F.1.A was tested at nominally 198, 297, 445, 667 and 1000 µg product/bee. The actual ingested doses were 198, 287,422, 641 and 961 µg product/bee. A control group, receiving untreated 50% (w/v) aqueous sucrose solution was tested in parallel.

A stock solution (= highest test solution) was prepared by dispersing 0.500 g test item in 10 mL sucrose solution. The lower test solutions were prepared by serial dilution (6.67 mL added to 10 mL sucrose solution each).

Contact toxicity test
DM.03503.F.1.A was tested at nominally 198, 297, 445, 667 and 1000 µg product/bee. A control group, receiving untreated 50% (w/v) aqueous sucrose solution (1% (v/v) Tween solution), was tested in parallel.

A stock solution (= highest test solution) was prepared by dispersing 5.00 g test item in 10 mL sucrose solution with 1% (v/v) Tween solution. The lower test solutions were prepared by serial dilution (6.67 mL added to 10 mL tween solution each).

Reference item

In the oral toxicity test, Dimethoate EC 400 was tested at nominally 0.086, 0.123, 0.175 and 0.250 µg product/bee. The actual ingested doses were identical to the nominal doses. For the contact toxicity test, Dimethoate EC 400 was tested at 0.105, 0.141, 0.188 and 0.250 µg product/bee.

For both tests, a stock solution was prepared by dispersing 0.325 g reference item either in sucrose solution (oral test) or Tween solution (contact test). The reference item solutions were prepared by serial dilution of the stock solution.

Treatment/Application

Oral toxicity test
A quantity of 200 µL of test or reference substance application solution was offered to each cage of ten bees. Bees within a cage shared the test solution and therefore were assumed to have each received a similar dose (social feeding behaviour). The actual amount of test solution consumed by each replicate was determined by weighing the food tubes (glass ampoules, half-open on longitudinal axis and 5 cm long) before and after feeding. After a period of approximately 5 hours, feeding tubes were obviously empty and were removed. Feeding tubes containing untreated sucrose solution were then introduced to the cages. In the control group, the bees were fed with 200 µL of 50% (w/v) aqueous sucrose solution and thereafter were fed *ad libitum* with 50% (w/v) aqueous sucrose solution.

Contact toxicity test
After the bees had been anaesthetised with carbon dioxide for approximately half a minute, they were treated individually by applying 2 µL Tween solution (wetting agent control), test or reference substance application solution dorsally to the thorax of the bee. According to the practical experience and to guarantee a good penetration of the test item this application volume is considered to be more appropriate than 1µL/bee as suggested in the test guideline. Application was performed using an Eppendorf micropipette. After treatment, the bees were returned to the test cages and fed with a 50% (w/v) aqueous sucrose solution *ad libitum.*

3. Observations and assessments

Mortality of the bees was assessed at 4, 24, 48, 72 and 96 hours after test start (start of feeding or after contact application). At the same observation times, any abnormal behaviour in comparison to the control bees was documented.

The consumption of application solution per replicate was determined by weighing the feeders at the start and at the end of the feeding application period.

The test temperature and relative humidity were continuously recorded.

4. Calculation of toxicity

Bee mortality was calculated for each treatment.

5. Statistics

The LD_{50} and its 95% confidence limits for the test item treatment was calculated by Probit analysis (contact test) and Weibull analysis (oral test). For the reference substance treatment, the LD_{50} was calculated by Probit analysis (linear maximum likelihood regression). Mortality data was statistically evaluated using Fisher's Exact Binomial Test with Bonferroni-Holm correction ($\alpha = 0.05$) for the test item and the reference item.

The statistical software program ToxRat Professional 3.3.0 (2018) was used for analysis.

II. RESULTS AND DISCUSSION

Oral toxicity test

The actual consumed doses of ADM.03503.F.1.A in the treatments of nominal 198, 297, 445, 667 and 1000 µg product/bee were 198, 287,422, 641 and 961 µg product/bee, respectively.

After 48 hours, significant increased mortalities of 76.7 and 20.0% were determined at consumed doses of 961 and 641 µg product/bee. Effects of 10, 6.7 and 3.3% mortality without any statistical significance were observed at dose rates of 422, 287 and 198 µg product/bee, respectively. The oral test was extended up to 96 hours due to the significant increase in bee mortality between the 24 hours and 48 hours assessments. After 96 hours, statistically significant increased mortalities of 76.7 and 36.7% were observed after oral consumption of 961 and 641 µg product/bee, respectively.

After 4 hours, effects on the behaviour of honeybees were observed at all dose rates ≥ 198 µg product/bee. In the following course up to 96 hours, no effects on behaviour at all dose rates up to 961 µg product/bee were observed.

Results of the oral toxicity test and relevant endpoints are summarized in the following table.

Table A 2.3.1.1.1.1-1: Oral toxicity of ADM.03503.F.1.A to honey bees (*Apis mellifera* L.)

Dose		Mean mortality [%]				
Target [µg product/bee]	Actual uptake [µg product/bee]	4 h	24 h	48 h	72 h	96 h
Control (Sucrose)						
-	-	0.0	0.0	0.0	0.0	0.0
Test item (ADM.03503.F.1.A)						
198	198	0.0	3.3	3.3	3.3	3.3
297	287	0.0	6.7	6.7	6.7	10.0
445	422	0.0	6.7	10.0	10.0	16.7
667	641	0.0	20.0 *	20.0 *	23.3 *	36.7 *
1000	961	0.0	70.0 *	76.7 *	76.7 *	76.7 *
Reference substance: Dimethoate EC 400						
0.086	0.086	0.0	16.7 *	20.0 *	n.a.	n.a.
0.123	0.123	0.0	56.7 *	63.3 *	n.a.	n.a.
0.175	0.175	0.0	80.0 *	86.7 *	n.a.	n.a.
0.250	0.250	0.0	100.0 *	100.0 *	n.a.	n.a.
Endpoint [a] [µg ADM.03503.F.1.A/bee]						
24-hour LD_{50} (95% Cl)	830 (731 – 973)					
48-hour LD_{50} (95% Cl)	788 (698 – 902)					
72-hour LD_{50} (95% Cl)	778 (689 – 891)					
96-hour LD_{50} (95% Cl)	721 (630 – 835)					

* Significantly different compared to control.
n.a.: not applicable
[a] values based on actual food consumption.

Contact toxicity test

After 48 hours, a significant increase in mortality was determined at the dose rate of 1000 µg product/bee. Mortalities of 10.0 and 3.3 %, without any statistical significance, occurred at the dose rates of 667 and 445 µg product/bee, respectively. The contact test was extended up to 96 hours due to the significant in-

crease in bee mortality between the 24 hours and 48 hours assessments. After 96 hours, statistically significant increased mortalities of 50.0 and 23.3 % were observed at the dose rates of 1000 and 667 µg product/bee, respectively.

After 4 hours, effects on the behaviour were observed at dose rates ≥ 297 µg product/bee. After 24 hours, only the bees treated with the highest dose rate of 1000 µg product/bee showed an affected behaviour compared to the control. In the following course up to 96 hours, no effects on behaviour at all dose rates up to 1000 µg product/bee.

Results of the contact toxicity test and relevant endpoints are summarized in the following table.

Table A 2.3.1.1.1.1-2: Contact toxicity of ADM.03503.F.1.A to honey bees (*Apis mellifera* L.)

Actual dose [µg product/bee]	Mean mortality [%]				
	4 h	24 h	48 h	72 h	96 h
Controls					
Water control	0.0	0.0	0.0	0.0	0.0
Tween solution control	0.0	0.0	0.0	0.0	0.0
Test item (ADM.03503.F.1.A)					
198	0.0	0.0	0.0	0.0	0.0
297	0.0	0.0	0.0	0.0	0.0
445	0.0	3.3	3.3	3.3	3.3
667	0.0	6.7	10.0	20.0 *	23.3 *
1000	0.0	13.3	40.0 *	50.0 *	50 *
Reference substance: Dimethoate EC 400					
0.105	0.0	10	16.7 *	n.a.	n.a.
0.141	0.0	43.3 *	53.3 *	n.a.	n.a.
0.188	0.0	73.3 *	83.3 *	n.a.	n.a.
0.250	0.0	93.3 *	96.7 *	n.a.	n.a.
Endpoint [µg product/bee]					
24-hour LD_{50} (95% Cl)	> 1000				
48-hour LD_{50} (95% Cl)	> 1000				
72-hour LD_{50} (95% Cl)	987 (853 – 1287)				
96-hour LD_{50} (95% Cl)	974 (842 – 1263)				

* Significantly different compared to control
n.a.: not applicable.
Cl: confidence limits.

Validity criteria

The oral toxicity test is considered to be valid since mortality in the control was 0.0% (required ≤ 10%) and the 24-hour LD_{50} of the reference item was 0.120 µg a.s./bee (required 0.10 - 0.35 µg a.s./bee).

The contact toxicity test is considered to be valid since mortality in the control was 0.0% for deionised water control and tween solution (required ≤ 10%) and the 24-hour LD_{50} of the toxic standard was 0.153 µg a.s./bee (required 0.10 - 0.30 µg a.s./bee).

III. CONCLUSION

The acute contact and oral toxicity on the honeybee *Apis mellifera* L. were investigated under laboratory conditions over a period of 96 hours. The 48-hour contact LD_{50} of ADM.03503.F.1.A was > 1000 µg product/bee and the 96-hour LD_{50} was 974 µg product/bee. The 48-hour oral LD_{50} of ADM.03503.F.1.A was 788 µg product/bee and the 96-hour LD_{50} was 721 µg product/bee. All validity criteria were fulfilled.

A 2.3.1.1.2 KCP 10.3.1.1.1 Acute oral toxicity to bees

Reference is made to A.2.3.1.1.

A 2.3.1.1.3 KCP 10.3.1.1.2 Acute contact toxicity to bees

Reference is made to A.2.3.1.1.

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.2.1 Study 1: Chronic toxicity to the honeybee

Comments of zRMS:	The study was conducted in line with OECD 245 with no deviation. The concentrations of the active ingredients in the applied test item feeding solutions were within the required range of ± 20 % of the nominal concentrations. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: LDD_{50} = 107 µg product/bee/day NOEDD = 49.2 µg product/bee/day LC_{50} = 5.534 (4.724 – 6.918) g product/kg food NOEC = 1.189 g product/kg food

Reference:	KCP 10.3.1.2/01
Report	Chronic toxicity of ADM.03503.F.1.A to the honeybee *Apis mellifera* L. under laboratory conditions, Dreßler, K., 2021, 20 48 BAC 0010 (report number), 000105073 (sponsor report number)
Guideline(s):	OECD 245 (2017)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	SC (Suspension concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)

Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle: aqueous sucrose solution (50%, w/v) and sucrose solution with 0.1% (w/v) xanthan (viscosifier control) Positive control: reference item
Reference item	Danadim® Progress
Description	Apricot liquid
Lot/Batch #	10214034
Purity	400.0 g/L dimethoate (nominal content) 411.20 g/L dimethoate (analysed content) Density: 1.069 g/mL
Stability of reference item	Stable under storage conditions (at room temperature) Expiry date: 6th September 2021

3. Test organism

Species	Honey bee, *Apis mellifera* L., ssp: *iberiensis* (Engel) (Insecta, Hymenoptera, Apoidea)
Source	Apiary: BioChem AGROLOGÍA S.L.U., Finca La Dehesilla, Sevilla, Spain
Age	Young adult worker bees (max. 2 days old)
Pre-treatment culturing conditions	Brood frames with capped cells were taken from outside hives and different colonies (day -2). Sufficient food supply was ensured either by honey and pollen which is on the same brood frame or by an additional frame containing food. These frames were placed without adult worker bees in a 'five frame hive body' and incubated under controlled environmental conditions in a climatic chamber at 33 ± 2 °C in darkness (until day -1). Afterwards, the newly hatched worker bees were transferred into the test cages in groups of 10 bees per cage. For the following 24 ± 2 hours (until day 0), bees were held in the test cages at 33 ± 2 °C and 50 – 70% relative humidity and provided with sugar solution for acclimatisation to the test conditions. Moribund and dead bees were rejected and replaced by healthy bees that were held in spare cages before starting the test.
Diet	The bees were fed *ad libitum* with a 50% (w/v) sucrose solution containing either the test item or the reference item or pure 50% (w/v) sucrose solution (untreated control group). The treated and untreated food was offered using syringes which were replaced daily by a new one containing fresh treated or untreated food.
Test units	Aluminium cages with the dimensions: 95 mm × 60 mm × 70 mm; with holes in the lateral walls for ventilation and two glass plates (one in front and one in the back) for observation of the bees.

4. Environmental conditions

Temperature	nominal: 33 ± 2 °C, actual: 33.3 – 33.8 °C
Relative humidity	nominal: 50 – 70%, actual: 60.0 – 63.9%
Photoperiod	During the test, the bees were kept in constant darkness except during observations (diffuse artificial light during handling and assessment).

B. STUDY DESIGN AND METHODS

1. In-life dates 5th May 2020 to 15th May 2020 (biological phase)

2. Experimental conditions

Test design

In a 10-day chronic test, young adults of *Apis mellifera* L. were exposed daily to five doses of ADM.03503.F.1.A in 50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan. In parallel, an untreated control (50% (w/v) aqueous sucrose solution), a sucrose/viscosifier control (50% (w/v) aqueous sucrose solution + 0.1% (w/v) xanthan) as well as one dose of the reference item were tested. Assessments of bee mortality and sub-lethal effects were done daily during the study.

Number of animals per treatment

Three replicates per test and reference substance treatment and untreated control were used with 10 bees per replicate.

Test doses

ADM.03503.F.1.A was tested at nominally 39.1, 86.0, 189, 416 and 916 µg product/bee/day, corresponding to 0.995, 2.189, 4.817, 10.60 and 23.328 g product/kg food, respectively. The control groups, receiving untreated 50% (w/v) aqueous sucrose solution or aqueous sucrose solution + 0.1% (w/v) xanthan, were tested in parallel. The test doses were determined based on a non-GLP range-finding test.

Reference item

The reference item, Danadim® Progress was tested at a single nominal dose of 27.3 ng dimethoate/bee/day corresponding to a concentration of 0.694 mg dimethoate/kg food.

Treatment/Application

The application took place for a period of 10 consecutive days. Test item solutions were prepared daily just before administration of food. The reference item stock solution was prepared once for the whole feeding period and stored in the refrigerator. The reference item feeding solutions were prepared at least every 4 days and stored in the refrigerator at 6 °C. The daily dose rates (administered solution) were based on a theoretical oral consumption of 33 µL per bee and day, which is described in literature.

The bees were fed with 50 % w/v aqueous sucrose solution + 0.1% (w/v) xanthan including the test item or the reference item. The control treatments were fed with either 50% w/v untreated aqueous sucrose solution or 50% w/v untreated aqueous sucrose solution + 0.1% (w/v) xanthan. The treated/untreated food was provided *ad libitum* in a plastic syringe, which had been weighed before application. The actual consumption was determined by reweighing the syringe containing the remaining test solution each day after removal from the test units.

Analytics

For verification of the exposure concentration, all test item feeding solutions as well as the viscosifier control solution were sampled in duplicate for analysis and retained directly after preparation, on each day of application (i.e. day 0 to day 9). Analysis was performed via reversed phase - HPLC with mass-spectrometric detection. The analytical method is summarized in Part B, Section 5.

An analytical verification of fluxapyroxad and prothioconazole in the feeding solutions was carried out. Recoveries were within the acceptable range.

Active ingredient	Specimen description	Nominal conc. of a.i. [mg/kg]	Analysed conc. of a.i. [mg/kg]	Recovery [%]
fluxapyroxad	20BAC0010-D0-BC-A	0.000	<30%LOQ	-
	20BAC0010-D1-BC-A		<30%LOQ	-
	20BAC0010-D2-BC-A		<30%LOQ	-
	20BAC0010-D3-BC-A		<30%LOQ	-
	20BAC0010-D4-BC-A		<30%LOQ	-
	20BAC0010-D5-BC-A		<30%LOQ	-
	20BAC0010-D6-BC-A		<30%LOQ	-
	20BAC0010-D7-BC-A		<30%LOQ	-
	20BAC0010-D8-BC-A		<30%LOQ	-
	20BAC0010-D9-BC-A		<30%LOQ	-
	20BAC0010-D0-ET-A	71.3	68.9	96.7
	20BAC0010-D1-ET-A		68.8	96.6
	20BAC0010-D2-ET-A		69.8	97.9
	20BAC0010-D3-ET-A		66.2	92.8
	20BAC0010-D4-ET-A		64.0	89.7
	20BAC0010-D5-ET-A		69.1	96.9
	20BAC0010-D6-ET-A		69.1	96.9
	20BAC0010-D7-ET-A		67.5	94.6
	20BAC0010-D8-ET-A		68.8	96.5
	20BAC0010-D9-ET-A		64.3	90.2
	20BAC0010-D0-AT-A	1673	1617	96.7
	20BAC0010-D1-AT-A		1569	93.8
	20BAC0010-D2-AT-A		1664	99.5
	20BAC0010-D3-AT-A		1476	88.2
	20BAC0010-D4-AT-A		1499	89.6
	20BAC0010-D5-AT-A		1766	106
	20BAC0010-D6-AT-A		1746	104
	20BAC0010-D7-AT-A		1667	99.7
	20BAC0010-D8-AT-A		1663	99.4
	20BAC0010-D9-AT-A		1551	92.7

Active ingredient	Specimen description	Nominal conc. of a.i. [mg/kg]	Analysed conc. of a.i. [mg/kg]	Recovery [%]
prothioconazole	20BAC0010-D0-BC-A	0.000	<30%LOQ	-
	20BAC0010-D1-BC-A		<30%LOQ	-
	20BAC0010-D2-BC-A		<30%LOQ	-
	20BAC0010-D3-BC-A		<30%LOQ	-
	20BAC0010-D4-BC-A		<30%LOQ	-
	20BAC0010-D5-BC-A		<30%LOQ	-
	20BAC0010-D6-BC-A		<30%LOQ	-
	20BAC0010-D7-BC-A		<30%LOQ	-
	20BAC0010-D8-BC-A		<30%LOQ	-
	20BAC0010-D9-BC-A		<30%LOQ	-
	20BAC0010-D0-ET-A	136	115	84.7
	20BAC0010-D1-ET-A		117	85.5
	20BAC0010-D2-ET-A		120	87.8
	20BAC0010-D3-ET-A		114	83.4
	20BAC0010-D4-ET-A		111	81.5
	20BAC0010-D5-ET-A		115	84.1
	20BAC0010-D6-ET-A		121	88.6
	20BAC0010-D7-ET-A		118	86.5
	20BAC0010-D8-ET-A		123	90.4
	20BAC0010-D9-ET-A		113	83.3
	20BAC0010-D0-AT-A	3196	2800	87.6
	20BAC0010-D1-AT-A		2788	87.2
	20BAC0010-D2-AT-A		2967	92.8
	20BAC0010-D3-AT-A		2597	81.3
	20BAC0010-D4-AT-A		2672	83.6
	20BAC0010-D5-AT-A		3123	97.7
	20BAC0010-D6-AT-A		3009	94.2
	20BAC0010-D7-AT-A		2901	90.8
	20BAC0010-D8-AT-A		2867	89.7
	20BAC0010-D9-AT-A		2644	82.7

LOQ: 33.5 mg/kg fluxapyroxad, corresponding to 18.4 µg/L in diluted extracts; 63.9 mg/kg prothioconazole, corresponding to 35.2 µg/L in diluted extracts

3. Observations and assessments

Mortality and behavioural abnormalities were assessed daily until test end at about the same time of day (every 24 ± 2 hours), over 10 days following start of exposure.

The actual amount of feeding solution consumed was determined daily by weighing the feeders before and after feeding. The feeding syringes were replaced daily. The difference of weight at start and end of each feeding period represents the food consumed by the bees in one cage for 24 hours. The evaporation figure, determined in three additional test cages without bees, was then subtracted from the calculated uptake to give the real uptake accounting for loss by evaporation. This amount of food was divided by the number of living bees at start of the corresponding exposure interval (in case of resulting in a negative value, food consumption of the respective day was considered to be zero).

4. Calculation of toxicity

The percentage of cumulative mortality was calculated for each treatment group and assessment from the number of dead individuals in relation to the number of introduced test organisms. Mortality was corrected for control mortality according to Abbott (1925) modified by Schneider-Orelli (1947).

The consumption of feeding solution per bee per day was calculated by dividing the total daily consumption per replicate by the number of living bees at the beginning of the respective feeding interval.

5. Statistics

For statistical calculation of mortality results, the Step-down Cochran-Armitage test was used ($\alpha = 0.05$, one-sided greater). The LDD_{50} along with its 95% confidence limits was determined by the Moving Average Computation after Thompson. LC_x values along with its 95% confidence limits were determined by Weibull analysis using linear maximum likelihood regression.

Statistical calculations were made by using the statistical program ToxRat Professional 3.3.0 (2018).

II. RESULTS AND DISCUSSION

Analytical recovery rates of the active substance fluxapyroxad were ranging between 88.2 and 106% (highest dose) or between 89.7 and 97.9% (lowest dose). Recovery rates of prothioconazole ranged between 81.3 and 97.7% (highest dose) or between 81.5 and 90.4% (lowest dose). Therefore, the concentrations of the active substances in the feeding solutions were verified and biological results were based on nominal concentrations.

In the course of the test, single bees were described as being affected in terms of uncoordinated movements in the three highest test item doses. At the consumed dose of 130 µg product/bee/day, one bee was observed as being moribund on day 3. No other behavioural abnormalities were observed in controls or test item concentration on any other assessment day.

Food intake in the test item group ranged between 5.56 and 31.3 mg solution/bee/day corresponding to 14.2 to 79.7% of the expected amount with tendency of lower food intake in higher test item doses indicating a repellent effect of test item at higher doses. In the control group, bees on average consumed 46.3 mg/bee/day (i.e. 117.9.9% of the expected daily amount) and in the viscosifier control average consumption was 47.8 mg/bee/day (i.e. 121.8% of the expected daily amount).

Results and relevant endpoints are summarized in the following table.

Table A 2.3.1.2-1: Mortality of bees in the chronic toxicity feeding test after 10 days

Treatment group	Test concentration [g product/kg food]	Dose level consumed		Cumulative mortality after 10 days	
		nominal [µg product/bee/day]	actual [µg product/bee/day]	absolute [%]	corrected [%] [a)]
Control	-	-	-	0.0	-
Viscosifier control	-	-	-	0.0	-
Test item ADM.03503.F.1.A	0.995	39.1	31.1	0.0	-
	2.189	86.0	49.2	3.3	-
	4.817	189	97.6	33.3 *	-
	10.600	416	139	100 *	-
	23.328	916	130	100 *	-
Reference item Danadim® Progress [b)]	0.694 mg a.s./kg food	27.3 ng a.s./bee/day	18.8 ng a.s./bee/day	100	-

10-day endpoints	[µg consumed product/bee/day] [c)]	[g product/kg food]
LDD_{50} (95% Cl)	107 (96.8 – 118)	-
NOEDD	49.2	-
LC_{50} (95% Cl)	-	5.534 (4.724 – 6.918)
NOEC	-	2.189

[a)] due to 0% mortality in both control groups, no correction is needed.
[b)] active substance: dimethoate.
[c)] taking into account the actual food uptake and evaporation.
Cl: confidence limits.

Validity criteria

The validity criteria were met (mean mortality in the control was below 15%, actual values: 0% in the sucrose control and 0% in the sucrose + xanthan control), mean mortality in the reference item treatment was ≥ 50% at the end of the test (actual value: 100%).

III. CONCLUSION

In this chronic toxicity feeding study with ADM.03503.F.1.A on the honey bee, the 10-day LDD_{50} was determined at 107 µg product/bee/day and the 10-day NOEDD was determined to be 49.2 µg product/bee/day (based on consumed doses). The validity criteria were fulfilled.

A 2.3.1.3 KCP 10.3.1.3 Effects on honeybee development and other honeybee life stages

A 2.3.1.3.1 Study 1: Toxicity to honeybee larvae

Comments of zRMS:	The study was conducted in line with OECD 239 with no deviation. Analytical recovery rates of the active substances in the test item stock solutions ranged between 103 and 117% for fluxapyroxad in each test item treatment group and between 111 and 118% for prothioconazole in each test item treatment group, except for the lowest concentration that was out of range and amounted to 129%. Therefore, the toxicity endpoints were 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: EC_{50} (D22) = 30.520 mg product/kg food NOEC = 3.207 mg product/kg food ED_{50} = 4.827 µg product/larva NOED (D22) = 0.507 µg product/larva **ED_{10}=0.179 µg product/larva** Since the derived ED_{10} is lower than NOED, the reliability of EC_{10} was checked in line with the recommendations of EFSA Supporting publication 2019:EN-1673. Normalised width of confidence interval: NW = 1.78; rating: poor Steepness: 0.037; rating: shallow curve Classification based on the relationship between ED_{10} and ED_{20}/ED_{50} confidence intervals, considering the steepness of the curve: ED_{10} is lower than ED_{20},low: Certainty of the protection level: High) The ED_{10} is suitable for consideration in the risk assessment.

Reference:	KCP 10.3.1.3/01
Report	ADM.03503.F.1.A – Repeated exposure of honeybee larvae (*Apis mellifera* L.) under laboratory conditions, Hänsel, M., 2021, 20 48 BLC 0012 (report number), 000105074 (sponsor report number) (Including Amendment No. 1)
Guideline(s):	OECD 239 (2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material ADM.03503.F.1.A
(Fluxapyroxad 75 Prothioconazole 150 g/L EC)

Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle: untreated diet (50% aqueous sucrose solution with 50% royal jelly) Positive control: reference item
Reference item	Dimethoate tech.
Description	Not stated
Lot/Batch #	778197
Purity	98.8% ± 0.5% (w/w)
Stability of reference item	Stable under storage conditions (chilled) Expiry date: 1st November 2021

3. Test organism

Species	Honey bee, *Apis mellifera* L., ssp: *iberiensis* (Engel) (Insecta, Hymenoptera, Apoidea)
Source	Apiary: BioChem AGROLOGÍA S.L.U., Spain
Age	First instar (L1) larvae
Pre-treatment culturing conditions:	The colonies producing the larvae were held under field conditions in hives including a healthy queen. Brood in egg, larval and pupal stages as well as filled food combs (with nectar and pollen) were present in the hives.
Method of producing L1 larvae:	Each of the three colonies used in the test was treated in parallel in the same way: On day -3, the queens of the colonies were caged on an empty brood comb in an excluder cage and placed in the hive. The caging time was approx. 24 hours. On day -2, the queen was released from the cage. The comb was checked for presence of freshly laid eggs, the queen was confined to the excluder again in order to avoid any further egg laying and was placed in the hive near to frames containing open brood. The eggs were incubated within the hive between day -2 and day 1.
Grafting:	On day 1, combs containing larvae were transported to an acclimatised laboratory room using a polystyrene box. Larvae were transferred to cells using a suitable grafting tool. During grafting, the larvae were placed on the surface of the artificial diet within the grafting cells, each replicate representing larvae originating from a different colony to exclude colony effects. Grafting was performed on a warming plate set to 32 °C.
Diet	The food was composed of three different artificial diets which were adapted to the needs of the larvae at different stages of development: - Diet A (day 1): 50% royal jelly + 50% aqueous solution containing 2% yeast extract, 12% glucose and 12% fructose - Diet B (day 3): 50% royal jelly + 50% aqueous solution contain-

	ing 3% yeast extract, 15% glucose and 15% fructose - Diet C (days 4 – 6): 50% royal jelly + 50% aqueous solution containing 4% yeast extract, 18% glucose and 18% fructose
Test units	Crystal polystyrene grafting cells (diameter 9 mm, depth 8 mm) were disinfected with ethanol (70%) followed by drying of the cells under laminar flow.
4. Environmental conditions	The test was performed in a climatic chamber.
Temperature	Nominal: 34.5 ± 0.5 °C; actual: 34.0 – 34.9°C
Relative humidity	Day 1 – 8**:** nominal: 95 ± 5%; actual: 97.2 – 100% Day 8 – 15: nominal: 80 ± 5%; actual: 75.9 – 84.3% Day 15 – 22: nominal: 50 – 80 %; actual: 60.1 – 69.8%
Photoperiod	During the test, the bees were kept in constant darkness except during handling and assessments (diffuse artificial light). The climatic chamber was ventilated by air-conditioning.

B. STUDY DESIGN AND METHODS

1. In-life dates 8th June 2020 to 29th June 2020 (biological phase)

2. Experimental conditions

Test design

The effects of the test substance ADM.03503.F.1.A to honey bee larvae (*Apis mellifera* L.) were assessed in a chronic toxicity test up to day 22 of their development. Honey bee larvae were either treated repeatedly with the test item at six concentrations, the reference item dimethoate tech. at a single concentration or remained untreated (control). Larval mortality and adult emergence were assessed. Additionally, other observations such as smaller body size or remaining food during the larval development were noted, if applicable.

Number of animals per treatment

12 larvae/replicate; 3 replicates/test and reference substance treatment and control

Test doses

The toxicity of ADM.03503.F.1.A was determined at 0.118, 0.355, 1.068, 3.207, 9.622 and 28.870 mg product/kg diet, equivalent to cumulative doses of 0.019, 0.056, 0.169, 0.507, 1.522 and 4.567 µg product/larva, respectively. A control group, receiving untreated artificial diet, was tested in parallel.

Reference item

The reference item, dimethoate tech. was tested at a concentration of 48 mg dimethoate tech./kg diet (equivalent to 7.6 µg dimethoate/larva).

Treatment/Application

The stock solution of the test item was prepared by weighing 0.083 g test item and filling up to 50 mL aqueous sugar solution of the respective diet. The other stock solutions were prepared by filling up of the base stock to 10 mL aqueous sucrose solution.

Applied volumes of diet per larva (individual feeding) were as follows: day 1 (pre-treatment): 20 µL untreated diet A; day 3: 20 µL untreated/treated diet B; day 4: 30 µg untreated/treated diet C; day 5: 40 µL untreated/treated diet C; day 6: 50 µL untreated/treated diet C.

3. Observations and assessments

The number of dead larvae and pupae (not reacting to contact stimulus) was assessed daily on days 4 to 8 (larvae) and on days 15 and 22. Adult emergence (i.e. the number of adult bees alive and dead) was determined on day 22. To aid the interpretation of mortality data, other observations as presence of unconsumed food or morphological differences to controls were noted during mortality assessments.

Analytical verification was performed on the highest to lowest test item stock solutions and the control (dilution medium) in samples collected directly after diet preparations on days 3, 4, 5 and 6. The analysis was performed via liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). The analytical method is summarized in Part B, Section 5.

Test conditions were continuously recorded.

4. Calculation of toxicity

For each dose/concentration mean cumulative mortality and corrected were calculated according to Abbott (1925) modified by Schneider-Orelli (1947) were determined for days 3 to 8 (larvae), days 8 to 15 (pupae) as well as for days 3 to 22 (larvae and pupae).

5. Statistics

The Step-down Cochran-Armitage test ($\alpha = 0.05$, one-sided greater) was used to determine the 22-day NOEC/NOED. The ED_x/EC_x values were determined by Weibull analysis.

Statistical calculations were made by using the statistical program ToxRat Professional 3.3.0 (2018).

II. RESULTS AND DISCUSSION

Analytical recovery rates of the active substances in the test item stock solutions ranged between 103 and 117% for fluxapyroxad in each test item treatment group and between 111 and 118% for prothioconazole in each test item treatment group, except for the lowest concentration that was out of range and amounted to 129%. Therefore, biological results were based on nominal concentrations.

Statistically significant differences in adult emergence and cumulative mortality on day 22 compared to the control occurred in two highest treatments (i.e. 1.522 and 4.567 µg product/larva).

No remaining food on day 8 or any other observations indicating sublethal effects were observed in any treatment group.

Results and relevant endpoints are summarized in the following table.

Table A 2.3.1.3-1: Cumulative mortality of larvae exposed to ADM.03503.F.1.A in a chronic toxicity test

Cumulative dosage	Test concentration	On day 8		On day 15		On day 22		
		Mean cumulative mortality [%] Days 3 – 8		Mean cumulative mortality [%] Days 8 – 15		Mean cumulative mortality [%] Days 3 – 22		Adult emergence rate [%]
[µg product/larva]	[mg product/kg food]	abs.	corr.	abs.	corr.	abs.	corr.	abs.
Control								
-	-	0.0	-	8.3	-	19.4	-	80.6
Test item (ADM.03503.F.1.A)								
0.019	0.118	0.0	0.0	8.3	0.0	19.4	0.0	80.6
0.056	0.355	0.0	0.0	11.1	3.0	27.8	10.3	72.2
0.169	1.068	0.0	0.0	13.9	6.1	30.6	13.8	69.4
0.507	3.207	0.0	0.0	16.7	9.1	30.6	13.8	69.4
1.522	9.622	0.0	0.0	22.2	15.2	36.1	20.7	63.9 *
4.567	28.870	0.0	0.0	33.3	27.3	63.9	55.2	36.1 *
Reference item: Dimethoate tech.								
7.6 µg a.s./larva	48 µg a.s./kg food	66.7	66.7	41.7	36.4	97.2	96.6	2.8

22-day endpoints	On day 22 (i.e. 19 days after first application)	
	[µg product/larva/test period]	[mg product/kg food]
ED_{50} (95% CI)	4.827 (2.642 – 13.524)	-
ED_{20} (95% CI)	0.664 (0.312 – 1.142)	-
ED_{10} (95% CI)	0.179 (0.046 – 0.366)	-
NOED	0.507	-
EC_{50} (95% CI)	-	30.520 (16.703 – 85.532)
EC_{20} (95% CI)	-	4.199 (1.971 – 7.218)
EC_{10} (95% CI)	-	1.129 (0.291 – 2.311)
NOEC	-	3.207

* Statistically significantly different compared to control.
corr.: corrected mortality to untreated control according to Schneider-Orelli (1947).
abs.: absolute mortality as counted from the results; negative values were set to zero.
CI: confidence limits.

Validity criteria

The test is considered valid since the cumulative larval mortality on Day 8 was 0% (required ≤ 15%), adult emergence rate in controls was 80.6% in untreated control across all replicates on Day 22 (required ≥ 70%) and cumulative larval mortality in the reference item was 66.7% (required ≥ 50%).

III. CONCLUSION

In this chronic larval toxicity study with ADM.03503.F.1.A, the 22-day ED_{50} was determined to be 4.827 µg product/larva equivalent to a 22-day EC_{50} of 30.520 mg product/kg food. The 22-day NOED was determined at 0.507 µg product/larva, corresponding to a NOEC of 3.207 mg product/kg diet. The validity criteria of the guideline were fulfilled.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

No additional data submitted.

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.5.1 Study 1: Tunnel test in Germany

Comments of zRMS:	The tunnel study was performed in accordance with OECD 75 (2007), OEPP/EPPO No. 170(4) (2010), US EPA OCSPP 850.3040 (2012) guidelines with the following deviations: - The weight of pupae was not assessed to determine any adverse effect - No information on the levels of Varroa is documented. The study was performed in a highly attractive crop (Phacelia tanacetifolia). Applications were conducted in the daytime (morning), during full bee flight and at full flowering of the crop (BBCH 65). Four bee colonies per treatment group were considered (+2 for sampling). Several assessments were carried out to address effects on mortality (non-woven sheets/dead bee traps), foraging activity, abnormal behaviour, the condition of the colonies (colony strength, presence of queen). It also included detailed brood assessments (brood termination rate, brood index and brood compensation index) as well as analysis of residues in flowers, nectar and pollen on the day of application and after treatment. The duration of the study was sufficient to cover one full brood cycle. Each tunnel with an effective Phacelia crop area of 90 m² (18 m x 5 m) correponded to one replicate. This effective area is considered sufficient. The duration of the study covers one full brood cycle. The duration does not cover effects that are likely to occur over a period longer than a single cycle and is not suitable to address effects on over-wintering survival. However, it is generally considered suitable for the scope of the current assessment. Colony sizes before set-up ranged from as low as 5525 to 8825 worker bees. The homogeneity of the colony size is considered within acceptable levels. There was no rainfall immediately prior or within the first day after application. The first rainfall after application was recorded in the night from DAT 2 of DAT 3 and the maximum rainfall per day was 26.8 mm on DAT 3. No significant effects of the reference item were seen on any of the adult parameters, meaning that the study cannot be used to draw conclusions on effects on adult honeybees. However, as significant effects of the reference item were seen at all parameters relevant for effects on honeybee larvae, i.e. number of pupae, brood development and brood termination rate. Therefore, the study is considered acceptable for the evaluation of effects on honeybee larvae, and is used in the risk assessment. Based on the study results it can be concluded that there were no effects on honeybee larvae from ADM.03503.F.1.A at an application rate of 1.25 L/ha in semi-field conditions.

Reference:	KCP 10.3.1.5/01
Report	Study on the Effect of ADM.03503.F.1.A on Honey Bee Colonies (*Apis mellifera* L.) under Semi-Field Conditions in Germany, Persigehl, M., Beinert M., Hotopp I., 2022a, B20F026 (report number), 000107305 (sponsor report number)
Guideline(s):	OECD 75 (2007), OEPP/EPPO No. 170(4) (2010), US EPA OCSPP 850.3040 (2012)
Deviations:	On day 3 and 4, *phacelia* covered the middle path after heavy rain in the night. As consequence, forager mortality assessments could only be carried out on the sheets at the front (position of bee hives) and backside of each assessment tunnel. Flight activity and abnormal behaviour assessments were

	conducted for 60 seconds at the beehive entrance instead. During the exposure phase, it was not possible to collect a few B samples on days 0, 3 and 6 because the bees did not carry enough pollen throughout the day. Due to low quantity of the A sample, an additional pollen sampling was carried out on day 1 which replaced the missing B sample from day 0.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 g/L + prothioconazole 150 g/L EC)
Description	Transparent liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Untreated control: tap water Positive control: reference item
Reference item	Insegar WG
Description	Not stated
Lot/Batch #	SSP9J022
Content of a.s.	250 g fenoxycarb/kg
Stability of reference item	Stable under storage conditions (dry, cool and well ventilated)
3. Test organism	
Species	Honey bee, *Apis mellifera* L (Insecta, Hymenoptera, Apoidea)
Source	Apiary of Dr. Pia Aumeier, Bochum, Germany
Age	All honey bee queens were of the same age and ≤ 2 years old
Set up of colonies:	In total, 16 honey bee colonies were prepared on day -7 and transferred from the apiary to the acclimatisation location. On day -3, the honey bee colonies were transferred to the study field and placed in the tunnels where 14 colonies were prepared with dead-bee traps and two with pollen traps in front of the bee hive entrance. On day -1, the 12 best and suitable colonies were selected and assigned to the treatment groups, the two back up colonies were excluded from the study
Diet	No additional food was provided. A water supply was placed in each tunnel
Test location	Pre-exposure and exposure: Reusrath, Langenfeld, North Rhine-Westphalia, Germany

	Post-exposure: Dierath, Burscheid, North Rhine-Westphalia, Germany
Test units	Tunnels constructed with a tubular steel frame (21 m length × 5.5 m width × 2.5 m height) that was covered with synthetic gauze (2 mm mesh). Each tunnel contained a crop area of 90 m^2 (18 m × 5 m). Non-woven sheets, for collection of dead bees, covered the outermost 50 cm of the front and back ends of the tunnel as well as the path that split down the crop area inside each tunnel.
Test plants	*Phacelia tanacetifolia* at full flowering (BBCH 65)
4. Environmental conditions	The study was performed under semi-field conditions.
Temperature	Pre-exposure and exposure: actual: 14.9 – 28.7 °C Post-exposure: actual: 13.2 – 22.6 °C
Relative humidity	Pre-exposure and exposure: actual: 54.1 – 85.6% air humidity Post-exposure: actual: 65.3 – 100 % air humidity
Max. rainfall per day:	Pre-exposure and exposure: actual: 26.8 mm Post-exposure: actual: 100.2 mm
Wind strength:	Pre-exposure and exposure: actual: mean 0.8 – 2 m/s Post-exposure: actual: mean < 1m/s

B. STUDY DESIGN AND METHODS

1. In-life dates 10th June 2021 to 16th July 2021 (biological phase)

2. Experimental conditions

Test design

The effects of the test substance ADM.03503.F.1.A to honey bees (*Apis mellifera* L.) were assessed under confined semi-field conditions in Germany for 28 days. The test item was applied once at a rate of 1.25 L/ha to flowering *Phacelia tanacetifolia* in tunnel tents via a spray application during bee flight. The reference item, Insegar WG, was applied at a single rate of 4.8 kg/ha and the control group remained untreated. Mortality, foraging activity, behaviour, colony condition and bee brood development were assessed throughout the 28-day study. In addition, samples of flowers, pollen and nectar were collected during the study to evaluate the magnitude of residues of fluxapyroxad and prothioconazole and its metabolite prothioconazole-desthio in these matrices.

Number of colonies per treatment

1 colony/replicate (tunnel); 4 replicates/test treatments, reference substance and control. Additionally, 2 replicates were used for the residue sampling of pollen and nectar during the exposure phase.

Test doses

The toxicity of ADM.03503.F.1.A was determined at a single application rate of 1.25 L/ha corresponding to 93.75 g fluxapyroxad/ha and 187.5 g prothioconazole/ha based on nominal content of active substances in the formulation. A control group treated with tap water was tested in parallel.

Reference item

The reference item, Insegar WG was tested at a rate of 4.8 kg/ha, corresponding to 1200 g fenoxycarb/ha.

Treatment/Application

Treatments were made to the *Phacelia* crop area with a hand-held portable boom sprayer (Schachtner PSG). Prior to application, the sprayer had been calibrated using tap water to ensure the exact amount of 400 L/ha ± 10 % spray solution per tunnel. Following calibration, treatments were applied in the order of control, test item and finally the toxic reference item.

After 7 days of exposure, the colonies were removed from the assessment tunnels in the study field and placed in a remote location. The colonies of the pollen and nectar sampling tunnels were removed from the study field after the exposure phase and were no longer part of the study.

3. Observations and assessments

Forager mortality was assessed daily during the pre-exposure (day -3 to 0) and exposure (day 0 to 7) phases by collecting dead bees from the non-woven sheets. The in-hive mortality was assessed daily from day -2 until test termination (day 28) by collecting dead bees from dead-bee traps in front of each hive. On day 0 (day of application), in-hive mortality was assessed four times, one time directly before the applications and three times after application. On day 7, one additional in-hive mortality assessment was conducted in the late evening before the colonies were translocated to the remote location. Throughout all assessments dead bees were subdivided in adult workers, larvae, pupae and drones.

Foraging activity and behavioural abnormality assessments were conducted, in 10 randomly selected observation areas of approx. 1 m^2 in the tunnels, one time daily throughout the pre-exposure and the exposure phases, with exception of day 3. On day 0, four assessments of foraging activity and behavioural abnormalities were conducted, one before application and three after treatments. Also, on day 1, the foraging activity was assessed three times: once in the morning, once at noon and once in the evening. Since no foraging activity assessment could be carried out on day 3, after a thunderstorm during the night, a flight activity assessment was carried out directly at the entrance instead, by counting the number of incoming bees for 60 seconds. Additionally, any behavioural abnormalities of adult bees returning to the hive and lingering on the hive were recorded that day.

The colony condition was determined on days -1, 9, 21 and 28 using the following parameters: strength of the colony, presence and vitality of the queen or eggs, comb area containing brood in different stages and comb area with pollen and nectar. The strength of the colony was determined by estimation of comb area and inner sides of the hive supers covered with bees under consideration of bee density and by estimation of the number of bees outside the hive at the moment of assessment; the number of incoming bees were counted for 60 seconds immediately before opening the hive. All assessments, except for the counting of the incoming bees, were conducted using the Liebefelder Schätzmethode. The total area of each comb side to be assessed was 8 dm^2 comprising eight imaginary units, each 1 dm^2. One comb side that was fully covered with a single layer of bees was deemed to be equivalent to 1000 bees (corresponding to 125 bees/dm^2). However, when the frames were covered with multiple layers of bees, the number of bees was higher. If only parts of the comb were covered with bees, those were conceptually pushed together into a cluster and their numbers were estimated based on the occupied area. The numbers of worker brood cells and cells used for food storage were calculated under the assumption that one unit contains 400 cells and for drone brood 260 cells.
Additionally, the presence and number of dead bees in the bottom of each hive were assessed, too during the colony condition checks.

The development of honey bee brood was assessed on days -1, 4, 9, 15 and 21 by taking photos of comb sides containing worker brood. After applying the software HoneybeeComplete 6.0, 200 cells containing eggs per colony on the photos made on day -1 (brood fixing day 0) were manually selected, automatically numbered and the position marked for the following assessments. For each subsequent brood assessment, the pre-selected cells were automatically found and manually determined according to different brood stage categories (i.e. empty, egg, young larvae, old larvae, pupae, nectar, and pollen). On this basis, Hon-

eybeeComplete 6.0 calculated the brood termination rate (BTR), the brood index (BI) and the compensation index (CI) for each assessment day and colony.

One retain sample of the test item spray solution was collected after application. Also, *Phacelia* flowers were sampled 1 day before test item application and after test item application on day 0 in the four control and four test item treated tunnels used for the assessments and in the two tunnels intended for sampling. Pollen from the two sampling tunnels was sampled after test item application on days 0, 3 and 6. An additional pollen sample was collected on day -2 from the two sampling tunnels and both samples were pooled. Nectar was sampled from captured honey bee nectar foragers whose honey stomachs were later dissected. Honey bee foragers were sampled from the two sampling tunnels after test item application on days 0, 3 and 6. The analysis was performed via liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). The analytical method is summarized in Part B, Section 5.

Air temperature, air humidity and precipitation were recorded hourly inside and outside of the tunnels at the study field and on the remote location. The wind strength and wind direction were recorded at approx. 2 m height by an anemometer located at the study field or on the remote location. The cloud cover was estimated during flight activity assessments.

4. Calculation of toxicity

The forager mortality and in-hive mortality were analysed separately. Since the data for both assessments was overdispersed, a negative binomial family was used. For the in-hive mortality, different random effects (e.g. colony and the location) were tested previously to finding the best fixed effect formula. For the forager mortality, this step could be skipped as the assessment on the sheets was only carried out in the tunnels. Tested fixed effects included the treatment group in interaction with a variable of the time as day after treatment and different powers of the time to account for the nonlinear temporal development of the mortality and possible nonlinear effects over time. The mortality assessed on day 0 in the morning was attributed to day 0, the other three assessments, as well as the one on day 1 were attributed to day 1 and summed up.
The mortality of dead pupae and larvae in dead bee traps was analysed together as a sum per colony and day after treatment. The data was zero-inflated and therefore, first the fixed effect formula was determined and then a formula for the zero-inflation model. A Poisson family was used in the model. The tested effects included the treatment group in interaction with a variable of the time as day after treatment and different powers of the time to account for the nonlinear temporal development of the mortality and possible nonlinear effects over time.

The count data for colony strength and number of worker brood cells were analysed using statistical models with a negative binomial family. For both parameters, multiple statistical models were tested that included different fixed effects. The fixed effects included the interaction between the treatment and the time. Furthermore, different powers of the time were tested to account for a nonlinear development. For the random effect, the colony was used.

The BTR was analysed using a model with a binomial family. This family can be used to analyse the number of successes (e.g. number of cells with normal developments) and failures (e.g. number of cells with terminated or unexpected development) out of a given number of trials. The BFD, the BFD in second power and the treatment group were used to find the best fixed effect formula. The colony was used as random effects. A further observation level random effect was added to account for overdispersion.

5. Statistics

Statistical calculations were made by using the statistical software R version 4.1.1. The evaluation of the data was performed using Generalized Linear Mixed Models (GLMMs). For each parameter, GLMMs with different model formulas were calculated. Then, the Akaike information criterion (AIC) was used to find the model formula that was the best fit for each parameter

II. RESULTS AND DISCUSSION

Analytical results

No fluxapyroxad, prothioconazole and its metabolite prothioconazole-desthio residues were found in samples from flowers, pollen and nectar collected before test item application. After test item applications, fluxapyroxad, prothioconazole and its metabolite were found in flower samples at the same concentration levels in assessment tunnels and sampling tunnels. Maximum concentrations of fluxapyroxad, prothioconazole and its metabolite in pollen and nectar were detected after application on day 0 and decreased throughout the exposure phase. Therefore, it can be concluded that the test item was applied evenly in all replicates, and honey bees were exposed to the test item.

Detailed analytical results are presented in the following table.

Table A 2.3.1.5.1-1: Concentrations of fluxapyroxad and prothioconazole in flowers, pollen and nectar

Nominal test concentration [L/ha]-replicates	Measured concentration of active substance ± SD [mg a.s./kg]			
	Sampling date (days)	Flowers	Pollen	Nectar
Active substance: fluxapyroxad				
Control	-1	< LOD	n.a.	n.a.
	0	< LOD	n.a.	n.a.
1.25 L/ha -assessment tunnel	-1	< LOD	n.a.	n.a.
	0	33.00 ± 7.35	n.a.	n.a.
1.25 L/ha -sampling tunnel	-1	< LOD	n.a.	n.a.
	0	31.00 ± 5.66	18.50 ± 0.71	0.06 ±0.00
	3	-	0.09 ± 0.01	< LOQ – 0.01
	6	-	0.04 ± 0.00	< LOQ
Active substance: prothioconazole				
Control	-1	< LOD	n.a.	n.a.
	0	< LOD	n.a.	n.a.
1.25 L/ha -assessment tunnel	-1	< LOD	n.a.	n.a.
	0	6.63 ± 2.30	n.a.	n.a.
1.25 L/ha -sampling tunnel	-1	< LOD	n.a.	n.a.
	0	7.25 ± 0.35	31.50 ± 2.12	0.07 ± 0.01
	3	-	0.09 ± 0.04	< LOD
	6	-	0.03 ± 0.01	< LOD
Metabolite: prothioconazole-desthio				
Control	-1	< LOD – < LOQ	n.a.	n.a.
	0	< LOQ	n.a.	n.a.
1.25 L/ha -assessment tunnel	-1	< LOD – < LOQ	n.a.	n.a.
	0	6.83 ± 1.87	n.a.	n.a.
1.25 L/ha -sampling tunnel	-1	< LOD – < LOQ	n.a.	n.a.
	0	6.15 ± 0.78	4.90 ± 0.28	0.03 ± 0.00
	3	n.a.	0.16 ± 0.01	< LOQ
	6	n.a.	0.09 ± 0.01	< LOQ

n.a.: not applicable.
LOQ = limit of quantification of 0.01 mg/kg.
LOD = limit of detection of 0.003 mg/kg.

Mortality:

The GLMM model revealed no statistically detectable treatment effect by the test item on the forager and in-hive survival (adults, larvae and pupae) with and without time interaction. Furthermore, the mortality of drones in the test item treatment groups was low throughout the experiment and in a similar range as for the control group.

For the reference group, the mortality of adult forager bees and in-hive bees was not detectably increased as the reference item, fenoxycarb, acts as an insect growth regulator, inhibiting the larval metamorphosis to the adult stage (imago) and disrupting the moult of early larvae stages. A significant difference in the in-hive mortality of the larvae and pupae was determined for the reference group when compared to the control.

Results on mortality are summarized in the following table.

Table A 2.3.1.5.1-2: Summary of forager and in-hive mortality

Treatment group	Maximum of daily means ± SD			Min./Max. range		
	Pre-exposure	Exposure	Post-exposure	Pre-exposure	Exposure	Post-exposure
Forager mortality (n° of workers)						
Control	34.8 ± 22.8	36.8 ± 24.4	n.a.	3 – 60	2 – 47	n.a.
Test item	37.0 ± 23.8	49.5 ± 20.1	n.a.	8 – 54	4 – 54	n.a.
Reference item	34.0 ± 17.8	42.8 ± 22.1	n.a.	2 – 58	20 – 84	n.a.
In-hive mortality – adult worker bees (n° of workers)						
Control	5.0 ± 3.6	5.8 ± 4.5	10.0 ± 6.4	1 – 14	0 – 12	0 – 19
Test item	6.0 ± 2.9	9.5 ± 3.8	7.8 ± 3.0	0 – 10	0 – 14	0 – 12
Reference item	8.0 ± 1.8	13.8 ± 9.8	20.3 ± 10.6	0 – 10	0 – 25	1 – 32
In-hive mortality – bee brood (n° of pupae)						
Control	0.5 ± 1.0	2.0 ± 1.6	1.8 ±1.3	0 – 2	0 – 4	0 – 5
Test item	0.2 ±0.5	2.5 ± 3.8	1.5 ± 1.3	0 – 1	0 – 8	0 – 4
Reference item	0.2 ± 0.5	4.5 ± 2.9	123.5 ± 31.9	0 – 1	0 – 11	0 – 195

n.a.: not applicable.

Foraging activity:

Throughout the experiment, the mean foraging activity of worker bees in the test item group was comparable or slightly higher to that of the colonies of the control group. Therefore, it can be concluded that the test item application or the weather conditions did not alter honey bee foraging activity and the colonies were exposed throughout the exposure phase to the treatments in the tunnel.

Results on foraging activity are summarized in the following table.

Table A 2.3.1.5.1-3: Summary of the foraging activity during the pre-exposure and exposure phases

Treatment group	Mean number of forager bees/m^2 ± SD													
			0 d					1 d						
	-2 d	-1 d	BA	1h AA	2 h AA	4 h AA	6 h AA	M	N	E	2 d	4 d	5 d	6 d	7 d
Control	9.3 ± 3.8	13.2 ± 5.6	12.5 ± 3.6	8.1 ± 3.4	14.1 ± 4.9	13.4 ± 4.9	10.9 ± 5.0	9.6 ± 3.7	11.3 ± 4.4	18.4 ± 5.1	17.3 ± 5.7	4.1 ± 1.8	7.8 ± 2.8	14.1 ± 4.8	17.2 ± 4.9
Test item	13.4 ± 3.1	13.2 ± 4.1	13.0 ± 3.1	7.2 ± 2.6	13.0 ± 2.6	10.9 ± 3.8	14.9 ± 2.3	19.2 ± 4.3	25.2 ± 4.6	22.6 ± 4.4	20.6 ± 5.0	4.7 ± 1.9	6.4 ± 2.1	12.1 ± 3.9	19.1 ± 3.9
Reference item	9.5 ± 3.5	13.6 ± 4.5	12.6 ± 4.9	7.6 ± 2.5	15.5 ± 6.0	9.5 ± 4.0	7.3 ± 3.5	16.6 ± 4.5	21.9 ± 5.9	20.7 ± 5.9	20.2 ± 4.3	2.2 ± 1.4	6.5 ± 2.2	9.4 ± 2.5	15.5 ± 3.8

AA = after treatment.
BT = before treatment.
E= evening.
M = morning.
N = noon.

Behavioural abnormalities:

No behavioural abnormalities of adult bees were observed during the foraging activity assessments or during the behavioural assessments throughout the complete exposure phase.

Condition of the colonies:

In all colonies, the initial bee queens were found regularly during colony assessments. If a queen was not found, her presence could always be verified by freshly laid eggs.

The GLMM model revealed no significant overall difference on the colony strength and brood development between the test item group and the control, with and without time interaction. A significant difference on the colony strength and brood development was determined in the reference group when compared with the control, with and without time interaction.

Results on colony strength and brood development are summarized in the following table.

Table A 2.3.1.5.1-4: Summary of the colony strength and brood development during the pre-exposure and post-exposure phases

Treatment group	Mean ± SD			
	-1 d	9 d	21 d	28 d
Number of worker bees				
Control	7912.5 ± 926.8	11721.9 ± 1664.6	13106.2 ± 1910.7	16834.4 ± 2991.5
Test item	8137.5 ± 601.0	10131.2 ± 2625.6	12309.4 ± 2192.7	14812.5 ± 2972.9
Reference item	7437.5 ± 1389.6	12153.1 ± 1120.3	9543.8 ± 793.8	10981.2 ± 1600.5
Number of cells with bee brood				
Control	19450 ± 3616.2	22700 ± 1311.5	28550 ± 1900.0	31200 ± 3898.7
Test item	19000 ± 4690.4	18750 ± 3205.7	24250 ± 2690.1	26550 ± 3304.0
Reference item	17900 ± 2754.4	12450 ± 2909.2	18300 ± 3000.0	22250 ± 3634.6

Brood termination rate (BTR), brood index (BI) and compensation index (CI):

There was no visible effect of the test item application on the BTR compared to the control, whereas the reference group showed a visible and significantly higher BTR at BFD22 and throughout the whole assessment period.

The mean BI and CI of the test item group was visible comparable to the control following the application, whereas the reference group lead to visible and significantly lower BI as most brood cells were terminated and replaced. Also, the low and visible and significant lower CI in the reference group is caused by the reference item mode of action.

Results on BI and CI are summarized in the following table.

Table A 2.3.1.5.1-5: Summary of the Brood index (BI) and Compensation index (CI)

Treatment group	Mean ± SD			
	BFD5 (4 d)	BFD10 (9 d)	BFD16 (15 d)	BFD22 (21 d)
Brood termination rate				
Control	6.75 ± 3.23	11.50 ± 6.96	12.25 ± 6.38	12.25 ± 6.38
Test item	6.75 ± 1.19	10.25 ± 3.30	10.38 ± 3.50	10.38 ± 3.50
Reference item	81.25 ± 8.25	86.63 ± 4.75	88.25 ± 4.05	88.38 ± 4.29
Brood index				
Control	2.76 ± 0.14	3.54 ± 0.28	3.51 ± 0.26	4.39 ± 0.32
Test item	2.77 ± 0.05	3.59 ± 0.13	3.59 ± 0.14	4.48 ± 0.17
Reference item	0.49 ± 0.20	0.54 ± 0.19	0.47 ± 0.16	0.59 ± 0.20
Compensation index				
Control	2.77 ± 0.14	3.59 ± 0.27	3.60 ± 0.18	4.63 ± 0.17
Test item	2.80 ± 0.03	3.62 ± 0.10	3.62 ± 0.11	4.63 ± 0.15
Reference item	0.72 ± 0.41	1.16 ± 0.95	1.84 ± 1.06	2.75 ± 1.03

Validity criteria

The test is considered valid since the mortality in the control group was not considerable and effects in the colonies exposed to the reference item were comparatively high.

III. CONCLUSION

In this semi-field study, the residue data collected throughout the exposure phase and the reported foraging activity proved a chronic exposure to the test item ADM.03503.F.1.A for the duration of the exposure phase. No effects on the mortality of adult honey bees and on the colony development from the application of the test item were detected. Throughout all experimental phases, the development of the colony strength and the BTR in the test item group was comparable to the control, indicating no short- or long-term effect of ADM.03503.F.1.A on the development of the colony strength and the numbers or composition of worker bee brood. The results for the reference group, together with additionally recorded parameters such as the analytical results show that the test system provided adequate exposure and sensitivity.

A 2.3.1.5.2 Study 2: Tunnel test in Spain

Comments of zRMS:	The tunnel study was performed according to relevant guidelines OECD 75 (2007), OEPP/EPPO No. 170(4) (2010), US EPA OCSPP 850.3040 (2012) with following deviation: - The weight of pupae was not assessed to determine any adverse effect - No information on the levels of Varroa is documented. The study is GLP-compliant and performed in a highly attractive crop (Phacelia tanacetifolia). Applications were conducted in the daytime (morning), during full bee flight and close to full flowering of the crop (BBCH 63-64). Four bee colonies per treatment group were considered (+2 for sampling). Several assessments were carried out to address effects on mortality (non-woven sheets/dead bee traps), foraging activity, abnormal behaviour, the condition of the colonies (colony strength, presence of queen). It also included detailed brood assessments (brood termination rate, brood index and brood compensation index) as well as analysis of residues in flowers, nectar and pollen on the day of applica-tion and after treatment. The duration of the study was sufficient to cover one full brood cycle. The selection of the reference item (fenoxycarb) is fitting to the scope of the study. The results on this treatment group show that the test system provided adequate exposure and sensitivity. Each tunnel with an effective Phacelia crop area of 105 m² (21 m x 5 m) corre-sponded to one replicate. This effective area is considered sufficient. The dura-tion of the study covers one full brood cycle. The duration does not cover effects that are likely to occur over a period longer than a single cycle and is not suitable to address effects on over-wintering survival. However, it is generally considered suitable for the scope of the current assessment. Mean colony sizes ranged from as low as 6070 to 6500 worker bees, before appli-cation. The homogeneity of the colony size is considered within acceptable lev-els. There was no rainfall immediately prior or within the first day after applica-tion. The first rainfall after application and also the maximum rainfall per day was recorded on DAT 2 with 16.4 mm. This precipitation event is not expected to have compromised the exposure of bees to the test item. No significant effects of the reference item were seen on any of the adult parameters, meaning that the study cannot be used to draw conclusions on effects on adult honeybees. However, as significant effects of the reference item were seen at all parameters relevant for effects on honeybee larvae, i.e. number of pupae, brood development and brood termination rate. Therefore, the study is considered acceptable for the evaluation of effects on honeybee larvae, and is used in the risk assessment. Overall, there were no effects on honeybee larvae from ADM.03503.F.1.A at an application rate of 1.25 L/ha in semi-field conditions.

Reference:	KCP 10.3.1.5/02
Report	Study on the Effect of ADM.03503.F.1.A on Honey Bee Colonies (*Apis mellifera* L.) under Semi-Field Conditions in Spain, Persigehl, M., Beinert M., Hotopp I., 2022b, B20F027 (report number), 000107306 (sponsor report number)
Guideline(s):	OECD 75 (2007), OEPP/EPPO No. 170(4) (2010), US EPA OCSPP 850.3040 (2012)
Deviations:	During the post-exposure phase, the data logger was set incorrectly and therefore, only the actual air temperature and humidity values were recorded hourly, but no minimum and maximum values are available as stated in OECD 75 (2007)
GLP:	Yes

Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 g/L + prothioconazole 150 g/L EC)
Description	Transparent liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/mL (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Untreated control: tap water Positive control: reference item
Reference item	Insegar WG
Description	Not stated
Lot/Batch #	SSP9J022
Content of a.s.	250 g fenoxycarb/kg
Stability of reference item	Stable under storage conditions (dry, cool and well ventilated)
3. Test organism	
Species	Honey bee, *Apis mellifera* L (Insecta, Hymenoptera, Apoidea)
Source	Apiary of Antonio Escrivà Moreno, Montroy, Valencia, Spain
Age	All honey bee queens were of the same age and ≤ 2 years old
Set up of colonies:	In total, 20 honey bee colonies were transferred 14 days before application (day -14) from the apiary to the acclimatisation location. On day -4, the honey bee colonies were transferred to the study field and placed in the tunnels (acclimatisation period). 12 colonies were placed in the assessment tunnels equipped with dead-bee traps in front of the bee hive entrance and two colonies were placed in the sampling tunnels with pollen traps in front of the bee hive entrance. On day -1, the 12 assessment colonies were assigned to the treatment groups. The remaining two tunnels, which served as backup, were excluded from the study.
Diet	No additional food was provided. A water supply was placed in each tunnel
Test location	Pre-exposure and exposure: Lliria, Valencia, Spain Post-exposure: Turis, Valencia, Spain
Test units	Tunnels constructed with a tubular steel frame (24 m length × 5.5 m width × 3.5 m height) that was covered with synthetic gauze (2 – 3 mm mesh). Each tunnel contained a crop area of 105 m^2 (21 m × 5 m). Non-woven sheets, for collection of dead bees, covered the outermost 50 cm of the front and back ends of the tunnel as well as the path that split down the crop area inside each tunnel.
Test plants	*Phacelia tanacetifolia* at full flowering (BBCH 63 – 64)

4. Environmental conditions	The study was performed under semi-field conditions.
Temperature	Pre-exposure and exposure: actual: 14.6 – 20.5 °C Post-exposure: actual: 8.2 – 25.5 °C
Relative humidity	Pre-exposure and exposure: actual: 63.2 – 93.1% air humidity Post-exposure: actual: 46.2 – 92.9 % air humidity
Max. rainfall per day:	Pre-exposure and exposure: actual: 16.4 mm Post-exposure: actual: 12 mm
Wind strength:	Pre-exposure and exposure: actual: mean 0 – 2 m/s Post-exposure: actual: mean 0 – 1 m/s

B. STUDY DESIGN AND METHODS

1. In-life dates 07th May 2021 to 18th June 2021 (biological phase)

2. Experimental conditions

Test design

The effects of the test substance ADM.03503.F.1.A to honey bees (*Apis mellifera* L.) were assessed under confined semi-field conditions in Spain for 28 days. The test item was applied once at 1.25 L/ha to flowering *Phacelia tanacetifolia* in tunnel tents via a spray application during bee flight. The reference item, Insegar WG, was applied at a single rate of 4.8 kg/ha and the control group remained untreated. Mortality, foraging activity, behaviour, colony condition and bee brood development were assessed throughout the 28-day study. In addition, samples of flowers, pollen and nectar for residue analysis were collected during the study to evaluate the magnitude of residues of fluxapyroxad and prothioconazole and its metabolite prothioconazole-desthio in these matrices.

Number of colonies per treatment

1 colony/replicate (tunnel); 4 replicates/test treatments, reference substance and control. Additionally, 2 replicates were used for the residue sampling of pollen and nectar during the exposure phase

Test doses

The toxicity of ADM.03503.F.1.A was determined at a single application rate of 1.25L/ha corresponding to 93.75 g fluxapyroxad/ha and 187.5 g prothioconazole/ha based on nominal content of active substances in the formulation. A control group treated with tap water was tested in parallel.

Reference item

The reference item, Insegar WG was tested at a rate of 4.8 kg/ha, corresponding to 1200 g fenoxycarb/ha.

Treatment/Application

Treatments were made to the *Phacelia* crop area with a hand-held portable boom. Prior to application, the sprayer had been calibrated using tap water to ensure the exact amount of 400 L/ha ± 10 % spray solution per tunnel. Following calibration, treatments were applied in the order of control, test item and finally the toxic reference item.

After 10 days of exposure, the colonies were removed from the assessment tunnels in the study field and placed in a remote location. The colonies of the pollen and nectar sampling tunnels were removed from the study field after the exposure phase and were no longer part of the study.

3. Observations and assessments

Forager mortality was assessed daily during the pre-exposure (day -3 to 0) and exposure (day 0 to 10) phases by collecting dead bees from the non-woven sheets. The in-hive mortality was assessed daily from day -3 until test termination (day 28) by collecting dead bees from dead-bee traps in front of each hive. On day 0 (day of application), in-hive mortality was assessed four times, one time directly before the applications and three times after the application. On day 10, one additional in-hive mortality assessment was conducted in the late evening before the colonies were translocated to the remote location. Throughout all assessments dead bees were subdivided in adult workers, larvae, pupae and drones.

Foraging activity and behavioural abnormality assessments were conducted, in 10 randomly selected observation areas of approx. 1 m^2 in the tunnels, one time daily throughout the pre-exposure and the exposure phases. On day 0, five assessments of foraging activity and behavioural abnormalities were conducted, one before application and four after treatments. Also, on day 1 the foraging activity was assessed three times: once in the morning, once at noon and once in the evening.

The colony condition was determined on days -1, 9, 21 and 26 using the following parameters: strength of the colony, presence and vitality of the queen or eggs, comb area containing brood in different stages and comb area with pollen and nectar. The strength of the colony was determined by estimation of comb area and inner sides of the hive supers covered with bees under consideration of bee density and by estimation of the number of bees outside the hive at the moment of assessment; the number of incoming bees were counted for 60 seconds immediately before opening the hive. All assessments, except for the counting of the incoming bees, were conducted using the Liebefelder Schätzmethode. The total area of each comb side to be assessed was 10.8 dm^2 comprising nine imaginary units, each 1.2 dm^2. One comb side that was fully covered with a single layer of bees was deemed to be equivalent to 1350 bees (corresponding to 125 bees/dm^2). However, when the frames were covered with multiple layers of bees, the number of bees was higher. If only parts of the comb were covered with bees, those were conceptually pushed together into a cluster and their numbers were estimated based on the occupied area. The numbers of worker brood cells and cells used for food storage were calculated under the assumption that 1 dm^2 contains 380 brood cells and for drone brood 260 cells. The presence of the queen and eggs were also recorded as indicators of the queen's health and hence, the colonies' vitality.
Additionally, the presence and number of dead bees in the bottom of each hive were assessed too during the colony condition checks.

The development of honey bee brood was assessed on days -1, 4, 9, 14 and 21 by taking photos of comb sides containing worker brood. After applying the software HoneybeeComplete 6.0, 200 cells containing eggs per colony on the photos made on day -1 (brood fixing day 0) were manually selected, automatically numbered and the position marked for the following assessments. For each subsequent brood assessment, the pre-selected cells were automatically found and manually determined according to different brood stage categories (i.e. empty, egg, young larvae, old larvae, pupae, nectar, and pollen). On this basis, HoneybeeComplete 6.0 calculated the brood termination rate (BTR), the brood index (BI) and the compensation index (CI) for each assessment day and colony.

One retain sample of the test item spray solution was collected after application. Also, *Phacelia* flowers were sampled 1 day before test item application and after test item application on day 0 in the four control and four test item treated tunnels used for the assessments and in the two tunnels intended for sampling. Pollen from the two sampling tunnels was sampled after test item application on days 0, 3 and 7. Nectar was sampled from captured honey bee nectar foragers whose honey stomachs were later dissected. Honey bee foragers were sampled from the two sampling tunnels after test item application on days 0, 3 and 7. The analysis was performed via liquid chromatography coupled to a tandem mass spectrometer (LC-MS/MS). The analytical method is summarized in Part B, Section 5.

Air temperature and air humidity were recorded hourly inside and outside of the tunnels at the study field and on the remote location. Precipitation was recorded hourly inside and outside of the tunnels at the field

location and once per day with a manual rain gauge during pre-exposure. The wind strength and wind direction were recorded hourly by an anemometer. During the post-exposure phase, the wind strength and wind direction were obtained from the nearest weather station in Godelleta. The cloud cover was estimated during flight activity assessments.

4. Calculation of toxicity

The forager mortality and in-hive mortality were analysed separately. Since the data for both assessments was overdispersed, a negative binomial family was used. For the in-hive mortality, different random effects (e.g. colony and the location) were tested previously to finding the best fixed effect formula. For the forager mortality, this step could be skipped as the assessment on the sheets was only carried out in the tunnels. Tested fixed effects included the treatment group in interaction with a variable of the time as day after treatment and different powers of the time to account for the nonlinear temporal development of the mortality and possible nonlinear effects over time. The mortality assessed on day 0 in the morning was attributed to day 0, the other three assessments, as well as the one on day 1 were attributed to day 1 and summed up.
The mortality of dead pupae and larvae in dead bee traps was analysed together as a sum per colony and day after treatment. The data of the post-exposure phase was analysed using a Poisson-GLMM with was zero inflation. The fixed effect included only the treatment group and the random effect consisted of the colony as random intercept.

The count data for colony strength and number of worker brood cells were analysed using statistical models with a negative binomial family. For both parameters, multiple statistical models were tested that included different fixed effects. The fixed effects included the interaction between the treatment and the time. Furthermore, different powers of the time were tested to account for a nonlinear development. For the random effect, the colony was used.

The BTR was analysed using a model with a binomial family. This family can be used to analyse the number of successes (e.g. number of cells with normal developments) and failures (e.g. number of cells with terminated or unexpected development) out of a given number of trials. The BFD, the BFD in second power and the treatment group were used to find the best fixed effect formula. The colony was used as random effects. A further observation level random effect was added to account for overdispersion.

5. Statistics

Statistical calculations were made by using the statistical software R version 4.1.1. The evaluation of the data was performed using Generalized Linear Mixed Models (GLMMs). For each parameter, GLMMs with different model formulas were calculated. Then, the Akaike information criterion (AIC) was used to find the model formula that was the best fit of each parameter

II. RESULTS AND DISCUSSION

Analytical results

No fluxapyroxad, prothioconazole and its metabolite prothioconazole-desthio residues were found in samples from flowers, pollen and nectar collected before test item application. After test item applications, fluxapyroxad, prothioconazole and its metabolite were found in flower samples at the same concentration levels in assessment tunnels and sampling tunnels. Maximum concentrations of fluxapyroxad, prothioconazole and its metabolite in pollen and nectar were detected after application on day 0 and decreased throughout the exposure phase. Therefore, it can be concluded that the test item was applied evenly in all replicates and honey bees were exposed to the test item.

Detailed analytical results are presented in the following table.

Table A 2.3.1.5.2-1: Concentrations of fluxapyroxad and prothioconazole in flowers, pollen and nectar

Nominal test concentration [L/ha]-replicates	Measured concentration of active substance ± SD [mg a.s./kg]			
	Sampling date (days)	Flowers	Pollen	Nectar
Active substance: fluxapyroxad				
Control	-1	< LOD	n.a.	n.a.
	0	< LOD	n.a.	n.a.
1.25 L/ha -assessment tunnel	-1	< LOD	n.a.	n.a.
	0	15.25 ± 1.71	n.a.	n.a.
1.25 L/ha -sampling tunnel	-1	< LOD	n.a.	n.a.
	0	15.0 ± 1.41	27.50 ± 6.36	0.10 ± 0.03
	3	-	0.38 ± 0.02	0.02 ± 0.00
	7	-	0.26 ± 0.01	< LOD
Active substance: prothioconazole				
Control	-1	< LOD	n.a.	n.a.
	0	< LOD	n.a.	n.a.
1.25 L/ha -assessment tunnel	-1	< LOD	n.a.	n.a.
	0	3.58 ± 0.38	n.a.	n.a.
1.25 L/ha -sampling tunnel	-1	< LOD	n.a.	n.a.
	0	4.30 ± 0.57	62.50 ± 12.02	0.12 ± 0.03
	3	n.a.	0.10 ± 0.02	< LOD
	7	n.a.	0.03 ± 0.00	< LOD
Metabolite: prothioconazole-desthio				
Control	-1	< LOD	n.a.	n.a.
	0	< LOD	n.a.	n.a.
1.25 L/ha -assessment tunnel	-1	< LOD	n.a.	n.a.
	0	4.80 ± 0.59	n.a.	n.a.
1.25 L/ha -sampling tunnel	-1	< LOD	n.a.	n.a.
	0	5.70 ± 0.85	6.45 ± 1.77	0.06 ± 0.01
	3	n.a.	0.55 ± 0.06	0.02 ± 0.00
	7	n.a.	0.09 ± 0.01	< LOD

n.a.: not applicable.
LOD = limit of detection of 0.003 mg/kg.

Mortality:

The GLMM model revealed no statistically detectable treatment effect by the test item on foragers or in-hive survival (adults, larvae and pupae) compared to the control, with and without time interaction. Furthermore, the mortality of drones in the test item treatment groups was low throughout the experiment and in a similar range as for the control group.

For the reference group, mortality of adult forager bees and in-hive bees was not detectably increased as fenoxycarb acts as an insect growth regulator, inhibiting the larval metamorphosis to the adult stage (imago) and disrupting the moult of early larvae stages. A significant difference in the in-hive mortality of the larvae and pupae was determined for the reference group when compared to the control.

Results on mortality are summarized in the following table.

Table A 2.3.1.5.2-2: Summary of forager and in-hive mortality

Treatment group	Maximum of daily means ± SD			Min./Max. range		
	Pre-exposure	Exposure	Post-exposure	Pre-exposure	Exposure	Post-exposure
Forager mortality (n° of workers)						
Control	39.5 ± 34.8	91.5 ± 83.1	n.a.	1 – 85	0 – 175	n.a.
Test item	33.0 ± 10.2	68.8 ± 105.3	n.a.	3 – 41	1 – 226	n.a.
Reference item	41.0 ± 38.9	51.2 ± 64.8	n.a.	0 – 97	0 – 146	n.a.
In-hive mortality – adult worker bees (n° of workers)						
Control	5.2 ± 8.5	8.0 ± 7.5	20.0 ± 14.2	0 – 18	0 – 15	0 – 41
Test item	7.8 ± 4.6	26.5 ± 18.4	28.2 ± 16.3	0 – 14	0 – 54	0 – 27
Reference item	8.0 ± 3.7	14.0 ± 12.6	26.5 ± 18.0	0 – 41	0 – 49	0 – 49
In-hive mortality – bee brood (n° of pupae)						
Control	0.0 ± 0.0	0.2 ± 0.5	0.2 ± 0.5	0 – 0	0 – 1	0 – 1
Test item	0.2 ± 0.5	0.2 ± 0.5	0.2 ± 0.5	0 – 1	0 – 1	0 – 1
Reference item	0.0 ± 0.0	7.2 ± 6.8	21.5 ± 13.9	0 – 1	0 – 16	0 – 53

n.a.: not applicable.

Foraging activity:

Throughout the experiment the mean foraging activity of worker bees in the test item group was comparable to colonies of the control group. Therefore, it can be concluded that the test item application or the weather conditions did not alter honey bee foraging activity and the colonies were exposed throughout the exposure phase to the treatments in the tunnel.

Results on foraging activity are summarized in the following table.

Table A 2.3.1.5.2-3: Summary of the foraging activity during the pre-exposure and exposure phases

Treatment group	Mean number of forager bees/m^2 ± SD										
	Pre- exposure phase			Exposure phase							
				0 d					1 d		
	-3 d	- 2 d	- 1 d	BA	1h AA	2 h AA	4 h AA	6 h AA	M	N	E
Control	6.0 ± 3.7	6.0 ± 2.8	13.7 ± 2.4	17.4 ±3.4	19.8 ±3.5	19.7 ± 2.7	20.0 ± 2.4	18.9 ± 2.0	10.3 ± 4.9	21.3 ± 4.5	7.3 ± 2.5
Test item	9.0 ± 4.3	7.9 ± 3.2	16.8 ± 4.8	21.4 ±3.4	22.6 ± 3.5	21.8 ± 3.1	20.0 ± 2.7	18.6 ± 1.7	13.1 ± 5.2	21.6 ± 3.3	7.4 ± 2.4
Reference item	7.8 ± 5.1	4.3 ± 1.6	15.5 ± 6.7	19.2 ± 4.4	19.7 ± 3.6	21.2 ± 5.4	16.9 ± 5.1	4.0 ± 2.3	9.1 ± 4.8	18.4 ± 5.0	6.7 ± 2.0
Treatment group	**Exposure phase**										
	2 d	**3 d**	**4 d**	**5 d**	**6 d**	**7 d**	**8 d**	**9 d**	**10 d**		
Control	0.4 ± 0.7	19.5 ± 5.1	19.1 ± 4.1	10.6 ± 3.7	16.4 ± 3.6	7.9 ± 2.9	18.6 ± 5.4	12.9 ± 3.2	15.4 ± 2.5		
Test item	0.6 ± 0.9	18.4 ± 4.9	19.2 ± 4.4	11.6 ± 3.8	14.1 ± 3.2	8.5 ± 2.7	15.5 ± 6.0	13.5 ± 3.0	14.8 ± 4.6		
Reference item	0.3 ± 0.6	17.6 ± 5.3	18.1 ± 3.4	11.4 ± 4.0	15.9 ± 3.4	6.9 ± 1.9	15.0 ± 4.2	14.6 ± 2.8	14.8 ± 3.8		

AA = after treatment.
BT = before treatment.
E= evening.
M = morning.
N = noon.

Behavioural abnormalities:

No behavioural abnormalities of adult bees were observed during the foraging activity assessments or during the behavioural assessments throughout the complete exposure phase.

Condition of the colonies:

In all colonies from the test item and control groups, the initial bee queens were found regularly during colony assessments. If a queen was not found, her presence could always be verified by freshly laid eggs. In the reference item group, one replicate lost their queen during the exposure phase. In this replicate,

neither the initial bee queen nor eggs were found during the colony assessment at the end of the exposure and post-exposure phases.

The GLMM model revealed no significant overall difference on the colony strength between the test item group and the control without time interaction. The model indicates a statistically significant difference on colony strength and colony development colonies in interaction with time compared to the control colonies. Thus, the development of the test item colonies over time differed from the one of the control colonies. However, the significant difference between test item group and control was caused by a higher decrease of the colony strength in the control colonies compared to the test item colonies during the post-exposure phase. Therefore, a treatment related effect of the test item is not indicated.

The GLMM model revealed no significant overall difference on brood development between the test item group and the control, with and without time interaction. No significant difference on the colony strength was determined in the reference group when compared with the control, with and without time interaction. A significant difference on brood development was determined in the reference group when compared with the control, with and without time interaction.

Results on colony strength and brood development are summarized in the following table.

Table A 2.3.1.5.2-4: Summary of the colony strength and brood development during the pre-exposure and post-exposure phases

Treatment group	Mean ± SD			
	-1 d	**9 d**	**21 d**	**26 d**
Number of worker bees				
Control	6070.0 ± 921.9	6512.5 ± 1132.9	5132.5 ± 949.1	4942.5 ± 1252.2
Test item	6468.8 ± 1183.5	6312.5 ± 1124.4	6576.2 ± 1278.5	6412.5 ± 1077.0
Reference item	6302.5 ± 326.0	6291.2 ± 749.5	5048.8 ± 1646.9	4800.0 ± 1377.1
Number of cells with bee brood				
Control	11115.0 ± 4295.2	7501.2 ± 1592.1	8743.8 ± 4577.8	10545.0 ± 5082.5
Test item	11536.8 ± 851.5	6338.4 ± 3005.2	9975.0 ± 2596.8	13064.4 ± 2077.5
Reference item	11673.6 ± 1881.2	2713.2 ± 810.2	6714.6 ± 3676.5	9484.8 ± 5104.1

Brood termination rate (BTR), brood index (BI) and compensation index (CI):

There was no visible effect of the test item application on the BTR compared to the control, whereas the reference group showed a visible and significantly higher brood termination at BFD22 and throughout the whole assessment period.

The mean BI and CI of the test item group was visible comparable to the control following the application, whereas the reference group lead to visible and significantly lower BI as most brood cells were terminated and replaced. Also, the low and visible and significantly lower CI in the reference group is caused by the reference item mode of action.

Results on BI and CI are summarized in the following table.

Table A 2.3.1.5.2-5: Summary of the brood termination rate (BTR), brood index (BI) and compensation index (CI)

Treatment group	Mean ± SD			
	BFD5 (4 d)	BFD10 (9 d)	BFD16 (15 d)	BFD22 (21 d)
Brood termination rate				
Control	5.38 ± 3.86	8.63 ± 2.36	11.50 ± 3.03	11.50 ±3.03
Test item	7.0 ±4.43	11.13 ± 5.81	12.0 ± 5.94	12.13 ± 5.84
Reference item	84.38 ± 28.92	93.00 ± 14.00	95.13 ± 9.75	95.25 ± 9.50
Brood index				
Control	2.82 ± 0.10	3.66 ± 0.09	3.54 ± 0.12	4.43 ± 0.015
Test item	2.71 ± 0.12	3.56 ± 0.23	3.52 ± 0.24	4.39 ± 0.29
Reference item	0.39 ± 0.74	0.28 ± 0.56	0.20 ± 0.39	0.24 ± 0.49
Compensation index				
Control	2.82 ± 0.10	3.66 ± 0.99	3.54 ±0.12	4.51 ± 0.17
Test item	2.73 ± 0.11	3.58 ± 0.23	3.56 ± 0.21	4.56 ± 0.22
Reference item	0.44 ± 0.74	0.31 ± 0.57	0.53 ± 0.46	1.60 ± 0.61

Validity criteria

The test is considered valid since the mortality in the control group was not considerable and effects in the colonies exposed to the reference item were comparatively high.

III. CONCLUSION

In this semi-field study, the residue data collected throughout the exposure phase and the reported foraging activity proved a chronic exposure to the test item ADM.03503.F.1.A for the duration of the exposure phase. No effects on the mortality of adult honey bees and on the colony development from the application of the test item were detected. During the exposure phase, the colony strength in the test item group was comparable to the control group. However, during the post-exposure phase, colonies of the test item treated group showed elevated increase in colony size and the colony strength of the colonies in the control group decreased, which led to a significant difference in colony strength between the test item group and the control group during the post-exposure phase. This statistically significant difference was caused by a higher colony strength in the test item group and does not indicate a treatment related effect. Throughout all experimental phases, the BTR in the test item group was comparable to the control, indicating no short- or long-term effect of ADM.03503.F.1.A on the numbers or composition of worker bee brood. The results for the reference group, together with additionally recorded parameters such as the analytical results show that the test system provided adequate exposure and sensitivity.

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

No additional data submitted.

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing

A 2.3.2.1.1 Study 1: Standard laboratory test with *Aphidius rhopalosiphi*

Comments of zRMS:	The study was conducted in line with Mead-Briggs et al. (2000)with no deviation. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: 48-hour LR_{50} = 0.954 L product/ha 48 h ER_{50} > 0.843 L product/ha

Reference:	KCP 10.3.2.1/01
Report	Effects of ADM.03503.F.1.A on the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) in a laboratory test, Röhlig, U., 2020a, 20 48 NAL 0004 (report number), 000105076 (sponsor report number)
Guideline(s):	Mead-Briggs *et al.* 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle control: 200 L/ha purified water Positive control: reference substance with 0.30 mL product per 200 L water/ha
Reference item	DANADIM PROGRESS
Description	EC (emulsifiable concentrate)
Lot/Batch #	10214034
Purity	400.0 g/L dimethoate (nominal content) 411.2 g/L dimethoate (analysed content)

Stability of reference item	Expiry date: 6th September2021

3. Test organism

Species	Parasitoid wasp, *Aphidius rhopalosiphi* (Destefani-Perez) (Hymenoptera: Braconidae)
Source	Purchased from Katz Biotech AG, Baruth, Germany (in the stage of mummies).
Age	Adult, within 48 h of their emergence
Acclimation period	Parasitised aphid mummies of a uniform age were placed in glass bottles for hatching.
Diet	During the acclimation period, a cotton wool pad, soaked with aqueous fructose solution, was fixed at one opening of the hatching bottle as source of food. The wasps were not fed 17 hours prior to exposure initiation. In the mortality phase, the emergent adult wasps were provided with a 1:3 (v/v) solution of honey and water. During the 24 hours of parasitisation in the reproduction part of the test, no food was provided.
Test units	Mortality/Repellence assessments: Two treated glass plates (13 cm × 13 cm) fitted to a square aluminium frame (13 cm × 13 cm × 1.4 cm). Three sides of the frame contained 6 holes each (1 cm diameter) that provided ventilation. The inside surface of the frame was coated with black tight cotton material to seal the ventilation holes. The fourth side of the frame contained an oval hole functioning as access hole for introduction of parasitoids, closed from the outside with black paper and adhesive tape. Reproduction assessments: Acrylic cylinders (approx. 11 cm diameter, 20 cm height), tops covered with nylon netting (0.5 mm × 0.5 mm mesh) for ventilation) containing approximately 8 days old wheat seedlings (*Triticum* var. Tambor) infested with host aphids (> 100 adults and nymphs).

4. Environmental conditions	The study was performed in a controlled-environment room.
Temperature	Nominal: 17 – 23 °C; actual: 19 – 22 °C
Relative humidity	Nominal: 50 – 90%; actual: 62 – 74%
Photoperiod	16 hours light to 8 hours dark photoperiod Light intensity: 1030 lux (exposure phase); 2410 lux (parasitisation phase); 6590 lux (reproduction phase)

B. STUDY DESIGN AND METHODS

1. In-life dates	6th July 2020 to 20th July 2020

2. Experimental conditions

Test design

Effects of the test substance on the parasitoid wasp *Aphidius rhopalosiphi* were assessed using five application rates in the laboratory. A control and a toxic reference were tested in parallel. Adult wasps were

exposed via contact to dry residues on glass plates. Assessments of mortality were carried out 2, 24 and 48 hours after test initiation.

To assess any significant sub-lethal effects on reproduction, assessments were then carried out for the control and for all treatment rates of the test item. Female wasps were confined individually for 24 hours over untreated wheat plants infested with adult and nymphal aphids (*Rhopalosiphum padi*). After a 24-hour parasitisation period, surviving female wasps were removed and the plants were kept for 11 days before the number of aphid mummies was assessed.

Number of animals per treatment

Mortality assessment:
Ten wasps (three males and seven females)/replicate; four replicates (i.e. a total of 40 wasps) per treatment.

Reproduction assessment:
Fifteen individually-confined female wasps per treatment.

Test doses

Following a range-finder test, ADM.03503.F.1.A was tested at rates equivalent to 0.172, 0.292, 0.496, 0.843 and 1.433 L product/ha. A control group was treated with deionised water.

Reference item

DANADIM PROGRESS was tested at nominally 0.3 mL product/ha (containing 400 g dimethoate/L).

Treatment/Application

For preparing the application solutions of ADM.03503.F.1.A, 0.773 g test item was diluted to 100 mL with deionised water to produce solution A (the highest test item concentration). The solution A was consecutively diluted to 100 mL with deionised water to produce the remaining solutions. The spray volume rate was 200 L spray solution/ha. For the reference substance, a stock solution was prepared by adding 0.160 g of reference item to 100 mL deionised water followed by one dilution of 0.1 mL of the stock solution to 100 mL with deionised water. For the reference substance, 0.30 mL product was sprayed per 200 L water/ha. The control test units were sprayed with deionised water only at 200 L/ha.

Treatments were applied using a laboratory track-sprayer (Schachtner, Ludwigsburg, Germany). The spray pressure was 3.4 bar. Prior to application, the sprayer had been calibrated using deionised water to confirm an application rate of 200 L/ha. Following calibration, treatments were applied in the order of control, test item (in ascending concentration order) and finally the toxic reference item.

After 48 hours, to determine the parasitisation capacity, 15 surviving females from the control and the treated groups (except the highest test item rate, mortality > 50 %), were transferred to single reproduction units using an aspirator. After 24 hours of parasitisation, the females were removed from the reproduction units and their condition was recorded. The plants bearing the aphids were maintained at test conditions for further eleven days.

3. Observations and assessments

Mortality and condition of the wasps were assessed at approximately 2, 24 and 48 hours after test initiation.

Eleven days after the 24-hour parasitation period, the number of parasitised aphids per reproduction test unit was counted in replicate units where wasps were found alive.

Test temperature and relative humidity were recorded continuously throughout the test. Light intensity was measured at the start of the mortality, parasitisation and reproduction phases.

4. Calculation of toxicity

Wasp mortality after 48 hours was calculated for each treatment as the number of moribund and dead wasps combined relative to the number of wasps at study initiation. The corrected percentage mortality was derived using Abbot (1925) formula.

The percentage change in numbers of mummies produced in individual test-item treatments relative to the control was calculated.

5. Statistics

Mortality was analysed for statistical significance using the Multiple Sequentially-rejective Fisher test after Bonferroni-Holm for test.

Reproductive capacity was analysed for statistical significance using Williams-t-test, following Shapiro-Wilk's test on normal distribution, Levene's test on variance homogeneity and trend analysis by contrasts to test the data for monotonicity of rate/response.

The calculation of the LR_{50} and ER_{50} was not possible since there were only minor effects on mortality and reproduction.

Statistical analyses were performed using the computer program ToxRat Professional 3.3.0.

II. RESULTS AND DISCUSSION

Results and relevant endpoints are summarized in the following table.

Table A 2.3.2.1.1-1: Mortality and reproduction of *A. rhopalosiphi*

Application rate [L product/ha]	Mortality at 48 hours [%]	Corrected mortality [%]	Reproduction	
			Mean number of mummies per female [a)]	Deviation to control [%] [b)]
Control				
Control	0	-	20.1	-
ADM.03503.F.1.A				
0.172	0	0	21.0	-4.5
0.292	5.0	5.0	20.5	-2.0
0.496	15 *	15	18.4	8.5
0.843	40 *	40	15.7	21.9
1.433	75 *	75	n.d.	-
Reference substance: DANADIM PROGRESS (active substance: dimethoate)				
0.3 mL product/ha	97.5 *	97.5	-	-
	Endpoints [L ADM.03503.F.1.A/ha] (95% confidence limits)			
48-hour LR_{50}	0.954 (0.821 – 1.149)			
NOER (mortality)	0.292			
ER_{50} (reproduction)	> 0.843			
NOER (reproduction)	0.843			

* Statistically significantly different from the control.

[a)] the mean number of mummies/female was calculated from the number of mummies per surviving female.

[b)] change in mean number of mummies per female, relative to control. A negative value indicates an increase, and a positive value indicates a decrease relative to the control.

n.d: not determined (corrected mortality > 50 %).

There was no requirement to assess reproductive performance in the reference item group, since the reference item treatment served only as an indicator for test species sensitivity.

Validity criteria

The test is considered to be valid since mortality in the control was ≤ 13% (actual: 0/40 wasps; i.e. 0.0%) and mortality in the toxic reference group was > 50% at 48 hours (actual corrected mortality: 97.5%). Furthermore, the reproductive capacity was ≥ 5 mummies per female (actual: 20.1 mummies per female) and no more than two females (actually one) failed to produce mummies in the control group.

III. CONCLUSION

Under laboratory conditions with exposure on glass plates, the 48-hour LR_{50} was estimated to be 0.954 L product/ha and the NOER for survival was 0.292 L product/ha. The ER_{50} for reproduction was estimated to be > 0.843 L product/ha and the NOER for reproduction was 0.843 mL product/ha.

A 2.3.2.1.2 Study 2: Standard laboratory test with *Typhlodromus pyri*

Comments of zRMS:	The study was conducted in line with Blümel et al. (2000) with no deviation. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment: The 7-day LR_{50}/ ER_{50} > 1.193 L product/ha.

Reference:	KCP 10.3.2.1/02
Report	Effects of ADM.03503.F.1.A on the predatory mite *Typhlodromus pyri* SCHEUTEN in a laboratory test, Röhlig, U., 2020b, 20 48 NTL 0004 (report number), 000105075 (sponsor report number)
Guideline(s):	Blümel *et al.* 2000
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle control: 200 L/ha deionised water Positive control: reference substance with 15 mL product per 200 L water/ha
Reference item	DANADIM PROGRESS
Description	EC (emulsifiable concentrate)
Lot/Batch #	10214034
Purity	400.0 g/L dimethoate (nominal content) 411.2 g/L dimethoate (analysed content)
Stability of reference item	Expiry date: 6th September 2021
3. Test organism	
Species	Predatory mite, *Typhlodromus pyri* SCHEUTEN (Acari: Phytoseiidae)
Source	Purchased from Katz Biotech AG, Baruth, Germany (in the stage of eggs)

Age	Protonymphs < 24 hours old
Acclimation period	Mites were reared at the laboratory of "Katz Biotech AG" at 20 – 25 °C and a relative humidity of 60 – 80 %. Eggs of the predatory mites were placed in cages for hatching and mites were cultured at 23 – 25 °C and a relative humidity of 67 – 73 % prior to the test start.
Diet	The mites were fed with untreated pollen of a 1:1 v/v mixture of pine and birch. Untreated pollen was provided as food and replenished at each assessment day.
Test units	Glass plates (50 × 22 mm) stuck together along their longitudinal sides with a barrier of sticky material on moistened filter paper on a sponge placed in a plastic tray (inside dimensions: about 165 mm × 120 mm × 60 mm) filled with tap water up to a height of approx. 15 mm

4. Environmental conditions	The bioassays were performed in a controlled test room.
Temperature	Nominal: 23 – 27 °C; actual: 23 – 25.6 °C
Relative humidity	Nominal: 60 – 90%; actual: 67 – 79%
Photoperiod	16 hours light (light intensity: 2040 lux)

B. STUDY DESIGN AND METHODS

1. In-life dates 2nd June 2020 to 16th June 2020

2. Experimental conditions

Test design

Lethal and sub-lethal effects on the predatory mite *Typhlodromus pyri* were assessed in a rate-response design at five rates of ADM.03503.F.1.A (dry spray residues) under standard laboratory conditions. A control and a reference substance were tested in parallel. Cumulative mortality was assessed after 3 and 7 days. From day 7 to day 14, cumulative reproduction was recorded by counting the number of eggs per female.

Number of animals per treatment

Twenty protonymphs/replicate; five replicates/test and reference substance treatment and control; i.e. in total 100 mites per treatment.

Test doses

Based on a range-finder experiment, ADM.03503.F.1.A was tested at rates equivalent to 0.143, 0.243, 0.413, 0.702 and 1.193 L product/ha. A control group was exposed to residues of deionised water.

Reference substance

DANADIM PROGRESS (containing 400 g dimethoate/L) was tested at nominally 15 mL product per 200 L water/ha.

Treatment/Application

For preparing the application solutions of ADM.03503.F.1.A, 0.644 g test item was diluted to 100 mL with deionised water to produce solution A (the highest test item concentration). The solution A was con-

secutively diluted to 100 mL with deionised water to produce the remaining solutions. The spray volume rate was 200 L spray solution/ha. For the reference substance, a stock solution was prepared by adding 0.802 g of reference item to 100 mL deionised water followed by one dilution of 1 mL of the stock solution to 100 mL with deionised water. For the reference substance, 15 mL product was sprayed per 200 L water/ha. The control test units were sprayed at 200 L/ha with deionised water only.

The diluted products were applied using a laboratory track-sprayer (Chr. Schachtner, Ludwigsburg, Germany). The spray pressure selected was 3.4 bar. Prior to application, the sprayer had been calibrated (by weighing spray deposits delivered on glass plates of known surface area) in order to achieve the application rate of 200 L/ha. The deviation in the spray deposit did not exceed ± 10 % for three consecutive applications without adjusting. Following calibration, treatments were applied in the order of control, test item (in ascending rate order) and finally the toxic reference.

The bioassays were initiated within one hour of treatment, once residues had dried on glass plates. After setting up the test units, protonymphs were placed on each treated glass plate using a fine hair brush.

3. Observations and assessments

The condition of the mites was assessed on days 3, 7, 9, 11 and 14 after test item application, dividing the conditions into alive, dead and escaped (trapped by the glue or water or not visible). Any dead mites were removed at the time of each assessment.

On days 3 and 7, after the application mortality assessments were conducted. Any eggs that were produced prior to 7 days after treatment were discarded. The number of eggs laid, and hatched juveniles present were determined on days 9, 11 and 14, these were removed on days 9 and 11. The final assessment for mortality was performed on day 7 after treatment and the final assessment for reproduction was made on day 14 after treatment.

The temperature and the relative humidity were recorded continuously during the test. Light intensity was measured at the start of the test

4. Calculation of toxicity

Mortality (sum of dead and escaped mites) was determined after 3 and 7 days of exposure and the mean percentage mortality was calculated. Mortality in the treatment groups was corrected by the mortality of the control group using the formula of Abbott (1925).

The mean cumulative number of eggs per female (reproduction) during the reproduction period was calculated for each test group by counting the number of females, eggs and juveniles (larvae) at the 3 assessment days following day 7. In addition, the reduction of reproduction was expressed as percentage in relation to the control value.

5. Statistics

Mortality was analysed for statistical significance using the Multiple Sequentially-rejective Chi^2-2×2 Table test after Bonferroni-Holm.

Reproduction was analysed for statistical significance using Williams-t-test, following Shapiro-Wilk's test for normal distribution, Levene's test procedure for variance homogeneity.

The calculation of the LR_{50} and ER_{50} was not possible since effects on mortality and reproduction in all test item treatment groups was less than 50 % compared to the control group.

Statistical analyses were performed using the computer program ToxRat Professional 3.3.0.

II. RESULTS AND DISCUSSION

Results and relevant endpoints are summarized in the following table.

Table A 2.3.2.1.2-1: Mortality and reproduction of *Typhlodromus pyri* exposed to ADM.03503.F.1.A

Application rate [L product/ha]	**Mean mortality after 7 days**		**Cumulative reproduction from day 7 to day 14**	
	[%]	**corrected [%]**	**[mean number of eggs per female]**	**[% effect rel. to control] a)**
Control	3.0	-	6.77	-
0.143	3.0	0.0	6.84	-1.0
0.243	6.0	3.1	6.87	-1.5
0.413	12.0 *	9.3	6.51	3.8
0.702	16.0 *	13.4	4.63	31.6
1.193	24.0 *	21.6	3.63	46.4
Toxic reference (15 mL product per 200 L water/ha)	71.0	70.1	-	-
	Endpoints [L ADM.03503.F.1.A/ha] (95% confidence limits)			
0-7-d LR_{50} (mortality)	> 1.193			
NOER (mortality)	0.243			
7- 14 d ER_{50} (reproduction)	> 1.193			
NOER (reproduction)	0.413			

* Statistically significantly different from the control.

a) change in mean number of eggs per female, relative to control. A positive value indicates a decrease, and a negative value indicates an increase relative to the control.

There was no requirement to assess reproductive performance in the reference item group, since the reference item treatment served only as an indicator for test species sensitivity.

Validity criteria

The test is considered to be valid since mortality in the control and toxic reference groups was ≤ 20% (actual: 3.0%) and 50 – 100% (actual: 70.1% corrected mortality), respectively. Furthermore, mean cumulative egg production in the control was at least 4 eggs per female (actual: 6.77 eggs per female).

III. CONCLUSION

After exposure of the predatory mite *Typhlodromus pyri* to freshly applied residues of ADM.03503.F.1.A on glass plates, the 7-day LR_{50} was estimated to be > 1.193 L product/ha. With respect to reproduction, the ER_{50} was estimated to be > 1.193 L product/ha. The NOER for mortality and reproduction was 0.413 L product/ha.

A 2.3.2.2 KCP 10.3.2.2 Extended laboratory testing and aged residue studies

No additional data submitted.

A 2.3.2.1 KCP 10.3.2.3 Semi-field studies

No additional data submitted.

A 2.3.2.2 KCP 10.3.2.4 Field studies

No additional data submitted.

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1.1 Study 1: Toxicity to *Eisenia fetida* of ADM.03503.F.1.A

Comments of zRMS:	The study was conducted in line with OECD 222 with no deviation. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment. The 56-day NOEC for reproduction was determined to be 30.9 mg product/kg dry soil. Since both the active substances Prothioconazole and Fluxapyroxad have a log Kow > 2, the endpoint should be corrected by a factor of 2 for the use in the risk assessment: $NOEC_{repr,corr}$ = 15.45 mg test item /kg soil dw

Reference:	KCP 10.4.1.1/01
Report	Effects of ADM.03503.F.1.A on the reproduction of the earthworm *Eisenia fetida* in artificial soil, Friedrich, S., 2020a, 20 48 TEC 0033 (report number), 000105077 (sponsor report number)
Guideline(s):	Yes, OECD 222 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)

Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle control: Deionised water Reference item: The reference substance Carbendazim (formulation Maypon Flow, SC 500) was tested in a separate study in January 2020. In this study, carbendazim showed statistically significant effects on reproduction (53 and 99% reduction of number of juveniles at 5 and 10 mg/kg dry soil carbendazim, respectively).
3. Test organism	
Species	Earthworm *Eisenia fetida* (Savigny, 1826)
Source	Purchased from Bias Labs Ltd, Fife, UK
Age	Adults, approximately 7 months old with clitellum; body weight at test start: 302 – 493 mg/worm
Acclimatisation	At least 24 hours in the artificial substrate with food
Diet	Air-dried and finely ground horse manure was fed to the worms during the test. One day after application, 5 g air-dried horse manure was scattered on the soil surface and sprinkled with 5 mL deionised water. The feeding interval was weekly during the first four weeks of the test with weekly amount of manure (5 g) dependent on feeding activity assessed by visual inspection. After removing the adult earthworms after 4 weeks, again 5 g of horse manure was carefully mixed into the soil (last feeding occasion).
Test units	Plastic vessels (inside dimensions: 16.5 × 12 × 6 cm) with lids pervious to air and light filled with 810 g soil (wet weight, corresponding to 600 g dry weight with water content corresponding to 40 – 60% of water holding capacity (WHC).
4. Environmental conditions	
Soil	Artificial soil was prepared with the following constituents: Sphagnum peat 10% Kaolin clay 20% Calcium carbonate ($CaCO_3$) 0.5% (for adjustment of pH) Industrial quartz sand 69.5% The maximum water holding capacity (WHC) was 62.4 g/100 g dry soil. One day prior to test start, the artificial soil was pre-moistened with deionised water to obtain approximately half of the final water content
Temperature	nominal: 20 ± 2°C; actual: 19.0 – 21.7 °C
Photoperiod	16-hour light (light intensity: nominal: 400 – 800 Lux; actual: 620 lux) to 8-hour dark photoperiod
Water content	nominal: 40 – 60% of WHC; actual: 54.6 – 56.1% of WHC
pH	nominal: 6.0 ± 0.5; actual: 5.78 – 6.08

B. STUDY DESIGN AND METHODS

1. In-life dates	11th June 2020 to 6th August 2020

2. Experimental conditions

Test design

Adult earthworms were exposed to soil treated with the test item at eight concentrations or remaining untreated (control) for a period of 28 days. After this period, the adults were removed from the test vessels and mortality, behavioural effects, and biomass development (body weight change) were determined. The reproduction rate was determined after an additional period of 4 weeks (on day 56) based on the number of juveniles.

Number of animals per treatment

Ten earthworms/replicate; four replicates/test substance treatment and eight replicates/control.

Test conditions

After application, the soil moisture content in each test vessel was adjusted to 34.9 – 35.0 g/100 g dry soil (55.9 – 56.1% of WHC) by addition of water. The soil moisture content at study end was 34.1 – 34.8 g/100 g dry soil (54.6 – 55.8% of WHC). The pH value in the test substance treatments and control was 6.04 – 6.08 at the start of the test and 5.78 – 5.87 at the end of the test. During the test period, the test temperature was 19.0 – 21.7 °C.

Test concentrations

ADM.03503.F.1.A was tested at 1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg product/kg dry soil. A control (receiving deionised water only) was tested in parallel. The reference item carbendazim was tested in a separate study.

Treatment/Application

The stock solutions A (equivalent to the highest test concentration) was prepared by adding deionised water to 1 g test item to a final volume of 1000 mL. Solution A served as a stock solution for the lower concentrations (55.6, 30.9, 17.1, 9.53, 5.29, 2.94 and 1.63 mL were diluted with deionised water to 250 mL, respectively). The solutions were mixed.

For each test item group, 60.0 mL of the respective test item solution was mixed into 750 g of substrate (wet weight). Thorough mixing was conducted at 240 rotations per minute for 2 × 2 minutes for each replicate with a mixer. Immediately after mixing, the test substrate of each treatment group was split, and 810 g (corresponding to 600 g dry substrate) were placed into the test units.

3. Sampling and measurements

At the beginning (prior to exposure) of the first four weeks of the test, the adult test organisms of each test vessel were weighed individually.

Observations of behavioural and pathological symptoms (including feeding activity) were made weekly.

After 4 weeks of exposure, surviving adult worms were counted per replicate and observations were made for behavioural and pathological symptoms (including morphological alterations). At the end of the first four weeks, the fresh weight of surviving earthworms was recorded for each replicate.
After 8 weeks, the number of living juveniles per test replicate and the number of unhatched cocoons were determined.

The pH and water content of the soil was determined for all treatment groups and the control at the start and end of the test. Temperature was recorded continuously by data logger.

4. Calculation of toxicity

Parameters reported are mortality, change of biomass (difference in fresh weight of surviving worms between test start and four weeks after treatment) and reproduction (number of juveniles present). The arithmetic mean and the standard deviation per treatment and per control for reproduction, mortality and biomass were calculated.

5. Statistics

The EC_X values (number of juveniles) and its 95% confidence limits were calculated by Probit analysis using the maximum likelihood method and normal approximation, respectively.

For identifying the NOEC values, the Multiple Sequentially-rejective Fisher test after Bonferonni-Holm and the Williams-t-test were used to compare the control with the independent test item groups. For statistical evaluation of the biomass change, the changed mean fresh weight of surviving worms per replicate was used.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (Ratte 2018).

II. RESULTS AND DISCUSSION

The test item caused no statistically significant effects on the change in biomass, mortality and number of unhatched cocoons. No pathological symptoms and no effects on behaviour (including feeding activity) of worms were observed during the test.

Statistically significant effects on the number of juveniles compared to the control group were recorded at concentrations ≥ 55.6 mg test item/kg dry weight.

Results and relevant endpoints are summarized in the following table.

Table A 2.4.1.1.1-1: Effects of ADM.03503.F.1.A on earthworm survival, growth and reproduction

Treatment [mg product/kg dry soil]	Mortality after 4 weeks of exposure [%]	Mean change in body fresh weight per replicate after 4 weeks of exposure		Reproduction rate after 8 weeks	
		mg weight/worm ± SD	Reduction compared to initial fresh weight [%]	Mean juveniles/test vessel ± SD	Reduction compared to control [%] [a)]
Control	0.0	110.4 ± 15.4	30.8	290.8 ± 34	-
1.63	2.5	111.3 ± 19.1	31.1	298.8 ± 39.3	-2.8
2.94	2.5	106.0 ± 8.0	29.7	279.0 ± 17.2	4.0
5.29	0.0	116.2 ± 12.7	32.5	290.5 ± 46.1	0.1
9.53	2.5	103.0 ± 19.2	28.8	283.8 ± 35.1	2.4
17.1	0.0	118.7 ± 11.3	33.2	282.0 ± 57.5	3.0
30.9	0.0	114.5± 8.5	31.9	279.8 ± 36.9	3.8
55.6	0.0	103.7 ± 25.0	29.0	214.5 * ± 16.0	26.2
100	0.0	109.8 ± 24.4	30.3	189.8 * ± 29.7	34.7
Endpoints [mg product/kg dry soil] (95% confidence limits)					
LC_{50} (mortality)		> 100			
EC_{10} (reproduction)		32.7 (21.5 – 49.8)			
EC_{20} (reproduction)		55.0 (43.0 – 70.4)			
EC_{50} (reproduction)		> 100			
NOEC (mortality, biomass)		≥ 100			
NOEC (reproduction)		30.9			

* Statistically significantly different from the control.
[a)] negative % values indicate an increase compared to the control.
SD: standard deviation.

Validity criteria

The validity of the test was fulfilled since adult mortality after 4 weeks in the controls was ≤ 10% (actual: 0%), control replicates produced 235 to 343 juveniles (required ≥ 30 juveniles/replicate) and the coefficient of variance of the reproduction rate per test vessel in the control was 11.7% (required ≤ 30%).

III. CONCLUSION

The 56-day EC_{10}, EC_{20}, and EC_{50} for reproduction of ADM.03503.F.1.A for *Eisenia andrei* were calculated to be 32.7, 55.0 and > 100 mg product/kg dry soil and the 56-day NOEC for reproduction was determined to be 30.9 mg product/kg dry soil. All validity criteria were fulfilled.

A 2.4.1.2 KCP 10.4.1.1 Earthworms - sub-lethal effects

No additional data submitted.

A 2.4.1.3 KCP 10.4.1.2 Earthworms - field studies

No additional data submitted.

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 Study 1: Toxicity to *Folsomia candida* for ADM.03503.F.1.A

Comments of zRMS:	The study was conducted in line with OECD 232 with no deviation. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment. 28-day NOEC of ADM.03503.F.1.A for the mortality and ≥ 100 mg product/kg dry soil. EC_{10}, EC_{20}, EC_{50} > 100 mg product/kg dry soil Since both the active substances Prothioconazole and Fluxapyroxad have a log Kow > 2, the endpoint should be corrected by a factor of 2 for the use in the risk assess-ment: $NOEC_{repr,corr}$ = 50 mg test item /kg soil dw

Reference:	KCP 10.4.2.1/01
Report	Effects of ADM.03503.F.1.A on the reproduction of the collembolan *Folsomia candida*, Friedrich, S., 2020b, 20 48 TCC 0023 (report number), 000105078 (sponsor report number)
Guideline(s):	Yes, OECD 232 (2016)
Deviations:	None
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle control: deionised water Reference item: Boric acid is routinely tested at the test facility. The most recent study from September 2020 determined a reproductive EC_{50} of 107 mg/kg soil dry weight (required in OECD 232: 50% reduction at about 100 mg/kg)

3. Test organism

Species	Collembolan *Folsomia candida* (Willem)
Source	In-house culture at the test facility, originally purchased from Biologische Bundesanstalt (BBA), Berlin-Dahlem, Germany
Age	Juvenile collembolans (9 – 12 days old)
Acclimatisation	Breeding under similar laboratory conditions (12:12 h light:dark cycle at 400 – 800 lux, temperature at 20 ± 1 °C)
Diet	At the start of the test and after 14 days, 2 mg of granulated dry yeast was added to each test unit
Test units	Glass container (approximately 150 mL) covered with a lid (18.9 cm^2 surface area). Each test unit was filled with 30 g wet weight of artificial soil. Test units were briefly opened for aeration twice a week.

4. Environmental conditions

Soil	Artificial soil was prepared with the following constituents:

Sphagnum peat	5%
Kaolinite clay	20%
Calcium carbonate	0.3%
Industrial quartz sand	74.7%

The maximum water holding capacity (WHC) was 42.8%.
Two days prior to test start, deionised water was added to the artificial soil to achieve approximately half of the final water content.

Temperature	Nominal: 20 ± 2 °C; actual: 19.0 – 21.0 °C
Photoperiod	16 hours light (light intensity: nominal: 400 – 800 lux, actual 640 lux) to 8 hours dark photoperiod
Water content	nominal: 40 – 60% of WHC; actual: 56.3 – 58.4% of WHC
pH	nominal: 6.0 ± 0.5; actual: 5.70 – 6.06

B. STUDY DESIGN AND METHODS

1. In-life dates 20th July 2020 to 17th August 2020

2. Experimental conditions

Test design

Juvenile collembolans were exposed to soil treated with the test substance at eight concentrations for a period of 28 days. A water control (deionised water) was tested in parallel. The reference item boric acid was tested in a separate study. After 4 weeks of exposure, the number of adults was counted, and mortality was determined. The reproduction output was determined by counting the number of juveniles.

Number of animals per treatment

Four replicates per test substance treatment and eight replicates each for the control were used with ten collembolans per replicate. Two additional vessels per treatment group were set up for pH and water content determination.

Test concentrations

ADM.03503.F.1.A was tested at 1.63, 2.94, 5.29, 9.53, 17.1, 30.9, 55.6 and 100 mg product/kg dry soil. A control (receiving deionised water only) was tested in parallel. The reference item boric acid was tested in a separate study.

Treatment/Application

An exact weighed amount (0.200 g) of test item was mixed with deionised water (to a total volume of 200 mL) to make stock solution A. 25 mL of stock solution A was added to 287.5 g artificial soil (equivalent to 250 g dry weight) to prepare the highest test concentration. The stock solution A was diluted with deionised water to prepare seven further test solutions (serial dilution; spacing factor: 1.8; i.e. 27.78, 15.43, 8.57, 4.76, 2.65, 1.47 and 0.82 mL each of the respective higher concentration to 50 mL deionised water). Afterwards, the solutions (25 mL) were thoroughly mixed with the artificial soil (287.5 g wet weight) separately for each treatment group by intensive stirring in a laboratory mixer. Applications were made in the following order: first untreated control and thereafter the test item in ascending order. Subsequently, 30 g (dry weight) of treated artificial soil were placed into each test vessel and collembolans were introduced to each vessel using an aspirator.

3. Sampling and measurements

Four weeks after introducing the test organisms the numbers of parental and juvenile collembolans in the test item and control vessels were determined per replicate. Observations on obvious physiological or pathological symptoms or distinct changes in behaviour were made. The test substrate of each replicate was poured into an individual container of about 200 mL and the test organisms were floated off the substrate by the addition of water. To improve the contrast between the white collembolans and surrounding water surface, the water was stained dark with ink. After gentle stirring, the numbers of parental and juvenile collembolans floating on the surface were determined. Missing parental collembolans were assumed to have died during the test period. Surviving adults and juveniles were counted using a digital image processing system (LemnaTec Scanalyzer), an automated counting technique based on a video camera connected to digital image storage and analysis system.

The extraction efficiency of the extraction method was determined to be 98% in a separate extraction run using vessels containing a known number of juveniles kept in untreated test substrate.

The soil water content was checked weekly by reweighing the additional test vessels. Water loss was compensated for by addition of deionised water if exceeding 2% of the initial water content. Water content was also determined at start and end of the study period.

At the start and end of the test, the pH of the artificial soil was measured. The test temperature was recorded continuously by data logger.

4. Calculation of toxicity

Mortality (number of dead adults) in % for each treatment group was calculated. Missing parental collembolans were counted as dead.

The reproductive output for each test item treatment group was calculated in % deviation from controls.

5. Statistics

Multiple Sequentially-rejective Fisher test after Bonferroni-Holm and Williams-t-test after Bonferroni-Holm were used to compare the control with the independent test item groups. EC_{10}, EC_{20} and EC_{50} could not be determined with values higher than the highest concentration tested.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018).

II. RESULTS AND DISCUSSION

No effects on behaviour of the collembolans were observed during the test. No statistically significant effects on mortality or number of juveniles compared to the control group were found at any concentration tested.

Results and relevant endpoints are summarized in the following table.

Table A 2.4.2.1.1-1: Effects of ADM.03503.F.1.A on survival and reproduction of *Folsomia candida*

Treatment [mg product/kg dry soil]	**Mortality after 4 weeks** [%]	**Reproduction output after 4 weeks**		
		Mean juveniles/ replicate ± SD	**Reduction in reproductive output [%]**	**Coefficient of variation [%]**
Control	1.3	1405 ± 113.8	-	8.1
1.63	2.5	1426 ± 53.3	-1.5	3.7
2.94	2.5	1464 ± 110.2	-4.2	7.5
5.29	0.0	1388 ± 176.7	1.2	12.7
9.53	0.0	1410 ± 191.8	-0.3	13.6
17.1	0.0	1415 ± 229.1	-0.7	16.2
30.9	2.5	1441 ± 98.8	-2.6	6.9
55.6	2.5	1417 ± 85.4	-0.9	6.0
100	0.0	1371 ± 124.7	2.4	9.1
Endpoints [mg product/kg dry soil] (95% confidence limits)				
LC_{50} (mortality)		> 100		
EC_{10} (reproduction)		> 100		
EC_{20} (reproduction)		> 100		
EC_{50} (reproduction)		> 100		
NOEC (mortality)		≥100		
NOEC (reproduction)		≥100		

No statistically significant differences from control.
SD: standard deviation.

Validity criteria

The validity of the test was fulfilled since mean mortality of adults in the control was 1.3% (required ≤ 20%) at the end of the test, the mean number of juveniles per replicate in controls was 1405 (required ≥ 100) and the maximum coefficient of variation for the mean number of juveniles was 8.1% in the controls (required ≤ 30%).

III. CONCLUSION

In this study the 28-day NOEC of ADM.03503.F.1.A for the mortality and reproduction of *Folsomia candida* was determined to be ≥ 100 mg product/kg dry soil. The EC_{10}, EC_{20}, EC_{50} were estimated to be > 100 mg product/kg dry soil. All validity criteria were fulfilled.

A 2.4.2.1.2 Study 2: Toxicity to *Hypoaspis aculeifer* of ADM.03503.F.1.A

Comments of zRMS:	The study was conducted in line with OECD 232 with no deviation. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment. $NOEC_{mortality,\ reproduction} \geq 40$ mg product/kg soil dry weight. EC_{10}, EC_{20} and $EC_{10} > 40$ mg product/kg soil dry weight. Since both the active substances Prothioconazole and Fluxapyroxad have a log Kow > 2, the endpoint should be corrected by a factor of 2 for the use in the risk assessment: $NOEC_{repr,corr}$ = 20 mg prod./kg soil dw

Reference:	KCP 10.4.2.1/02
Report	Effects of ADM.03503.F.1.A on the reproduction of the predatory mite *Hypoaspis aculeifer*, Schulz, L., 2020a, 20 48 THC 0019 (report number), 000105079 (sponsor report number)
Guideline(s):	Yes, OECD 226 (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material — ADM.03503.F.1.A
(Fluxapyroxad 75 Prothioconazole 150 g/L EC)

Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021

2. Vehicle and/or positive control — Vehicle control: deionised water
Positive control: The reference item dimethoate (98.8 % ± 0.5 %, analysed) was tested in a separate study in September – October 2019 and resulted in an EC_{50} of 6.3 mg a.s./kg dry soil (required according to OECD 226: 3.0 – 7.0 mg a.s./kg dry soil)

3. Test organism

Species	Predatory mite *Hypoaspis aculeifer* Canestrini
Source	Received synchronised from Katz Biotech AG, Baruth, Germany.
Age	Adults from a synchronised culture with a maximum age difference of 2 days

Acclimatisation	Synchronised culture was maintained at a temperature of approximately 21 – 24 °C.
Diet	The mites were fed with *Tyrophagus putrescentiae* (Schrank) 2 – 3 times a week during breeding and likewise every 2 – 3 days during the test (~ 20 mg/vessel)
Test units	160 mL WECK-jar with glass lid (inside dimensions: 4.7 cm diameter, 8 cm high) containing 20 g (dry weight) of treated or untreated soil. Test units were briefly opened every 2 – 3 days for aeration and feeding

4. Environmental conditions

Soil	Artificial soil was prepared with the following constituents:

Sphagnum peat	5%
Kaolin clay	20%
Industrial quartz sand	74.75%
Calcium carbonate	0.25% (to adjust pH)

	The maximum water holding capacity (WHC) of the soil was determined to be 43.16 g/100 g soil dry weight. Final moistening was achieved with application of the test item in volume water required to hydrate the soil to 40 – 60% of WHC
Temperature	Nominal: 20 ± 2 °C; actual: 19.4 – 21.4 °C
Photoperiod	16 hours light (light intensity: nominal 400 – 800 lux; actual 513 lux) to 8 hours dark photoperiod
Water content	nominal: 40 – 60% of WHC; actual: 45.47 – 48.37% of WHC
pH	nominal: 6.0 ± 0.5; actual: 6.0 – 6.4

B. STUDY DESIGN AND METHODS

1. In-life dates 10th June 2020 to 2nd July 2020

2. Experimental conditions

Test design

Adult female mites were exposed to soil treated with the test substance at eight test item concentrations for a period of 14 days. Deionised water was used as a control treatment. The reference item dimethoate was tested in a separate study. At the end of the exposure period, the surviving individuals were extracted from the test units. The number of juveniles per test unit and additionally the number of surviving females were determined. The reproductive output and the mortality in the test item group were compared to that of the control group.

Number of animals per treatment

Ten female mites per replicate; four replicates per test substance treatment and eight for controls.

Two additional replicates without mites were prepared each for treatment groups and control to determine pH and water content.

Test concentrations

ADM.03503.F.1.A was tested at soil concentrations of 0.65, 1.18, 2.12, 3.81, 6.86, 12.3, 22.2 and 40.0 mg product/kg soil dry weight. A control (untreated substrate) was tested in parallel. The reference item dimethoate was tested in a separate study.

Treatment/Application
An exact weighed amount (0.100 g) of test item was mixed with deionised water (to a total volume of 250 mL) to prepare stock solution A without addition of solubility mediators immediately before application. 20 mL of the stock solution was added to 223.16 g artificial soil (equivalent to 200 g dry weight) to prepare the highest test concentration. The stock solution A was diluted with deionised water to prepare seven further test solutions (serial dilution; spacing factor: 1.8; i.e. 138.89 mL each of the respective higher concentration to 20 mL with deionised water). Afterwards, the solutions (20 mL) were thoroughly mixed with the artificial soil (223.16 g wet weight) separately for each treatment group by means of a hand stirrer. Applications were made in the following order: first untreated control and thereafter the test item in ascending order. Subsequently, 20 g (dry weight) of treated artificial soil were placed into each test vessel and mites were introduced to each vessel by means of a moistened brush.

3. Sampling and measurements

On day 14 after application of the test item and introduction of the test organisms, surviving mites and juveniles of *Hypoaspis aculeifer* were extracted from each test replicate using a MacFadyen high-gradient extractor (heat/light extraction method). This was achieved by adding the soil substrate from each test vessel into a canister placed inverted onto the extraction system. Soil substrate was retained within the canister using a plastic net (1 mm mesh size) on the bottom. Beneath the canister was a funnel attached to a collecting flask with 25 mL of a fixing liquid. A temperature gradient was created between the upper part (where the samples were) and the lower part of the system (where the collecting flasks were placed). The temperature gradient was obtained by circulating heated air in the canister area (upper part of the system) and cooled air on the collecting area (lower part of the system). The duration of extraction was 48 hours at the following heating regime: 25 °C for 12 hours, 35 °C for 12 hours, 45 °C for 24 hours. During this time, adult and juvenile mites moved down through the soil substrate away from the heat source, until they fell from the substrate into the funnel/ fixing liquid.

Following extraction, all juveniles and adults present in the fixing liquid were counted. Any adult mites not found after extraction were recorded as dead. From these data, the mortality of the adult females and the reproductive output were calculated.

The efficiency of the method used to extract the mites in this test should be > 90%. The extraction efficiency of the extractor was determined to be 91.5 % in a separate extraction run using vessels containing a known number of juveniles and adult mites kept in untreated test substrate.

The soil water content was measured at test start and end for each treatment group. The water content of the soil substrate in the test vessels was maintained throughout the test based on reweighing additional test vessels and compensating the water loss, if necessary.

The pH was checked at the beginning and end of the test. Temperature was recorded continuously.

4. Calculation of toxicity

Mortality (number of dead adults) in % for each treatment group was calculated. Missing mites were counted as dead.

The reproductive output for the test item treatment group was calculated in % compared to the control.

5. Statistics

Multiple Sequentially-rejective Fisher test after Bonferroni-Holm and Dunnett's Multiple t-test were used to compare the control with the independent test item groups. EC_{10}, EC_{20} and EC_{50} could not be determined with values higher than the highest concentration tested.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018).

II. RESULTS AND DISCUSSION

Results and relevant endpoints are summarized in the following table.

Table A 2.4.2.1.2-2: Effects of ADM.03503.F.1.A on survival and reproduction of *Hypoaspis aculeifer*

Treatment [mg product/kg dry soil]	Mean adult mortality after 2 weeks [%]	Reproduction output after 2 weeks		
		Mean juveniles/ test vessel ± SD	Reproduction [% of control]	Coefficient of variation [%]
Control	0.0	267.5 ± 20.9	100	7.8
0.65	0.0	266.8 ± 19.6	100	7.4
1.18	2.5	278.3 ± 6.7	104	2.4
2.12	5.0	273.0 ± 22.6	102	8.3
3.81	0.0	282.8 ± 10.1	106	3.6
6.86	0.0	294.5 ± 11.2	110	3.8
12.30	2.5	269.0 ± 24.1	101	9.0
22.2	0.0	268.0 ± 28.2	100	10.5
40.0	0.0	253.8 ± 16.5	95	6.5
Endpoints [mg product/kg soil dry weight] (95% confidence limits)				
LC_{50} (mortality) [a)]	> 40.0			
EC_{10} (reproduction) [a)]	> 40.0			
EC_{20} (reproduction) [a)]	> 40.0			
EC_{50} (reproduction) [a)]	> 40.0			
NOEC (mortality)	≥ 40.0			
NOEC (reproduction)	≥ 40.0			

[a)] based on estimation of the data.
No statistically significant differences from control.
SD: standard deviation.

Validity criteria

The validity of the test was fulfilled since the mortality of female adults in the control was 0.0% (required ≤ 20%) at the end of the test, the mean number of juveniles per replicate was 267.5 (required ≥ 50) and the coefficient of variation for the mean number of juveniles in the control was 7.8% (required ≤ 30%).

III. CONCLUSION

In this 14-day test on chronic toxicity to *Hypoaspis aculeifer*, ADM.03503.F.1.A the NOEC for mortality and reproduction was determined to be ≥ 40 mg product/kg soil dry weight. The EC_{10}, EC_{20} and EC_{10} were estimated to be > 40 mg product/kg soil dry weight. All validity criteria were fulfilled in the study.

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

No additional data submitted.

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

A 2.5.1 Study 1: Toxicity to the soil microflora of ADM.03503.F.1.A

Comments of zRMS:	The study was conducted in line with OECD 216. ~~with no the deviation.~~ During the interval 7-14 days an effect > 25 % was observed (+ 31.1 %). But after 28 days no deviation were noted. Based on the study results no adverse effects (i.e. deviation from control < 25%) were seen at the end of the 28-day incubation period at 0.350 and 3.50 mg product/kg soil d.w. Based on the deviation from the control the results 3.50 mg product/kg dws should be treated with caution.

Reference:	KCP 10.5/01
Report	Effects of ADM.03503.F.1.A on the activity of soil microflora (Nitrogen transformation test), Schulz, L., 2020b, 20 48 SMN 0020 (report number), 000105080 (sponsor report number)
Guideline(s):	Yes, OECD 216 (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIAL AND METHODS

A. MATERIALS

1. Test material

ADM.03503.F.1.A
(Fluxapyroxad 75 Prothioconazole 150 g/L EC)

Description: Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)

Lot/Batch #: 1162-230719-011

Purity: Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w)
Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w)
Density: 1.0792 g/cm³ (20 °C)

Stability of test material: Stable under storage conditions (dark and dry conditions)
Expiry date: 5th September 2021

2. Vehicle and/or positive control

Vehicle control: untreated artificial soil
Positive control: the method is validated by routinely testing the inhibition of nitrogen transformation caused by dicyandiamide (purity: 99.6 % analysed). The results of the latest positive control test performed from October to November 2019 confirmed the sensitivity of the test system (i.e. deviation from control > 25%) on nitrogen turnover after 28 days was observed when applied at 100 and 200 mg/kg soil dry weight (d.w.).

3. Test soil

Soil type	Loamy sand (DIN 4220) Sandy loam (USDA)

Batch No	1/2020	
C_{org}	1.45%	
Humus content	2.49%	
Microbial biomass	31.39 mg C/100 g soil d.w. (2.16% of C_{org})	
N_{min}	1.05 mg/100 g soil d.w.	
Total nitrogen	0.14%	
pH	6.0	
Particle size distribution	USDA	DIN ISO 11277
Clay	9.4%	10.0%
Silt	37.7%	36.6%
Sand	52.9%	53.4%
Max. water holding cap. (WHC)	38.76 g/100 g dry soil	
Water content	11.21 g/100 g soil d.w.	
Cation exchange capacity	8.2 $cmol^+$/kg soil d.w.	

Source Wassergut Canitz, Schlag 34/3, Germany

Soil history The soil is from a fallow ground and was not subjected to any pesticide treatment since 1990. No fertilizer had been applied to the site since 2003

Soil sampling 21st February 2020
The soil was sampled at a depth of ~20 cm and sieved (2 mm)

Soil preparation 200 g soil dry weight (= one sub-sample) per test vessel was weighed. The soil was mixed with 0.5% (i.e. 1.0 g/200 g soil d.w.) lucerne meal (C/N ratio 13.2:1) in order to stimulate nitrogen transformation by means of a hand stirrer. One additional soil sample without lucerne meal was used for determination of initial NH_4-N content and NO_3-N-content (1.88 mg/100 g soil d.w.)

Test units Wide-mouth glass flasks (500 mL) with screw caps permitting air exchange. Once a week, the amount of moisture loss was determined and adjusted to the required range of 40 – 50% WHC

4. Environmental conditions The test was performed in darkness in a climatic room.

Temperature nominal: 20 ± 2°C; actual: 19.7 – 21.5 °C

Soil moisture/Water content actual: 16.43 – 17.16 g/100 g soil d.w. (equivalent to 42.40 – 44.26% of the soil WHC)

pH actual: 5.8 – 6.1

B. STUDY DESIGN AND METHODS

1. In-life dates 13th May 2020 to 24th June 2020

2. Experimental conditions

Test design

Nitrogen transformation (NO_3-nitrogen production) in test item treated soil at two soil concentrations was compared to non-treated soil, using soil enriched with lucerne meal. NH_4-nitrogen, NO_3-nitrogen and NO_2-nitrogen were determined at 0, 7, 14, 28 and 42 days after treatment. Based on the analysed contents, nitrate formation rates were calculated.

Number of replicates per treatment

Three replicates per treatment and control units were set up.

Test concentrations

ADM.03503.F.1.A was tested at soil concentrations of 0.350 mg product/kg soil d.w. (low concentration) and 3.50 mg product/kg soil d.w. (high concentration). A control treatment was tested in parallel.

Treatment/Application

In order to obtain the target concentrations of 0.350 and 3.50 mg product/kg soil d.w., 175.0 mg product were weighed and filled up to a final volume of 100 mL with deionised water (1.75 mg/mL stock solution). The stock solution was diluted with deionised water with dilution factors of 100 and 10 resulting in application solution concentrations of 0.0175 and 0.1750 mg/mL, respectively. Test item was mixed with deionized water (4.00 mL application solution each) and the test solution was subsequently mixed with the soil by means of a hand stirrer. Water (8.46 mL) was added to achieve a water content of approximately 45% of WHC (amount of wet soil per test vessel: 222.42 g, equivalent to 200 g soil d.w.).

3. Sampling and measurements

Soil samples (10 g soil d.w./replicate) were taken at 3 hours, 7, 14, 28 and 42 days after application and NH_4-N, NO_3-N and NO_2-N contents were determined.

Soil was extracted by adding 1 M KCl solution (50 mL) and mixing on a rotator at 150 rpm for 60 minutes. The mixtures were centrifuged and stored deep-frozen prior to analysis at -20 ± 5°C. For the quantitative determination of the mineralized part of nitrogen the calibrated autoanalyzer (continuous flow analysis system) produced by SEAL Analytical was used. The autoanalyzer was calibrated before each measurement series by establishing a calibration curve.

The test temperature was recorded throughout the test. Water content of soils was determined at test start and adjusted once a week to the required range of 40 – 50 % of WHC. The pH was measured at test start and at the sampling on day 28 and 42, respectively.

4. Calculation of toxicity

The mean nitrogen-content, standard deviation and coefficient of variation were calculated for each treatment group and sampling date. Furthermore, the nitrogen transformation rate per time interval and the nitrogen transformation rate per time interval per day (for days 0 – 7, 7 – 14, 14 – 28 and 28 – 42) are calculated for each treatment group. The % deviations in quantities of nitrogen formed between control and test item groups was determined.

5. Statistics

The statistical evaluation was performed by means of a 2-sided Student-t-test (for homogeneous variances at 5 % significance level).

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018).

II. RESULTS AND DISCUSSION

No adverse effects (trigger value of 25% deviation) of the test item on nitrogen transformation in soil were observed at both test concentrations (0.350 and 3.50 mg/kg soil d.w.) after 28 days (time interval 14 – 28 days). The extension of the test to 42 days, demonstrated no adverse effects of the test item on nitrogen transformation rate in soil in two consecutive intervals for both test concentrations compared to the control.

Results are summarized in the following table.

Table A 2.5.1-1: Effects of ADM.03503.F.1.A on nitrogen transformation in soil

Sampling date	Control		0.350 mg product/kg soil d.w.		3.50 mg product/kg soil d.w.	
	Mean Nitrate-N [mg/kg soil d.w.]	CV [%]	Mean Nitrate-N [mg/kg soil d.w.]	CV [%]	Mean Nitrate-N [mg/kg soil d.w.]	CV [%]
Day 0	21.5	6.3	21.3	3.1	20.7	8.4
Day 7	45.7	8.8	48.0	4.4	44.4	7.9
Day 14	50.2	5.0	52.1	5.3	50.3	2.9
Day 28	72.0	2.3	72.0	1.4	71.3	1.2
Day 42	82.2	2.6	80.9	0.4	80.1	2.6
Nitrate formation rates [mg product/kg dry soil/day] at different time intervals						
	Control		0.350 mg product/kg soil d.w.		3.50 mg product/kg soil d.w.	
Time interval [days]	Mean Nitrate-N [mg/kg soil d.w./day]		Mean Nitrate-N [mg/kg soil d.w./day]	[%] difference to controls [a)]	Mean Nitrate-N [mg/kg soil d.w./day]	[%] difference to controls [a)]
Day 0 – 7	3.46		3.82	+10.5	3.39	-1.9
Day 7 – 14	0.64		0.58	-10.4	0.84	+31.1
Day 14 – 28	1.56		1.42	-8.9	1.50	-3.7
Day 28 – 42	0.73		0.64	-11.8	0.63	-13.4

[a)] positive values = stimulating effect and negative values = inhibitory effect.
CV: coefficient of variation.
SD: standard deviation.

Validity criteria

The coefficient of variation in the controls was less than ± 15% for all test parameters and sampling times (actual maximum: 8.8%). Therefore, the validity criterion of the test was fulfilled.

III. CONCLUSION

Based on the results of this study, ADM.03503.F.1.A caused no adverse effects (i.e. deviation from control < 25%) at the end of the 28-day incubation period at 0.350 and 3.50 mg product/kg soil d.w. The NOAEC (defined as less than 25% effect at ≤ 100 days) is therefore determined at 3.50 mg product/kg soil d.w. The validity criterion was fulfilled.

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

No screening data submitted. Reference is made to rate-response data provided under A 2.6.2.

A 2.6.2 KCP 10.6.2 Testing on non-target plants

A 2.6.2.1 Study 1: Effects on Seedling emergence and growth of ADM.03503.F.1.A

Comments of zRMS:	The study was conducted in line with OECD 208. Some minor deviations from the OECD were recorded but they didn't affect the outcome of the study: -The relative humidity in greenhouse chamber 1 for about 4 to 5 hours was < 45 % for all test species (outside the range of 70 % ± 25 % of the OECD). -The light intensity in the greenhouse chamber 1 was below 300 µmol/m²/s before sunrise and after sunset for 4 hours for onion and tomato. -The light intensity in the greenhouse chamber 1 was below 300 µmol/m²/s for 10 hours for onion, lettuce, sunflower and tomato. No visible phytotoxic effects were observed for all tested plant species at test end. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment. ER_{50} >1.193 L product/ha

Reference: KCP 10.6.2/01

Report: Effect of ADM.03503.F.1.A on seedling emergence and seedling growth of six non-target terrestrial plant species under greenhouse conditions, Friedemann, A., 2021a, 20 46 PSE 0004 (report number), 000105081 (sponsor report number)

Guideline(s): Yes, OECD 208 (2006)

Deviations: Deviations in test conditions were reported with light intensities below 300 µmol/m²/s on two occasions for 10 hours (for onion, lettuce, sunflowers and tomato) and 4 hours (before and after sunset for onion and tomato) which was attributed to a leaf shading the light sensor and wrong placing of the sensor, respectively. Relative humidity was below 45% for 4 to 5 hours due to wrong air conditioning for all species. These deviations did not have an impact on the study as all plants of each species were exposed to the same environmental conditions.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study): -

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: ADM.03503.F.1.A
(Fluxapyroxad 75 Prothioconazole 150 g/L EC)

Description: Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)

Lot/Batch #: 1162-230719-011

Purity: Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w)
Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w)
Density: 1.0792 g/cm³ (20 °C)

Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle control: deionised water No positive control required
3. Test plants	Dicotyledonous species: Lettuce – *Lactuca sativa* (Asteraceae) Sunflower – *Helianthus annuus* (Asteraceae) Tomato – *Solanum lycopersicum* (Solanaceae) Soybean – *Glycine max* (Fabaceae) Monocotyledonous species: Onion – *Allium cepa* (Liliaceae) Wheat – *Triticum aestivum* (Poaceae)
Source	Not stated
Test containers	Non-porous plastic pots with a diameter of 15 cm with bottom watering by pot saucers
4. Test soil	Soil type: Loamy sand Batch No: G 02/2019 TOC: 0.78% Salt content: 29.6 mg KCl/100 g dry soil pH: 6.0 Particle size distribution: 10.4 % (clay), 28.4% (silt) and 61.2% (sand) Max. water holding cap. (WHC): 26.9 g/100 g dry soil
Source	Gerichshain, Germany
Soil history	The soil was from a natural field and was not subjected to any pesticide or fertiliser treatment since at least 3 years
5. Environmental conditions	Test plants were cultivated in a greenhouse, with a concrete floor, under controlled climatic conditions.
Temperature	Nominal: 22 ± 10 °C; actual: 15.1 – 31.9 °C
Relative humidity	Nominal: 70 ± 25%; actual: 31.9 – 90.1 %
Photoperiod	Minimum 16 hours light and maximum 8 hours dark with artificial light intensity (daily mean) of 350 ± 50 µmol/m²/s (mean actual light intensity: 424 µmol/m²/s)

B. STUDY DESIGN AND METHODS

1. In-life dates 9th September 2020 to 9th October 2020 (experimental phase)

2. Experimental conditions

Test design

The inhibitory effect of ADM.03503.F.1.A on seedling emergence of six crop species, four dicotyledons (lettuce, sunflower, tomato and soybean) and two monocotyledons (onion and wheat), was investigated in a single rate study. Following the application, all plants were grown for 21 days. ADM.03503.F.1.A was applied pre-emergence to the soil surface. Assessments for phytotoxicity, seedling emergence and survival were carried out at weekly intervals (on days 7, 14 and 21) for all plants. At test termination, the plant

height and the plant dry weight of the plant biomass above ground per replicate were determined. A deionised water control was tested in parallel.

Number of replicates per treatment

Each treatment group consisted of five (onion and wheat), ten (lettuce and tomato) or 15 (sunflower and soybean) replicates, each with six, three and two seeds per replicate, respectively, for a total of 30 seeds per treatment.

Test concentrations

The test plants were treated with ADM.03503.F.1.A at a nominal application rate of 1.193 L product/ha. For each test species, a control receiving deionised water was tested in addition.

Treatment/Application

The spray solution was prepared by mixing 12.875 g test item to 2000 mL with deionised water, without addition of solubility mediators. The control group was treated with deionised water only.

The test item was prayed once onto the soil surface in an automatic application cabin at a spray volume equivalent to 200 L/ha. Before application of test item, the application system was calibrated three times with deionised water. Each application was made by an application run of the nozzle (Teejet 9502 EVS, nozzle pressure: 2.88 bar; distance between nozzle and average stand of plants: 40.0 cm; driving speed of nozzle: 2.25 km/h).

3. Sampling and measurements

To verify the active substance concentrations, the control and the test item solution were sampled directly after preparation and immediately before application. The concentration of prothioconazole and fluxapyroxad was analysed by HPLC method (with UV-Diode-Array detection). More details on the analytical method are given in Part B, Section 5.

The plants were observed for BBCH stage, seedling emergence, plant survival and phytotoxicity at 7, 14 and 21 days after 50% seedling emergence in the control group. Phytotoxicity was rated in % following the EPPO scale (0% = no injury or effect on plants; 20% = slight symptom(s); 40% = moderate symptom(s); 60% = severe symptom(s); 80% = symptom(s) on nearly the total plant; 100% = moribund plants).

At test termination, plants were cut directly above soil surface and plant height per plant and the plant dry weight per replicate were determined. For determination of plant dry weight, plants were dried in an oven to constant weight at 60 °C.

Test temperature, air humidity and illumination were recorded continuously throughout the test.

4. Calculation of toxicity

Mean and standard deviation of assessment data were calculated and rounded by Excel. The measurements and observations were compared to those of untreated control plants.

5. Statistics

A two-sample test was chosen to test the limit rate in wheat for emergence (Fisher`s Exact Binomial Test, ($\alpha = 0.05$, one-sided greater).

For statistical evaluation of metric data of plant dry weight in wheat the data were tested for normal distribution by Shapiro-Wilk´s test ($\alpha = 0.01$) and variance homogeneity by Levene´s test ($\alpha = 0.01$). Based on the outcome of the pre-testing sequences, the Student-t test for homogenous variances (one-sided smaller, $\alpha = 0.05$) was used.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018).

II. RESULTS AND DISCUSSION

A. VERIFICATION OF APPLICATION RATE

The analysis of the active substances in the test item solution yielded an analytical recovery of 89.1% of nominal for both, prothioconazole and fluxapyroxad. In the applied control solutions (only deionised water), no active substance of the test item could be detected.

B. SEEDLING EMERGENCE AND PLANT SURVIVAL

No effect on seedling emergence and plant survival could be detected after pre-emergence application at a rate of 1.193 L product/ha compared to the control.

Results on seedling emergence and plant survival are summarized in the following table.

Table A 2.6.2.1-1: Effect of ADM.03503.F.1.A on seedling emergence and plant survival after 21 days

Plant species	Application rate [L product/h]	Emergence [%]	Survival of emerged plants [%]
Onion	Control	-	100
Allium cepa	1.193	100	100
Wheat	Control	-	100
Triticum aestivum	1.193	97	100
Lettuce	Control	-	100
Lactuca sativa	1.193	100	100
Sunflower	Control	-	100
Helianthus annuus	1.193	100	100
Tomato	Control	-	100
Solanum lycopersicum	1.193	104	100
Soybean	Control	-	100
Glycine max	1.193	100	100

C. PHYTOTOXICITY

The pre-emergence application at a rate of 1.193 product/ha caused no visible phytotoxic effects 21 days after emergence on tested plant species.

D. PLANT HEIGHT

No significant reduction in plant height was determined for all plant species after pre-emergence application at a rate of 1.193 L product/ha compared to the control.

Results on plant height are summarized in the following table.

Table A 2.6.2.1-2: Effect of ADM.03503.F.1.A on plant height 21 days after emergence

Plant species	Application rate [L product/h]	Mean plant height ± SD [cm]	Reduction compared to the control [%] [a)]
Onion	Control	21.8 ± 1.8	-
Allium cepa	1.193	22.5 ± 2.1	-2.9
Wheat	Control	31.7 ± 2.2	-
Triticum aestivum	1.193	31.7 ± 2.6	-0.2
Lettuce	Control	13.9 ± 1.0	-
Lactuca sativa	1.193	14.0 ± 0.7	-0.5
Sunflower	Control	55.2 ± 4.9	-
Helianthus annuus	1.193	55.8 ± 3.9	-1.2
Tomato	Control	15.8 ± 0.8	-
Solanum lycopersicum	1.193	16.5 ± 0.9	-4.4
Soybean	Control	39.7 ± 3.0	-
Glycine max	1.193	40.3 ± 2.4	-1.5

[a)] negative values indicate an increase compared to the control.
SD: standard deviation.

E. BIOMASS (PLANT DRY WEIGHT)

No significant reduction in plant dry weight was determined for all plant species after pre-emergence application at a rate of 1.193 L product/ha compared to the control.

Results on plant dry weight are summarized in the following table.

Table A 2.6.2.1-3: Effect of ADM.03503.F.1.A on plant dry weight 21 days after emergence

Plant species	Application rate [L product/h]	Mean plant dry weight ± SD [cm]	Reduction compared to the control [%] [a)]
Onion	Control	0.261 ± 0.0	-
Allium cepa	1.193	0.274 ± 0.1	-4.7
Wheat	Control	4.155 ± 0.4	-
Triticum aestivum	1.193	4.128 ± 0.5	0.6
Lettuce	Control	2.650 ± 0.5	-
Lactuca sativa	1.193	3.19.1 ± 0.4	-20.4
Sunflower	Control	16.235 ± 1.3	-
Helianthus annuus	1.193	16.479 ± 2.0	-1.5
Tomato	Control	3.752 ± 0.8	-
Solanum lycopersicum	1.193	4.409 ± 0.6	-17.5
Soybean	Control	7.676 ± 0.6	-
Glycine max	1.193	8.232 ± 0.4	-7.2

[a)] negative values indicate an increase compared to the control.
SD: standard deviation.

Validity criteria

The control seedling emergence was ≥ 70% (actual: 83 – 100%). Control mortality was ≤ 10% (actual: 0%) and plants remained healthy throughout the complete test period. Furthermore, environmental conditions and growing media for a particular species were identical. Therefore, the validity criteria of the guideline were met.

III. CONCLUSION

Based on the results of this seedling emergence and growth test with ADM.03503.F.1.A, no effect on seedling emergence, plant survival, plant height, plant dry weight and phytotoxicity could be detected after pre-emergence application of 1.193 L product/ha to all tested plant species. All validity criteria in the study were fulfilled.

A 2.6.2.2 Study 2: Effects on vegetative vigour of ADM.03503.F.1.A

Comments of zRMS:	The study was conducted in line with OECD 227. A minor deviation from the OECD was recorded but it didn't affect the outcome of the study: - The relative humidity in greenhouse chamber 1 for about 4 to 5 hours was < 45 % for all test species. (Outside the range of 70 % ± 25 % of the OECD). Some visible phytotoxic effects were detected in sunflower, tomato and soybean; however, effects did not exceed the EPPO rating value of 10%. All the validity criteria were met and the study is considered acceptable with the following endpoints relevant for the risk assessment. ER_{50} >1.193 L product/ha

Reference:	KCP 10.6.2/02
Report	Effects of ADM.03503.F.1.A on vegetative vigour of six non-target terrestrial plant species under greenhouse conditions, Friedemann, A., 2021b, 20 46 PVV 0006 (report number), 000105082 (sponsor report number)
Guideline(s):	Yes, OECD 227 (2006)
Deviations:	Relative humidity was below 45% for 4 to 5 hours due to wrong air conditioning for all species. This deviation did not have an impact on the study as all plants of each species were exposed to the same environmental conditions.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material	ADM.03503.F.1.A (Fluxapyroxad 75 Prothioconazole 150 g/L EC)
Description	Transparent yellowish to brownish liquid, EC (Emulsifiable Concentrate)
Lot/Batch #	1162-230719-011
Purity	Fluxapyroxad: 75 g/L nominal; 77.4 g/L analysed (7.17% w/w) Prothioconazole: 150 g/L nominal; 148 g/L analysed (13.7% w/w) Density: 1.0792 g/cm³ (20 °C)
Stability of test material	Stable under storage conditions (dark and dry conditions) Expiry date: 5th September 2021
2. Vehicle and/or positive control	Vehicle control: deionised water No positive control required
3. Test plants	Dicotyledonous species:

	Lettuce	*Lactuca sativa* (Asteraceae)
	Sunflower	*Helianthus annuus* (Asteraceae)
	Tomato	*Solanum lycopersicum* (Solanaceae)
	Soybean	*Glycine max* (Fabaceae)
	Monocotyledonous species:	
	Onion	*Allium cepa* (Liliaceae)
	Wheat	*Triticum aestivum* (Poaceae)
Source	Not stated	
Test containers	Non-porous plastic pots with a diameter of 15 cm with bottom watering by pot saucers	
4. Test soil	Soil type	Loamy sand
	Batch No	G 02/2019
	TOC	0.78%
	Salt content	29.6 mg KCl/100 g dry soil
	pH	6.0
	Particle size distribution	10.4 % (clay), 28.4% (silt) and 61.2% (sand)
	Max. water holding cap. (WHC)	26.9 g/100 g dry soil
Source	Gerichshain, Germany	
Soil history	The soil was from a natural field and was not subjected to any pesticide or fertiliser treatment since at least 3 years	
5. Environmental conditions	Test plants were cultivated in a greenhouse, with a concrete floor, under controlled climatic conditions	
Temperature	Nominal: 22 ± 10 °C; actual: 15.1 – 31.9 °C	
Relative humidity	Nominal: 70 ± 25%; actual: 31.9 – 90.1%	
Photoperiod	Minimum 16 hours light and maximum 8 hours dark with artificial light intensity (daily mean) of 350 ± 50 µmol/m^2/s (mean actual light intensity: 450 µmol/m^2/s)	

B. STUDY DESIGN AND METHODS

1. In-life dates 9th September 2020 to 5th October 2020 (experimental phase)

2. Experimental conditions

Test design

The inhibitory effect of ADM.03503.F.1.A on vegetative vigour of six crop species, four dicotyledons (lettuce, sunflower, tomato and soybean) and two monocotyledons (onion and wheat), was investigated in a single rate study during 21 days. ADM.03503.F.1.A was applied at plant growth stage (BBCH) 12 – 14. The test plants were assessed for mortality and phytotoxicity symptoms on days 7, 14 and 21. Furthermore, the plant shoot dry weight and shoot height were determined at test termination. A deionised water control was tested in parallel.

Number of replicates per treatment

Each treatment group consisted of a total of 32 (onion and wheat) or 30 (lettuce, sunflower, tomato and soybean) plants with eight or 15 replicates, each with four or two plants per pot after thinning, respectively.

Test concentrations

The test plants were treated with ADM.03503.F.1.A at nominal application rate of 1.193 L product/ha. For each test species, a control receiving deionised water was tested in addition.

Treatment/Application

The spray solution was prepared by mixing 12.875 g test item to 2000 mL with deionised water, without addition of solubility mediators. The control group was treated with deionised water only.

The test item was prayed once onto the plants at BBCH stage 12 – 14 in an automatic application cabin at a spray volume equivalent to 200 L/ha. Before application of test item, the application system was calibrated three times with deionised water. Each application was made by an application run of the nozzle (Teejet 9502 EVS; nozzle pressure: 2.88 bar; distance between nozzle and average stand of plants: 40.0 cm; driving speed of nozzle: 2.25 km/h).

3. Sampling and measurements

To verify the active substance concentrations, the control and the test item solution were sampled directly after preparation and immediately before application. The concentration of prothioconazole and fluxapyroxad was analysed by HPLC method (with UV-Diode-Array detection). More details on the analytical method are given in Part B, Section 5.

The plants were observed for BBCH stage, plant survival and phytotoxicity at 7, 14 and 21 days after-treatment. Phytotoxicity was rated in % following the EPPO scale (0% = no injury or effect on plants; 20% = slight symptom(s); 40% = moderate symptom(s); 60% = severe symptom(s); 80% = symptom(s) on nearly the total plant; 100% = moribund plants).

At test termination, plants were cut directly above soil surface and plant height per plant and the plant dry weight per replicate were determined. For determination of plant dry weight, plants were dried in an oven to constant weight at 60 °C.

Test temperature, air humidity and illumination were recorded continuously throughout the test.

4. Calculation of toxicity

Mean and standard deviation of assessment data were calculated and rounded by Excel. The measurements and observations were compared to those of untreated control plants.

5. Statistics

For statistical evaluation of metric data of plant length and plant dry weight the data were tested for normal distribution by Shapiro-Wilk´s test ($\alpha = 0.01$) and variance homogeneity by Levene´s test ($\alpha = 0.01$). Based on the outcome of the pre-testing sequences, the Student test for homogenous variances (one-sided smaller, $\alpha = 0.05$) was used.

For statistical evaluation of the visual injury, the rank sum test Two-sample Mann-Whitney-U-test procedure (one-sided greater, $\alpha=0.05$) was used.

The statistical analysis was performed with the software ToxRat Professional 3.3.0 (2018).

II. RESULTS AND DISCUSSION

A. VERIFICATION OF APPLICATION RATE

The analysis of the active substances in the item solution yielded an analytical recovery of 93.1 and 93.0% of nominal for prothioconazole and fluxapyroxad, respectively. In the applied control solutions (only deionised water), no active substance of the test item could be detected.

B. PLANT SURVIVAL

Survival in all plants tested was 100% at the end of the test.

C. PHYTOTOXICITY

The post-emergence application at BBCH stage 12 – 14 at a rate of 1.193 product/ha caused no visible phytotoxic effects after 21 days on onion, wheat and lettuce.

Necrosis and chlorosis were observed in sunflower, tomato and soybean and deformations could be found in tomato and soybean. The average phytotoxicity score (EPPO rating) was ≤ 10%.

Results on phytotoxicity are summarized in the following table.

Table A 2.6.2.2-1: Effect of ADM.03503.F.1.A on phytotoxicity after 21 days

Plant species	Application rate [L product/h]	Phytotoxicity				EPPO rating [%]
		Chlorosis	Necrosis	Deformation	Stunting	
Onion	Control	-	-	-	-	0.0
Allium cepa	1.193	-	-	-	-	0.0
Wheat	Control	-	-	-	-	0.0
Triticum aestivum	1.193	-	-	-	-	0.0
Lettuce	Control	-	-	-	-	0.0
Lactuca sativa	1.193	-	-	-	-	0.0
Sunflower	Control	-	-	-	-	0.0
Helianthus annuus	1.193	x	x	-	-	10.0 *
Tomato	Control	-	-	-	-	0.0
Solanum lycopersicum	1.193	x	x	x	-	10.0 *
Soybean	Control	-	-	-	-	0.0
Glycine max	1.193	x	x	x	-	10.0 *

* Significant difference between control and treatment (Two-sample Mann-Whitney U-test Procedure, one-sided greater, $\alpha = 0.05$).

D. PLANT HEIGHT

No significant reduction in plant height was determined for all plant species after application at BBCH stage 12 – 14 at a rate of 1.193 L product/ha compared to the control.

Results on plant height are summarized in the following table.

Table A 2.6.2.2-2: Effect of ADM.03503.F.1.A on plant height after 21 days

Plant species	Application rate [L product/h]	Mean plant height ± SD [cm]	Reduction compared to the control [%] [a)]
Onion	Control	48.2 ± 3.1	-
Allium cepa	1.193	49.8 ± 5.0	-3.4
Wheat	Control	35.0 ± 1.3	-
Triticum aestivum	1.193	35.0 ± 0.9	0.0
Lettuce	Control	22.3 ± 4.1	-
Lactuca sativa	1.193	22.0 ± 5.2	1.0
Sunflower	Control	57.1 ± 3.8	-
Helianthus annuus	1.193	57.1 ± 6.8	0.0
Tomato	Control	43.4 ± 2.4	-
Solanum lycopersicum	1.193	42.2 ± 2.2	2.8
Soybean	Control	129.7 ± 7.4	-
Glycine max	1.193	123.5 ± 14.9	4.8

[a)] negative values indicate an increase compared to the control.
SD: standard deviation.

E. BIOMASS (PLANT DRY WEIGHT)

No significant reduction in plant dry weight was determined for all plant species after application at BBCH stage 12 – 14 at a rate of 1.193 L product/ha compared to the control.

Results on plant dry weight are summarized in the following table.

Table A 2.6.2.2-3: Effect of ADM.03503.F.1.A on plant dry weight after 21 days

Plant species	Application rate [L product/h]	Mean plant dry weight ± SD [cm]	Reduction compared to the control [%] [a)]
Onion	Control	10.027 ± 1.1	-
Allium cepa	1.193	10.653 ± 1.8	-6.2
Wheat	Control	6.796 ± 0.4	-
Triticum aestivum	1.193	7.060 ± 0.1	-3.9
Lettuce	Control	5.746 ± 0.4	-
Lactuca sativa	1.193	5.945 ± 0.7	-3.5
Sunflower	Control	21.587 ± 2.2	-
Helianthus annuus	1.193	22.091 ± 1.3	-2.3
Tomato	Control	14.437 ± 1.3	-
Solanum lycopersicum	1.193	13.818 ± 1.8	4.3
Soybean	Control	25.230 ± 1.4	-
Glycine max	1.193	24.448 ± 1.5	3.1

[a)] negative values indicate an increase compared to the control.
SD: standard deviation.

Validity criteria

The control seedling emergence was ≥ 70% (actual: 87 – 97%). Control mortality was ≤ 10% (actual: 0%) and plants remained healthy throughout the complete test period. Furthermore, environmental conditions and growing media for a particular species were identical. Therefore, the validity criteria of the guideline were met.

III. CONCLUSION

Based on the results of this seedling emergence and growth test with ADM.03503.F.1.A, no effect on plant survival, plant height, plant dry weight could be detected after application at BBCH stage 12 – 14 of 1.193 L product/ha to all tested plant species. Visible phytotoxic effects ≤ 10% were determined in sunflower, tomato and soybean. All validity criteria in the study were fulfilled.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No additional data submitted.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No additional data submitted.

A 2.8 KCP 10.8 Monitoring data

No additional data submitted.