FINAL REGISTRATION REPORT

Part B

Section 9

Ecotoxicology

Detailed summary of the risk assessment

Product code: SHA 7216 A

Product name: CIAZ

Chemical active substances:

Boscalid, 233 g/L

Difenoconazole, 66 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

Applicant: SHARDA Cropchem España S.L.

Submission date: August 2021

MS Finalisation date: February 2022, December 2022

Version history

When	What
February 2022	Finalization of the assessment by zRMS
December 2022	Final Version of the report after commenting perood

Table of Contents

9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 9.1-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: develop-mental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-[illegible]	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	CEU	**Winter wheat**	F	*Septoria spp.*	Foliar Spray	BBCH 30-59	a) 2 b) 2	14	a) 1.5 b) 3	a) 0.35 boscalid + 0.1 difenoconazole b) 0.7 boscalid + 0.2 difenoconazole	200-400	-								
2	CEU	**Winter wheat**	F	*Puccinia spp.*	Foliar Spray	BBCH 30-59	a) 2 b) 2	14	a) 1.5 b) 3	a) 0.35 boscalid + 0.1 difenoconazole b) 0.7 boscalid + 0.2 difenoconazole	200-400	-								
3	CEU	**Winter wheat**	F	*Fusarium spp.*	Foliar Spray	BBCH 39-59	a) 2 b) 2	14	a) 1.5 b) 3	a) 0.35 boscalid + 0.1 difenoconazole b) 0.7 boscalid + 0.2 difenoconazole	200-400	-								

* 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

Explanation for column 15 – 21 “Conclusions”

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

Remarks table:

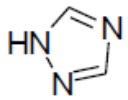
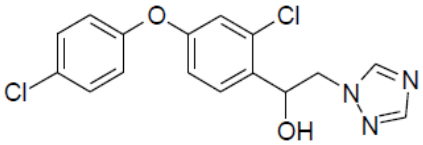
(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field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

9.1.1 Overall conclusions

zRMS comment:

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. The changes are introduced directly as text in blue.

9.1.1.1 Table 9.1-4 Metabolites of Difenoconazole

9.1.1.2 Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
1,2,4-triazole (CGA 71019)		69.065 g/mol	Soil: 23.4% Water/sediment: 9.6% (worst case assumption calculated by RMS)	Yes, for: - aquatic organisms - earthworms and other non-target soil organisms - soil microbial activity
Difenoconazole-alcohol (CGA 205375)		350 g/mol	Soil: 11.9% Water/sediment: 11.6%	Yes, for: - aquatic organisms - earthworms and other non-target soil organisms - soil microbial activity

9.1.1.3 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)

- Birds

According to the screening and first-tier assessment for winter wheat, all the TERa and TERlt values for active substances Boscalid and Difenoconazole are greater than the Annex VI trigger of 10 and 5, respectively, indicating that CIAZ presents no unacceptable cute and long-term risk to birds accord-ing to the intended uses on winter wheat. In addition, no unacceptable acute and long-term risk is ex-pected due to combined exposure.
Difenoconazole has been shown to have the potential for bioaccumulation, however, there is no risk to earthworm-eating and fish-eating birds according to the intended uses of CIAZ.

- Mammals

According to the screening and first-tier assessment for winter wheat, all the TERa and TERlt values for active sub-stances Boscalid and Difenoconazole are greater than the Annex VI trigger of 10 and 5, respectively, indicating that CIAZ presents no unacceptable acute and long-term risk to mammals according to the intended uses on winter wheat. In addition, no unacceptable acute and long-term risk is expected due to combined exposure.

Difenoconazole have been shown to have the potential for bioaccumulation, however, there is no risk to earthworms-eating and fish-eating mammals according to the intended uses of CIAZ.

9.1.1.4 Effects on aquatic organisms (KCP 10.2)

Boscalid

For **metabolites** 1,2,4-triazole and CGA 205375 step 1 calculations reached an acceptable risk for aquatic organisms in all crops.

Difenoconazole

For the intended uses, calculated PEC/RAC ratios did not indicate a acceptable risk for the most sensitive group of aquatic organisms (~~risk for invertebrate as characterized by a NOEC for *Daphnia magna* of 5.6 µg/L in connection with an assessment factor of 10).~~
The PECsw/RAC ratio for the most sensitive organism – long term exposure for fish for difenconazole for scenarios D1 (ditch and stream), D2 (ditch and stream) and D3 (ditch), D4 (stream), D5 (stream), D6 (ditch) and R1 and R3 stream scenario on winter cereals indicated an acceptable risk for aquatic organisms with 5-meter buffer zone to surface water bodies, but for R4 stream scenario on winter cereals an acceptable risk for aquatic organisms 5-meter vegetative buffer zone, calculated by FOCUS STEP 4 programme is identified.
For remained scenarios the risk for difenconazole is acceptable already with PEC_{sw} FOCUS STEP 3 calculations. Taking into account the PEC_{sed}/RAC ratio for Cironomus riparius for difenconazole for R1 (stream) and R4 (stream) scenarios on winter cereals an acceptable risk for aquatic organisms with **5-meter** vegetative buffer zone, calculated by FOCUS STEP 4 programme is indicated.
The PEC_{sw}/RAC ratio for boscalid for all scenarios passed the trigger below 1 with PEC_{sw} STEP1-3 calculations on winter cereals.
The PEC/RAC ratio for metabolites of difenconazole and boscalid for all scenarios passed the trigger below 1 with PEC_{sw} STEP 1-2 calculations on winter cereals.

The final risk mitigation measures for aquatic organism should be decided at MSs level.

CIAZ

Regarding the formulation, calculated PEC/RAC ratios indicated an acceptable risk for the most sensitive group of aquatic organisms (risk for acute fish as characterised by a LC_{50} for *Oncorhynchus mykiss* of 14030 µg f.p./L in connection with an assessment factor of 100). No mitigation measures will be needed.

In addition, a risk assessment for the combinations of a.s. in the formulation was performed and no unacceptable risk was obtained.

Conclusions

Winter cereals – Spe 3: *To protect aquatic organisms respect an unsprayed vegetative buffer zone of 5m to surface water bodies.*

9.1.1.5 Effects on bees (KCP 10.3.1)

First-tier assessments indicate that no unacceptable risk for bees exposed to CIAZ is expected according to the intended uses.
According to EU Reg. 284 /2009, the chronic toxicity test for adult bees, the chronic test for larvae should be provided for authorization of plant protection product. However, the final decision of the date of sub-

mission these studies by the Applicant should be considered at MSs level.

9.1.1.6 Effects on arthropods other than bees (KCP 10.3.2)

No in-field and off-field risk to non-target arthropods is expected after the application of CIAZ according to the proposed GAP.

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

The acute and chronic TER values for Boscalid and Difenoconazole were above the relevant Annex VI trigger of 10 and 5, respectively. Therefore, it is concluded that the active substances do not pose an acute and long-term risk to earthworms and other soil macro- and mesofauna when applied according to the proposed uses rates.

Morevover, an application of CIAZ in respect of the GAP should not represent an acute and long term risk to earthworm and the other soil meso/microfauna.

Risk assessment conducted with relevant PEC_{soil} for the active substances Boscalid and Difenoconazole indicate a low risk to soil microorganisms when applied according to the proposed use rates.

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

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for CIAZ shows that the Annex VI trigger value of 5 is not exceeded, indicating that CIAZ poses a low risk to non-target plants when applied according to the proposed use rates.

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

Not relevant.

9.1.2 Grouping of intended uses for risk assessment

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

Table 9.1-2: Critical use pattern of CIAZ grouped according to criterion

Grouping according to criterion			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
Winter cereals	Winter wheat	application rate	Same application rate: 2 x 1.5 L f.p./ha (0.35 L boscalid/ha + 0.1 difenoconazole L/ha)

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 CIAZ is indicated in the table.

Table 9.1-3 Metabolites of Boscalid

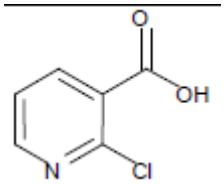
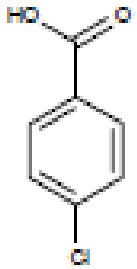
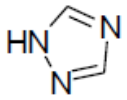
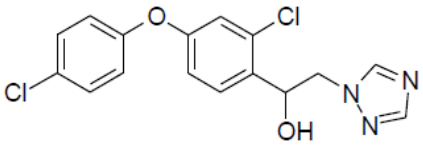
Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
M510F47		157.6 g/mol	Soil: anaerobic conditions. 2.6 % after 3 d, 6 % after 62 d, 5.9 % after 90 d, 6.7 % after 120 d	No
M510F64		156.56 g/mol	Sediment: under outdoor conditions. 7.3 % after 7 d 9 % after 14 d 9.4 % after 30 d 1.9 % after 120 d	No

Table 9.1-4 Metabolites of Difenoconazole

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
1,2,4-triazole (CGA 71019)		69.065 g/mol	Soil: 23.4% Water/sediment: 9.6% (worst case assumption calculated by RMS)	Yes, for: - aquatic organisms - earthworms and other non-target soil organisms - soil microbial activity
Difenoconazole-alcohol (CGA 205375)		350 g/mol	Soil: 11.9% Water/sediment: 11.6%	Yes, for: - aquatic organisms - earthworms and other non-target soil organisms - soil microbial activity

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with Boscalid and Difenoconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of CIAZ were not evaluated as part of the EU assessment of Boscalid and Difenoconazole.

However, the provision of further data on the CIAZ is not considered essential, because active substance data on toxicity to birds can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Bobwhite quail *(Colinus virginianus)*	Boscalid	Oral Acute	**LD_{50} > 2000 mg/kg bw**	SANCO/3919/2007 – rev. 5
Anas platyrhynchos *Colinus virginianus*	Boscalid	Dietary Short-term	LC_{50} > 5000 mg/kg feed	SANCO/3919/2007 – rev. 5
Colinus virginianus	Boscalid	Dietary Reproductive toxicity	**NOEL = 24.1 mg/kg bw/d**	SANCO/3919/2007 – rev. 5
Japanese quail	Difenoconazole	Oral Acute	LD_{50} > 2000 mg/kg bw	EFSA Journal 2011;9(1):1967
~~Mallard duck *(Anas platyrhynchos)*~~ Bobwhite quail *(Colinus virginianus*	Difenoconazole	Dietary Short-term	**LC_{50} > 349 mg/kg bw/d**	EFSA Journal 2011;9(1):1967
Mallard Duck	metabolite CGA131013	Dietary 5 d Short-term	LC_{50} >1342 mg/kg bw/day	EFSA J 2011; 9(1):1967
Mallard duck *(Anas platyrhynchos)*	Difenoconazole	Dietary Reproductive toxicity	**NOEL = 9.71 mg/kg bw/d**	EFSA Journal 2011;9(1):1967

9.2.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints.

Selection of the endpoints used for acute risk assessment:
According to the Guidance EFSA/2009/1438, where the dietary LC_{50} is lower than the acute LD_{50}, the dietary value should be used in the acute risk assessment. Therefore, LC_{50} > 349 mg/kg bw/d was used in the acute risk assessment as worst-case for Difenoconazole.

The Applicant considers that the acute risk assessment on birds for the metabolite CGA131013 is covered by the acute risk assessment of the parental Difenoconazole.

9.2.2 Risk assessment for spray applications

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

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

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

Table 9.2-2: First-tier assessment of the acute and long-term/reproductive risk for birds due to the use of CIAZ in winter wheat

Intended use	**Winter wheat**				
Active substance/product	Boscalid				
Application rate (g/ha)	2 x 350				
Acute toxicity (mg/kg bw)	> 2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening – Cereals	Small omnivorous bird	158.8	1.2	66.70	30.0
Reprod. toxicity (mg/kg bw/d)	24.1				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small omnivorous bird 'lark' Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	1.4 x 0.53	0.86	28.1
Cereals BBCH 30-39	Small omnivorous bird 'lark' Combination (invertebrates with interception) 25% crop leaves 25% weed sedds 50% ground arthropods	5.4	1.4 x 0.53	1.40	17.2
Cereals Late season- Seed heads	Small granivorous/insectivorous bird "bunting"	4.7	1.4 x 0.53	1.22	19.7
Active substance/product	Difenoconazole				
Application rate (g/ha)	2 x 100				
Acute toxicity (mg/kg bw)	> 349				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening - Cereals	Small omnivorous bird	158.8	1.2	19.06	18.3
Reprod. toxicity (mg/kg bw/d)	9.71				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 40	Small omnivorous bird 'lark' Combination (invertebrates with interception). 25% crop leaves 25% weed seeds 50% ground arthropods	3.3	1.4 x 0.53	0.24	39.7
Cereals BBCH 30-39	Small omnivorous bird 'lark' Combination (invertebrates with interception). 25% crop leaves	5.4	1.4 x 0.53	0.40	24.2

	25% weed sedds 50% ground arthropods				
Cereals Late season- Seed heads	Small granivorous/insectivorous bird "bunting". Grains/ear. 100% cereal seeds	~~12.5~~ 4.7	1.4 x 0.53	~~0.93~~ 0.35	~~10.5~~ 27.8

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.

Risk Assessment for combined exposure

The simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

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

With:

$X(\text{a.s.}_i)$ = fraction of each a.s. in the mixture

$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for each a.s.

Acute risks from combined exposure

The active substance content of the formulation CIAZ addressed in this dossier is 23.3 % Boscalid and 6.6 % Difenoconazole, making up a total of 299 g a.s./L product. According to GAP, the maximum application rate is 1.5 L product/ha, therefore, an application rate of 448.5 g a.s./ha was considered in the assessment.

Table 9.2-3 shows the calculation of the predicted LD_{50} (mix) of Boscalid and Difenoconazole when mixed in these proportions (step 1 in Appendix B to the EFSA GD 2009).

Table 9.2-3: Avian LD_{50} (mix) for boscalid and Difenoconazole when combined as CIAZ (step 1 in EFSA GD 2009, Appendix B)

	Boscalid	Difenoconazole
Content in the formulation CIAZ	23.3%	6.6%
Fraction in the a.s. mixture	0.7793	0.2207
LD_{50} of a.s. [mg/kg bw]	>2000	>349
Fraction / LD_{50}	0.000390	0.000632
Sum	0.001022	
1/ sum = predicted LD_{50} (mix)	978.37 mg mix/kg bw	

It is obvious from the comparison of the (low) acute oral toxicity of the active substances, and their relative proportions of the formulated product CIAZ.

Table 9.2-4: Avian "tox per fraction" for the CIAZ (step 1 in EFSA GD 2009, Appendix B)

	Boscalid	Difenoconazole	"mix"
Content in the formulation CIAZ	23.3%	6.6%	29.9 %
Fraction in mixture	0.7793	0.2207	1.0
LD_{50} (mg/kg bw)	>2000	>349	978.37
Tox per fraction	2566.52	1581.08	978.37
Contribution to predicted toxicity	38.12 %	61.88 %	

Boscalid contributes to 38.12 % to mixture toxicity, while the Difenoconazole have an impact on the predicted risk of 61.88 %, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.2-5: First-tier assessment of the acute risk for birds due to the use of CIAZ in winter wheat

Intended use	Winter wheat				
Active substance/product	CIAZ				
Application rate (g/ha)	2 x 448.5				
LD_{50} (mix) (mg/kg bw)	978.37				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening - Cereal	Small omnivorous bird	158.8	1.2	85.47	11.4

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.

According to reults, no unacceptable acute risk is obtained in winter wheat acording to the proposed GAP for combined exposure.

Regarding chronic risk assessment, the Applicant considers that, according to EFSA/2009/1438, the calculation of a combined toxicity is not applicable to the risk assessment for reproductive effect. Due to differences in evaluated endpoints and the dependency of the derived NOEL of the test design, any calculated TERmix value can only be used for illustrating purposes. Hence, in the case of an unacceptable TERmix, it has to be discussed if the results of the toxicity studies present any evidence for a possible concentration additivity of the effects and risks.

In addition, the combined toxicological effect of these two active substances has not been investigated with regard to repeated dose toxicity. Possibly, the combined exposure to these active substances may lead to a different toxicological profile than the profile(s) based on the individual substances.

Despite all of this, the reproductive risk from combined exposure has been performed by the Applicant:

Reproductive risks from combined exposure

Table 9.2-6: Avian NOEL (mix) for Boscalid and Difenoconazole when combined as CIAZ(step 1 in EFSA GD 2009, Appendix B)

	Boscalid	Difenoconazole
Content in the formulation CIAZ	23.3%	6.6%
Fraction in the a.s. mixture	0.7793	0.2207
NOEL of a.s. [mg/kg bw]	24.1	9.71
Fraction / NOEL	0.032335	0.022733
Sum	0.055067	
1/ sum = predicted NOEL (mix)	18.16 mg mix/kg bw	

It is obvious from the comparison of the (low) long- term oral toxicity of the active substances, and their relative proportions of the formulated product CIAZ, that any risk of long-term effects would very much be similar to toxicity of both active substances.

Table 9.2-7: Avian "tox per fraction" for the CIAZ (step 1 in EFSA GD 2009, Appendix B)

	Boscalid	Difenoconazole	"mix"
Content in the formulation CIAZ	23.3%	6.6%	29.9 %
Fraction in mixture	0.7793	0.2207	1.0
NOEL (mg/kg bw)	24.1	9.71	18.16
Tox per fraction	30.93	43.99	18.16
Contribution to predicted toxicity	58.72%	41.28%	

Boscalid contributes to 58.72 % to mixture toxicity, while the Difenoconazole have an impact on the predicted risk of 41.28 %, therefore, surrogate NOEL was used in the long-term risk assessment.

Table 9.2-8: First-tier assessment of the long-term risk for birds due to the use of CIAZ in winter wheat

Intended use	Winter wheat				
Active substance/product	CIAZ				
Application rate (g/ha)	2 x 448.5				
NOEL (mix) (mg/kg bw)	18.16				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**$SV_{90\ m}$**	**$MAF_{90\ m}$**	**$DDD_{90\ m}$ (mg/kg bw/d)**	**$TER_{a\ lt}$**
Cereals BBCH 30-39	Small omnivorous bird 'lark' Combination (invertebrates with interception) 25% crop leaves 25% weed sedds 50% ground arthropods	5.4	1.4 x 0.53	1.80	10.1
Cereals BBCH ≥ 40	Small omnivorous bird 'lark' Combination (invertebrates with interception) 25% crop leaves 25% weed seeds 50% ground	3.3	1.4 x 0.53	1.10	16.5

	arthropods				
Cereals Late season- Seed heads	Small granivorous/insectivorous bird "bunting"	12.5	1.4 x 0.53	4.16	**4.4**

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.

According to results, a unacceptable reproductive risk from combined exposure is obtained in winter wheat for small granivorous/insectivorous bird "bunting". Therefore, a refinement of the risk is needed.

COMBINED EXPOSURE REFINEMENT

The refinement of combitox long risk assessment was provided taking into account the refined PT value of 0.87 for yellowhammer – species recommended in the EFSA GD for B$M 2009, (90 th percentile value, consumer only), obtained from Prosper study: ,,Consolidation of bird and mammal PT data for use in risk assessment", March 2010.

Table 9.2-9: Higher-tier assessment of the long-term risk for mammals due to the use of CIAZ in cereals – refined parameters (*) are further described and justified in the text

Intended use	Winter wheat					
Active substance/product	CIAZ					
Application rate (g/ha)	2 x 448.5					
NOEL (mix) (mg/kg bw)	18.16					
TER criterion	5					
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals Late season- Seed heads	Small granivorous/insectivorous bird "bunting"	12.5	1.4 x 0.53	0.87	3.6	5.04

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.

zRMS comments:

The risk assessment is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438). The refinement with PT value was considered. Safe use of boscalid and difenconazole solo and in the combined exposure of two substances and for ppp Ciaz for birds were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.

9.2.2.2 Higher-tier risk assessment

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

Puddle scenario

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

With a K(f)oc of 771.5, Boscalid belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha)	=	490	App. Rate x MAF = 350 x 1.4		
Acute toxicity (mg/kg bw)	=	2000	quotient	=	0.25
Reprod. toxicity (mg/kg bw/d)	=	24.1	quotient	=	20.3

With a Koc of 3759.4 (mean value) L/kg, difenoconazole belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha)	=	140	App. Rate x MAF = 100 x 1.4		
Acute toxicity (mg/kg bw)	=	349	quotient	=	0.40
Reprod. toxicity (mg/kg bw/d)	=	9.71	quotient	=	14.4

As the ratios do not exceed the value of 3000 for Boscalid and Difenoconazole, it is not necessary to conduct a drinking water risk assessment for birds.

zRMS comments:

Since is not a for spray applications / 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. Therefore, the leaf scenario does not have to be considered taking onto account the proposed uses (cereals).

Evaluation of exposing for birds through the drinking water Puddle scenario for the active substances, demonstrate that the acceptable risk for birds for proposed use pattern of Ciaz.

9.2.2.4 Effects of secondary poisoning

The log P_{ow} of Boscalid amounts to 2.96 (at pH 7.1, 21°C) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Difenoconazole amounts to 4.4 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating birds via secondary poisoning

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

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

Parameter	Winter wheat	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.051	
log P_{ow} / P_{ow}	4.4 / 25118.86	EFSA J 2011; 9(1):1967
Koc	3759.4	Mean value. EFSA J 2011; 9(1):1967
foc	0.02	Default
BCF_{worm}	4.020	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.21	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.22	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	9.71	EFSA J 2011; 9(1):1967
TER_{lt}	45.1	No risk, TERlt>5

TER values shown in bold fall below the relevant trigger.

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.

Table 9.2-11: Assessment of the risk for fish-eating birds due to exposure to Difenoconazole via bioaccumulation in fish (secondary poisoning) for the intended use in winter wheat

Parameter	Winter wheat	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.000201	Step 3 (scenario D1 ditch – 2 applications)
BCF_{fish}	330	(based on total ^{14}C). EFSA J 2011; 9(1):1967
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.07	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.011	$DDD = PEC_{fish} \times 0.159$ x twa
NOEL (mg/kg bw/d)	9.71	EFSA J 2011; 9(1):1967
TER_{lt}	920.7	No risk, TERlt>5

TER values shown in bold fall below the relevant trigger.

zRMS comments:

No unacceptable risks are expected for secondary poisoning (residue uptake from drinking water or bio-accumulation in food chains) for CIAZ for difenconalzole as TER_{LT} values are above trigger value of 5.

9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.2.4 Overall conclusions

According to the screening and first-tier assessment for winter wheat, all the TER_a and TER_{lt} values for active substances Boscalid and Difenoconazole are greater than the Annex VI trigger of 10 and 5, respectively, indicating that CIAZ presents no unacceptable acute and long-term risk to birds according to the intended uses on winter wheat. In addition, no unacceptable acute and long-term risk is expected due to combined exposure.
Difenoconazole has been shown to have the potential for bioaccumulation, however, there is no risk to earthworm-eating and fish-eating birds according to the intended uses of CIAZ.

zRMS comment:

According to the screening and first-tier assessment for winter wheat, all the TER_a and TER_{lt} values for active substances Boscalid and Difenoconazole are greater than the Annex VI trigger of 10 and 5, respectively, indicating that Ciaz presents no unacceptable acute and long-term risk to birds according to the intended uses on winter wheat.
In addition, no unacceptable acute and long-term risk is expected due to combined exposure.
Difenoconazole has been shown to have the potential for bioaccumulation, however, there is no risk to earthworm-eating and fish-eating birds according to the intended uses of Ciaz.

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 Boscalid and Difenoconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of CIAZ were not evaluated as part of the EU assessment of Boscalid and Difeno-

conazole. New data submitted with this application are listed in Appendix 1 and summarised in Section 6 (Mammalian Toxicology) of this report.

However, the provision of further data on the formulation CIAZ is not considered essential, because active substance data on toxicity to mammals can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Boscalid	Acute	**LD_{50} > 5000 mg/kg bw**	SANCO/3919/2007 – rev. 5
Rat	Boscalid	Dietary Reproductive toxicity Two-generation study	**NOAED = 67 mg/kg bw/d**	SANCO/3919/2007 – rev. 5
Rat	Difenoconazole	Acute	**LD_{50} > 1453 mg/kg bw**	EFSA Journal 2011;9(1):1967
Rat	Divident 030 FS	Acute	LD_{50} >3000 mg/kg bw	EFSA Journal 2011;9(1):1967
Rat	Score 250 EC	Acute	LD_{50} >3000 mg/kg bw	EFSA Journal 2011;9(1):1967
Rat	Metabolite CGA 131013	Acute	LD_{50} >5000 mg/kg bw	EFSA Journal 2011;9(1):1967
Rat	Difenoconazole	Long-term Reproductive toxicity Two-generation study	**NOAEL = 17.3 mg/kg bw/d**	EFSA Journal 2011;9(1):1967
Rat	Metabolite CGA 131013	Long-term	NOAEL = 100 mg/kg bw/d	EFSA Journal 2011;9(1):1967

9.3.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints.

The Applicant considers that the acute and long-term risk assessments on mammals for the metabolite CGA131013 are covered by the acute and log-term risk assessments of the parental Difenoconazole.

9.3.2 Risk assessment for spray applications

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

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

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

bles.

Table 9.3-2: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CIAZ in winter wheat

Intended use	**Winter wheat**				
Active substance/product	Boscalid				
Application rate (g/ha)	2 x 350				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening - Cereals	Small herbivorous mammal	118.4	1.2	49.73	100.5
Reprod. toxicity (mg/kg bw/d)	67				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening - Cereals	Small herbivorous mammal	48.3	1.4 x 0.53	12.54	5.34
Active substance/product	Difenoconazole				
Application rate (g/ha)	2 x 100				
Acute toxicity (mg/kg bw)	> 1453				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening - Cereals	Small herbivorous mammal	118.4	1.2	14.21	102.3
Reprod. toxicity (mg/kg bw/d)	17.3				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
~~Screening – Cereals~~	~~Small herbivorous mammal~~	~~48.3~~	~~1.4 x 0.53~~	~~0.36~~	~~48.27~~
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew". Ground dwelling invertebrates with interception. 100% ground arthropods	1.9	1.4 x 0.53	0.14	122.7
Cereals BBCH ≥ 40	Small herbivorous mammal "vole". Grass + cereals. 100% grass	21.7	1.4 x 0.53	1.61	10.7
Cereals BBCH 30 - 39	Small omnivorous mammal “mouse”. Combination (invertebrates with interception). 25% weeds 50% weed seeds 25% ground arthropods	3.9	1.4 x 0.53	0.29	59.8
Cereals BBCH ≥ 40	Small omnivorous mammal “mouse”. Combination (invertebrates with interception). 25% weeds	2.3	1.4 x 0.53	0.17	101.4

	50% weed seeds 25% ground arthropods				

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

Risk Assessment for combined exposure

The simultaneous exposure of animals to residues of two or more potential toxic substances should be considered in the risk assessment. Therefore, for the assessment of acute effects, a surrogate LD_{50} for the mixture of active substances with known toxicity was derived assuming dose additivity of toxicity. For the calculation, the following equation was used:

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

With:

$X(\text{a.s.}_i)$ = fraction of each a.s. in the mixture

$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for each a.s.

Acute risks from combined exposure

The active substance content of the formulation CIAZ addressed in this dossier is 23.3 % Boscalid and 6.6 % Difenoconazole, making up a total of 299 g a.s./L product. According to GAP, the maximum application rate is 1.5 L product/ha, therefore, an application rate of 448.5 g a.s./ha was considered in the assessment.

Table 9.3-3 shows the calculation of the predicted LD_{50} (mix) of Boscalid and Difenoconazole when mixed in these proportions (step 1 in Appendix B to the EFSA GD 2009).

Table 9.3-3: Mammalian LD_{50} (mix) for boscalid and Difenoconazole when combined as CIAZ (step 1 in EFSA GD 2009, Appendix B)

	Boscalid	Difenoconazole
Content in the formulation CIAZ	23.3%	6.6%
Fraction in the a.s. mixture	0.7793	0.2207
LD_{50} of a.s. [mg/kg bw]	>5000	>1453
Fraction / LD_{50}	0.000156	0.000152
Sum	0.000308	
1/ sum = predicted LD_{50} (mix)	3249.18 mg mix/kg bw	

Table 9.3-4: Mammalian "tox per fraction" for CIAZ (step 1 in EFSA GD 2009, Appendix B)

	Boscalid	Difenoconazole	"mix"
Content in the formulation CIAZ	23.3%	6.6%	29.9 %
Fraction in mixture	0.7793	0.2207	1.0
LD_{50} (mg/kg bw)	>5000	>1453	3249.18
Tox per fraction	6416.31	6582.53	3249.18
Contribution to predicted toxicity	50.64%	49.36%	

Boscalid contributes to 50.64 % to mixture toxicity, while the Difenoconazole have an impact on the predicted risk of 49.36%, therefore, surrogate LD_{50} was used in the acute risk assessment.

Table 9.3-5: First-tier assessment of the acute risk for mammals due to the use of CIAZ in winter wheat

Intended use	Winter wheat				
Active substance/product	CIAZ				
Application rate (g/ha)	2 x 448.5				
LD_{50} (mix) (mg/kg bw)	3249.18				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening - Cereals	Small herbivorous mammal	118.4	1.2	63.72	51.00

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.

According to reults, no unacceptable acute risk is obtained in winter wheat for combined exposure acording to the proposed GAP.

Regarding chronic risk assessment, the Applicant considers that, according to EFSA/2009/1438, the calculation of a combined toxicity is not applicable to the risk assessment for reproductive effect. Due to differences in evaluated endpoints and the dependency of the derived NOEL of the test design, any calculated TERmix value can only be used for illustrating purposes. Hence, in the case of an unacceptable TERmix, it has to be discussed if the results of the toxicity studies present any evidence for a possible concentration additivity of the effects and risks.

In addition, the combined toxicological effect of these two active substances has not been investigated with regard to repeated dose toxicity. Possibly, the combined exposure to these active substances may lead to a different toxicological profile than the profile(s) based on the individual substances.

Despite all of this, the reproductive risk from combined exposure has been performed by the Applicant:

Reproductive risks from combined exposure

Table 9.3-6: Mammalian NOEL (mix) for Boscalid and Difenoconazole when combined as CIAZ (step 1 in EFSA GD 2009, Appendix B)

	Boscalid	Difenoconazole
Content in the formulation CIAZ	23.3%	6.6%
Fraction in the a.s. mixture	0.7793	0.2207
NOEL of a.s. [mg/kg bw]	67	17.3
Fraction / NOEL	0.011631	0.012759
Sum	0.02439	
1/ sum = predicted NOEL (mix)	41.00 mg mix/kg bw	

Table 9.3-7: Mammalian "tox per fraction" for the CIAZ (step 1 in EFSA GD 2009, Appendix B)

	Boscalid	Difenoconazole	"mix"
Content in the formulation CIAZ	23.3%	6.6%	29.9 %
Fraction in mixture	0.7793	0.2207	1.0
NOEL (mg/kg bw)	67	17.3	41.00
Tox per fraction	85.98	78.37	41.00
Contribution to predicted toxicity	47.69%	52.31%	

Boscalid contributes to 47.69 % to mixture toxicity, while the Difenoconazole have an impact on the predicted risk of 52.31%, therefore, surrogate NOEL was used in the long-risk assessment.

Table 9.3-8: First-tier assessment of the acute and long-term/reproductive risk for mammals due to the use of CIAZ in winter wheat

Intended use	Winter wheat
Active substance/product	CIAZ
Application rate (g/ha)	2 x 448.5
NOEL (mix) (mg/kg bw/d)	41.00
TER criterion	5

Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Cereals BBCH ≥ 20	Small insectivorous mammal "shrew" 100% ground arthropods	1.9	1.4 x 0.53	0.63	64.8
Cereals BBCH ≥ 40	Small herbivorous mammal "vole" Grass + cereals 100% grass	21.7	1.4 x 0.53	7.22	5.7
Cereals BBCH 30-39	Small omnivorous mammal "mouse" Combination (invertebrates with interception) 25% weeds 50% weed seeds 25% ground arthropods	3.9	1.4 x 0.53	1.30	31.6

Cereals BBCH ≥ 40	Small omnivorous mammal "mouse" Combination (inverte-brates with interception) 25% weeds 50% weed seeds 25% ground arthropods	2.3	1.4 x 0.53	0.77	53.6

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.

According to reults, no unacceptable long-term risk is obtained in winter wheat for combined exposure acording to the proposed GAP.

zRMS comments:

The risk assessment at Tier 1 is considered acceptable. The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

Safe use of boscalid and difenconazole solo and in the combined exposure of two substances and for ppp Ciaz for mammals were confirmed based on TER_A and TER_{LT} above the trigger values of 10 and 5, respectively, indicating the acute and long-term risk is acceptable.

9.3.2.2 Higher-tier risk assessment

Not required.

9.3.2.3 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Puddle scenario

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

With a K(f)oc of 771.5, Boscalid belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha) =	490	App. Rate x MAF = 350 x 1.4		
Acute toxicity (mg/kg bw) =	5000	quotient =	0.1	
Reprod. toxicity (mg/kg bw/d) =	67	quotient =	7.3	

With a Koc of 3759.4 (mean value) L/kg, difenoconazole belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied.

Effective application rate (g/ha) =	140	App. Rate x MAF = 100 x 1.4

Acute toxicity (mg/kg bw)	=	1453	quotient	=	0.10
Reprod. toxicity (mg/kg bw/d)	=	17.3	quotient	=	8.09

As the ratios do not exceed the value of 3000 for Boscalid and Difenoconazole, it is not necessary to conduct a drinking water risk assessment.

9.3.2.4 Effects of secondary poisoning

The log P_{ow} of Boscalid amounts to 2.96 (pH 7.1, 21°C) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

The log P_{ow} of Difenoconazole amounts to 4.4 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

Risk assessment for earthworm-eating mammals via secondary poisoning

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

Table 9.3-9: Assessment of the risk for earthworm-eating mammals due to exposure to Difenoconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in winter wheat

Parameter	Difenoconazole	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.051	
log P_{ow} / P_{ow}	4.4 / 25118.86	EFSA J 2011; 9(1):1967
Koc	3759.4	Mean value. EFSA J 2011; 9(1):1967
foc	0.02	Default
BCF_{worm}	4.020	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.21	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.26	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	17.3	EFSA J 2011; 9(1):1967
TER_{lt}	65.9	No risk, TERlt>5

TER values shown in bold fall below the relevant trigger.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Table 9.3-10: Assessment of the risk for fish-eating mammals due to exposure to Difenoconazole via bioaccumulation in fish (secondary poisoning) for the intended use in winter wheat

Parameter	Difenoconazole	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.000201	Step 3 (scenario D1 ditch – 2 applications)
BCF_{fish}	330	(based on total ^{14}C). EFSA J 2011; 9(1):1967

BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC_{fish}	0.07	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.009	$DDD = PEC_{fish} \times 0.142$ x twa
NOEL (mg/kg bw/d)	17.3	EFSA J 2011; 9(1):1967
TER_{lt}	1836.7	No risk, TERlt>5

TER values shown in bold fall below the relevant trigger.

zRMS comments:

No unacceptable risks are expected for secondary poisoning (residue uptake from drinking water or bio-accumulation in food chains) for Ciaz and for difenconalzole and as TER_{LT} values are above trigger value of 5.

9.3.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

9.3.4 Overall conclusions

According to the screening and first-tier assessment for winter wheat, all the TER_a and TER_{lt} values for active substances Boscalid and Difenoconazole are greater than the Annex VI trigger of 10 and 5, respectively, indicating that CIAZ presents no unacceptable acute and long-term risk to mammals according to the intended uses on winter wheat. In addition, no unacceptable acute and long-term risk is expected due to combined exposure.

Difenoconazole have been shown to have the potential for bioaccumulation, however, there is no risk to earthworms-eating and fish-eating mammals according to the intended uses of CIAZ.

zRMS comments:

According to the screening and first-tier assessment for winter wheat, all the TER_a and TER_{lt} values for active substances Boscalid and Difenoconazole are greater than the Annex VI trigger of 10 and 5, respectively, indicating that Ciaz presents no unacceptable acute and long-term risk to mammals according to the intended uses on winter wheat.

In addition, no unacceptable acute and long-term risk is expected due to combined exposure.

Difenoconazole has been shown to have the potential for bioaccumulation, however, there is no risk to earthworm-eating and fish-eating mammals according to the intended uses of Ciaz.

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

No data available.

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 Boscalid, Difenoconazole and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

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

However, the provision of further data on the CIAZ is not considered essential, because active substance data on toxicity to aquatic organisms can be used.

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

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

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	Boscalid	96 h, s	**EC_{50} = 2.7 mg a.s./L**	SANCO/3919/2007 – rev. 5
Oncorhynchus mykiss	Boscalid	97 d, f	**NOEC = 0.125 mg a.s./L**	SANCO/3919/2007 – rev. 5
Daphnia magna	Boscalid	48 h, s	**EC_{50} = 5.33 mg a.s./L**	SANCO/3919/2007 – rev. 5
Daphnia magna	Boscalid	21 d, ss	**NOEC = 131 mg a.s./L**	SANCO/3919/2007 – rev. 5
Chironomus riparius	Boscalid	28 d, s	**NOEC = 1 mg a.s./L**	SANCO/3919/2007 – rev. 5
Pseudokirchneriella subcapitata	Boscalid	96 h, s	**EC_{50} = 1.34 mg a.s./L**	SANCO/3919/2007 – rev. 5
Higher-tier studies (micro- or mesocosm studies)				
No data.				

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

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

Species	Substance	Exposure System	Results	Reference
Fish				
Oncorhynchus mykiss	Difenoconazole	96 h, f	**EC_{50} = 1.1 mg a.s./L** (0.98-1.1)	EFSA Journal 2011;9(1):1967
Fathead minnow	Difenoconazole	34 d, f	**NOEC = 0.0076 mg a.s./L**	EFSA Journal 2011;9(1):1967
Pimephales promelas	Difenoconazole	Full life-cycle test	**NOEC = 0.0036 mg/L**	EFSA supporting publication 2014:EN-680
Oncorhynchus mykiss	1,2,4-triazole (CGA 71019)	96 h, s	EC_{50} = 498 mg/L (378-657)	EFSA Journal 2011;9(1):1967
Oncorhynchus mykiss	CGA 205375	96 h, s	EC_{50} = 0.74 mg/L (0.58-0.95)	EFSA Journal 2011;9(1):1967
Oncorhynchus mykiss	1,2,4-triazole (CGA 71019)	28 d, s	NOEC = 3.2 mg/L	EFSA Journal 2011;9(1):1967
Aquatic invertebrate				
Daphnia magna	Difenoconazole	48 h, s	EC_{50} = 0.77 mg a.s./L (0.59-0.95)	EFSA Journal 2011;9(1):1967
Mysidopsis bahia	Difenoconazole	96 h ,f	**EC_{50} = 0.15 mg a.s./L** (0.11-0.22)	EFSA Journal 2011;9(1):1967
Crassostrea virginica	Difenoconazole	96 h, f	EC_{50} > 0.30 mg a.s./L	EFSA Journal 2011;9(1):1967
Daphnia magna	Difenoconazole	21 d, f	**NOEC = 0.0056 mg a.s./L**	EFSA Journal 2011;9(1):1967
Daphnia magna	1,2,4-triazole (CGA 71019)	48 h, s	EC_{50} > 100 mg/L	EFSA Journal 2011;9(1):1967
Daphnia magna	CGA 205375	48 h, s	EC_{50} = 1.4 mg/L	EFSA Journal 2011;9(1):1967
Sediment dwelling organisms				
Chironomus riparius	Difenoconazole	28 d, s	**NOEC = 0.015 mg a.s./L (water)**	EFSA Journal 2011;9(1):1967
Chironomus riparius	Difenoconazole	28 d, s	**NOEC = 0.0525 mg a.s./kg dw (sediment)**	Addendum to the DAR, 2010
Chironomus riparius	CGA 205375	28 d, s	NOEC = 0.4 mg/L (water)	EFSA Journal 2011;9(1):1967
Chironomus riparius	CGA 205375	28 d, s	NOEC = 10 mg/kg dw (sediment)	EFSA Journal 2011;9(1):1967
Algae				
Scenedesmus subspicatus	Difenoconazole	72 h, s	**E_bC_{50} = 0.032 mg a.s./L** (0.026-0.039)	EFSA Journal 2011;9(1):1967
Scenedesmus subspicatus	1,2,4-triazole (CGA 71019)	96 h, s	E_bC_{50} = 8 mg/L E_rC_{50} > 31 mg/L	EFSA Journal 2011;9(1):1967

Species	Substance	Exposure System	Results	Reference
Scenedesmus subspicatus	CGA 205375	72 h, s	E_bC_{50} = 1.2 mg/L (1.2-1.3) E_rC_{50} = 3.1 mg/L (3.0-3.2)	EFSA Journal 2011;9(1):1967
Higher-tier studies (micro- or mesocosm studies)				
No reliable data, not required.				

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

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

Species	Substance	Exposure System	Results	Reference
Oncorhynchus mykiss	CIAZ	96 h, ss	**LC_{50} = 14.03 mg f.p./L $_{nom}$ (equivalent to 2.708 mg Boscalid/L + 0.764 mg Difenoconazole/L)^{geomean}**	KCP 10.2.1-01 Nierzędska, E. 2018 W/95/17
Daphnia magna	CIAZ	48 h, ss	**EC_{50} = 18.26 mg f.p./L $_{nom}$ (equivalent to 4.35 mg Boscalid/L + 1.20 mg Difenoconazole/L)^{nom}**	KCP 10.2.1-02 Nierzędska, E. 2018 W/97/17
Pseudokirchneriella subcapitata	CIAZ	72 h	**E_rC_{50} = 9.80 mg f.p./L $_{nom}$ (equivalent to 2.284 mg Boscalid/L + 0.647 mg Difenoconazole/L)$_{nom}$** E_yC_{50} = 6.23 mg f.p./L $_{nom}$ (equivalent to 1.451 mg Boscalid/L and 0.411 mg Difenoconazole/L)	KCP 10.2.1-03 Nierzędska, E. 2017 W/96/17

Species	Substance	Exposure System	Results	Reference
Lemna gibba	CIAZ	7d, ss	**Frond number** **E_rC_{50} = 245.55 mg f.p./L $_{nom}$ (equivalent to 49.526 mg Boscalid/L and 14.386 mg Difenoconazole/L)$_{geomean}$** E_yC_{50} = 23.65 mg f.p./L $_{nom}$ (equivalent to 4.971 mg Boscalid/L and 1.458 mg Difenoconazole/L) $_{geomean}$ Dry weight E_rC_{50} = 266.00 mg f.p./L $_{nom}$ (equivalent to 54.158 mg Boscalid/L and 15.605 mg Difenoconazole/L) $_{geomean}$ E_yC_{50} = 18.09 mg f.p./L $_{nom}$ (equivalent to 3.726 mg Boscalid/L and 1.117 mg Difenoconazole/L) $_{geomean}$	KCP 10.2.1-04 Nierzędska, E. 2018 W/98/17

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

9.5.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to CIAZ formulation.

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", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

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

Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for CIAZ for each organism group for the use of CIAZ in winter cereals

Group			**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
Test species			***O. mykiss***	***D. magna***	***P. subcapitata***	***L. gibba***
Endpoint (µg/L)			LC_{50} 14030	EC_{50} 18260	E_rC_{50} 9800	E_rC_{50} 245550
AF			100	100	10	10
RAC (µg/L)			140.3	182.6	980	24555
Distance	**% Drift**	**PEC $_{gl\text{-}max}$ (µg/L)**				

Group			**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic plants**
1m	2.38	26.44	0.188	0.145	0.027	0.001

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

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.

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

9.5.2.1 Boscalid

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

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Daphnia magna***	***Pseudokirchn. subcapitata***	***Chironomus riparius***
Endpoint		LC_{50}	NOEC	EC_{50}	NOEC	E_rC_{50}/E_yC_{50}	NOEC
(µg/L)		2700	125	5330	131000	1340	1000
AF		100	10	100	10	10	10
RAC (µg/L)		27	12.5	53.3	13100	134	100
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**						
Step 1							
	61.841 / 123.683	**2.290/4.581**	**4.947/9.895**	**1.160/2.321**	0.005/0.009	0.462/0.923	0.618/1.237
Step 2							
S-Europe	20.445 / 39.580	0.757/**1.466**	**1.636/3.166**	0.384/0.743	0.002/0.003	0.153/0.295	0.204/0.396
N-Europe	11.190 / 21.491	0.414/0.796	0.895/1.719	0.210/0.403	0.001/0.002	0.084/0.160	0.112/0.215
Step 3							
D1/ditch	8.093 / 16.140	0.300/0.598	0.647/**1.291**	0.152/0.303	0.001/0.001	0.060/0.120	0.081/0.161
D1/stream	5.066 / 10.090	0.188/0.374	0.405/0.807	0.095/0.189	<0.001/0.001	0.038/0.075	0.051/0.101
D2/ditch	10.260 / 22.190	0.380/0.822	0.821/**1.775**	0.192/0.416	0.001/0.002	0.077/0.166	0.103/0.222
D2/stream	6.404 / 13.840	0.237/0.513	0.512/**1.107**	0.120/0.260	<0.001/0.001	0.048/0.103	0.064/0.138

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**
D3/ditch	2.215 / 1.939	0.082/0.072	0.177/0.155	0.042/0.036	<0.001/<0.001	0.017/0.014	0.022/0.019
D4/pond	1.094 / 2.648	0.041/0.098	0.088/0.212	0.021/0.050	<0.001/<0.001	0.008/0.020	0.011/0.026
D4/stream	1.637 / 3.468	0.061/0.128	0.131/0.277	0.031/0.065	<0.001/<0.001	0.012/0.026	0.016/0.035
D5/pond	0.694 / 1.434	0.026/0.053	0.056/0.115	0.013/0.027	<0.001/<0.001	0.005/0.011	0.007/0.014
D5/stream	1.784 / 2.387	0.066/0.088	0.143/0.191	0.033/0.045	<0.001/<0.001	0.013/0.018	0.018/0.024
D6/ditch	2.977 / 4.937	0.110/0.183	0.238/0.395	0.056/0.093	<0.001/<0.001	0.022/0.037	0.030/0.049
R1/pond	0.243/ 0.619	0.009/0.023	0.019/0.050	0.005/0.012	<0.001/<0.001	0.002/0.005	0.002/0.006
R1/stream	1.688 / 4.751	0.063/0.176	0.135/0.380	0.032/0.089	<0.001/<0.001	0.013/0.035	0.017/0.048
R3/stream	2.305 / 5.029	0.085/0.186	0.184/0.402	0.043/0.094	<0.001/<0.001	0.017/0.038	0.023/0.050
R4/stream	3.281 / 7.416	0.122/0.275	0.262/0.593	0.062/0.139	<0.001/0.001	0.024/0.055	0.033/0.074

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, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish prolonged as characterised by a NOEC for *Onchorhynchus mykiss* of 125 µg/L in connection with an assessment factor of 10) in D1 ditch, D2 ditch and D2 stream. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-6: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Boscalid based on FOCUS Step 4 calculations and toxicity data for fish prolonged with mitigation of spray drift and run-off for the use of CIAZ in winter wheat

Intended use		**Winter wheat**		
Active substance		Boscalid		
Application rate (g/ha)		2 x 350		
Nozzle reduction	**No-spray buffer (m)**	1	10	20
	Vegetated filter strip (m)	None	None	None
None	D1 ditch	- / 16.140	- / 16.140	- / 16.140
50 %		- / 16.140	- / -	- / -
75 %		- / 16.140	- / -	- / -
90 %		- / 16.140	- / -	- / -
None	D2 ditch	- / 22.190	- / 22.190	- / 22.190
50 %		- / 22.190	- / -	- / -
75 %		- / 22.190	- / -	- / -
90 %		- / 22.190	- / -	- / -
None	D2 stream	- / 13.840	- / 13.840	- / 13.840
50 %		- / 13.840	- / -	- / -
75 %		- / 13.840	- / -	- / -
90 %		- / 13.840	- / -	- / -
RAC (µg/L)				
12.5		**PEC/RAC ratio**		
None	D1 ditch	- / **1.291**	- / **1.291**	- / **1.291**
50 %		- / **1.291**	- / -	- / -
75 %		- / **1.291**	- / -	- / -
90 %		- / **1.291**	- / -	- / -
None	D2 ditch	- / **1.775**	- / **1.775**	- / **1.775**
50 %		- / **1.775**	- / -	- / -
75 %		- / **1.775**	- / -	- / -
90 %		- / **1.775**	- / -	- / -
None	D2 stream	- / **1.107**	- / **1.107**	- / **1.107**
50 %		- / **1.107**	- / -	- / -
75 %		- / **1.107**	- / -	- / -
90 %		- / **1.107**	- / -	- / -

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

9.5.2.2 Difenoconazole

Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Difenoconazole for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CIAZ in winter wheat

Group		**Fish acute**	**Fish prolonged**		**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**		**Sed. dwell. prolonged**
Test species		***Oncorhynchus mykiss***	~~***Fathead minnow***~~	***Pimephales promelas***	~~***Daphnia magna***~~ ***M.bahia***	***Daphnia magna***	***Scenedescmus subspicatus***	***Chironomus riparius***		***Chironomus riparius***
Endpoint (µg/L)		LC_{50} 1100	~~NOEC 7.6~~	NOEC 3.6	EC_{50} 150	NOEC 5.6	E_bC_{50} 32	NOEC 15		NOEC 52.5
AF		100	~~10~~	10	100	10	10	10		10
RAC (µg/L)		11	~~0.76~~	0.36	1.5	0.56	3.2	1.5		5.25*
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**								**PEC $_{sed\ plateau}$ (µg/L)**	
Step 1										
	7.69 / 15.38	0.699/**1.398**	~~**10.118/20.237**~~	**21.361/42.722**	**5.127/10.253**	**13.732/27.464**	**2.403/4.806**	**5.127/10.253**	370.39 / 740.77	**70.550/141.099**
Step 2										
S-Europe	2.38 / 4.53	0.216/0.412	~~**3.132/5.961**~~	**6.611/12.583**	**1.587/3.020**	**4.250/8.089**	0.744/**1.416**	**1.587/3.020**	122.79 / 234.93	**23.389/44.749**
N-Europe	1.32 / 2.49	0.120/0.226	~~**1.737/3.276**~~	**3.667/6.917**	0.880/**1.660**	**2.357/4.446**	0.413/0.778	0.880/**1.660**	66.33 / 126.09	**12.634/12.634**
Step 3										
D1/ditch	0.636 / 0.562	0.058/0.051	~~0.837/0.739~~	**1.767/1.561**	0.424/0.375	**1.136/1.004**	0.199/0.176	0.424/0.375	1.258 / 4.221	0.240/0.804
D1/stream	0.495 / 0.472	0.045/0.043	~~0.651/0.621~~	**1.375/1.311**	0.330/0.315	0.884/0.843	0.155/0.148	0.330/0.315	0.036 / 0.203	0.007/0.039
D2/ditch	0.640 / 0.573	0.058/0.052	~~0.842/0.754~~	**1.778/1.592**	0.427/0.382	**1.143/1.023**	0.200/0.179	0.427/0.382	1.739 / 4.537	0.331/0.864
D2/stream	0.544 / 0.492	0.049/0.045	~~0.716/0.647~~	**1.511/1.367**	0.363/0.328	0.971/0.879	0.170/0.154	0.363/0.328	0.290 / 3.106	0.055/0.592
D3/ditch	0.634 / 0.555	0.058/0.050	~~0.834/0.730~~	**1.761/1.542**	0.423/0.370	**1.132**/0.991	0.198/0.173	0.423/0.370	0.796 / 1.175	0.152/0.224

Group		**Fish acute**	**Fish prolonged**		**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Sed. dwell. prolonged**		**Sed. dwell. prolonged**
D4/pond	0.022 / 0.026	0.002/0.002	~~0.029/0.034~~	0.061/0.072	0.015/0.017	0.039/0.046	0.007/0.008	0.015/0.017	0.419 / 0.740	0.080/0.141
D4/stream	0.468 / 0.419	0.043/0.038	~~0.616/0.551~~	**1.300/1.164**	0.312/0.279	0.836/0.748	0.146/0.131	0.312/0.279	0.034 / 0.085	0.006/0.016
D5/pond	0.022 / 0.030	0.002/0.003	~~0.029/0.039~~	0.061/0.083	0.015/0.020	0.039/0.054	0.007/0.009	0.015/0.020	0.471 / 0.767	0.090/0.146
D5/stream	0.506 / 0.483	0.046/0.044	~~0.666/0.636~~	**1.406/1.342**	0.337/0.322	0.904/0.863	0.158/0.151	0.337/0.322	0.027 / 0.082	0.005/0.016
D6/ditch	0.626 / 0.557	0.057/0.051	~~0.824/0.733~~	**1.739/1.547**	0.417/0.371	**1.118**/0.995	0.196/0.174	0.417/0.371	0.375 / 1.552	0.071/0.296
R1/pond	0.027 / 0.066	0.002/0.006	~~0.036/0.087~~	0.075/0.183	0.018/0.044	0.048/0.118	0.008/0.021	0.018/0.044	1.275 / 2.923	0.243/0.557
R1/stream	0.418 / 0.361	0.038/0.033	~~0.550/0.475~~	**1.161/1.003**	0.279/0.241	0.746/0.645	0.131/0.113	0.279/0.241	2.906 / 6.899	0.554/**1.314**
R3/stream	0.586 / 0.510	0.053/0.046	~~0.771/0.671~~	**1.628/1.417**	0.391/0.340	**1.046**/0.911	0.183/0.159	0.391/0.340	2.741 / 4.847	0.522/0.923
R4/stream	0.419 / 0.546	0.038/0.050	~~0.551/0.718~~	**1.164/1.517**	0.279/0.364	0.748/0.975	0.131/0.171	0.279/0.364	3.878 / 7.806	0.739/**1.487**

*** it should be noted that the NOEC value was based on very conservative estimate of the concentration in the sediment (see Addendum B.9, 0ctober 2010), and no effects were seen at the highest test concentration.**

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 all intended uses. calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for aquatic invertebrates as characterised by an NOEC of 5.6 µg/L for *Daphnia magna* in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

Table 9.5-8: Aquatic organisms: PEC calculations and acceptability of risk (PEC/RAC < 1) for Difenoconazole based on FOCUS Step 4 calculations and toxicity data for invertebrates with mitigation of spray drift and run-off for the use of CIAZ in winter wheat for single/multiple applications

Intended use		Winter wheat	
Active substance		Difenoconazole	
Application rate (g/ha)		2 × 100	
		PEC_{sw} (µg/L)	
Nozzle reduction	**No-spray buffer (m)**	5	5
	Vegetated filter strip (m)	None	5
None	D1 ditch	0.172 / 0.146	- / -
None	D1 stream	0.181 / 0.167	- / -
None	D2 ditch	0.174 / 0.284	- / -
None	D2 stream	0.199 / 0.179	- / -
None	D3 ditch	0.172 / 0.144	- / -
None	D4 stream	0.171 / 0.148	- / -
None	D5 stream	0.185 / 0.171	- / -
None	D6 ditch	0.170 / 0.145	- / -
None	R1 stream	0.153 / 0.359	- / -
None	R3 stream	0.214 / 0.309	- / -
None	R4 stream	0.243 / 0.545	- / 0.356
RAC (µg/L)			
~~0.56~~ 0.36		**PEC/RAC ratio**	
None	D1 ditch	~~0.307 / 0.261~~ 0.478/0.406	- / -
None	D1 stream	0.503/0.464	- / -
None	D2 ditch	~~0.311 / 0.507~~ 0.483/0.789	- / -
None	D2 stream	0.553/0.497	- / -
None	D3 ditch	~~0.307 / -~~ 0.478/0.400	- / -
None	D4 stream	0.475/0.411	- / -
None	D5 stream	0.514/0.475	- / -
None	D6 ditch	~~0.304 / -~~ 0.472/0.403	- / -
None	R1 stream	0.425/0.997	- / -
None	R3 stream	~~0.382 / -~~ 0.594/0.858	- / -
None	R4 stream	0.675/**1.514**	- / 0.989

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

Metabolites

Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for 1,2,4-triazole (CGA 71019) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CIAZ in winter wheat

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Algae**
Test species		***Oncorhynchus mykiss***	***Oncorhynchus mykiss***	***Daphnia magna***	***Scenedesmus subspicatus***
Endpoint		LC_{50}	NOEC	EC_{50}	E_bC_{50}
(µg/L)		~~489000~~ 498 000	3200	100000	8000
AF		100	10	100	10
RAC (µg/L)		~~4890~~ 498000	320	1000	800
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**				
Step 1					
	1.70 / 3.40	<0.001/0.001	0.005/0.011	0.002/0.003	0.002/0.004
Step 2					
S-Europe	0.53 / 1.00	<0.001/<0.001	0.002/0.003	0.001/0.001	0.001/0.001
N-Europe	0.027 / 0.051 0.27 / 0.51	<0.001/<0.001	0.001/0.002	<0.001/0.001	<0.001/0.001

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 9.5-10: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for CGA 205375 for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of CIAZ in winter wheat

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Sed. dwell. prolonged**		**Sed. dwell. prolonged**
Test species		***O. mykiss***	***D. magna***	***P. subcapitata***	***C. riparius***		***C. riparius***
Endpoint		LC_{50}	EC_{50}	E_bC_{50}	NOEC		NOEC
(µg/L)		740	1400	1200	400		10000
AF		100	100	10	10		10
RAC (µg/L)		7.4	14	120	40		1000
FOCUS Scenario	**PEC $_{gl\text{-}max}$ (µg/L)**					**PEC $_{sed\text{-}max}$ (µg/L)**	
Step 1							
	1.58 / 3.15	0.214/0.426	0.113/0.225	0.013/0.026	0.040/0.079	39.96 / 79.91	0.040/0.080
Step 2							
S-Europe	0.49 / 0.93	0.066/0.126	0.035/0.066	0.004/0.008	0.012/0.023	12.83 / 24.48	0.013/0.024
N-Europe	0.26 / 0.49	0.035/0.066	0.019/0.035	0.002/0.004	0.007/0.012	6.68 / 12.71	0.007/0.013

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

zRMS comments:

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", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The calculations based on PEC_{sw}/RAC for active substances – boscalid and difenconalzole and their metabolites have been accepted by zRMS-PL.

Difenconazole:

The PEC_{sw}/RAC ratio for the most sensitive organism – long term exposure for fish for difenconazole for scenarios D1 (ditch and stream), D2 (ditch and stream) and D3 (ditch), D4 (stream), D5 (stream), D6 (ditch) and R1 and R3 stream scenario on winter cereals indicated an acceptable risk for aquatic organisms with 5-meter buffer zone to surface water bodies, but for R4 stream scenario on winter cereals an acceptable risk for aquatic organisms **5-meter** vegetative buffer zone, calculated by FOCUS STEP 4 programme is identified.

For remained scenarios the risk for difenconazole is acceptable already with PEC_{sw} FOCUS STEP 3 calculations.

Taking into account the PEC_{sed}/RAC ratio for Cironomus riparius for difenconazole for R1 (stream) and R4 (stream) on winter cereals indicated an acceptable risk for aquatic organisms with **5-meter** vegetative buffer zone, calculated by FOCUS STEP 4 programme.

Table 9.5-7$_{corr}$: Aquatic organisms: PEC calculations and acceptability of risk (PEC/RAC < 1) for Difenoconazole based on FOCUS Step 4 calculations and toxicity data for invertebrates with mitigation of spray drift and run-off for the use of Ciaz in winter wheat for single/multiple applications for Chironomus riparius.

Intended use		Winter wheat
Active substance		Difenoconazole
Application rate (g/ha)		2 × 100
		PEC $_{sed\text{-}max}$ (µg/L)
Nozzle reduction	**No-spray buffer (m)**	**5 meter vegetative buffer zone**
	Vegetated filter strip (m)	None
None	R1 scenario	- / 4.205
None	R4 scenario	- / 4.776
RAC 5.25*		**PEC/RAC ratio**
None	R1 scenario	0.80
None	R4 scenario	0.90

-/ for one/and multiple applications
*RAC for Chironomus riparius

Taking into account the PEC_{sed}/RAC ratio for Cironomus riparius for difenconazole for R1 (stream) and R4 stream on winter cereals indicated an acceptable risk for aquatic organisms with **5-meter vegetative buffer zone,** calculated by FOCUS STEP 4 programme.

Boscalid

The PEC_{sw}/RAC ratio for boscalid for all scenarios passed the trigger below 1 with PEC_{sw} STEP1-3 calculations on winter cereals.

The PEC/RAC ratio for metabolites of difenconazole and boscalid for all scenarios passed the trigger below 1 with PEC_{sw} STEP 1- 2 calculations on winter cereals.

Ciaz

The risk assessment for the combinations of a.s. in the formulation CIAZ indicated an acceptable risk for aquatic organism.

Risk assessment for the combinations of a.s. in the formulation

Following the dilution and spraying of the formulated product, much of the formulation constituents are likely to be lost by volatilisation. Therefore, shortly after application of a formulated product, aquatic organisms are mainly exposed to the active substance present in the formulation. In addition, as demonstrated in the short-term studies here above there are no indications for interactions of the active substances (no synergisms or additional toxicity occurs due to the co-formulants) given that the formulation does not cause an (unexpected) increased toxicity compared to the active substances. An evaluation of the risk posed by the intact formulation is therefore relevant only for the acute/short-term assessment. The long-term risk was assessed considering data for the active substances in the formulation and no chronic combined risk assessment has been performed.

According to the new EFSA Scientific Opinion (EFSA, 2013) measured and calculated mixture toxicity should be compared to determine synergistic, additive or antagonistic effects of the formulation. In the following the concentration addition (CA) model is used as proposed by EFSA.

To determine the respective formulation effect, EFSA proposed to calculate the model deviation ratio (MDR), which divides the calculated mixture toxicity ($LC_{50}/EC_{50\ mix\text{-}CA}$) by the measured mixture toxicity ($LC_{50}/EC_{50\ CIAZ}$). Ecotoxicity studies are biological test systems which underlie a certain natural biological variability when repeating a study. Hence, a threshold has to be defined when an increased/decreased mixture toxicity effect cannot be seen as only additive any longer. EFSA proposes a factor of 5, *i.e.* if the MDR is between 0.2 and 5 the observed and calculated mixture toxicities are considered in agreement.

Active susbtance / species	**Test system**	**Endpoint (mg a.s./L)**
Boscalid		
Oncorhynchus mykiss	LC_{50} 96h	2.7
Daphnia magna	EC_{50} 48h	5.33
P. subcapitata	EC_{50} 72h	1.34
Difenoconazole		
Oncorhynchus mykiss	LC_{50} 96h	1.1
~~*Daphnia magna*~~ *M.bahia*	EC_{50} 48h	0.15
Scenedesmus subspicatus	E_bC_{50} 72h	0.032

The calculated MDR values are between 0.2 and 5 for each organism except daphnia and algae (see Table 9.5-11), indicating that the formulation does not cause an (unexpected) increased toxicity compared to the active substances for these organisms. No synergisms or additional toxicity occurs due to the co-formulants. The apparent antagonism for daphnia and algae (toxicity of the formulation lower than expected) can be ex-

plained by the fact that endpoints for individual active substances are "higher than" values.

Table 9.5-11: Summary of results obtained in the studies with the formulated product CIAZ and comparison of calculated and measured mixture toxicity

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]			
		Measured toxicity of CIAZ ($LC_{50\ CIAZ}$ or $EC_{50\ CIAZ}$) (mg/L)	Measured toxicity of CIAZ (converted to be a.i. based) ($LC_{50\ CIAZ}$ or $EC_{50\ CIAZ}$) (mg a.s./L)	Calculated mixture toxicity[a] $LC_{50\ mix-CA}$ or $EC_{50\ mix-CA}$	Model deviation ratio (MDR = $EC_{50\ mix-CA}$ / $EC_{50\ CIAZ}$)
O. mykiss	LC_{50}, acute, 96 h	14.03	3.776	2.044	0.541
~~*D. magna*~~ *M.bahia*	EC_{50}, acute, 48 h	18.26	4.914	0.618	0.126
Algae	E_bC_{50}, 72 h	9.8	2.637	0.134	0.051

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Boscalid (233 g/L) and Difenoconazole (66 g/L) within the formulation and based on a density of formulation of 1.111 g/mL.

The calculated factors fall outside 0.8-1.2 for fish (see Table 9.5-11), indicating that the mixture composition in the formulation study giving the measured mixture toxicity is not similar to the mixture composition at the PECmix.

Table 9.5-12: Comparison of mixture composition in the formulation study (giving the measured mixture toxicity) and mixture composition at the PEC_{mix}

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in CIAZ) $LC_{50\ mix-CA}$ or $EC_{50\ mix-CA}$	Calculated mixture toxicity (a.s. in PEC_{mix})[b] $LC_{50\ mix-CA}$ or $EC_{50\ mix-CA}$ at lower exposure tier	Factors ($EC_{50\ mix-CA}$ (a.s. in CIAZ)/$EC_{50\ mix-CA}$ (a.s. in PEC_{mix})) at lower exposure tier
O. mykiss	LC_{50}, acute, 96 h	2.044	2.409	0.848
~~*D. magna*~~ M.bahia	EC_{50}, acute, 48 h	0.618	1.378	0.448
Algae	E_bC_{50}, static, 72 h	0.134	0.305	0.438

[a] The mixture toxicity of the formulation was re-calculated based on the nominal contents of Boscalid (233 g/L) and Difenoconazole (66 g/L) within the formulation and based on a density of formulation of 1.111 g/mL.

[b] The mixture toxicity of the formulation was re-calculated based on the mixture composition at the PEC_{mix} for Boscalid (0.000243 mg/L at Step 3 for R1 pond scenario) and Difenoconazole (0.000022 mg/L at Step 3 for D4 and D5 pond scenarios).

Table 9.5-13: Comparison of calculated mixture toxicity and toxicity per fraction of a single a.s.

Test species	Endpoint & Test system	LC_{50} / EC_{50} [mg/L]		
		Calculated mixture toxicity (a.s. in CIAZ) $LC_{50\ mix\text{-}CA}$ or $EC_{50\ mix\text{-}CA}$	Calculated toxicity per fraction of CIAZ (based on each a.s.) $(1/TU_i)$[a]	Deviation from mixture toxicity (1-$EC_{x\ mix\text{-}CA}$ x $(1/EC_{x\ mix\text{-}CA} - TU_i)$) [%]
O. mykiss	LC_{50}, acute, 96 h	2.044	Boscalid: 3.465 Difenoconazole: 4.983	Boscalid: 58.99% Difenoconazole: 41.01%
~~*D. magna*~~ *M.bahia*	EC_{50}, acute, 48 h	0.618	Boscalid: 6.840 Difenoconazole: 0.680	Boscalid: 9.04% Difenoconazole: 90.96%
Algae	E_bC_{50}, static, 72 h	0.134	Boscalid: 1.720 Difenoconazole: 0.145	Boscalid: 7.78% Difenoconazole: 92.22%

[a] TUi is defined as the concentration of the i^{th} a.s. at the $EC_{50\ CIAZ}$ (re-caculated to the sum of a.s.) divided by the respective single-substance toxicity ($EC_{50\ a.s.}$). This is calculated based on the nominal contents of Boscalid (233 g/L) and Difenoconazole (66 g/L) within the formulation and based on a density of formulation of 1.111 g/mL

With regard to the mixture risk assessment EFSA further states that if the toxicity of the mixture is largely explained by the toxicity of a single active substance, a sufficient protection level might be achieved by simply basing the RA on the toxicity data for that single 'driver'.

Regarding CIAZ, no active substance is clearly driving the acute risk for fish. The studies performed with the formulated product do not reflect the toxicity of one particular active substance, as the formulation toxicity – endpoint recalculated to each active substance concentrations – does not come for 90 % (of more) from the toxicity per fraction of a single a.s. (TUi) (see Table 9.5-12).

However, regarding daphnia and algae, the risk assessments based on single-substance toxicity data for Boscalid and Difenoconazole respectively are sufficient given that they were identified as the drivers of the mixture toxicity.

Table 9.5-14: Conduct a mixture RA based on calculated mixture toxicity according to 10.3.8 from EFSA AGD in winter wheat for fish

Exposure	Lower exposure tier		Higher exposure tier	
	Boscalid	Difenoconazole	Boscalid	Difenoconazole
Exposure tier (FOCUS step)	Step 3 (R1 Pond)	Step 3 (D4 and D5 pond)	Step3 (D2 ditch)	Step3 (D2 ditch)
PECsw [mg a.s./L]	0.000243	0.000022	0.022190	0.000640
Relative proportions of the individual mixture components in the environment (pi PEC)	0.917	0.083	0.972	0.028
Total exposure concentration of the mixture (a.s. based) (PECmix) [mg/L]	0.000265		0.022830	
Calculated mixture toxicity (a.s. in PECmix) (ECx mix-CA = ∑ (pi PEC/ECx i)) [mg a.s./L]	2.409		2.594	
ETRmix = PECmix/ECx PPP	0.0001		0.0088	
Trigger	0.01			

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for fish.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxCIAZ) and a.s. (ECxa.s.):	Please refer to table 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxCIAZ) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxCIAZ) by means of the model deviation ratio (MDR = ECxmix-CA/ECxCIAZ).	MDR = 0.2–5 (CA approximately holds for the mixture)	Please refer to table 9.5- 11	Go to 3
3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxCIAZ) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxCIAZ with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) is between 0.8 and 1.2	Please refer to table 9.5- 12	Go to 4
4	Conduct a mixture RA based on measured mixture toxicity, with the exposure-toxicity ratio (ETRmix) being defined as the PECmix divided by the measured ECxPPP and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	If $ETR_{mix-} < 0.10$ for fish: Low risk	Please refer to table 9.5-14	**Low risk**

Applicability of such approach is justified following the EFSA AGD *Decision scheme for mixture toxicity risk assessment* for daphnia and *algae*.

Step	EFSA AGD provisions	Option	Justification	Outcome
1	Are measured toxicity data (ECx) available for the given endpoint (typically chronic data available only for a.s.)?	For both formulation (ECxCIAZ) and a.s. (ECxa.s.):	Please refer to table 9.5-4	Go to 2
2	Check the plausibility of the measured formulation toxicity (ECxCIAZ) against the calculated mixture toxicity ECxmix-CA (assuming CA, Equation 13) for exactly the mixture composition of the a.s. in the formulation (ECxCIAZ) by means of the model deviation ratio (MDR = ECxmix-CA/ECxCIAZ).	MDR < 0.2 (CA approximately holds for the mixture)	Please refer to table 9.5-11	Go to 9
9	Carefully recheck the apparent antagonism as observed in the measured mixture toxicity data (ECx PPP) regarding potential impacts of the default assumption of CA and/or heterogeneous input data used for the CA calculation. Does the apparent antagonism remain and no toxicologically plausible explanation is available (e.g. special feature of the formulation type)?	No (measured mixture toxicity plausible):		Go to 3

3	Check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxCIAZ) in terms of the relative proportions of the individual a.s. is similar to the mixture composition at the PECmix. As a direct comparison on the basis of the relative proportions of the a.s. at the ECxCIAZ with the relative proportion at the PECmix is not informative as such, the comparison is done based on calculated mixture toxicity (assuming CA) for both mixture compositions. Therefore, calculate ECxmix-CA (see Equation 13) for the mixture composition of the a.s. at the PECmix and compare with the estimate calculated for the formulation (as already done in step 2 above).	ECx mix-CA (a.s. in product)/ECx mix-CA (a.s. in PECmix) is <0.8 or >1.2	Please refer to table 9.5-12	Go to 5
5	Check whether one mixture component clearly drives the toxicity if considering the measured mixture toxicity (ECx PPP), that is, does the largest part of the sum of toxic units (Equation 14) calculated for the formulation (≥ 90 %) comes from a single a.s. (TUi)?	Deviation from mixture toxicity = 1-ECx mix-CA x (1/ECx mix-CA-TUi) [%] >=90% for one a.s.	Please refer to table 9.5- 13	Go to 6
6	Conduct a RA based on single-substance toxicity data (ECx a.s.) for the identified 'driver' of mixture toxicity, with the exposure-toxicity ratio (ETRa.s.) being defined as the PECa.s. divided by the measured ECx a.s. and compare the outcome with the acceptability criterion (trigger value) decisive for the specific endpoint/exposure scenario combination.	Covered by active substance assessment		**Low risk**

zRMS comments:

The calculations and conclusion provided in the Tables above of the combined risk assessment have been accepted by zRMS-PL.

The risk assessment for the combinations of active substances – boscalid and difenconazole in the formulation CIAZ indicated an acceptable risk for aquatic organism.

9.5.3 Overall conclusions

Boscalid

For the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish prolonged as characterised by a NOEC for *Oncorhynchus mykiss* of 125 µg/L in connection with an assessment factor of 10) in D1 ditch, D2 ditch and D2 stream. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{sw} considering reduced exposure of surface water bodies. However, scenarios D1 ditch, D2 ditch and D2 stream on winter cereals did not indicate an acceptable risk for aquatic organisms with 20-m buffer zone. These scenarios are not relevant for CEU.

Difenoconazole

For the intended uses, calculated PEC/RAC ratios did not indicate a acceptable risk for the most sensitive group of aquatic organisms (chronic risk for ~~invertebrate~~ fish as characterised by a NOEC for ~~*Daphnia magna*~~ *Pimephales promelas* of ~~5.6~~ 3.6 µg/L in connection with an assessment factor of 10) in D1 ditch, D1

stream, D2 ditch, D2 stream, D3 ditch, D4 stream, D5 stream, D6 ditch, R1 stream, R3 stream and R4 stream. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies.

After Step 4 calculations, an acceptable risk with an unsprayed vegetated buffer zone of 5 m was obtained for ~~D1 ditch, D2 ditch, D3 ditch, D6 ditch and~~ R3~~4~~4 stream for ~~*Daphnia*~~ *Pimephales promelas*.

For **metabolites** 1,2,4-triazole and CGA 205375 step 1 calculations reached an acceptable risk for aquatic organisms in all crops.

CIAZ
Regarding the formulation, calculated PEC/RAC ratios indicated an acceptable risk for the most sensitive group of aquatic organisms (risk for acute fish as characterised by a LC_{50} for *Oncorhynchus mykiss* of 14030 µg f.p./L in connection with an assessment factor of 100). No mitigation measures will be needed.

In addition, a risk assessment for the combinations of a.s. in the formulation was performed and no unacceptable risk was obtained.

Conclusions
Winter cereals – Spe 3: *To protect aquatic organisms respect an unsprayed vegetated buffer zone of 5m to surface water bodies.*

zRMS comments:

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", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).

The calculations based on PEC_{sw}/RAC for active substances – boscalid and difenconalzole and their metabolites have been accepted by zRMS-PL.

DIFENCONAZOLE

The PEC_{sw}/RAC ratio for the most sensitive organism – long term exposure for fish for difenconazole for scenarios D1 (ditch and stream), D2 (ditch and stream) and D3 (ditch), D4 (stream), D5 (stream), D6 (ditch) and R1 and R3 stream scenario on winter cereals indicated an acceptable risk for aquatic organisms with 5-meter buffer zone to surface water bodies, but for R4 stream scenario on winter cereals an acceptable risk for aquatic organisms **5-meter** vegetative buffer zone, calculated by FOCUS STEP 4 programme is identified.

The PECsw/RAC ratio for boscalid for all scenarios passed the trigger below 1 with PEC_{sw} STEP1-3 calculations on winter cereals.

The PEC/RAC ratio for metabolites of difenconazole and boscalid for all scenarios passed the trigger below 1 with PEC_{sw} STEP1- 2 calculations on winter cereals.

CIAZ

The risk assessment for the combinations of a.s. in the formulation CIAZ indicated no unacceptable risk for the Ciaz.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with Boscalid and Difenoconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of CIAZ were not evaluated as part of the EU assessment of Boscalid and Difenoconazole.

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

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

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Boscalid	Oral	LD_{50} = 166 µg/bee	SANCO/3919/2007 – rev. 5
Apis mellifera	Boscalid	Contact	LD_{50} = 200 µg/bee	SANCO/3919/2007 – rev. 5
Apis mellifera	Difenoconazole	Oral	LD_{50} > 177 µg/bee	EFSA Journal 2011;9(1):1967
Apis mellifera	Difenoconazole	Contact	LD_{50} > 100 µg/bee	EFSA Journal 2011;9(1):1967
Apis mellifera	CIAZ	Oral	LD_{50} > 400 µg f.p./bee (equivalent to >85.9 µg Boscalid/bee + >23.7µg Difenoconazole/bee)	KCP 103.1.1.1 Lemańska, N. 2018 B/80/16
Apis mellifera	CIAZ	Contact	LD_{50} > 400 µg f.p./bee (equivalent to >85.9 µg Boscalid/bee + >23.7µg Difenoconazole/bee)	KCP 103.1.1.2 Lemańska, N. 2018 B/81/16
Higher-tier studies (tunnel test, field studies)				
Boscalid: None Difenoconazole: No significant effects on bee mortality, foraging behaviour, flight activity or brood health in semi-field study with the formulation SCORE 250 EC.				

9.6.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to CIAZ formulation.

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).

9.6.2.1 Hazard quotients for bees

Table 9.6-2: First-tier assessment of the risk for bees due to the use of CIAZ in winter wheat

Intended use	**Winter wheat**		
Active substance	Boscalid		
Application rate (g/ha)	2 x 350		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	166	350	2.11
Contact toxicity	200		1.75
Active substance	Difenoconazole		
Application rate (g/ha)	2 x 100		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	177	100	0.56
Contact toxicity	100		1.00
Product	CIAZ		
Application rate (g/ha)	2 x 1.5 L f.p./ha (2 x 1666.5* g f.p./ha)		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>400	1666.5	<4.17
Contact toxicity	>400		<4.17

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
* Based on the density of the formulation = 1.111 g/mL

zRMS comments:

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).

Therefore, the risk assessment has been conducted according to EPPO/OEPP (2003) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)).

Based on the acute risk assessment with the consideration SANCO/10329/2002 rev.2 (final), October 17, 2002), HQ values for adult bees from exposure of CIAZ are< 50, indicating un acceptable risk to adult bees. The HQ values are lower than the trigger of 50, indicating low risk to bees from following application of CIAZ.

According to EU Reg. 284 /2009, the chronic toxicity test for adult bees, the chronic test for larvae should be provided for authorisation of plant protection product.

However, the final decision of the date of submission these studies by the Applicant should be considered at MSs level.

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

Not relevant.

9.6.3 Effects on bumble bees

Not relevant.

9.6.4 Effects on solitary bees

Not relevant.

9.6.5 Overall conclusions

First-tier assessments indicate that no unacceptable risk for bees exposed to CIAZ is expected according to the intended uses.

ZRMS comments:

The risk to bees for CIAZ was assessed in line with the Terrestrial Guidance document (2002).

Both hazard quotients for oral and contact toxicity for honey bees are considerably lower than 50, indicating that the proposed uses of CIAZ poses an acceptable risk.

According to EU Reg. 284 /2009, the chronic toxicity test for adult bees, the chronic test for larvae should be provided for authorisation of plant protection product. However, the final decision of the date of submission these studies by the Applicant, should be considered at MSs level.

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 Boscalid and Difenoconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of CIAZ were not evaluated as part of the EU assessment of Boscalid and Difenoconazole.

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

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

Species	Substance	Exposure System	Results	Reference
Boscalid				

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	BAS 510 01 F (Boscalid 50%)	Laboratory test glass plates (2D) 14 d	LR_{50} > 1800 g a.s./ha	SANCO/3919/2007 – rev. 5
Aphidius rhopalosiphi (imagines)	BAS 510 01 F (Boscalid 50%)	Laboratory test glass plates (2D) 48 h	LR_{50} > 1800 g a.s./ha	SANCO/3919/2007 – rev. 5
Typhlodromus pyri (protonymphs)	BAS 510 01 F (Boscalid 50%)	Extended laboratory test (2D) 14 d	Mortality (after 1 week): 0 % at 1800 g a.s./ha Fertility (after 4 weeks): 3 % at 180 g a.s./ha	SANCO/3919/2007 – rev. 5
Aphidius rhopalosiphi (imagines)	BAS 510 01 F (Boscalid 50%)	Extended laboratory test (2D) 48 h	Mortality (after 1 week): 11 % at 1800 g a.s./ha Fertility (after 4 weeks): 34% at 1800 g a.s./ha	SANCO/3919/2007 – rev. 5
Difenoconazole				
Typhlodromus pyri	Difenoconazole	Laboratory test glass plates (2D) 14 d	LR_{50} = 112 g a.s./ha	EFSA Journal 2011;9(1):1967
Aphidius rhopalosiphi	Difenoconazole	Laboratory test glass plates (2D) 14 d	LR_{50} = 178 g a.s./ha	EFSA Journal 2011;9(1):1967
Aleochara bilineata (adults)	DIVIDEND 030FS	Laboratory test 14d Quartz sand containing plastic box (2D)	At 23.2 g ai/ha: LR_{50} > 23.2 g a.s./ha (22.2% effect)	EFSA Journal 2011;9(1):1967
Poecilus cupreus (adults)	DIVIDEND 030FS	14 d Exposition to wheat seeds	At 18.8 g ai/ha: LR_{50} > 18.8 g a.s./ha (no effect)	EFSA Journal 2011;9(1):1967
Poecilus cupreus (larvae)	DIVIDEND 030FS	48-51 d Exposition to wheat seeds	At 56.4 g ai/ha: LR_{50} > 56.4 g a.s./ha (no effect)	EFSA Journal 2011;9(1):1967
Aphidius rhopalosiphi (juveniles)	SCORE 250 EC	Laboratory test 14 d Glass plate (2D)	At 5, 127, 253 g ai/ha: $LR_{50} \geq 253$ g a.s./ha NOER = 5.06 g a.s./ha	EFSA Journal 2011;9(1):1967
Typhlodromus pyri (proto-nymphs)	SCORE 250 EC	Laboratory test 14 d Glass plate (2D)	At 5, 127, 253 g ai/ha: $ER_{50} \geq 127$ g a.s./ha NOER = 5.06 g a.s./ha	EFSA Journal 2011;9(1):1967
Chrysoperla carnea (larvae)	SCORE 250 EC	Laboratory test 22 or 27 d Glass plate (2D)	At 4, 100, 200 g ai/ha: ER_{50} > 200 g a.s./ha NOER = 101 g a.s./ha	EFSA Journal 2011;9(1):1967
Pardosa spp. (adults)	SCORE 250 EC	Laboratory test 14d moist sand containing plastic box (2D)	At 4, 100, 200 g ai/ha: ER_{50} > 200 g a.s./ha (no effect)	EFSA Journal 2011;9(1):1967
Poecilus cupreus (adults)	SCORE 250 EC	Laboratory test 14d moist sand containing plastic box (2D)	At 6, 30, 150, 300 g ai/ha: ER_{50} > 300 g a.s./ha	EFSA Journal 2011;9(1):1967

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (proto-nymphs)	SCORE 250 EC	Extended laboratory test 14 d	At 6, 30, 150, 300 g ai/ha: ER_{50} = 210 (165-267) g a.s/ha NOER = 30 g a.s./ha	EFSA Journal 2011;9(1):1967
Chrysoperla carnea (larvae)	SCORE 250 EC	Extended laboratory test 50 d Glass plate (2D)	At 14, 28, 75, 125, 202, 288 g ai/ha: ER_{50} > 288 g a.s./ha NOER: No reliable	EFSA Journal 2011;9(1):1967
Orius laevigatus (nymphs)	SCORE 250 EC	Extended laboratory test 10 d exposure, 10 d re-production	At 6, 30, 150, 300 g ai/ha: 0%, 7%, 10%, 18% LR_{50} > 304 g a.s./ha	DAR, 2006 and EFSA Journal 2011;9(1):1967
Semi-field studies				
Trichogramma cacoeciae (adults)	SCORE 250 EC	Broad bean under field condition and reproductive phase under laboratory condition 2-5 d exposure	At 15, 75, 125, 288 g ai/ha: ER_{50} > 288 g a.s./ha NOER = 125 g a.s./ha	EFSA Journal 2011;9(1):1967
Coccinella septempunctata (larvae)	SCORE 250 EC	Broad bean under field condition and reproductive phase under laboratory condition Exposure: until pupation of the larvae Reproduction phase: up to 7 weeks	At 4 applications of 125 g ai/ha with 14-day intervals: ER_{50} > 4 x 125 g a.s./ha NOER: No reliable	EFSA Journal 2011;9(1):1967
Episyrphus balteatus (larvae)	SCORE 250 EC	Broad bean under field condition and reproductive phase under laboratory condition	At 15, 75, 125, 288 g ai/ha: LR_{50} > 288 g a.s./ha for aged residues based on mortality. Results from fresh residues not reliable dut to high control mortality. NOER: No reliable	EFSA Journal 2011;9(1):1967
Episyrphus balteatus (larvae)	SCORE 250 EC	Broad bean under field condition and reproductive phase under laboratory condition	At 288 g ai/ha: 62% effect when an outlier was excluded	EFSA Journal 2011;9(1):1967
Field studies				

Boscalid:
None.

Difenoconazole:
SCORE 250 EC, field study on predatory mites in apple orchards in Italy. 4 applications of 79.5 g as/ha at intervals of 10 or 11 days. No significant effect on population density of predatory mites up to 28 days after the last applications, excepts for an increased population on day 28 after the last application.

SCORE 250 EC, filed study on predatory mites in apple orchards in Germany. 4 applications of 59.6 g as/ha at intervals of 9 or 12 days. No significant effect on population density of predatory mites up to 28 days after the last application.

Table 9.7-2: Endpoints and effect values relevant for the risk assessment for non-target arthropods regarding formulation CIAZ

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri (protonymphs)	CIAZ	Laboratory test (2D)	LR_{50} > 7.7 L f.p./ha	KCP 10.3.2.1-01 Angayarkanni, V. 2019 5651/2019
Aphidius rhopalosiphi (adult)	CIAZ	Laboratory test (2D)	LR_{50} > 7.7 L f.p./ha	KCP 10.3.2.1-02 Angayarkanni, V. 2019 5652/2019
Typhlodromus pyri (protonymphs)	CIAZ	Extended study on bean leaves (2D)	LR_{50} > 10.20 L f.p./ha ER_{50} > 10.20 L f.p./ha	KCP 10.3.2.2-01 Lemańska, N. 2018 B/83/16
Aphidius rhopalosiphi (adult)	CIAZ	Extended study on barley seedlings (3D)	LR_{50} > 8.0 L f.p./ha ER_{50} > 8.0 L f.p./ha	KCP 10.3.2.2-02 Lemańska, N. 2018 B/82/16

9.7.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to CIAZ formulation.

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), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

Table 9.7-3: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of CIAZ in winter wheat

Intended use	**Winter wheat**		
Active substance/product	CIAZ		
Application rate (L f.p./ha)	2 x 1.5		
MAF	1.7 (foliar)		
Test species Tier I	**LR_{50} (lab.) (g/ha)**	**$PER_{in\text{-}field}$ (g/ha)**	**$HQ_{in\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 7.7	2.55	<0.33
Aphidius rhopalosiphi	> 7.7		<0.33

Test species Higher-tier	**Rate with ≤ 50 % effect* (L f.p./ha)**	**$PER_{in-field}$ (L f.p./ha)**	**$PER_{in-field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	>10.20	2.55	yes
Aphidius rhopalosiphi	>8.00		yes
Active substance/product	CIAZ		
Application rate (L f.p./ha)	2 x 1.5 (2 x 0.3** L f.p./ha)		
MAF	1.9 (soil)		
Test species Higher-tier	**Rate with ≤ 50 % effect* (L f.p./ha)**	**$PER_{in-field}$ (L f.p./ha)**	**$PER_{in-field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	>10.20	0.57	yes
Aphidius rhopalosiphi	>8.00		yes

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

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

** Rate with a 80% of interception.at BBCH from 30-59. According to the interception values of FOCUS (2012)[1].

9.7.2.2 Risk assessment for off-field exposure

Table 9.7-4: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of CIAZ in winter wheat

Intended use	**Winter wheat**
Active substance/product	CIAZ
Application rate (g/ha)	2 x 1.5
MAF	1.7 (foliar)
vdf	10 (Tier 1) / 1 (Higher-tier)

Test species Tier I	**LR_{50} (lab.) (g/ha)**	**Drift rate**	**$PER_{off-field}$ (g/ha)**	**CF**	**$HQ_{off-field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	> 7.7	0.0238	0..006	10	0.01
Aphidius rhopalosiphi	> 7.7				0.01
Test species Higher-tier	**Rate with ≤ 50 % effect* (L f.p./ha)**	**Drift rate**	**$PER_{off-field}$ (L f.p./ha)**	**CF**	**corr. $PER_{off-field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	>10.20	0.0238	0.006	5	yes
Aphidius rhopalosiphi	>8.00		0.061		yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

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

zRMS comments:

The calculations of the risk assessment for in – field for CIAZ for two indicator species were accepted by ZRMS-PL. HQ in - field and HQ-off field are below 2 based on laboratory studies (Tier1).

[1] FOCUS (2012) "Focus groundwater scenarios in the EU review of active substances" Report of the FOCUS Groundwater Scenarios Workgroup, EC Document Reference Sanco/321/2000 rev.2, 202 pp.

The PER-in and PER_{off}-field corrected for T.Pyri and A. rhopalosiphi (based on the extended laboratory studies) are below the rate with ≤ 50 % effects. Therefore, this assessment indicates that CIAZ poses low risk to in-field and off-field non-target arthropods following application according to the proposed use patterns.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

No in-field and off-field risk to non-target arthropods is expected after the application of CIAZ according to the proposed GAP.

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

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with Boscalid, Difenoconazole and its relevant 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 CIAZ were not evaluated as part of the EU assessment of Boscalid and Difenoconazole.

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

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

Species	Substance	Exposure System	Results	Reference
Boscalid				
Eisenia fetida	Boscalid	Mixed into substrate 14 d, acute	LC_{50} > 1000 mg a.s./kg dw **$LC_{50,corr}$ > 500 mg a.s./kg dw***	SANCO/3919/2007 – rev. 5

Species	Substance	Exposure System	Results	Reference
Eisenia fetida	BAS 510 01 F (Boscalid 500 g/kg)	Mixed into substrate 14 d, acute	LC_{50} > 1000 mg prod./kg dw $LC_{50, corr}$ > 500 mg prod./kg dw*	SANCO/3919/2007 – rev. 5
Eisenia fetida	BAS 510 01 F (Boscalid 500 g/kg)	Mixed into substrate 56 d, chronic	NOEC = 3.6 kg prod./ha $NOEC_{,corr}$ = 1.8 kg prod./ha* **$NOEC_{,corr}$ = 1.197 mg a.s./kg dw***	SANCO/3919/2007 – rev. 5
Folsomia candida	BAS 510 01 F (Boscalid 500 g/kg)	Mixed into substrate 28 d, chronic	NOEC (reproduction) >1000 mg BAS 510 01 F /kg soil (>500 mg a.s./kg soil) **$NOEC_{,corr}$** >500 mg BAS 510 01 F /kg soil* **(>250 mg a.s./kg soil*)**	SANCO/3919/2007 – rev. 5
Difenoconazole				
Eisinia fetida	Difenoconazole	14 d, acute	LC_{50} > 610 mg a.s./kg dw soil $LC_{50\ corr}$ > 305 mg a.s./kg dw soil*	EFSA Journal 2011;9(1):1967
Eisinia fetida	Difenoconazole	56 d, chronic	No reliable data, assessment based on representative formulation studies	EFSA Journal 2011;9(1):1967
Eisinia fetida	DIVIDEND 030FS	56 d, chronic, reproduction	NOEC = 0.2 mg a.s./kg dw soil $NOEC_{corr}$ = 0.1 mg/kg dw*)	EFSA Journal 2011;9(1):1967
Eisenia fetida	Difenoconazole	56 d, chronic, reproduction	NOEC = 0.5 mg a.s./kg dw soil **$NOEC_{corr}$ = 0.25 mg/kg dw*)**	Addendum to DAR following submission of confirmatory data, 2014
Eisinia fetida	SCORE 250 EC	14 d, acute	LC_{50} 40 mg as/kg dw **$LC_{50,corr}$ = 20 mg as/kg dw***	EFSA Journal 2011;9(1):1967
Eisinia fetida	SCORE 250 EC	56 d, chronic, reproduction	No reliable data	EFSA Journal 2011;9(1):1967
Eisinia fetida	CGA 71019 (1,2,4-triazole)	14 d, acute	LC_{50} >1000 mg met/kg dw **$LC_{50,corr}$ >500 mg as/kg dw***	EFSA Journal 2011;9(1):1967
Eisinia fetida	CGA 71019 (1,2,4-triazole)	56 d, chronic, reproduction	NOEC = 1 mg met/kg **$NOEC_{corr}$ = 0.5 mg/kg dw*)**	EFSA Journal 2011;9(1):1967

Species	Substance	Exposure System	Results	Reference
Eisinia fetida	CGA 205375	14 d, acute	LC_{50} = 312 mg met/kg dw **$LC_{50,corr}$ =156 mg as/kg dw***	EFSA Journal 2011;9(1):1967
Folsomia candida	Difenoconazole	28 d, chronic	NOEC = 500 mg as/kg dw **$NOEC_{corr}$ = 250 mg/kg dw***	EFSA Journal 2011;9(1):1967
Folsomia candida	CGA 71019 (1,2,4-triazole)	28 d, chronic	NOEC = 1.8 mg as/kg dw **$NOEC_{corr}$ = 0.9 mg/kg dw***	EFSA Journal 2011;9(1):1967
CIAZ				
Eisenia andrei	CIAZ	Mixed into substrate 56 d, chronic 5 % peat content	NOEC = 100 mg/kg dw (equivalent to 21.5 mg Boscalid/kg dw + 5.9 mg Difenoconazole/kg dw soil) **$NOEC_{corr}$ = 50 mg/kg dw* (equivalent to 10.75 mg Boscalid/kg dw + 2.95 mg Difenoconazole/kg dw soil)** EC_{10} = 117.7 mg/kg dw (equivalent to 25.3 mg Boscalid/kg dw + 7.0 mg Difenoconazole/kg dw soil)	KCP 10.4.1.1 Pieczka, P. 2018 G/129/17

Species	Substance	Exposure System	Results	Reference
Folsomia candida	CIAZ	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 560 mg/kg dw (equivalent to 120.3 mg Boscalid/kg dw + 33.16 mg Difenoconazole/kg dw soil) EC_{10} = 357.6 mg/kg dw (equivalent to 76.8 mg Boscalid/kg dw + 21.2 mg Difenoconazole/kg dw soil) **$EC_{10\ corr}$ = 178.8 mg/kg dw* (equivalent to 38.4 mg Boscalid/kg dw + 10.6 mg Difenoconazole/kg dw soil)**	KCP 10.4.2.1-01 Pieczka, P. 2018 G/130/17
Field studies				
Boscalid: None Difenoconazole: None				
Litter bag test				
Boscalid: None Difenoconazole: In a litter bag study with SCORE 250 EC, a 17% reduction in decomposition rate was observed at direct overspray of 506 g a.s/ha compared to the control after 168 days. Exposure conditions considered as worst case wompared to the representative use of Difenoconazole in carrots, pome fruits and as seed treatment.				

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

9.8.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to CIAZ formulation.

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).

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, Table 8.7-3 to 8.7-7. According to the assessment of environmental-fate data, multi-annual accumulation in soil is considered for Boscalid, Difenoconazole and its metabolites.

Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of CIAZ in winter wheat

Intended use	**Winter wheat**			
Acute effects on earthworms				
Product/active substance	**LC_{50} (mg/kg dw)**	**$LC_{50, corr}$ (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Boscalid	1000	500	0.202	2475.25
Difenoconazole	40	20	0.060	333.3
1,2,4-triazole	1000	500	0.002	250000
CGA 205375	312	156	0.005	31200
Chronic effects on earthworms				
Product/active substance	**NOEC (mg/kg dw)**	**$NOEC_{corr}$ (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Boscalid	2.394	1.197	0.202	5.93
Difenoconazole	0.5	0.25	0.060	4.2
1,2,4-triazole	1.0	0.5	0.002	250
CIAZ	100	50	0.889	56.24
CIAZ[1]	21.5	10.75	0.202	53.22
CIAZ[2]	5.9	2.95	0.060	49.2
Chronic effects on other soil macro- and mesofauna – *Folsomia candida*				
Product/active substance	**NOEC/ EC_{10} (mg/kg dw)**	**$NOEC_{corr}$ (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Boscalid	500	250	0.202	1237.62
Difenoconazole	500	250	0.060	4166.7
1,2,4-triazole	1.8	0.9	0.002	450
CIAZ	357.6	178.8	0.889	201.12
CIAZ[1]	76.8	38.4	0.202	190.10
CIAZ[2]	21.2	10.6	0.060	176.7

TER values shown in bold fall below the relevant trigger.
[1]Risk assessment based on an endpoint expressed as mg Boscalid/kg dw from CIAZ study.
[2]Risk assessment based on an endpoint expressed as mg Difenoconazole/kg dw from CIAZ study.

Chronic studies with CIAZ on earthworms and collembolan were submitted by the Applicant and no unacceptable risk was obtained after the risk assessment. Moreover, the risk assessment for NTA with the formulation was acceptable for indicator species and including with endpoints from Monograph for the ground dwelling arthropods *Poecilus* and *Pardosa* sp for difenoconazole according to the proposed uses rates. Therefore, the Applicant considers that an acceptable risk to *Hypoaspis aculeifer* for formulation CIAZ can be concluded on the basis that low risks to earthworms and other soil macro-organisms, and ground dwelling arthropod with formulation were concluded.

Therefore, it is expected that chronic toxicity on *Hypoaspis* will result from prolonged exposure and the formulation is not expected to remain intact in the environment.

However, the available long term study on the active ingredient from Addendum to DAR was used for the risk assessment and did not achieve the TER ≥ 5. It was not considered as representative based on recom-

mendations in the Guidance Document On Terrestrial Ecotoxicology (SANCO/10329/2002), which states that certain study types (for example non-target arthropod studies, the earthworm reproduction test and the soil micro-flora test) may be conducted with a formulated product instead of the active substance. Hence, it is considered appropriate to use the more reliable NOEC from available formulation studies, as CIAZ in this case, for the risk assessment.

Higher-tier risk assessment

Not relevant.

zRMS comments:

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate). The risk assessment considering the agreed PEC_s value was presented by zRMS-PL below:

Table 9.8-2$_{corr}$: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of CIAZ in winter wheat.

Intended use	**Winter wheat**			
Acute effects on earthworms				
Product/active substance	**LC_{50} (mg/kg dw)**	**$LC_{50, corr}$ (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Boscalid	1000	500	0.182	747.25
Difenoconazole	40	20	0.052	884.61
Chronic effects on earthworms				
Product/active substance	**NOEC (mg/kg dw)**	**$NOEC_{corr}$ (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Boscalid	2.394	1.197	0.182	6.57
Difenoconazole	0.5	0.25	0.052	**4.80**
Ciaz [1]	21.5	10.75	0.182	59.06
Ciaz [2]	5.9	2.95	0.052	56,73
Chronic effects on other soil macro- and mesofauna – *Folsomia candida*				
Product/active substance	**NOEC/EC_{10} (mg/kg dw)**	**$NOEC_{corr}$ (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Boscalid	500	250	0.182	373.62
Difenoconazole	500	250	0.052	807.69
CIAZ [1]	76.8	38.4	0.182	210.98
Ciaz [2]	21.2	10.6	0.052	203.84

TER values shown in bold fall below the relevant trigger.
[1]Risk assessment based on an endpoint expressed as mg Boscalid/kg dw from CIAZstudy.
[2]Risk assessment based on an endpoint expressed as mg Difenoconazole/kg dw from CIAZstudy.

zRMS comments:

TER_{LT} values for active substance – boscalid and its metabolites are above trigger value of 5, indicating an acceptable risk for earthworm.

TER_{LT} value for active substance – difenconazole is slight below trigger value of 5, (being 4.80), indicating further refinement. The field study on the difenoconazole formulation SCORE 250 EC (Hamberger 2015) which was discussed at the PPR TC 58 on difenoconazole (28-30.06.2021) with the NOEC of 1 L product/ha (corresponding to 250 g a.s./ha) is used by zRMS for conclude an acceptable risk from difeconazole in Ciaz formulation.

In addition, TER_{LT} for ppp based on formulation study with Ciaz (converted to the a.s.- difenconazole) for earthworm is above trigger of 5, indicating acceptable risk for earthworm.

The chronic TER values for Boscalid and Difenoconazole and their metabolites for Folsomia candida were above the relevant Annex VI trigger of 10 and 5, respectively.

Therefore, it is concluded that the active substances and formulation Ciaz do not pose an acute and long-term risk to earthworms and other soil macro- and mesofauna when applied according to the proposed uses rates.

9.8.3 Overall conclusions

The acute and chronic TER values for Boscalid and Difenoconazole were above the relevant Annex VI trigger of 10 and 5, respectively. Therefore, it is concluded that the active substances do not pose an acute and long-term risk to earthworms and other soil macro- and mesofauna when applied according to the proposed uses rates.

Morevover, an application of CIAZ in respect of the GAP should not represent an acute and long-term risk to earthworm and the other soil meso/microfauna.

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 Boscalid, Difenoconazole and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of CIAZ were not evaluated as part of the EU assessment of Boscalid and Difenoconazole.

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

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

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	BAS 510 01 F (Boscalid 500 g/kg)	28 d, aerobic Loamy sand / Loamy silt	No effect up to 12 kg product/ha (equivalent to 6 kg a.s./ha or 8 mg a.s./kg soil)	SANCO/3919/2007 – rev. 5
C-mineralisation	BAS 510 01 F (Boscalid 500 g/kg)	28 d, aerobic Loamy sand / Loamy silt	No effect up to 12 kg product/ha (equivalent to 6 kg a.s./ha or 8 mg a.s./kg soil)	SANCO/3919/2007 – rev. 5
N-mineralisation	difenoconazole	28 d, aerobic silty loam	< 25% effect at day 28 at 1.67 and 16.7 mg a.s./kg dw soil in silty loam, 60% increase in loamy sand	EFSA Journal 2011;9(1):1967
N-mineralisation	CGA 71019 (1,2,4-triazole)	28 d, aerobic sandy loam	< 25% effect at day 28 at 0.035 and 0.353 mg a.s./kg dw soil	EFSA Journal 2011;9(1):1967
N-mineralisation	CGA 205375	28 d, aerobic sandy loam	< 25% effect at day 28 at 0.09 and 0.22 mg a.s./kg dw soil	EFSA Journal 2011;9(1):1967
N-mineralisation	SCORE 250 EC	28 d, aerobic 2 field soils	< 25% effect at day 28 at 0.33 and 1.67 mg a.s./kg dw soil	EFSA Journal 2011;9(1):1967
C-mineralisation	difenoconazole	28 d, aerobic loamy sand	< 25% effect at day 28 at 1.67 and 16.7 mg a.s./kg dw soil	EFSA Journal 2011;9(1):1967
C-mineralisation	CGA 71019 (1,2,4-triazole)	28 d, aerobic sandy loam	< 25% effect at day 28 at 0.035 and 0.353 mg a.s./kg dw soil	EFSA Journal 2011;9(1):1967
C-mineralisation	CGA 205375	28 d, aerobic sandy loam	< 25% effect at day 28 at 0.09 and 0.22 mg a.s./kg dw soil	EFSA Journal 2011;9(1):1967
C-mineralisation	SCORE 250 EC	28 d, aerobic 2 field soils	< 25% effect at day 28 at 0.33 and 1.67 mg a.s./kg dw soil	EFSA Journal 2011;9(1):1967
N-mineralisation	CIAZ	84 d, aerobic agricultural soil	< 25% effect at day 42 at 6.648 mg f.p./kg dw soil (equivalent to 1.4 mg boscalid/kg dw soil + 0.4 mg difenoconazole/kg dw soil) < 25% effect at day 84 at 33.24 mg f.p./kg dw soil (equivalent to 7.0 mg boscalid/kg dw soil + 2.0 mg difenoconazole/kg dw soil)	KCP 10.5.1 Pieczka, P. 2018 G/128/17

Endpoint	Substance	Exposure System	Results	Reference
C-mineralisation	CIAZ	28 d, aerobic agricultural soil	< 25% effect at day 28 at 6.648 and 33.24 mg f.p./kg dw soil (equivalent to 1.4 mg boscalid/kg dw soil + 0.4 mg difenoconazole/kg dw soil and 7.0 mg boscalid/kg dw soil + 2.0 mg difenoconazole/kg dw soil, respectively)	KCP 10.5.2 Pieczka, P. 2017 G/127/17

9.9.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to CIAZ formulation.

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).

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of CIAZ in winter wheat

Intended use	**Winter wheat**		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Boscalid	8.0 (at 28 d)	0.182	yes
Difenoconazole	16.7 (at 28 d)	0.052	yes
CGA 71019 (1,2,4-triazole)	0.353 (at 28 d)	0.002	yes
CGA 205375	0.22 (at 28 d)	0.005	yes
CIAZ	33.24 (at 84 d)	0.889	yes
CIAZ[1]	7.0 (at 84 d)	0.182	yes
CIAZ[2]	2.0 (at 84 d)	0.052	yes
C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Boscalid	8.0 (at 28 d)	0.182	yes
Difenoconazole	16.7 (at 28 d)	0.052	yes
CGA 71019 (1,2,4-triazole)	0.353 (at 28 d)	0.002	yes

CGA 205375	0.22 (at 28 d)	0.005	yes
CIAZ	33.24 (at 84 d)	0.889	yes
CIAZ[1]	7.0 (at 84 d)	0.182	yes
CIAZ[2]	2.0 (at 84 d)	0.052	yes

[1]Risk assessment based on an endpoint expressed as mg Boscalid/kg dw from CIAZ study.
[2]Risk assessment based on an endpoint expressed as mg Difenoconazole/kg dw from CIAZ study.

zRMS comment:

The risk assessment for soil micro-organism after exposure of both active substances and their metabolites has been verified and accepted by the zRMS. The effects on the nitrogen transformations are acceptable (<25%) at concentration which is higher than the maximum relevant PEC_s for the maximum application rate of active substances and the product Ciaz.

9.9.3 Overall conclusions

Risk assessment conducted with relevant PEC_{soil} for the active substances Boscalid and Difenoconazole indicate a low risk to soil microorganisms when applied according to the proposed use rates.

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 Boscalid and Difenoconazole. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of CIAZ were not evaluated as part of the EU assessment of Boscalid and Difenoconazole.

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

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

Species	Substance	Exposure System	Results	Reference
Daucus carota $_d$ [1)] *Brassica oleracea* $_d$ [2)] *Pisum sativum* $_d$ [3)] *Zea mays* $_m$ [4)] *Avena sativa* $_m$ [5)] *Allium cepa* $_m$ [6)]	BAS 510 01 F (Boscalid 500 g/kg)	14 d Vegetative vigour 1.2 kg a.s./ha	[1)] 2.5 [2)] 0 [3)] 0 [4)] 0 [5)] 0 [6)] 0 Mean phytotoxicity (% control)	Monograph Boscalid, 2002
Daucus carota $_d$ [1)] *Brassica oleracea* $_d$ [2)] *Pisum sativum* $_d$ [3)] *Zea mays* $_m$ [4)] *Avena sativa* $_m$ [5)] *Allium cepa* $_m$ [6)]	BAS 510 01 F (Boscalid 500 g/kg)	14 d Vegetative vigour 3.6 kg a.s./ha	[1)] 5 [2)] 0 [3)] 0 [4)] 0 [5)] 0 [6)] 0 Mean phytotoxicity (% control)	Monograph Boscalid, 2002
Daucus carota $_d$ [1)] *Brassica oleracea* $_d$ [2)] *Pisum sativum* $_d$ [3)] *Zea mays* $_m$ [4)] *Avena sativa* $_m$ [5)] *Allium cepa* $_m$ [6)]	BAS 510 01 F (Boscalid 500 g/kg)	14 d Vegetative vigour 1.2 kg a.s./ha	[1)] 109.4 [2)] 100.2 [3)] 91.2 [4)] 101.9 [5)] 105.7 [6)] 108.2 Mean fresh weight (% control)	Monograph Boscalid, 2002
Daucus carota $_d$ [1)] *Brassica oleracea* $_d$ [2)] *Pisum sativum* $_d$ [3)] *Zea mays* $_m$ [4)] *Avena sativa* $_m$ [5)] *Allium cepa* $_m$ [6)]	BAS 510 01 F (Boscalid 500 g/kg)	14 d Vegetative vigour 3.6 kg a.s./ha	[1)] 110.6 [2)] 98.9 [3)] 97.5 [4)] 103.5 [5)] 99.5 [6)] 113.6 Mean fresh weight (% control)	Monograph Boscalid, 2002
Avena sativa, *Brassica napus*, *Glycine maxima*	Difenoconazole	Emergence Vegetative vigour	$ER_{50\ emergence}$ > 10 mg a.s./kg dw soil (incorporation) $ER_{50\ vegetative\ vigour}$ > 10 mg a.s./kg dw soil (incorporation)	EFSA Journal 2011;9(1):1967
Glycine maxima	SCORE 250 EC	Emergence Vegetative vigour	**$ER_{50\ emergence}$ = 100 g a.s./ha (spray application)** $ER_{50\ vegetative\ vigour}$ > 100 g a.s./ha (spray application)	EFSA Journal 2011;9(1):1967

Species	Substance	Exposure System	Results	Reference
Pisum sativum $_d$ *Helianthus annuus* $_d$ *Brassica oleracea var. capitata* $_d$ *Daucus carota* $_d$ *Allium cepa* $_m$ *Avena sativa* $_m$.	CIAZ	14 d Seedling emergence	**ER_{50} > 6000 mL f.p./ha (equivalent to >1428.0 g boscalid/ha + 393.6 g difenoconazole/ha)**	KCP 10.6.2-01 Pieczka, 2018 G/132/17
Pisum sativum $_d$ *Helianthus annuus* $_d$ *Brassica oleracea var. capitata* $_d$ *Daucus carota* $_d$ *Allium cepa* $_m$ *Avena sativa* $_m$.	CIAZ	21 d Vegetative vigour	ER_{50} > 6000 mL f.p./ha (equivalent to >1428.0 g boscalid/ha + 393.6 g difenoconazole/ha)	KCP 10.6.2-02 Pieczka, 2018 G/133/17

m: monocotyledonous; d: dicotyledonous

zRMS comment:

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area. The deterministic risk based on the ER_{50} > 6000 mL product/ha values and PER_{off}- field the risk is considered acceptable.

9.10.1.1 Justification for new endpoints

Not relevant as there is no deviation to the EU agreed endpoints, except for formulation, corresponding to data proper to CIAZ formulation.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

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). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Table 9.10-2: Assessment of the risk for non-target plants due to the use of CIAZ in winter wheat

Intended use	**Winter wheat**
Active substance/product	Boscalid

Application rate (g a.s./ha)	2 x 350			
MAF	1.7			
Test species	**ER_{50} (g a.s./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**TER criterion: TER ≥ 5**
Daucus carota *Brassica oleracea* *Pisum sativum* *Zea mays* *Avena sativa* *Allium cepa*	>1428	0.0238	14.16	>100.84
Active substance/product	Difenoconazole			
Application rate (g a.s./ha)	2 x 100			
MAF	1.7			
Test species	**ER_{50} (g a.s./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g a.s./ha)**	**TER criterion: TER ≥ 5**
Glycine maxima	100	0.0238	4.05	24.7
Active substance/product	CIAZ			
Application rate (mL f.p./ha)	2 x 1500			
MAF	1.7			
Test species	**ER_{50} (mL f.p./ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (mL f.p./ha)**	**TER criterion: TER ≥ 5**
Pisum sativum *Helianthus annuus* *Brassica oleracea var. capitata* *Daucus carota* *Allium cepa* *Avena sativa*	>6000	0.0238	60.69	>98.86

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

9.10.2.3 Higher-tier risk assessment

Not relevant.

9.10.2.4 Risk mitigation measures

No risk mitigation needed.

9.10.3 Overall conclusions

Risk assessment conducted with relevant toxicity data on non-target terrestrial plants for CIAZ shows that the Annex VI trigger value of 5 is not exceeded, indicating that CIAZ poses a low risk to non-target plants when applied according to the proposed use rates.

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

No additional data are available.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

	~~CIAZ~~
~~Common name~~	~~CIAZ~~
~~Classification and proposed labelling~~	
~~With regard to ecotoxicological endpoints (according to the criteria in Reg. 1272/2008, as amended)~~	~~Hazard classes (s), categories:~~ ~~Code(s) for hazard pictogram(s):~~ ~~Signal word: No signal word~~ ~~Hazard statement(s):~~ ~~Precautionary statement: P273, P391, P501~~

CIAZ

With regard to ecotoxicological endpoints (according to the criteria in Reg. 1272/ based on the lowest acute aquatic toxicity endpoint obtained with Ciaz no acute aquatic hazard category is given according to (EC) No 1272/2008 (CLP).
Regarding chronic classification of difenconazole (a.i. content of 6.6% w/w within the product), classified as chronic hazard cat. 1, is considered for the summation method in the 1st equation according to CLP (*M x hazard cat. 1*), yielding a value which is above the trigger of 25%.
Hence, Ciaz is classified as Chronic aquatic hazard category 1 (H410).
Chronic classification of Ciaz using the summation method is summarized in Table 9.13-1.

Table 9.13-1: Chronic classification of CIA using the summation method according to (EC) No 1272/2008.

Chronic classification of CIAZ						
Formulation component				Result (% Content x M-Factor)		
Name	Chronic Category	M-Factor	Content in CIAZ[%]			
difenconazole	1	100	6.6.	660		
1st equation	SUM *(M x Chronic 1)*				**≥ 25 %**	**Ciaz Aquatic Chronic Hazard Category 1**

Precautionary statement: P273, P391, P501

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

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	xxxxxxxx	2018	Boscalid 23.3% + Difenoconazole 6.6% SC:, Acute Toxicity Test Report No. W/95/17 xxxxxxxxxx GLP, Unpublished	Y	Sharda Cropchem Limited
KCP 10.2.1-02	Ewa Nierzędska	2018	Boscalid 23.3% + Difenoconazole 6.6% SC: *Daphnia magna*, acute immobilization test Report No. W/97/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.2.1-03	Ewa Nierzędska	2017	Boscalid 23.3% + Difenoconazole 6.6% SC: *Pseudokirchneriella subcapitata* SAG 61.81 Growth Inhibition Test Report No. W/96/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.2.1-04	Ewa Nierzędska	2018	Boscalid 23.3% + Difenoconazole 6.6% SC *Lemna gibba* L. CPCC 310, Growth inhibition test Report No. W/98/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	Owner
KCP 10.3.1.1.1	Natalia Lemańska	2018	Boscalid 23.3% + Difenoconazole 6.6% SC Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test Report No. B/80/16 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.3.1.1.2	Natalia Lemańska	2018	Boscalid 23.3% + Difenoconazole 6.6% SC Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test Report No. B/81/16 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1-01	Angayarkanni, V.	2019	A laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the predatory mite, *Typhlodromus pyri* (Scheuten) Report No. 5651/2019 Bioscience Research Foundation GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.1-02	Angayarkanni, V.	2019	A laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the parasatic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) Report No. 5652/2019 Bioscience Research Foundation GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.2-01	Natalia Lemańska	2018	An extended laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the predatory mite, *Typhlodromus pyri* (Sch.) Report No. B/83/16 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.3.2.2-02	Natalia Lemańska	2018	An extended laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez) Report No. B/82/16 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited

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 10.4.1.1	Paweł Pieczka	2018	Boscalid 23.3% + Difenoconazole 6.6% SC: Earthworm Reproduction Test (*Eisenia andrei*) Report No. G/129/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.4.2.1-01	Paweł Pieczka	2018	Boscalid 23.3% + Difenoconazole 6.6% SC: Collembolan (*Folsomia candida*) Reproduction Test Report No. G/130/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.5.1	Paweł Pieczka	2018	Boscalid 23.3% + Difenoconazole 6.6% SC: Soil Microorganisms: Nitrogen Transformation Test Report No. G/128/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.5.2	Paweł Pieczka	2017	Boscalid 23.3% + Difenoconazole 6.6% SC: Soil Microorganisms: Carbon Transformation Test Report No. G/127/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.6.2-01	Paweł Pieczka	2018	Boscalid 23.3% + Difenoconazole 6.6% SC: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test Report No. G/132/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited
KCP 10.6.2-02	Paweł Pieczka	2018	Boscalid 23.3% + Difenoconazole 6.6% SC: Terrestrial Plant Test: Vegetative Vigour Test Report No. G/133/17 Institute of Industrial Organic Chemistry Branch Pszczyna GLP, Unpublished	N	Sharda Cropchem Limited

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

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

The following tables are to be completed by MS

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

A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

A 2.2 KCP 10.2 Effects on aquatic organisms

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

A 2.2.2 Algae and macrophytes

Comments of zRMS:	The study is considered acceptable. The study is considered valid. • the mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used); • dissolved oxygen concentrations were within the range of 89 – 99% of air saturation value (obligatory above 60% of air saturation value). The determined concentrations of boscalid in fresh samples were in the range of 90.86 – 114.98% of the nominal concentration. The determined concentrations of difenoconazole in fresh samples were in the range of 90.88 – 99.96% of the nominal concentration. The results confirm correct preparation of the test item concentrations. The determined concentrations of boscalid in old samples were in the range of 75.30 – 98.14% of the nominal concentration. The determined concentrations of difenoconazole in old samples were in the range of 73.82 – 96.28% of the nominal concentration. Therefore, concentrations of boscalid and difenoconazole were not stable under the

	test conditions. **Agreed endpoints:** The endpoint values determined on the basis of the nominal test item concentrations: The LC_{50}/96 h =14.03 mg/L (95% confidence intervals: 11.37 – 17.40). The endpoint values determined on the basis of the nominal concentrations of boscalid: The LC_{50}/96 h = 3.016 mg/L (95% confidence intervals: 2.444 – 3.740). The endpoint values determined on the basis of the geometric means of determined concentrations of boscalid: The LC_{50}/96 h = 2.708 mg/L (95% confidence intervals: 2.212 – 3.339). The endpoint values determined on the basis of the nominal concentrations of difenoconazole: The LC_{50}/96 h = 0.830 mg/L (95% confidence intervals: 0.673 – 10.030). The endpoint values determined on the basis of the geometric means of determined concentrations of difenoconazole The LC_{50}/96 h =0.764 mg/L (95% confidence intervals: 0.612 – 0.960).

Reference:	KCP 10.2.1 - 01
Report	"Boscalid 23.3% + Difenoconazole 6.6% SC: Rainbow Trout, Acute Toxicity Test". xxxxxxxxxxxx, 2018, W/95/17. xxxxxxxxxxxxxx
Guideline(s):	Yes, OECD Guideline No. 203 (1992)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes

Materials and methods

Test item:

Description:	Boscalid 23.3% + Difenoconazole 6.6% SC
Production batch:	SCL - 20245;
A.i. content:	boscalid 23.8% w/v, difenoconazole 6.56% w/v

Test system:

Species:	Rainbow trout (*Oncorhynchus mykiss*)
Strain:	Walb.
Age:	2.5 months
Average weight:	0.51 g ± 0.08 g
Average length:	4.10 cm ± 0.11 cm
Source:	'The Culture of Salmonidae Fish in Zawoja', Poland.
Acclimation period:	7-day quarantine, 12-day acclimatization.

Diet:	During the adaptation the fish were fed with standard granulated fish food. Feeding of the fish was terminated 24 h before exposure initiation.

Experimental conditions:

Temperature:	14.2 – 14.9°C
Dissolved O_2:	89 – 99% ASV
Hardness:	75.1 mg/L $CaCO_3$
pH:	7.68 – 7.98
Light and photoperiod:	16h light and 8h dark
Loading:	Ten fish per aquarium. The ratio of fish weight per volume (10 L) was 0.36 g/L.
Test procedure:	The test was performed in temperature-controlled aquaria and incubated in a temperature-controlled room.

Experimental period: 96h

Test design and treatment

Static system (96 h of exposure).
According to the preliminary test results, the main final test included the test item concentrations of 80, 40, 20, 10, 5.0, and 2.5 mg/L and the control. The fish were observed for intoxication symptoms and mortality 3, 6, 24, 48, 72 and 96 h of exposure.
The concentration of boscalid and difenoconazole in water was determined using the validated high performance liquid chromatographic method with DAD. The determined concentrations of boscalid in fresh samples were in the range of 90.86 – 114.98% of the nominal concentration. The determined concentrations of difenoconazole in fresh samples were in the range of 90.88 – 99.96% of the nominal concentration. The results confirm correct preparation of the test item concentrations. The determined concentrations of boscalid in old samples were in the range of 75.30 – 98.14% of the nominal concentration. The determined concentrations of difenoconazole in old samples were in the range of 73.82 – 96.28% of the nominal concentration. Therefore, concentrations of boscalid and difenoconazole were not stable under the test conditions.

Results

The following validity criteria specified in the OECD Guideline No. 203 (1992) were met:

- the mortality in the control was 0% at exposure termination (should not exceed 10% or 1 fish if less than 10 fish are used);
- dissolved oxygen concentrations were within the range of 89 – 99% of air saturation value (obligatory above 60% of air saturation value).

Results of mortality during the test.

Nominal test item concentration [mg/L]	Number of fish	Number of dead fish in each observation term						Total of dead after 96 h of exposure [%]
		3 h	6 h	24 h	48 h	72 h	96 h	
Control	7	0	0	0	0	0	0	0
2.5	7	0	0	0	0	0	0	0
5.0	7	0	0	0	0	0	0	0
10	7	0	0	0	0	0	0	0
20	7	0	0	2	7	7	7	100
40	7	0	3	7	7	7	7	100
80	7	7	7	7	7	7	7	100

Time of exposure: 06.11.2017 – 10.11.2017

In the control and in the test item concentration of 2.5 mg/L neither mortality of fish nor symptoms of intoxication were observed during exposure.
In test item concentration of 5.0 mg/L, nontypical swimming and respiratory problems for five fish were observed after 6 h of exposure. After 24 and 48 h of exposure, nontypical swimming, respiratory problems and pigmentation change for five fish were observed. After 72 and 96 h of exposure, nontypical swimming and respiratory problems for six fish and pigmentation change for all fish were observed.
In the test item concentration of 10 mg/L, nontypical swimming and respiratory problems for two fish and were observed after 3 h of exposure. After 6 h of exposure, loss of balance for three fish and nontypical swimming for five fish as well as respiratory problems for all fish were observed. After 24 h of exposure, loss of balance, nontypical swimming, respiratory problems and pigmentation change for all fish were observed. After 48 h of exposure, loss of balance for four fish, nontypical swimming, respiratory problems and pigmentation change for all fish were observed. After 72 and 96 h of exposure, loss of balance for six fish, nontypical swimming, respiratory problems and pigmentation change for all fish were observed.
In the test item concentration of 20 mg/L, loss of balance and nontypical swimming for six fish and respiratory problems as well as pigmentation change for all fish were observed after 3 h of exposure. After 6 h of exposure, loss of balance, nontypical swimming, respiratory problems and pigmentation change for all fish were observed. After 24 h of exposure, two fish were dead, and loss of balance, nontypical swimming, respiratory problems as well as pigmentation change for five fish were observed. After 48 h of exposure, all fish were dead.
In the test item concentration 40 mg/L, loss of balance, nontypical swimming, respiratory problems and pigmentation change for all fish were observed after 3 h of exposure. After 6 h of exposure, three fish were dead, and loss of balance, nontypical swimming, respiratory problems as well as pigmentation change for four fish were observed. After 24 h of exposure, all fish were dead.
In the test item concentration 80 mg/L, all fish were dead after 3 h of exposure.

Conclusion
<u>The endpoint values for mortality of fish determined on the basis of the nominal test item concentrations:</u>
The LC_{50}/96 h value is 14.03 mg/L (95% confidence intervals: 11.37 – 17.40).
The Fisher's Exact Binomial Test with Bonferroni Adjustment performed with data for mortality at 96 h showed a significant difference between the test item concentrations of 80, 40, and 20 mg/L compared to the control. Therefore, the LOEC/96 h value is 20 mg/L, the NOEC/96 h value is 10 mg/L.
<u>The endpoint values for mortality of fish determined on the basis of the nominal concentrations of boscalid:</u>
The LC_{50}/96 h value is 3.016 mg/L (95% confidence intervals: 2.444 – 3.740).
The Fisher's Exact Binomial Test with Bonferroni Adjustment performed with data for mortality at 96 h showed a significant difference between the test item concentrations of 17.200, 8.600, and 4.300 mg/L compared to the control. Therefore, the LOEC/96 h value is 4.300 mg/L, the NOEC/96 h value is 2.150

mg/L.
The endpoint values for mortality of fish determined on the basis of the geometric means of determined concentrations of boscalid:
The LC_{50}/96 h value is 2.708 mg/L (95% confidence intervals: 2.212 – 3.339).
The Fisher's Exact Binomial Test with Bonferroni Adjustment performed with data for mortality at 96 h showed a significant difference between the geometric means of determined concentrations of boscalid 14.692, 7.788 and 3.812 mg/L compared to the control. Therefore, the LOEC/96 h value is 3.812 mg/L, the NOEC/96 h value is 1.957 mg/L.
The endpoint values for mortality of fish determined on the basis of the nominal concentrations of difenoconazole:
The LC_{50}/96 h value is 0.830 mg/L (95% confidence intervals: 0.673 – 10.030).
The Fisher's Exact Binomial Test with Bonferroni Adjustment performed with data for mortality at 96 h showed a significant difference between the test item concentrations of 4.736, 2.368, and 1.184 mg/L compared to the control. Therefore, the LOEC/96 h value is 1.184 mg/L, the NOEC/96 h value is 0.592 mg/L.
The endpoint values determined on the basis of the geometric means of determined concentrations of difenoconazole and mortality of fish:
The LC_{50}/96 h value is 0.764 mg/L (95% confidence intervals: 0.612 – 0.960).
The Fisher's Exact Binomial Test with Bonferroni Adjustment performed with data for mortality at 96 h showed a significant difference between the time-weighing means of determined concentrations of difenoconazole 4.231, 2.205, and 1.127 mg/L compared to the control. Therefore, the LOEC/96 h value is 1.127 mg/L, the NOEC/96 h value is 0.528 mg/L.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • the percentage of immobilization of Daphnia magna in the control was 0% (Criterion: not more than 10%), • the dissolved oxygen concentrations in the test vessels were within the range of 7.6 – 8.5 mg/L (criterion: not less than 3 mg/L). The determined fresh concentrations of boscalid in samples collected at exposure initiation and during renewal were in the range 88.73 – 104.99% of the nominal concentration. The determined concentrations of difenoconazole in fresh samples d at exposure initiation and during renewal were in the range 85.71 – 109.64% of the nominal concentration. The results confirm correct preparation of the test item concentrations. The determined concentration of boscalid in spent samples collected during renewal and at exposure termination were in the range 88.11 – 103.92% of the nominal concentration. The determined concentrations of difenoconazole in spent samples collected during renewal and at exposure termination were in the range 93.41 – 104.34% of the nominal concentration. re, concentrations of boscalid and difenoconazole were stable under the test conditions. The endpoint values were determined based on nominal test item concentrations and nominal concentrations of boscalid and difenoconazole. **Agreed endpoints:** The endpoint values determined based on the nominal test item concentrations: The EC_{50} = 18.26 mg/L (95% confidence limits: 14.97 – 22.24). The endpoint values based on nominal concentrations of boscalid: The EC_{50} =4.35 mg/L (95% confidence limits: 3.56 – 5.29). The endpoint values based on nominal concentrations of difenoconazole: The EC_{50} =1.20 mg/L (95% confidence limits: 0.99 – 1.46) .

Reference:	KCP 10.2.1-02
Report	"Boscalid 23.3% + Difenoconazole 6.6% SC: Daphnia magna, Acute immobilization test", Ewa Nierzędska (2018), Report No. W/97/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	OECD Guideline No. 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

Immobilization of *Daphnia magna* exposed to the test item Boscalid 23.3% + Difenoconazole 6.6% SC (batch number: SCL – 20245) was investigated during a 48-hour semi-static test. The test was performed in the following test item concentrations: 100, 45.45, 20.66, 9.39, 4.27 mg/L plus the control. Four replicates of each test item concentration and the control with five *Daphnia magna* per replicate were used.

The concentrations of boscalid and difenoconazole were chemically determined using a validated high performance liquid chromatographic method with DAD detection. Samples of all fresh test item concentrations and the control were determined at exposure initiation and during renewal and for all spent test item concentrations during the renewal and at exposure termination.
The determined fresh concentrations of boscalid in samples collected at exposure initiation and during renewal were in the range 88.73 – 104.99% of the nominal concentration. The determined concentrations of difenoconazole in fresh samples collected at exposure initiation and during renewal were in the range 85.71 – 109.64% of the nominal concentration. The results confirm correct preparation of the test item concentrations. The determined concentration of boscalid in spent samples collected during renewal and at exposure termination were in the range 88.11 – 103.92% of the nominal concentration. The determined concentrations of difenoconazole in spent samples collected during renewal and at exposure termination were in the range 93.41 – 104.34% of the nominal concentration. Therefore, concentrations of boscalid and difenoconazole were stable under the test conditions.
The endpoint values were determined based on nominal test item concentrations and nominal concentrations of boscalid and difenoconazole.

Results

Preliminary test
In the preliminary test four test item concentrations of 100, 10, 1.0, and 0.1 mg/L plus the control were used.
In the preliminary test, in the test item concentrations of 0.1 and 1.0 mg/L plus the control no immobilization was observed during exposure. In the test item concentration of 10 mg/L, 20% immobilization of *Daphnia magna* was observed at exposure termination. In the test item concentration of 100 mg/L, 100% of immobilization of *Daphnia magna* was observed at exposure termination.

Definitive test
In the definitive test *Daphnia magna* was exposed to the test item concentrations: 100, 45.45, 20.66, 9.39, and 4.27 mg/L plus the control. The results are summarized in the table below.

Table 10.2.1-02-01 Immobilization of *Daphnia magna*, definitive test

Nominal test	Number	Number of immobilized *Daphnia magna*	Total of immobi-

item concentration [mg/L]	of *Daphnia magna*	24 h				48 h				lized *Daphnia magna* [%]	
		Replicates									
		R1	R2	R3	R4	R1	R2	R3	R4	**24 h**	**48 h**
Control	20	0	0	0	0	0	0	0	0	0	0
4.27	20	0	0	0	0	0	0	0	0	0	0
9.39	20	1	0	0	0	1	0	0	0	5	5
20.66	20	3	3	0	3	3	3	2	4	45	60
45.45	20	5	5	5	5	5	5	5	5	100	100
100.00	20	5	5	5	5	5	5	5	5	100	100

Validity criteria

In the definitive test the validity criteria were met according to OECD Guideline No. 202 (2004):

- the percentage of immobilization of Daphnia magna in the control was 0% (criterion: not more than 10%),
- the dissolved oxygen concentrations in the test vessels were within the range of 7.6 – 8.5 mg/L (criterion: not less than 3 mg/L).

Conclusion

The endpoint values determined based on the nominal test item concentrations:
The EC_{50} value after 48 h of exposure is 18.26 mg/L (95% confidence limits: 14.97 – 22.24).
The EC_{20} value after 48 h of exposure is 13.27 mg/L (95% confidence limits: 9.49 – 16.01), and EC_{10} value after 48 h of exposure is 11.23 mg/L (95% confidence limits: 7.24 – 13.91).
The data on immobilization of the Daphnia magna at exposure termination were analyzed using the Fisher Exact Binomial Test with Bonferroni Correction which showed a significant difference between the nominal test item concentrations of 100, 45.45, and 20.66 mg/L and the control. Therefore, the lowest observed effect concentration (LOEC/48 h) is 20.66 mg/L. The highest test item concentration causing no immobilization (NOEC/48 h) is 9.39 mg/L.
The endpoint values based on nominal concentrations of boscalid:
The EC_{50} value after 48 h of exposure is 4.35 mg/L (95% confidence limits: 3.56 – 5.29).
The EC_{20} value after 48 h of exposure is 3.15 mg/L (95% confidence limits: 2.25 – 3.81), and EC_{10} value after 48 h of exposure is 2.67 mg/L (95% confidence limits: 1.72 – 3.31).
The data on immobilization of the Daphnia magna at exposure termination were analyzed using the Fisher Exact Binomial Test with Bonferroni Correction which showed a significant difference between the nominal concentrations of boscalid: 23.80, 10.82, and 4.92 mg/L and the control. Therefore, the lowest observed effect concentration (LOEC/48 h) is 4.92 mg/L. The highest test item concentration causing no immobilization (NOEC/48 h) is 2.23 mg/L.
The endpoint values based on nominal concentrations of difenoconazole:
The EC_{50} value after 48 h of exposure is 1.20 mg/L (95% confidence limits: 0.99 – 1.46).
The EC_{20} value after 48 h of exposure is 0.87 mg/L (95% confidence limits: 0.63 – 1.05), and EC_{10} value after 48 h of exposure is 0.74 mg/L (95% confidence limits: 0.48 – 0.92).
The data on immobilization of the Daphnia magna at exposure termination were analyzed using the Fisher Exact Binomial Test with Bonferroni Correction which showed a significant difference between the nominal concentrations of difenoconazole: 6.56, 2.98, and 1.36 mg/L and the control. Therefore, the lowest observed effect concentration (LOEC/48 h) is 1.36 mg/L. The highest test item concentration causing no immobilization (NOEC/48 h) is 0.62 mg/L.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • the biomass in the control increased by a factor of 197.0 within the 72-hour test period (criterion: at least a 16-fold growth), • the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 2.7% (criterion: it must not exceed 7%). • the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.3% (criterion: it must not exceed 35%). **Agreed endpoints:** The endpoint values determined on the basis of the nominal test item concentrations: <u>Growth rate</u> ErC_{50}/72 h =9.80 mg/L (with 95% confidence interval: 9.34 – 10.26). EyC_{50}/72 h = 6.23 mg/L (with 95% confidence interval: 5.84 – 6.63). The LOErC/72 h and the NOErC/72 h could not be determined on basis of the obtained results. The LOE_yC/72 h =6.25 mg/L and the NOE_yC/72 h =3.13 mg/L. The endpoint values determined on the basis of the nominal concentrations of boscalid: ErC_{50}/72 h = 2.284 mg/L (with confidence interval: 2.177 – 2.391) EyC_{50}/72 h value is 1.451 mg/L (with 95% confidence interval: 1.362 – 1.546) The LOE_yC/72 h =1.456 mg/L and the NOE_yC/72 h value for yield is 0.729 mg/L The LOErC/72 h and the NOErC/72 h could not be determined on basis of the obtained results. The endpoint values determined on the basis of the nominal concentrations of difenoconazole: ErC_{50}/72 h = 0.647 mg/L (with confidence interval: 0.617 – 0.677). EyC_{50}/72 h =0.411 mg/L (with confidence interval: 0.386 – 0.438). The LOEyC/72 h = 0.413 mg/L and the NOEyC/72 h =0.207 mg/L. LOErC/72 h and NOErC/72 h could not be determined on basis of the obtained results.

Reference:	KCP 10.2.1-03
Report	"Boscalid 23.3% + Difenoconazole 6.6% SC: *Pseudokirchneriella subcapitata* SAG 61.81 Growth Inhibition Test", Ewa Nierzędska (2017), Report No. W/96/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	OECD Guideline No. 201 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

The growth of the green algae *Pseudokirchneriella subcapitata* exposed to the test item Boscalid 23.3% + Difenoconazole 6.6% SC was investigated during a 72-hour test. The test was performed in glass flasks

with a capacity of 250 mL. Each of them contained 100 mL of a given test item concentration and the control. The initial density of the algae was 1 x 104 cells/mL. The following
test item concentrations were used: 50, 25, 12.5, 6.25, 3.13 mg/L plus the control.
Density of algae cells was determined in each replicate after 24, 48 and 72 h of exposure using direct method. Morphology observations of the algae cells were performed at exposure termination.
The concentrations of boscalid and difenoconazole were chemically determined using a validated
liquid chromatographic method with DAD detection. Samples of all test item concentrations the control collected at exposure initiation and at exposure termination were chemically analyzed.
In samples collected at exposure initiation the determined concentrations of boscalid were in the range of 81.91 – 93.82% of nominal concentration. In samples collected at exposure initiation the determined concentrations of difenoconazole were in the range of 81.76 – 90.42% of nominal concentration. Therefore, the results confirm correct preparation of the test item concentrations.
In samples collected at exposure termination in the determined concentrations of boscalid were in the range of 85.99 – 105.72% of initial concentration. In samples collected at exposure termination the determined concentrations of difenoconazole were in the range of 91.15 – 104.23% of initial concentration. Therefore, the concentrations of boscalid and difenoconazole were stable under test conditions.
The endpoint values were determined on the basis of the nominal test item concentrations, the nominal boscalid concentrations, and the difenoconazole nominal concentrations.

Results

Preliminary test
The preliminary test was conducted with the test item concentrations: 100, 10, 1.0, and 0.1 mg/L plus control.

Table 10.2.1-03.1 Growth rate and yield inhibition, preliminary test (non-GLP)

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.00	0.00
0.1	-2.08*	-12.36
1.0	-2.33*	-11.87*
10	51.60	92.64
100	220.20**	100.20**

* - calculated inhibition values are lower than 0%, what means that the algae cell density at observation during exposure is higher than the algae cell density in the control
**-calculated inhibition values are higher than, what means that the algae cell density at observation during exposure is lower than the initial algae cell density

Definitive test
In the definitive test, the algae, *Pseudokirchneriella subcapitata*, with an initial cell density of 1 x 10^4 cells/mL were exposed to the test item concentrations: 50, 25, 12.5, 6.25, 3.13 mg/L (with a separation factor 2.0) plus the control.
Morphology observations of the algae were performed at exposure termination. In the test item concentrations of 3.13, 6.25, 12.5 mg/L no differences in shape, size and colour of algae cells were reported as compared to the algae cells in the control. In the test item concentrations of 25 and 50 mg/L bigger and opalescent algal cells were observed as compared to the algal cells in the control.
The average section-by-section specific growth rates and the yield increase during exposure were
calculated on the basis of the density of the algal cells after 24, 48 and 72 h of exposure.

Table X.2 Growth rate and yield inhibition, definitive test

Nominal test item concentration [mg/L]	% inhibition after 72 h of exposure (growth rate)	% inhibition after 72 h of exposure (yield)
Control	0.00	0.00
3.13	1.90	10.09
6.25	12.14	47.89
12.5	73.84	98.45

25	93.33	99.78
50	169.80*	100.40*

* - calculated inhibition values are higher than 100%, what means that the algae cell density at observation during exposure is lower than the initial algae cell density

Validity criteria

In the definitive test, the following validity criteria specified in OECD Guideline No. 201 (2006) were met:

- the biomass in the control increased by a factor of 197.0 within the 72-hour test period (criterion: at least a 16-fold growth),
- the coefficient of variation of the mean specific growth rate after the 72-hour test period (exposure initiation – exposure termination) in the control culture was 2.7% (criterion: it must not exceed 7%).
- the mean coefficient of variation for the section-by-section growth rate in the control culture was 30.3% (criterion: it must not exceed 35%).

Conclusion

The endpoint values determined on the basis of the nominal test item concentrations:
The concentration causing a 50% inhibition of the growth rate of *Pseudokirchneriella subcapitata*, i.e. the ErC_{50}/72 h value is 9.80 mg/L (with 95% confidence interval: 9.34 – 10.26).
The concentration causing a 50% inhibition of yield of *Pseudokirchneriella subcapitata*, i.e. the EyC_{50}/72 h value is 6.23 mg/L (with 95% confidence interval: 5.84 – 6.63).
The LOEC/72 h value for yield is 6.25 mg/L and the NOEC/72 h value for yield is 3.13 mg/L. The LOEC/72 h and the NOEC/72 h values for growth rate could not be determined on basis of the obtained results.

The endpoint values determined on the basis of the nominal concentrations of boscalid:
The concentration causing a 50% inhibition of the growth rate of *Pseudokirchneriella subcapitata*, i.e. the ErC_{50}/72 h value is 2.284 mg/L (with confidence interval: 2.177 – 2.391).
The concentration causing a 50% inhibition of yield of *Pseudokirchneriella subcapitata*, i.e. the EyC_{50}/72 h value is 1.451 mg/L (with 95% confidence interval: 1.362 – 1.546).
The LOEC/72 h value for yield is 1.456 mg/L and the NOEC/72 h value for yield is 0.729 mg/L. The LOEC/72 h and the NOEC/72 h values for growth rate could not be determined on basis of the obtained results.

The endpoint values determined on the basis of the nominal concentrations of difenoconazole:
The concentration causing a 50% inhibition of the growth rate of *Pseudokirchneriella subcapitata*, i.e. the ErC_{50}/72 h value is 0.647 mg/L (with confidence interval: 0.617 – 0.677).
The concentration causing a 50% inhibition of yield of *Pseudokirchneriella subcapitata*, i.e. the EyC_{50}/72 h value is 0.411 mg/L (with confidence interval: 0.386 – 0.438).
The LOEC/72 h value for yield is 0.413 mg/L and the NOEC/72 h value for and yield is 0.207 mg/L. LOEC/72 h and the NOEC/72 h values for growth rate could not be determined on basis of the obtained results.

zRMS comments:

The study is considered acceptable. All validity criteria were met.

- The doubling time of frond number in the control was 2.0 days, criterion: less than 2.5 days (the factor of frond number in the control between 0 and 7 day was 11.8)
- The average specific growth rate in the control between day 0 and day 7 was 0.351 d-1 (minimum requirement: higher than 0.275 d-1)

Analytical results:

In fresh samples the determined concentrations of boscalid were in the range of 85.3 – 117.3% of the nominal concentration. In fresh samples the determined concentrations of difenoconazole were in the range of 84.8 – 114.3% of the nominal concentration. The results confirm correct preparation of the test item concentrations. In old samples the determined concentrations of boscalid were in the range of 50.7 – 119.2% of the nominal concentration.
In old samples the determined concentrations of difenoconazole were in the range of 58.0 – 121.0% of the nominal concentration. Therefore, the concentrations of boscalid were stable and concentrations of difenoconazole were unstable during renewals.

Agreed endpoints:

The endpoint values based on the nominal test item concentrations [mg/L]				
Endpoint (mg/L)	**Yield inhibition based on the frond number**	**Growth rate inhibition based on the frond number**	**Yield inhibition based on the dry weight**	**Growth rate inhibition based on the dry weight**
EyC_{10}/7d / ErC_{10}/7d	0.88 (0.02 – 3.19)	2.37 (0.11 – 7.99)	1.70 (0.08 – 4.73)	2.67 (0.00 – 12.00)
EyC_{20}/7d / ErC_{20}/7d	2.72 (0.22 – 7.44)	11.65 (1.96 – 27.67)	3.82 (0.43 – 8.66)	12.97 (0.40 – 39.84)
EyC_{50}/7d / ErC_{50}7d	23.65 (9.08 – 69.11)	245.55 (102.75 – 1508.38)	18.09 (7.72 – 42.91)	266.00 (85.12 – 10873.57)
LOEC/7d	10.00	10.00	10.00	32.00
NOEC/7d	3.20	3.20	3.20	10.00
The endpoint values based on the nominal concentrations of of boscalid [mg/L]				
Endpoint (mg/L)	**Yield inhibition based on the frond number**	**Growth rate inhibition based on the frond number**	**Yield inhibition based on the dry weight**	**Growth rate inhibition based on the dry weight**
EyC_{10}/7d / ErC_{10}/7d	0.210 (0.004 – 0.764)	0.520 (0.017 – 1.804)	0.434 (0.013 – 1.158)	0.578 (0.000 - 2.641)
EyC_{20}/7d / ErC_{20}/7d	0.623 (0.040 – 1.705)	2.483 (0.357 – 6.080)	0.908 (0.079 - 1.988)	2.747 (0.058 – 8.733)
EyC_{50}/7d / ErC_{50}/7d	4.971 (1.858 – 15.638)	49.526 (19.842 – 385.233)	3.726 (1.579 – 9.216)	54.158 (16.582 – 4116.664)
LOEC/7d	2.184	2.184	2.184	6.194
NOEC/7d	0.819	0.819	0.819	2.184
The endpoint values based on the geometric mean of determined concentrations of difenoconazole [mg/L]				
Endpoint (mg/L)	**Yield inhibition based on the frond number**	**Growth rate inhibition based on the frond number**	**Yield inhibition based on the dry weight**	**Growth rate inhibition based on the dry weight**
EyC_{10}/7d / ErC_{10}/7d	0.059 (0.001 – 0.213)	0.152 (0.006 – 0.510)	0.115 (0.005 – 0.312)	0.170 (0.000 - 0.752)
EyC_{20}/7d / ErC_{20}/7d	0.177 (0.013 – 0.483)	0.724 (0.117 – 1.715)	0.251 (0.027- 0.556)	0.801 (0.022 – 2.440)
EyC_{50}/7d / ErC_{50}/7d	1.458 (0.556 – 4.286)	14.386 (6.029 – 92.648)	0.251 (0.027- 0.556)	15.605 (5.029 – 730.761)
LOEC/7d	0.624	0.624	0.624	1.961
NOEC/7d	0.220	0.220	0.220	0.624

Reference:	KCP 10.2.1 - 04
Report:	"Boscalid 23.3% + Difenoconazole 6.6% SC: *Lemna gibba* CPCC 310 Growth Inhibition Test". Ewa Nierzędska, 2018, W/98/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	OECD Guideline No. 221 (2006)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:

Description:	Boscalid 23.3% + Difenoconazole 6.6% SC
Batch number:	SCL-20245
A.i. content:	boscalid: 23.8% w/v; difenoconazole 6.56% w/v

Test system:

Species:	*Lemna gibba*
Strain:	CPCC 310
Age:	-
Source:	Cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna
Medium:	20X AAP

Experimental conditions:

Temperature:	23.2 – 23.9ºC
pH values:	7.49 – 9.91
Mean light intensity:	7265 – 7990 lux, constant illumination
Test vessels:	glass vessels with 150 mL test volume
Initial frond number:	9 (3 plants consisting of 3 fronds each)

Experimental period: 7 d

Test design: Semi-static (7 days); daily renewal, three replicates of each test item concentration; six replicates of the control.
Test item concentrations: 320, 100, 32, 10, 3.2, 1.0, 0.32 and 0.10 mg/L plus the control.
In order to quantify the test item related effects on vegetative growth over a period of 7 days, the number of fronds in each replicate was counted twice during exposure and at exposure termination. At the same time observations of plant development were performed. The dry weight of the representative sample of the duckweed culture used as the inoculum was measured at exposure initiation. The dry weight of all plants from each test vessel was measured after exposure termination.
The concentrations of pendimethalin were chemically determined with a validated gas chromatographic method with DAD detection.

In fresh samples the determined concentrations of boscalid were in the range of 85.3 – 117.3% of the nominal concentration. In fresh samples the determined concentrations of difenoconazole were in the range of 84.8 – 114.3% of the nominal concentration. The results confirm correct preparation of the test item concentrations. In old samples the determined concentrations of boscalid were in the range of 50.7 – 119.2% of the nominal concentration. In old samples the determined concentrations of difenoconazole were in the range of 58.0 – 121.0% of the nominal concentration. Therefore, the concentrations of boscalid were stable and concentrations of difenoconazole were unstable during renewals.

Statistics: Probit method calculations and analysis by Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Wetch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment, Williams Multiple Sequential t-test Procedure.

Results: The endpoint values were determined on the basis of the nominal test item concentrations and the geometric means of determined concentrations of boscalid and difenoconazole were calculated. Due to decrease the content of boscalid and difenoconazole below 80% of nominal concentration the geometric means of determined concentrations of boscalid and difenoconazole were calculated.

The endpoint values based on the nominal test item concentrations [mg/L]				
Endpoint (mg/L)	**Yield inhibition based on the frond number**	**Growth rate inhibition based on the frond number**	**Yield inhibition based on the dry weight**	**Growth rate inhibition based on the dry weight**
EyC_{50}/7d / ErC_{50}/7d	0.88 (0.02 – 3.19)	2.37 (0.11 – 7.99)	1.70 (0.08 – 4.73)	2.67 (0.00 – 12.00)
EyC_{20}/7d / ErC_{20}/7d	2.72 (0.22 – 7.44)	11.65 (1.96 – 27.67)	3.82 (0.43 – 8.66)	12.97 (0.40 – 39.84)
EyC_{10}/7d / ErC_{10}/7d	23.65 (9.08 – 69.11)	245.55 (102.75 – 1508.38)	18.09 (7.72 – 42.91)	266.00 (85.12 – 10873.57)
LOEC/7d	10.00	10.00	10.00	32.00
NOEC/7d	3.20	3.20	3.20	10.00
The endpoint values based on the nominal concentrations of of boscalid [mg/L]				
Endpoint (mg/L)	**Yield inhibition based on the frond number**	**Growth rate inhibition based on the frond number**	**Yield inhibition based on the dry weight**	**Growth rate inhibition based on the dry weight**
EyC_{50}/7d / ErC_{50}/7d	0.210 (0.004 – 0.764)	0.520 (0.017 – 1.804)	0.434 (0.013 – 1.158)	0.578 (0.000 - 2.641)
EyC_{20}/7d / ErC_{20}/7d	0.623 (0.040 – 1.705)	2.483 (0.357 – 6.080)	0.908 (0.079 - 1.988)	2.747 (0.058 – 8.733)
EyC_{10}/7d / ErC_{10}/7d	4.971 (1.858 – 15.638)	49.526 (19.842 – 385.233)	3.726 (1.579 – 9.216)	54.158 (16.582 – 4116.664)
LOEC/7d	2.184	2.184	2.184	6.194
NOEC/7d	0.819	0.819	0.819	2.184
The endpoint values based on the geometric mean of determined concentrations of difenoconazole [mg/L]				
Endpoint (mg/L)	**Yield inhibition based on the frond number**	**Growth rate inhibition based on the frond number**	**Yield inhibition based on the dry weight**	**Growth rate inhibition based on the dry weight**
EyC_{50}/7d / ErC_{50}/7d	0.059 (0.001 – 0.213)	0.152 (0.006 – 0.510)	0.115 (0.005 – 0.312)	0.170 (0.000 - 0.752)
EyC_{20}/7d / ErC_{20}/7d	0.177 (0.013 – 0.483)	0.724 (0.117 – 1.715)	0.251 (0.027- 0.556)	0.801 (0.022 – 2.440)
EyC_{10}/7d / ErC_{10}/7d	1.458 (0.556 – 4.286)	14.386 (6.029 – 92.648)	0.251 (0.027- 0.556)	15.605 (5.029 – 730.761)
LOEC/7d	0.624	0.624	0.624	1.961
NOEC/7d	0.220	0.220	0.220	0.624

A 2.2.3 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.4 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • the average mortality for the control was 0% at the end of the experiment (criterion: it must not exceed 10%), • the LD50/24h of the reference item (dimethoate) was 0.1 µg/bee (criterion: 0.10 - 0.35 µg a.i./bee). **Agreed endpoint:** **LD_{50}/48 h $_{oral}$ > 400 µg product /bee**

Reference:	KCP 10.3.1.1.1
Report	"Boscalid 23.3% + Difenoconazole 6.6% SC: Honeybees (*Apis mellifera* L.), Acute Oral Toxicity Test". Natalia Lemańska, 2018, B/80/16. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	Yes, OECD Guideline for the Testing of Chemicals No. 213 (1998) and the EU Method C.16. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Yes

Materials and methods

Test item:

Description:	Boscalid 23.3%+ Difenoconazole 6.6% SC
Production batch:	SCL - 20245
A.i. content:	Boscalid 23.8 % + Difenoconazole 6.56% (w/v)

Test system:

Species:	*Apis mellifera*
Strain:	carnica
Age:	approximately 3 weeks
Average weight:	-
Average length:	-

Source: An apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna
Acclimation period: -
Diet: 50% (w/v) aqueous sucrose solution

Experimental conditions:

Temperature: 24 – 25.5°C
Humidity: 62 – 64%
Hardness: -
pH: -
Light and photoperiod: 24h darkness (except during observations).
Loading: 3 replicates per dose, 10 bees per replicate
Test procedure: For the oral toxicity test, the test substance was added to a 50% w/v sucrose solution reaching the concentration of 100 µg a.i./bee. Feeders were filled with the dilution. Bees were kept unfed for approximately 2 hours.

Experimental period: 48h

Test design and treatment

Plastic cages with an opening on each side to allow the feeding with micropipettes. The bees were observed for mortality and behavioural abnormalities after 4, 24 and 48 h of exposure.
A preliminary test was conducted with doses of 8.0, 40.0 and 200.0 µg of a.i./bee. According to the results, the following nominal test item concentrations were used: 25.0, 50.0, 100.0, 200.0 and 400.0 µg test item/bee (5.4 µg boscalid/bee + 1.5 µg difenoconazole/bee; 10.7 µg boscalid/bee + 3.0 µg difenoconazole/bee; 21.5 µg boscalid/bee + 5.9 µg difenoconazole/bee; 43.0 µg boscalid/bee + 11.8 µg difenoconazole/bee and 85.9 µg boscalid/bee + 23.7 µg difenoconazole/bee) and a control (0.0 µg/bee).
The LD_{50} expressed in µg of the test item per bee or in µg of the active ingredient contained in the reference item per bee, was calculated with the log-probit method using ToxRat Professional software, version 3.2.1.

Results

The acute contact toxicity study of the test item, Boscalid 23.3% + Difenoconazole 6.6% SC on honeybees (*Apis mellifera* L.) in the laboratory test are summarized below.

Oral toxicity test results

Dosage			**Number of tested bees [no.]**	**Mortality after 48 h**		**LD_{50} after 48 h**		
µg/bee[a]	**a.i.[c]**	**a.i.[d]**		**Total**		**µg/bee**	**a.i.[c]**	**a.i.[d]**
	µg a.i./bee[b]			**[no.]**	**[%]**		**µg a.i./bee**	
0.0 (Control)			30	0	0.0	> 400	> 85.9	> 23.7
25	5.4	1.5	30	0	0.0			
50	10.7	3.0	30	0	0.0			
100	21.5	5.9	30	0	0.0			
200	43.0	11.8	30	2	6.7			
400	85.9	23.7	30	4	13.3			

[a]: µg test item/bee
[b]: µg active ingredient/bee
[c]: boscalid
[d]: difenoconazole

The median lethal doses LD_{50}/24 h and LD_{50}/48 h are equal to 5.1 and 5.0 µg t.i./honeybee, respectively.

The following validity criteria were met during the test:

- the average mortality for the control was 0% at the end of the experiment (criterion: it must not

exceed 10%),
- the LD50/24h of the reference item (dimethoate) was 0.1 µg/bee (criterion: 0.10 - 0.35 µg a.i./bee).

Conclusion
With respect to the test results, it can be concluded that the test item, Boscalid 23.3% + Difenoconazole 6.6% SC had no adverse effect on mortality and bevaviour of honeybees (*Apis mellifera* L.).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • the average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%) • the LD_{50}/24 h of the reference item (dimethoate) was 0.24 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee). **Agreed endpoint:** **$LD_{50}/48h_{contact}$> 400 µg product /bee**

Reference:	KCP 10.3.1.1.2
Report	"Boscalid 23.3% + Difenoconazole 6.6% SC: Honeybees (*Apis mellifera* L.), Acute Contact Toxicity Test". Natalia Lemańska, 2018, B/81/16. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	Yes, OECD Guideline for the Testing of Chemicals No. 214 (1998) and the EU Method C.17. (2008)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:

Description:	Boscalid 23.3% + Difenoconazole 6.6% SC
Production batch:	SCL - 20245
A.i. content:	Boscalid: 23.8% [w/v], Difenoconazole: 6.56% [w/v]

Test system:

Species:	*Apis mellifera*
Strain:	carnica
Age:	approximately 3 weeks
Average weight:	-
Average length:	-
Source:	An apiary at the Institute of Industrial Organic Chemistry, Branch Pszczyna
Acclimation period:	20 hours
Diet:	50% (v/v) sucrose solution

Experimental conditions:

Temperature: 23 – 25°C
Humidity: 62 – 66%
Hardness: -
pH: -
Light and photoperiod: 24h darkness (except during observations).
Loading: 3 replicates per dose, 10 bees per replicate
Test procedure: The honeybees were anaesthetized with carbon dioxide, transferred to plastic trays and dosed on the dorsal side of the thorax with 1 µl of test solution containing the test substance or reference substance.

Experimental period: 48h

Test design and treatment

Plastic cages with an opening on each side to allow the feeding with syringes.
A preliminary test was done at the dose of 16.0, 80, and 400 µg test item/bee. According to the results, the following nominal test item concentrations were used: 25, 50, 100, 200 and 400 µg test item/bee (5.4 µg boscalid/bee + 1.5 µg difenoconazole/bee; 10.7 µg boscalid/bee + 3.0 µg difenoconazole/bee; 21.5 µg boscalid/bee + 5.9 µg difenoconazole/bee; 43.0 µg boscalid/bee + 11.8 µg difenoconazole/bee and 85.9 µg boscalid/bee + 23.7 µg difenoconazole/bee) and a control (0.0 µg/bee). The honeybees were observed for mortality and behavioural abnormalities after 4, 24 and 48 h of exposure.
The LD_{50}, expressed in µg test item/bee or µg active ingredient/bee, was calculated with the log-probit method. The ToxRat Professional 3.2.1 software was used.

Results

The median lethal doses (LD_{50}) after 24 and 48 hours of exposure are higher than the maximum used dose, i.e. 400 µg test item/honeybee.

Contact toxicity test results

Dosage		**Number of tested bees [no.]**	**Mortality after 48 h**		**LD_{50} after 48 h**	
			Total			
µg/bee	**µg a.i./bee[a]**		**[no.]**	**[%]**	**µg/bee**	**µg a.i./bee**
0.0 (Control)		30	0	0.0	> 400	> 85.9 + 23.7
25	5.4 + 1.5	30	0	0.0		
50	10.7 + 3.0	30	0	0.0		
100	21.5 + 5.9	30	0	0.0		
200	43.0 + 11.8	30	0	0.0		
400	85.9 + 23.7	30	0	0.0		

[a]:: boscalid + difenoconazole

The following validity criteria were met during the test:

- the average mortality for the total number of controls was 0.0% after 48 h (criterion: it must not exceed 10%),
- the LD_{50}/24 h of the reference item (dimethoate) was 0.24 µg a.i./bee (criterion: 0.10 - 0.30 µg a.i./bee).

Conclusion

With respect to the test results, it can be concluded that the test item, Boscalid 23.3% + Difenoconazole 6.6% SC had no adverse effect on mortality and bevaviour of honeybees (*Apis mellifera* L.).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on non-target arthropods other than bees

A 2.3.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropods

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • mortality of the control group was 0.0% on day 7 of exposure (criterion: a maximum of 20%), • mortality of the mites exposed to the reference item at the rate of 5.0 mL/ha was 96.0% on day 7 of exposure (criterion: from 50 to 100%), • the mean number of eggs per female in the control group was 4.56 (required: ≥ 4 eggs per female). **Agreed endpoints:** **ER_{50}>7.7 L product/ha** **$NOER_{reproduction}$ 1.9 L product/ha**

Reference:	KCP 10.3.2.1-01
Report	"A laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the predatory mite, Typhlodromus pyri (Sch.)". Dr. V. Angayarkanni, 2019, 5651/2019.
Guideline(s):	ESCORT 1 Guidance Document (Barrett K.L. et al., 1994) ESCORT 2 Guidance Document (Candolfi M.P. et al., 2001) Guidelines developed by the BIOSCIENCE RESEARCH FOUNDATION IOBC, BART and EPPO Joint Initiative (Blumel S. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

~~The extended laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on mortality and reproduction of the predatory mite, *T. pyri* (Sch.) was conducted for Sharda Cropchem Ltd, India at BIOSCIENCE RESEARCH FOUNDATION.~~

The study was carried out based on the Sponsor recommended rates for the test item as the definite test. There were 1.2, 1.9, 3.0, 4.8 and 7.7 L/ha. 24 hours old (protonymphal stage) of predatory mites *T. pyri* were exposed to the test item applied to bean leaf discs and fed with pine pollen (Pinus sp.) during the experimental period.

To verify the sensitivity of the mites and the precision of the test procedure, the insecticide, ROGORIN (30% dimethoate) was used as a reference item. The rate of the reference item was 5.0 mL/ha (1.5 g a.i./ha). The control group was treated with distilled water.

Mortality was observed after 7 days of post treatment of the test item. Observations of reproduction in the control and other groups treated with the test item were made after 8, 11 and 14 days post treatment of the test item.

Endpoints based on mortality of T. *pyri* was 7 days and reproduction reduction (Pr) was 14 days post-test item treatment.

Results

The effects of Boscalid 23.3% + Difenoconazole 6.6% SC on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below.

Mortality and reproduction of *T. pyri* in the laboratory test

Study group (application rate) [L/ha]	**Parameter (endpoint)**				
	Mortality		**Fecundity**		
	Total [%]	**LR_{50}**	**Mean no. of eggs/female (Rr) [No]**	**Reproduction reduction Pr [%]**	**ER_{50}**
Control	0.0	-	4.56	-	-
Boscalid 23.3% + Difenoconazole 6.6% SC					
1.2	1.0	**>7.7 L/ha** >(1832.6[a] + 505.1[b] g a.i./ha)	4.16	8.88	**>7.7 L/ha** >(1832.6[a] + 505.1[b] g a.i./ha)
1.9	6.0		4.08	10.51	
3.0	13.0		3.48	23.64	
4.8	27.0		3.39	25.61	
7.7	33.0		2.98	34.61	
$NOER_{mortality}$		**1.9 L/ha** >(452.2[a] + 124.6[b] g a.i./ha)	$NOER_{reproduction}$		**1.9 L/ha** >(452.2[a] + 124.6[b] g a.i./ha)
Reference item	**ROGORIN**				
5.0 mL/ha	96.0	-	-		-

a – ~~Azoxystrobin~~ Boscalid
b – ~~Folpet~~ Difenconazole

Findings

- Mortality of the control group after 7 days of exposure was 0.0%. After 7 days of exposure to Boscalid 23.3% + Difenoconazole 6.6% SC at rates of 1.2, 1.9, 3.0, 4.8 and 7.7 L/ha, the percentages of *T. pyri* mortalities, corrected using the formula of Abbott, were 1.0, 6.0, 13.0, 27.0 and 33.0 %, respectively.
- There were statistically no significant differences in mortality between group treated with the test item at rates of 3.0, 4.8 and 7.7 L/ha and the control group.

- On the basis of the obtained mortality results, the LR_{50} value is >7.7 L Boscalid 23.3% + Difenoconazole 6.6% SC/ha, i.e., >(1832.6 g Boscalid + 505.1 g Difenoconazole/ha). The $NOER_{mortality}$ value is equal to 1.9 L Boscalid 23.3% + Difenoconazole 6.6% SC/ha, i.e., >(452.2 g Boscalid + 124.6 g Difenoconazole/ha).
- For the reference item Rogorin (dimethoate 30% w/w), the mortality of mites after 7 days of exposure at the rate of 5.0 mL/ha, was 96.0%, hence the criterion specified in the method description was met. The results showed that the test organisms were sensitive to dimethoate.
- The mean reproduction rate (Rr) in the control group was 4.56 eggs/female. The mean reproduction rates (Rr) after 14 days of exposure to Boscalid 23.3% + Difenoconazole 6.6% SC at rates 1.2, 1.9, 3.0, 4.8 and 7.7 L/ha were 4.16, 4.08, 3.48, 3.39 and 2.98 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by rates of 1.2, 1.9, 3.0, 4.8 and 7.7 L/ha were 8.88, 10.51, 23.64, 25.61 and 34.61 % respectively.
- There were statistically significant differences in reproduction between group treated with the test item at rates of 3.0, 4.8 and 7.7 L/ha and the control group.
- On the basis of the obtained reproduction results, the ER_{50} value is >7.7 L Boscalid 23.3% + Difenoconazole 6.6% SC/ha, i.e., >(1832.6 g Boscalid + 505.1 g Difenoconazole/ha). The $NOER_{reproduction}$ value is equal to 1.9 L Boscalid 23.3% + Difenoconazole 6.6% SC/ha, i.e., >(452.2 g Boscalid + 124.6 g Difenoconazole/ha).

Conclusion
On the basis of the obtained results it can be concluded that Boscalid 23.3% + Difenoconazole 6.6% SC at the rate of 1.2, and 1.9 L/ has no adverse effect on mortality and reproduction of the mites.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. – after 48 hours, mortality in the control group was 0.0% (criterion: a maximum of 10.0%), – after 48 hours, mortality in the group treated with the reference item at a rate of 5.0 mL/ha was 83.3% (criterion: a minimum of 50%), – the mean number of mummies per female in the control was 27.1 (criterion: a minimum of 5.0 mummies/female), – all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring). **Agreed endpoints:** **ER_{50}>7.7 L product/ha** **$NOER_{reproduction}$ = 1.9 L product/ha**

Reference:	KCP 10.3.2.1-02
Report:	"A laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani - Perez)". Dr. V. Angayarkanni, 2019, 5652/2019. BIOSCIENCE RESEARCH FOUNDATION.
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000)

Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study):	No

SUMMARY

The laboratory test involved the evaluation of the effects of the test item, Boscalid 23.3% + Difenoconazole 6.6% SC on mortality and fecundity of the parasitic wasp, *A. rhopalosiphi.* In the definitive test, five rates of the test item were used. These were 1.2, 1.9, 3.0, 4.8 and 7.7 L/ha.

Adult wasps were exposed in exposure units to glass plates treated with the test item. The parasitoids were confined for 48 h and their condition was assessed after 2, 24, and 48 hours. Then, females which survived the 48-hour exposure to Boscalid 23.3% + Difenoconazole 6.6% SC and the ones from the control group were subjected to fecundity assessments. To allow the oviposition, 15 female wasps from the groups treated with the test item at rates of 1.2, 1.9, 3.0, 4.8 and 7.7 L/ha and the ones from the control group were individually introduced into the fecundity units containing the barley plants infested with the aphid, *Rhopalosiphum padi*. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which the wasp pupae were developing) was recorded.

Mortality of the wasps after 48 hours of the exposure and the percentage of fecundity reduction (Pr) relative to the control group recorded 12 days after the oviposition were the endpoints.

To assess the susceptibility of the test system and the sensitivity of the test method, an insecticide, ROGORIN (30% dimethoate) was used as a reference item. The rate of the reference item was 5.0 mL/ha (1.5 g dimethoate/ha). The control group was comprised of wasps having contact with glass plates sprayed with distilled water.

<u>Materials</u> and methods:

Test item:	name: Boscalid 23.3% + Difenoconazole 6.6% SC; content: 23.8% (w/v) of Boscalid and 6.56% (w/v) of Difenoconazole; batch no.: SCL-33540; manufacturing date: March 20, 2019; expiry date: March 19, 2021.
Biological test system:	the parasitic wasp, *Aphidius rhopalosiphi* (De Stefani-Perez); Hymenoptera: Braconidae, Aphiidinae.
– age:	larvae (24 - 48 hours after emerging from mummies)
– source:	BRF Insectary
Experimental design:	7 test groups: – a control group (0.0 mL/ha) – Boscalid 23.3% + Difenoconazole 6.6% SC at the rate of 1.2 L/ha – Boscalid 23.3% + Difenoconazole 6.6% SC at the rate of 1.9 L/ha – Boscalid 23.3% + Difenoconazole 6.6% SC at the rate of 3.0 L/ha – Boscalid 23.3% + Difenoconazole 6.6% SC at the rate of 4.8 L/ha – Boscalid 23.3% + Difenoconazole 6.6% SC at the rate of 7.7 L/ha – ROGORIN at the rate of 5.0 mL/ha (1.5 g a.i./ha) 5 replicates/group 30 wasps/replicate
Test conditions:	
– temperature:	19.8 – 20.5°C

– relative air humidity: 65-70%
– photoperiod: 16 hours light (mortality assessment and oviposition: 580 lx; fecundity assessment: 4220 lx): 8 hours dark

Statistical analyses: The endpoint values for mortality and reproduction were determined by using a Probit analysis in the NCSS (Number Cruncher Statistical System) and one-way ANOVA using Graphpad Prism 8.0. The means and standard deviations were calculated using validated Excel sheets.

Endpoints: LR_{50}, ER_{50}, $NOER_{mortality}$ and $NOER_{reproduction}$

RESULTS AND DISCUSSION:

Results: The effects of **Boscalid 23.3% + Difenoconazole 6.6% SC** on mortality and fecundity of *Aphidius rhopalosiphi* in a laboratory test are summarized in Table below.

Study group (application rate) (L test item/ha)	Parameter (endpoint)				
	Mortality after 48 h		Fecundity		
	Total (%)	LR_{50} [L test item/ha] [g a.i./ha]	Mean no. of mummies/ female	Fecundity reduction Pr [%]	ER_{50} [L test item/ha] [g a.i./ha]
Control	0.0	–	27.1	-	–
Boscalid 23.3% + Difenoconazole 6.6% SC					
1.2	0.0	**> 7.7 L/ha > (1832.6[a] + 505.1[b] g a.i./ha)**	26.3	3.0	**> 7.7 L/ha > (1832.6[a] + 505.1[b] g a.i./ha)**
1.9	3.3		25.0	7.7	
3.0	13.3		22.7	16.2+	
4.8	23.3+		21.1	22.1+	
7.7	40.0+		18.6	31.4+	
$NOER_{mortality}$		**3.0 L/ha (714.0[a] + 196.8[b] g a.i./ha)**	**$NOER_{fecundity}$**		**1.9 L/ha (452.2[a] + 124.6[b] g a.i./ha)**
ROGORIN	**Mortality after 24 h**				
5.0 mL/ha	**53.3%**				

+: statistically significant differences at $p < 0.05$
a: Boscalid
b: Difenoconazole

Mortality of the control wasps was 0.0% after 48 hours of the exposure. The mortality of *A. rhopalosiphi* after 48 hours of the exposure to Boscalid 23.3% + Difenoconazole 6.6% SC at the rates of 1.2, 1.9, 3.0, 4.8 and 7.7 L/ha. was 0.0, 3.3, 13.3, 23.3, and 40.0% respectively. The median lethal rate, LR_{50} (the application rate at which 50% mortality of wasps is observed) of Boscalid 23.3% + Difenoconazole 6.6% SC with 95% confidence intervals after 48 hours of the exposure was >7.7 L/ha (1832.6 g Boscalid + 505.1 g Difenoconazole/ha).

Wasp mortality after 48 hours of the exposure to ROGORIN was 83.3%. The results obtained in the reference item group showed that the insects were sensitive to dimethoate.

The percentages of fecundity reduction (Pr) after the exposure to Boscalid 23.3% + Difenoconazole 6.6% SC at the rates 1.2, 1.9, 3.0, 4.8 and 7.7 L/ha were 3.0, 7.7, 16.2, 22.1, and 31.4%, respectively.

TEST VALIDITY CRITERIA

The following validity criteria were met during the study:

– after 48 hours, mortality in the control group was 0.0% (criterion: a maximum of 10.0%),
– after 48 hours, mortality in the group treated with the reference item at a rate of 5.0 mL/ha was 83.3% (criterion: a minimum of 50%),
– the mean number of mummies per female in the control was 27.1 (criterion: a minimum of 5.0 mummies/female),
– all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

A 2.3.2.2 KCP 10.3.2.2. Extended laboratory testing, aged residue studies with non-target arthropods

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • mortality of the control group was 3.3% on day 7 of exposure (criterion: a maximum of 20%), • corrected mortality of the mites exposed to the reference item at the rate of 9.0 mL/ha was 93.1% on day 7 of exposure (criterion: from 50 to 100%), • the mean number of eggs per female in the control group was 4.2 (required: ≥ 4 eggs per female). **Agreed endpoints:** The ER_{50} could not be determined due to mathematical reasons and it may be presumed > 10.20 l/ha The $NOER_{reproduction}$ ≥10.20 L/ha. The LR_{50} value could not be determined due to mathematical reasons, and it may be presumed > 10.20 l/ha The $NOER_{mortality}$ ≥10.20 L/ha.

Reference:	KCP 10.3.2.2-01
Report	"An extended laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the predatory mite, *Typhlodromus pyri* (Sch.)". Natalia Lemańska, 2018, Study Code B/83/16. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Blümel S. et al., 2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

The aim of the laboratory test was to evaluate the effects of the test item, Boscalid 23.3% + Difenoconazole 6.6% SC on mortality and reproduction of the predatory mite, *T. pyri* (Sch.).
On the basis of the preliminary test results, it was decided to use five rates of the test item in the definitive

test. These were 1.28, 2.55, 5.10 and 10.20 L/ha.

The mites, *T. pyri* at the protonymphal stage (24 hours old) were exposed to the test item applied to leaf discs. The mites were fed with pine pollen (Pinus sp.). Mortality observations were made after 7 days of the treatment. Observations of reproduction of the control group and all groups treated with the test item were made after 10, 12, and 14 days of the treatment.
Mortality of *T.pyri* after 7 days of the treatment and the reproduction reduction (Pr) after 14 days of the treatment were test endpoints.
To verify the sensitivity of the mites and the precision of the test procedure, an insecticide, Danadim 400 EC (400 g dimethoate/L) was used as a reference item. The rate of the reference item was 9.0 mL/ha (3.6 g a.i./ha). The control group was treated with distilled water.

Results

The effects of Boscalid 23.3% + Difenoconazole 6.6% SC on mortality and reproduction of *Typhlodromus pyri* in the definitive test are summarized below.

Study group [application rate]	**Parameter (endpoints)**					
	Mortality			**Reproduction**		
Test item [L/ha]	**Total [%]**	**Corrected* [%]**	**LR_{50} [L/ha]**	**Mean number of eggs/female (Rr) [no.]**	**Reproduction reduction Pr [%]**	**ER_{50} [L/ha]**
Control (0.0)	3.3	-	-	4.2	-	-
Boscalid 23.3% + Difenoconazole 6.6% SC						
1.28	8.3	5.2	> 10.20	4.5	$(-5.0)^{-}$	> 10.20
2.55	11.7	8.6		3.4	19.4	
5.10	5.0	1.7		2.7	36.8	
10.20	13.3	10.3		3.3	21.9	
$NOER_{mortality}$			≥ 10.20	**$NOER_{reproduction}$**		≥ 10.20
Reference item [mL/ha]	**Danadim 400 EC**					
9.0	93.3	93.1	not determined	not assessed		

-: The negative value means that in the treated group there was more eggs/female than in the control group
*: Abbot correction

Findings

- In the definitive test, mortality of the control group after 7 days of exposure was 3.3%. After 7 days of exposure to Boscalid 23.3% + Difenoconazole 6.6% SC at the rates of 1.28, 2.55, 5.10 and 10.20 L/ha, the percentages of *T. pyri*, mortality corrected using the formula of Abbott, were 5.2, 8.6, 1.7 and 10.3%, respectively.
- There were statistically no significant differences in mortality between all groups treated with the test item at rates of 1.28, 2.55, 5.10 and 10.20 L/ha and the control group (Chi^2 2x2 Table Test with Bonferroni Correction, $p< 0.1$).
- On the basis of the obtained results the endpoints regarding mortality could not be utterly determined. The LR_{50} value could not be determined due to mathematical reasons and it may be presumed that it is higher than the maximum rate used in the studies. The $NOER_{mortality}$ value is higher or equal to 10.20 L/ha.
- After 7 days of exposure to Danadim 400 EC at the rate of 9.0 mL/ha (3.6 g a.i./ha), mortality corrected using the formula of Abbott was 93.1%. Therefore, the validity criterion specified in the Method description was met. The results obtained in the reference item group showed that the test organisms were sensitive to dimethoate.
- The mean reproduction rate (Rr) in the control group was 4.2 eggs/female. The mean Rr after 14 days of exposure to Boscalid 23.3% + Difenoconazole 6.6% SC at rates 1.28, 2.55, 5.10 and 10.20 L/ha were 4.5, 3.4, 2.7 and 3.3 eggs/female, respectively. The percentages of reproduction reduction (Pr) caused by at the rates of 1.28, 2.55, 5.10 and 10.20 L/ha were (-5.0), 19.4, 36.8 and

21.9%, respectively. The negative value means that in the treated group there was more eggs/female than in the control group. At the significance level of 0.1, there were no statistically significant differences in reproduction between the group treated with the test item at the rates of 1.28, 2.55, 5.10 and 10.20 L/ha and the control group (Wiliams Multiple Sequential t-test Procedure, |t|<|t*|).

- On the basis of the obtained results the endpoints regarding mortality could not be utterly determined. The ER_{50} could not be determined due to mathematical reasons and it may be presumed that it is higher than the maximum rate used in the studies. The $NOER_{reproduction}$ value is higher or equal to 10.20 L/ha.
- Based on the results it can be stated that Boscalid 23.3% + Difenoconazole 6.6% SC at the rates of 1.28, 2.55, 5.10 and 10.20 L/ha has no adverse effect on mortality of the mites. The LR_{50} value could not be determined due to mathematical reasons, though it is presumed that it may be higher than 10.20 L/ha. The $NOER_{mortality}$ value is higher or equal to 10.20 L/ha.
- The rates of 1.28, 2.55, 5.10 and 10.20 L of Boscalid 23.3% + Difenoconazole 6.6% SC /ha have no significant effect on the fecundity of the tested organisms. Based on the results received from the study, the ER_{50} could not be determined due to mathematical reasons though it is presumed that it may be higher than 10.20 L/ha. The $NOER_{reproduction}$ value is higher or equal to 10.20 L/ha.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • after 48 hours, mortality of the control group was 6.7% (criterion: a maximum of 10.0%), • after 48 hours, mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 64.3% (criterion: a minimum of 50%) • all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity) • the mean number of mummies per female in the control group was 39.9 (criterion: a minimum of 5.0 mummies/female) • all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring) **Agreed endpoints:** **$LR_{50}/ER_{50} > 8$ L/ha** **$NOER_{reproduction} \geq 8$ L/ha**

Reference:	KCP 10.3.2.2-02
Report	"An extended laboratory test for evaluating the effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the parasitic wasp, Aphidius *rhopalosiphi* (De Stefani - Perez)". Natalia Lemańska, 2018, B/82/16. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	ESCORT 1 (Barrett K.L. et al., 1994) and the ESCORT 2 (Candolfi M.P. et al., 2001) guidance documents and the guidelines developed by the IOBC, BART, and EPPO Joint Initiative (Mead-Briggs M.A. et al., 2000; Mead-Briggs M.A. et al., 2010)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

Materials and methods

SUMMARY

The extended laboratory test involved the evaluation of the effects of the test item, Boscalid 23.3% + Difenoconazole 6.6% SC on mortality and fecundity of the parasitic wasp, *Aphidius rhopalosiphi*.

On the basis of the results of the preliminary test, it was decided to use three rates of the test item in the definitive test. These were 8.0, 4.0 and 2.0 L/ha (i.e. 476.0 g boscalid/ha+131.2 g difenoconazole/ha, 952.0 g boscalid/ha+262.4 g difenoconazole/ha, 1904 g boscalid/ha+524.8 g difenoconazole/ha).

Adult female wasps were exposed to the test item applied to barley plants. Observations of settling behaviour were made during the initial 3 hours of exposure. The aims were to determine repellent effects of Boscalid 23.3% + Difenoconazole 6.6% SC and to check if the test insects had contact with barley plants sprayed with the test item. Settling behaviour of females from each replicate was observed five times. Mortality was determined 2, 24, and 48 hours after the introduction of the wasps to the test arenas.
Females which survived the 48-hour exposure to Boscalid 23.3% + Difenoconazole 6.6% SC and the ones from the control group were subjected to fecundity assessments. Fifteen female wasps from the each group treated with Boscalid 23.3% + Difenoconazole 6.6% SC and the control were individually introduced into the fecundity units containing barley plants infested with the aphid, *Rhopalosiphum padi*. After the 24-hour oviposition, the wasps were removed from the test arenas. After 12 days, the number of mummies (parasitized aphids in which wasp pupae were developing) was recorded.

Mortality of the wasps after 48 hours of exposure and the percentage of fecundity reduction (Pr) 12 days after the oviposition were the endpoints.

To verify the sensitivity of the biological test system and the precision of the test procedure, Danadim 400 EC (400 g dimethoate/L), which is an insecticide, was used as a reference item. The rate of the reference item was 5.0 mL/ha (2.0 g dimethoate/ha). The control group was treated with distilled water.

Results

The effects of the test item, Boscalid 23.3% + Difenoconazole 6.6% SC on mortality and fecundity of *Aphidius rhopalosiphi* in the extended laboratory test are summarized below.

Mortality and fecundity of *Aphidius rhopalosiphi* in the laboratory test

Study group [application rate]		**Parameter (endpoint)**							
		Mortality after 48 h of exposure				**Reproduction**			
Test item		**Total**	**Corrected**	**LR_{50}**		**Mean no. of mummies/ female**	**Fecundity reduction Pr [%]**	**ER_{50}**	
[L/ha][a]	**[g a.i./ha][b]**	**[%]**	**[%]**	**[L/ha][a]**	**[g a.i./ha][b]**			**[L/ha][a]**	**[g a.i./ha][b]**
Control (0.0)		6.7	-	-		39.9	-	-	
2.0	476.0 + 131.2*	6.7	0.0	>8.0	>1904 + 524.8*	40.7	(-2.0)	>8.0	>1904 + 524.8*
4.0	952.0 + 262.4*	3.3	(-3.6)			40.0	(-0.2)		
8.0	1904 + 524.8*	0.0	(-7.1)			48.4	(-21.0)		
$NOER_{mortality}$				≥ 8.0		**$NOER_{fecundity}$**		≥ 8.0	
Reference item		-							
[mL/ha][c]	**[g a.i./ha][c]**								
5.0	2.0	66.7	64.3	not determined		not assessed			

a: [L test item/ha]
b: [g active ingredient/ha]
c: [mL test item/ha]
*:g boscalid/ha + g difenoconazole/ha

**: statistically significant difference

Findings

- The results of the assessments of settling behaviour are presented in Table 1. Three hours after the introduction of the insects into the test units, the mean percentages of wasps settled on the plants were 67.3% in the control group, 37.3, 52.0 and 29.3% in the group treated with Boscalid 23.3% + Difenoconazole 6.6% SC at rates 2, 4 and 8 L/ha, and 56% in the reference item group.
- Repellent properties of the test item and the reference item were assessed. The results of Shapiro-Wilk's test ($p > 0.05$) confirmed normal data distribution in the group treated with the test item at all tested rates. Levene's test ($p < 0.05$) did not confirm variances homogenity in all the study groups. At the significance level of 0.05, there were statistically significant differences in the mean percentages of wasps settled on the plants between the treated at rates 2 and 8 L/ha and the control groups (non parametric Kruskall-Wallis test, $p < 0.05$). On the basis of the obtained results, it can be concluded that the test item at rates 2 and 8 L/ha had repellent effects on the wasps.
- After 48 hours of exposure, there was no dead wasps in the control group and in the group treated with Boscalid 23.3% + Difenoconazole 6.6% SC at all tested rates.
- Wasp mortality recorded in the definitive test is presented in Tables 3-5. After 48 hours of exposure, there were 2 dead wasps in the control group and it refers to 6.67% of mortality. In the group treated with Boscalid 23.3% + Difenoconazole 6.6% SC at the rates 2, 4, 8 L/ha the mortality corrected according to the Abbott formula was equal to 0.0, (-3.6) and (-7.1)%, respectively.
- It can be concluded that the LR_{50} is higher than the maximum application rate of Boscalid 23.3% + Difenoconazole 6.6% SC, i.e. > 8 L/ha (1904.0 g boscalid + 524.8 g difenoconazole/ha).
- Wasp mortality after 48 hours of exposure to Danadim 400 EC was 64.3%. The relation between Danadim 400 EC and wasp mortality showed that the insects were sensitive to dimethoate.
- All wasps survived the 24-hour oviposition period. The fecundity assessment showed that the mean number of mummies per female in the control group was 39.9. The mean number of mummies per female in the group treated with Boscalid 23.3% + Difenoconazole 6.6% SC at the rate of 2, 4 and 8 L/ha was 40.7, 40.0 and 48.4, respectively. Reduction in wasp fecundity (Pr) caused by Boscalid 23.3% + Difenoconazole 6.6% SC at the rates previous mentioned was (-2.0), (-0.2) and (-21.2)%.
- At the significance level of 0.05, no statistically significant differences in fecundity between the wasps exposed to the test item at all rates and the control group were stated (the Williams Multiple Sequential t-test procedure t, $p > 0.05$).
- The ER_{50} value and $NOER_{fecundity}$ could not be determined on the basis of the obtained fecundity results. It can be concluded that the ER_{50} is higher than the maximum application rate of Boscalid 23.3% + Difenoconazole 6.6% SC, i.e. > 8 L/ha (1904.0 g boscalid + 524.8 g difenoconazole/ha).

Validity criteria

The following validity criteria were met during the study:

- after 48 hours, mortality of the control group was 6.7% (criterion: a maximum of 10.0%),
- after 48 hours, mortality of the group treated with the reference item at the rate of 5.0 mL/ha was 64.3% (criterion: a minimum of 50%),
- all wasps survived the 24-hour oviposition period (criterion: only wasps that survive oviposition can be examined for fecundity),
- the mean number of mummies per female in the control group was 39.9 (criterion: a minimum of 5.0 mummies/female),
- all wasps in the control group gave offspring (criterion: a maximum of 2 females giving no offspring).

Conclusion
Corrected mortality of the wasps after 48 hours of exposure to the test item at the rate of 2.0, 4.0 and 8.0 L/ha was 0.0, (-3.7) and (-7.1)%, respectively. The negative value means that in the tested item rate there was higher number of live wasps than in the control group.
Corrected mortality of the wasps exposed to Danadim 400 EC at the rate of 5.0 mL/ha was 64.3% after 48 hours. Therefore, the validity criterion was met [5]. The results showed that the insects were sensitive to dimethoate.
The fecundity assessment showed that the mean number of mummies per female in the control group was 39.9. As for the wasps treated with Boscalid 23.3% + Difenoconazole 6.6% SC at the rates of 2, 4, and 8 L/ha the number of mummies/female were 40.7, 40.0 and 48.4, respectively. Fecundity reduction (Pr) caused by Boscalid 23.3% + Difenoconazole 6.6% SC at the rate of 2.0, 4.0 and 8.0 L/ha was respectively (-2.0), (-0.2) and (-21.0)%. The negative values indicate that a mean number of mummies per female in the treated group were higher than in the control. At the significance level of 0.05, there were no statistically significant differences in fecundity between the wasps exposed to the test item at the all rates and the control group.
Based on the above test results, it can be concluded that Boscalid 23.3% + Difenoconazole 6.6% SC had no negative effects on the mortality and on fecundity of *Aphidius rhopalosiphi*.

A 2.3.2.3 KCP 10.3.2.3. Semi-field studies with non-target arthropods

A 2.3.2.4 KCP 10.3.2.4. Field studies with non-target arthropods

A 2.3.2.5 KCP 10.3.2.5. Other routes of exposure for non-target arthropods

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

Comments of zRMS: The study is considered acceptable. All validity criteria were met.

- each replicate produced 46.4 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 24.3% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 8.7% (criterion: ≤ 10%).

Agreed endpoints:

Parameter	Value [mg of test item/kg dry weight of artificial soil]	Value [mg of boscalid/kg dry weight of artificial soil]	Value [mg of difenoconazole/kg dry weight of artificial soil]
EC_{10}	117.7 (102.6 – 131.6)	25.3 (22.0 – 28.3)	7.0 (6.1 – 7.8)
EC_{20}	159.4 (143.9 – 173.4)	34.2 (30.9 – 37.2)	9.4 (8.5 – 10.3)
EC_{50}	284.5 (268.1 – 301.8)	61.1 (57.6 – 64.8)	16.8 (15.9 – 17.9)
NOEC	100.0	21.5	5.9
LOEC	180.0	38.7	10.7
LC_{50} (mortality)	>1000	>214.8	>59.2

Reference	KCP 10.4.1.1
Report	"Boscalid 23.3% + Difenoconazole 6.6% SC: Earthworm Reproduction Test (*Eisenia fetida*)" Paweł Pieczka (2018) Study code: G/129/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s)	OECD Guideline No. 222 (2004)
Deviations	Yes; The study finished in August 2018 and not in May 2018 as it was planned. The deviation did not affect the study results.
GLP	Yes
Acceptability	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item	Boscalid 23.3% + Difenoconazole 6.6% SC, batch number: SCL – 20245, active substance: Boscalid 23.8% w/v, Difenoconazole 6.56% w/v
Artificial soil	5% sphagnum peat, 20% kaolin clay, 75% industrial sand
Test organism	Earthworm, *Eisenia fetida* obtained from a standard laboratory culture cultivated at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Department of Ecotoxicology, Laboratory of Soil Toxicology
Test design	Test duration: 8 weeks; number of replicates: 4 replicates/concentration + 8 replicates/control; number of earthworms: 10 earthworms/replicates
Concentration of the	A control; 18, 32, 56, 100, 180, 320, 560 and 1000 mg/kg dry weight of

test item	the artificial soil
Test conditions	temperature: 18 – 21.5°C; pH at the beginning of the experiment: 6.12 – 6.21; pH at the end of the experiment: 6.15 – 6.20; soil moisture content at the beginning of the experiment: 15.28 – 16.00% (51.19 – 53.59% of the maximum water holding capacity); soil moisture content at the end of the experiment: 16.44 – 17.21% (55.08 – 57.65 % of the maximum water holding capacity); light-dark cycle: 16h : 8h; light intensity: 676 – 712 lux at the beginning of the experiment, light intensity: 692 – 721 lux at the end of the experiment
Statistical analysis	EC_{10}, EC_{20}, EC_{50}: the probit method NOEC (reproduction) – the Shapiro-Wilk's Test on Normal Distribution, the Levene's Test on Variance Homogeneity (with Residuals), and the Williams Multiple Sequential t-test Procedure NOEC (survival) – Fisher's Exact Binomial Test with Bonferroni Correction
Endpoints	EC_{10}, EC_{20}, EC_{50}, NOEC

Results and discussions

On the basis of the results, it was concluded that after 4 weeks, at the control group the mortality of adult earthworm was noticed and it was equal to 8.7%. At concentrations ranging from 18 to 1000.00 mg of the test item/kg dry weight of artificial soil, after 4 weeks of exposure to the test item, mortality of the adult earthworms was ranging from 5.0 to 20.0%.

After 4 weeks of the experiment, the treated earthworms did not exhibit any changes in appearance and behaviour.

After the application of the test item at the concentrations ranging from 18 to 1000.00 mg/kg dry soil, the body weight increase was between 5.1 to 14.5%. As for the control group, it was equal to 13.6%.

After the application of the test item at the concentrations ranging from 18 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 1.5 – 49.5 per replicate. The mean number of juveniles in the control group was equal to 46.4 per replicate.
The obtained results made it possible to conclude that Boscalid 23.3% + Difenoconazole 6.6% SC had a statistically significant impact on reproduction of the earthworms at concentrations ranging from 180 to 1000 mg/kg dry weight of artificial soil.

- The concentration of the test item causing a 10% reduction in the number of juveniles produced within the exposure period (EC_{10}) is 117.7 mg/kg dry weight of artificial soil (25.3 mg of boscalid + 7.0 mg of difenoconazole/kg dry weight of artificial soil).
- The concentration of the test item causing a 20% reduction in the number of juveniles produced within the exposure period (EC_{20}) is 159.4 mg/kg dry weight of artificial soil (34.2 mg of boscalid + 9.4 mg of difenoconazole/kg dry weight of artificial soil).
- The concentration of the test item causing a 50% reduction in the number of juveniles produced within the exposure period (EC_{50}) is 284.5 mg/kg dry weight of artificial soil (61.1 mg of boscalid + 16.8 mg of difenoconazole/kg dry weight of artificial soil).
- The highest concentration at which the test item is observed to have no statistically significant effects on reproduction (NOEC) is 100 mg/kg dry weight of artificial soil (21.5 mg of boscalid + 5.9 mg of difenoconazole/kg dry weight of artificial soil).
- The lowest concentration at which the test item is observed to have a statistically significant effect on reproduction (LOEC) is 180 mg/kg dry weight of artificial soil (38.7 mg of boscalid + 10.7 mg of difenoconazole/kg dry weight of artificial soil).

Validity criteria
The results are considered valid because the following criteria were satisfied in the controls:

- each replicate produced 46.4 juveniles (mean) at the end of the experiment - (criterion: ≥ 30 juveniles by the end of the experiment),
- the coefficient of variation of reproduction was 24.3% (criterion: ≤ 30%),
- adult mortality over the initial 4 weeks of the experiment was 8.7% (criterion: ≤ 10%).

Conclusion:

Endpoints are presented in Table given below.

Parameter	Value [mg of test item/kg dry weight of artificial soil]	Value [mg of boscalid/kg dry weight of artificial soil]	Value [mg of difenoconazole/kg dry weight of artificial soil]
EC_{10}	117.7 (102.6 – 131.6)	25.3 (22.0 – 28.3)	7.0 (6.1 – 7.8)
EC_{20}	159.4 (143.9 – 173.4)	34.2 (30.9 – 37.2)	9.4 (8.5 – 10.3)
EC_{50}	284.5 (268.1 – 301.8)	61.1 (57.6 – 64.8)	16.8 (15.9 – 17.9)
NOEC	100.0	21.5	5.9
LOEC	180.0	38.7	10.7
LC_{50} (mortality)	>1000	>214.8	>59.2

A 2.4.1.2 KCP 10.4.1.2 Earthworms - field studies

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

A 2.4.2.1 KCP 10.4.2.1 Species level testing

Comments of zRMS:

The study is considered acceptable. All validity criteria were met.

- mean adult mortality: 15.0% (criterion: ≤ 20%),
- the mean number of juveniles per vessel at the end of the test: 546.4 (criterion: ≥ 100 juveniles at the end of the test),
- the coefficient of variation calculated for the number of juveniles: 24.8 (criterion: ≤ 30%).

Agreed endpoints:

LC_{10}, LC_{20}, LC_{50}, NOEC and LOEC values for mortality

Endpoint	Value [mg /kg dry weight of the artificial soil]	Value [mg of boscalid /kg dry weight of the artificial soil]	Value [mg of difenoconazole /kg dry weight of the artificial soil]
LC_{10}	> 1000.0*	> 214.8	> 59.2
LC_{20}	> 1000.0*	> 214.8	> 59.2
LC_{50}	> 1000.0*	> 214.8	> 59.2
NOEC	≥ 1000.0*	≥ 214.8	≥ 59.2
LOEC	> 1000.0*	> 214.8	> 59.2

EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC values for reproduction.

Endpoint	Value [mg /kg dry weight of the artificial soil]	Value [mg of boscalid /kg dry weight of the artificial soil]	Value [mg of difenoconazole /kg dry weight of the artificial soil]
EC_{10}	**357.6** (0.7 – 619.7)	**76.8** (0.2 – 133.1)	**21.2** (0.04 – 36.7)
EC_{20}	**811.6** (423.2 – >1000)	**174.3** (90.9 – >218.8)	**48.1** (25.1 – >59.2)
EC_{50}	**> 1000**	**> 214.8**	**> 59.2**
NOEC	**560.0**	**120.3**	**33.16**
LOEC	**1000.0**	**214.8**	**59.21**

Reference: KCP 10.4.2.1 - 01

Report "Boscalid 23.3% + Difenoconazole 6.6% SC: Collembolan (*Folsomia candida*) reproduction test". Paweł Pieczka, 2018, G/130/17. Institute of Industrial Organic Chemistry Branch Pszczyna

Guideline(s): OECD Guideline No. 232 (2016)

Deviations: Yes. At the end of the test the soil moisture content was determined by drying small sample of the artificial soil in 105°C instead of weighing the test vessels as it is mentioned in OECD Guideline No. 232 (2016). Physiological or pathological symptoms or distinct changes in behavior were not described. The study was finished in May 2018 and not in April 2018 as it was planned.
The deviations did not affect the study results.

GLP: Yes

Acceptability: Yes

Duplication (if vertebrate study) No

Materials and methods

Test item: Boscalid 23.3% + Difenoconazole 6.6% SC; Batch Number SCL- 20245; active substance: Boscalid 23.8%, Difenoconazole 6.56% (w/w)

Test species: *Folsomia candida* obtained from a standard laboratory culture at the Institute of Industrial Organic Chemistry, Branch Pszczyna, Laboratory of Soil Toxicology. The collembolans used in the study were 9 – 12 days old.

Soil: 5% sphagnum peat, 20% kaolin clay, and 75% air-dried industrial sand

Study design: Number of replicates: 4 replicates / concentration + 8 replicates / control
Number of collembolans: 10 / replicate
Test duration: 28 days

Application rates: Control, 10, 18, 32, 56, 100, 180, 320, 560, and 1000 mg of the test item/kg of dry weight of the artificial soil

Test conditions: Temperature: 18.0 – 22.0℃; humidity: 11.8 – 13.5%; lighting: 16 h light : 8 h dark; light intensity: 592 – 682 lux; pH: 6.37 – 6.83

Statistical analysis: EC_{10}, EC_{20}, and EC_{50} – a probit analysis
LC_{10}, LC_{20}, and LC_{50} - a probit analysis
NOEC:
- Shapiro-Wilk's Test on Normal Distribution,

- Bartlett's Test Procedure on Variance Homogeneity,
- Williams Multiple Sequential t-test Procedure (significance of differences – offspring number),
- Fisher's Exact Binomial Test with Bonferroni Correction (significance of differences – survival of adults)

Endpoints: EC_{10}, EC_{20}, EC_{50}, NOEC, LOEC
LC_{10}, LC_{20}, LC_{50}, NOEC

Results and Conclusions

Mortality at the concentrations ranging from 10 to 1000 mg/kg dry weight of the artificial soil ranged from 2.5 to 20.0%. As for the control group, it was equal to 15%.
The endpoint values showing the impact of the test item on the survival of adult collembolans are presented in the table given below.

LC_{10}, LC_{20}, LC_{50}, NOEC and LOEC values

Endpoint	Value [mg /kg dry weight of the artificial soil]	Value [mg of boscalid /kg dry weight of the artificial soil]	Value [mg of difenoconazole /kg dry weight of the artificial soil]
LC_{10}	> 1000.0*	> 214.8	> 59.2
LC_{20}	> 1000.0*	> 214.8	> 59.2
LC_{50}	> 1000.0*	> 214.8	> 59.2
NOEC	≥ 1000.0*	≥ 214.8	≥ 59.2
LOEC	> 1000.0*	> 214.8	> 59.2

After the exposure of collembolans to the test item at the concentrations ranging from 10 to 1000 mg/kg dry weight of the artificial soil, the mean number of juveniles was between 412.0 – 675.5 per replicate. As for the control group, the number of juveniles was equal to 546.4 per replicate.
The endpoint values showing the impact of the test item on reproduction of *Folsomia candida* are presented in the table given below.

EC_{10}, EC_{20}, EC_{50}, NOEC and LOEC values

Endpoint	Value [mg /kg dry weight of the artificial soil]	Value [mg of boscalid /kg dry weight of the artificial soil]	Value [mg of difenoconazole /kg dry weight of the artificial soil]
EC_{10}	**357.6** (0.7 – 619.7)	**76.8** (0.2 – 133.1)	**21.2** (0.04 – 36.7)
EC_{20}	**811.6** (423.2 – >1000)	**174.3** (90.9 – >218.8)	**48.1** (25.1 – >59.2)
EC_{50}	**> 1000**	**> 214.8**	**> 59.2**
NOEC	**560.0**	**120.3**	**33.16**
LOEC	**1000.0**	**214.8**	**59.21**

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • the variation among replicates of control samples was less than 15% The coefficients of variation (CV) in the control group were 9.1, 11.1, 12.2, 6.3, 4.0, 4.0, 1.2 and 0.7%, after 0, 7, 14, 28, 42, 56, 70, 84 days of incubation. The validity criterion was met, because the variation between replicate control samples is less than ± 15%. **Agreed endpoints:** On the basis of the results, it was concluded that at the concentrations 6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) and 33.24 mg of

	the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil), Boscalid 23.3% + Difenoconazole 6.6% SC did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Reference **KCP 10.5-01**

Report "Boscalid 23.3% + Difenoconazole 6.6% SC : Soil Microorganisms: Nitrogen Transformation Test".Paweł Pieczka, 2018. Study code: G/128/17. Institute of Industrial Organic Chemistry Branch Pszczyna

Guideline(s) OECD Guideline No. 216 (2000) / EU Method C.21

Deviations Yes.
The temperature in the test room was 18 – 23°C. According to the study plan should be 18 – 22°C. The temperature changes were short-lived and did not affect the result of the experiment.
According to the study plan, the experiment could be finished on day 42 of the analysis, because the difference in the nitrate formation rate between the control and the lower concentration, i.e. 6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) was lower than 25%. Upon the Sponsor's request, the experiment was continued until the difference in the nitrate formation rate between the control and the highest concentration, i.e. 33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil) was lower or equal to 25%.
According the Guideline, the soil extraction should be conducted at 150 rpm for 60 min. However, in this study, the extraction was performed at 90 rpm for 24 hours. The modification resulted from the optimization of the nitrate extraction which showed that the extraction was more effective when the shaking rate was lower and the extraction lasted longer.
These deviations did not affect the results of the study.

GLP Yes

Acceptability Yes

Duplication (if vertebrate study) No

Material and methods

Test material	Boscalid 23.3% + Difenoconazole 6.6% SC
Soil	Agricultural soil collected from a place belonging to the Institute of Industrial Organic Chemistry, Branch Pszczyna
Test design	Three portions of soil (3 x 1500 g), i.e. one control group and two treated groups. Every portion was divided into three replicates (3 x 500 g). The soil was enriched with the organic substrate, i.e. lucerne at dose of 5 g/kg dry weight of soil. Test duration: 84 days.
Concentrations of the test material	Control; 6.648 mg of the test item/kg soil (1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) and 33.24 mg of the test item/kg soil (7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil).
Test conditions	Temperature: 18 – 23°C, soil moisture: 41.2% – 48.8% of the maximum water holding capacity, incubation in darkness.
Endpoints	The concentration of nitrate [mg/kg dry soil] after 0, 7, 14, 28, 42, 56, 7 and 84 days of incubation. The nitrate formation rate [mg/kg dry weight of soil/day] for selected time intervals of soil incubation, i.e. 0 – 7, 0 – 14, 0 – 28, 0 – 42, 0 – 56, 0 – 70, 0 – 84 days. Percent deviation from the control in nitrate formation rate calculated for selected time intervals i.e. 0 – 7, 0 – 14, 0 – 28, 0 – 42, 0 – 56, 0 – 70, 0 – 84 days.
Statistical analysis	- Williams Multiple Sequential t-test Procedure.

Study design

The aim of the study was to detect long-term adverse effects of Boscalid 23.3% + Difenoconazole 6.6% SC on the processes of nitrogen transformation in aerobic surface soils.

Agricultural soil was used. It was manually cleared of large objects and sieved to a particle size of 2 mm.

The concentrations of the test item were 6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) and 33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil).

The treated and the control soils were divided into three replicates.

On days 0, 7, 14, 28, 42, 56, 70 and 84 days of incubation, soil samples were collected to determine the quantities of nitrate.

The method involves a measurement of the nitrates ions concentration in a soil extract obtained by using 0.1 M KCl. The pH/ION 7320 digital meter and the NO 800 nitrate electrode were used.

The nitrate formation rate in each treated group was compared with that in the control, and the percent deviation of the treated from the control was calculated.

Results:

The concentration of the nitrate after 7 days of incubation.

Concentration	**Control**			**6.648 mg of the test item/kg soil** (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)			**33.24 mg of the test item/kg soil** (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)		
Replicate	I	II	III	I	II	III	I	II	III
Reading [mg/L]**	39.46	37.07	45.86	27.00	27.58	27.40	27.84	34.27	28.01
Nitrate ion concentration [mg/kg of dry soil]	197.30	185.35	229.30	135.00	137.90	137.00	139.20	171.35	140.05
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	**203.98 ± 22.72**			**136.63* ± 1.48**			**150.20* ± 18.32**		
CV	**11.1**			**1.1**			**12.2**		

*- statistically significant difference between the mean nitrate ion concentration in the control group and in the treated one

**- values adjusted for the value of the blank sample, i.e. 4.94 mg/L

The concentration of the nitrate after 14 days of incubation.

Concentration	**Control**			**6.648 mg of the test item/kg soil** (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)			**33.24 mg of the test item/kg soil** (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)		
Replicate	I	II	III	I	II	III	I	II	III
Reading [mg/L]**	16.70	14.77	13.09	12.12	9.95	10.93	12.88	12.77	13.42
Nitrate ion concentration [mg/kg of dry soil]	83.50	73.85	65.45	60.60	49.75	54.65	64.40	63.85	67.10
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	**74.27 ± 9.03**			**55.00* ± 5.43**			**65.12* ± 1.74**		
CV	**12.2**			**9.9**			**2.7**		

*- statistically significant difference between the mean nitrate ion concentration in the control group and in the treated one

** - values adjusted for the value of the blank sample, i.e. 3.23 mg/L

The concentration of the nitrate after 28 days of incubation

Concentration	Control			6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)			33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	23.26	24.27	21.40	26.00	22.23	26.63	19.45	22.67	26.75
Nitrate ion concentration [mg/kg of dry soil]	116.30	121.35	107.00	130.00	111.15	133.15	97.25	113.35	133.75
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	114.88 ± 7.28			124.77 ± 11.90			114.78 ± 18.29		
CV	6.3			9.5			15.9		

* - values adjusted for the value of the blank sample, i.e. 8.85 mg/L

The concentration of the nitrate after 42 days of incubation

Concentration	Control			6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)			33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	55.23	54.52	58.74	59.43	56.67	57.54	66.11	61.80	61.65
Nitrate ion concentration [mg/kg of dry soil]	276.14	272.59	293.69	297.14	283.34	287.69	330.54	308.99	308.24
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	280.81 ± 11.30			289.39 ± 7.06			315.92 ± 12.66		
CV	4.0			2.4			4.0		

* - values adjusted for the value of the blank sample, i.e. 1.63 mg/L

The concentration of the nitrate after 56 days of incubation

Concentration	Control			6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)			33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)		
Replicate	I	II	III	I	II	III	I	II	III
Reading** [mg/L]	38.22	35.98	35.45	32.79	31.71	31.94	35.59	38.67	36.32
Nitrate ion concentration [mg/kg of dry soil]	191.11	179.91	177.26	163.96	158.56	159.71	177.96	193.36	181.61
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	182.76 ± 7.35			160.74* ± 2.84			184.31 ± 8.05		
CV	4.0			1.8			4.4		

*- statistically significant difference between the mean nitrate ion concentration in the control group and in the treated one

** - values adjusted for the value of the blank sample, i.e. 4.26 mg/L

The concentration of the nitrate after 70 days of incubation

Concentration	Control			6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)			33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	47.57	48.64	48.48	49.94	48.36	49.30	51.34	51.85	50.68
Nitrate ion concentration [mg/kg of dry soil]	237.87	243.22	242.42	249.72	241.82	246.52	256.72	259.27	253.42
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	241.17 ± 2.89			246.02 ± 3.97			256.47 ± 2.93		
CV	1.2			1.6			1.1		

* - values adjusted for the value of the blank sample, i.e. 1.0 mg/L

The concentration of the nitrate after 84 days of incubation

Concentration	Control			6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)			33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)		
Replicate	I	II	III	I	II	III	I	II	III
Reading* [mg/L]	53.05	53.79	53.37	52.06	51.57	51.45	57.54	54.62	51.75
Nitrate ion concentration [mg/kg of dry soil]	265.27	268.97	266.87	260.32	257.87	257.27	287.72	273.12	258.77
Mean nitrate ion concentration [mg/kg of dry soil] ± SD	267.04 ± 1.86			258.49 ± 1.62			273.20 ± 14.46		
CV	0.7			0.6			5.3		

* - values adjusted for the value of the blank sample, i.e. 3.55 mg/L

Nitrate formation rate [mg nitrate/kg dry weight of soil/day] for selected time intervals.**

Time interval [d]	Control				6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)				33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)			
	Replicate			Mean ± SD	Replicate			Mean ± SD	Replicate			Mean ± SD
	I	II	III		I	II	III		I	II	III	
0 - 7	9.23	7.52	13.8	10.18 ± 3.25	3.35	3.76	3.63	3.58* ± 0.21	4.27	8.86	4.39	5.84* ± 2.62
0 - 14	-3.52	-4.21	4.81	-4.18 ± 0.65	-3.64	-4.42	-4.07	-4.04 ± 0.39	-3.21	-3.25	-3.01	-3.16 ± 0.12
0 - 28	-0.59	-0.41	-0.92	-0.64 ± 0.26	0.66	-0.02	0.77	0.47 ± 0.42	-0.43	0.15	0.87	0.20 ± 0.65
0 - 42	3.42	3.33	3.83	3.53 ± 0.27	4.42	4.09	4.19	4.23 ± 0.17	5.27	4.76	4.74	4.92 ± 0.30
0 - 56	1.04	0.84	0.80	0.89 ± 0.13	0.94	0.84	0.86	0.88 ± 0.05	1.22	1.50	1.29	1.34 ± 0.14
0 – 70	1.50	1.58	1.57	1.55 ± 0.04	1.97	1.86	1.93	1.92 ± 0.57	2.10	2.14	2.06	2.10 ± 0.04
0 – 84	1.58	1.62	1.60	1.60 ± 0.22	1.77	1.74	1.74	1.75 ± 0.02	2.12	1.95	1.78	1.95 ± 0.17

* statistically significant difference between the control and the treated group (α=0.05)

** - Rate of nitrate ions formation per a day = [(mg nitrate / kg of soil dry weight on sampling day 'a') - (mg nitrate / kg of soil dry weight on day 0)]/ 'a' day; 'a' = 7, 14, 28, 42, 56, 70, 84 day

The difference in the nitrate formation rate between the control soil and the one treated with the test item at the concentrations 6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)

and 33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil) of soil did not exceed 25% on 84 day of analysis.

Conclusions

On the basis of the results, it was concluded that at the concentrations 6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) and 33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil), Boscalid 23.3% + Difenoconazole 6.6% SC did not have any long-term adverse effects on the process of nitrogen transformation in aerobic surface soils.

Comments of zRMS:	The study is considered acceptable. All validity criteria were met. • the variation among replicates of control samples was less than 15% (4.2, 8.0, 2.3 and 6.5% on 0, the 7th, 14th and 28th day of soil incubation, respectively. **Agreed endpoints:** On the basis of the results, it was concluded that Boscalid 23.3% + Difenoconazole 6.6% SC at the concentrations 6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) and 33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil) can be perceived as having no long-term influence on carbon transformations in soil.

Reference:	KCP 10.5-02
Report	"Boscalid 23.3% + Difenoconazole 6.6% SC. Soil Microorganisms: Carbon Transformation Test", Paweł Pieczka, 2017, G/127/17. Institute of Industrial Organic Chemistry Branch Pszczyna.
Guideline(s):	OECD Guideline No. 217 (2000) / EU Method C.22
Deviations:	The study finished in October and not in August as it was planned. The address of the Sponsor was changed. The temperature in the test room was 20.0 – 23°C. According to the study plan should be 18 – 22°C. The temperature changes were short-lived and did not affect the result of the experiment.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	-

Materials and methods

Materials

Test item:	
Description:	Boscalid 23.3% + Difenoconazole 6.6% SC
Production batch:	SCL – 20245
Active ingredients content:	Boscalid 23.8% w/v, Difenoconazole 6.56 % w/v
Vehicle and control:	Distilled water
Test system:	
Species:	Microorganisms

Source:	Agricultural soil taken from the area belonging to the Institute of Industrial Organic Chemistry, Branch Pszczyna.
Experimental conditions:	
Temperature:	20.0 – 23°C
Humidity:	42.7 – 47.8% of MWHC
Air changes:	-
Light and photoperiod:	Dark (24/24h)

Study design and methods

Experimental period:	25/07/2018 – 23/08/2018
Test design and treatment:	3 portions of soil weighing 1500 g each: one control group and two groups containing the test item. Every portion was divided into three replicates weighing 500 g each. Test duration: 28 days. Concentrations of the test material: Control; 6.648 mg of the test item/kg soil (1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) and 33.24 mg of the test item/kg soil (7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil). The mean respiration rate in the treated soil samples was compared with that in the control, and the percent deviation of the treated from the control was calculated after 0, 7, 14, and 28 days of incubation.
Statistics:	In order to determine significance in the soil respiration rate of differences between the control and the treated groups the Williams Multiple Sequential t-test Procedure was used.

Results

The difference in the soil respiration rate between the control soil and the one treated with the test item at the concentrations 6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) and 33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil) did not exceed 25% on any day of analysis.

Oxygen (O_2) consumption - deviations from the control [%]:

Day	**Control**	**PEC** 6.648 mg of the test item/kg soil (1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil)	**5 x PEC** 33.24 mg of the test item/kg soil (7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil)
0	17.7 ± 0.76	15.7^{+} ± 0.38	16.0^{+} ± 0.65
7	21.6 ± 1.71	22.3 ± 1.65	22.9 ± 0.39
14	17.5 ± 0.40	17.3 ± 0.66	17.8 ± 1.02
28	10.7 ± 0.67	10.8 ± 0.68	11.2 ± 0.78

+ statistically significant difference between the control and the treated group (α=0.05)

Conclusion

On the basis of the results, it was concluded that Boscalid 23.3% + Difenoconazole 6.6% SC at the concentrations 6.648 mg of the test item/kg soil (i.e. 1.4 mg boscalid/kg soil + 0.4 mg difenoconazole/kg soil) and 33.24 mg of the test item/kg soil (i.e. 7 mg boscalid/kg soil + 2 mg difenoconazole/kg soil) can be perceived as having no long-term influence on carbon transformations in soil.

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

A 2.6.2 KCP 10.6.2 Testing on non-target plants

zRMS comments:

The study is considered valid. All validity criteria were met.

- the seedling emergence in the control (validity criterion: at least 70%) was as follows:

- 95.2% - pea,
- 95.2% - sunflower,
- 95.2% - cabbage,
- 75% –carrot,
- 70% – onion,
- 95% – oats,

- the mean survival of the emerged control seedlings was 100% for all tested species (validity criterion: at least 90%);
- the control seedlings did not exhibit any visible phytotoxic effects;.
- environmental conditions for all plants of the same species were identical.

Phytotoxicity:

Only one phytotoxic symptom such as stunted growth of onion was observed during the experiment after 14 days.

Agreed endpoints:
ER_{10} and NOER values (as g of test item/ha).

	Pea ***Pisum sativum***	**Sunflower** ***Helianthus annuus***	**Cabbage** ***Brassica oleracea var. capitata***	**Carrot** ***Daucus carota***	**Onion** ***Allium cepa***	**Oats** ***Avena sativa***
Plant number at the end of the experiment						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
Shoot length (plants without roots)						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
Plant dry weight (plants without roots)						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000

Reference:	KCP 10.6.2-01
Report	"Boscalid 23.3% + Difenoconazole 6.6% SCTerrestrial Plant Test: Seedling Emergence and Seedling Growth Test". Paweł Pieczka, 2018, G/132/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	OECD Guideline No. 208 (2006)
Deviations:	The ER_{10} endpoints were determined additionally on the Sponsor's request. The study was finished in June 2018 and not in April 2018 as it was planned. According to OECD Guideline No. 208 (2006), the light intensity should be 350 ± 50µE/m²/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 87.2 – 127.0 µE/m²/s. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The deviations did not affect the results of the study.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Boscalid 23.3% + Difenoconazole 6.6% SC; Batch Number SCL-20245; active substance: Boscalid 23.8% w/v, Difenoconazole 6.56% w/v
Test species:	pea (*Pisum sativum*), sunflower (*Helianthus annuus*), cabbage (*Brassica oleracea var. capitata*), carrot (*Daucus carota*), onion (*Allium cepa*), oats (*Avena sativa*)
Soil:	Agricultural soil collected from the place belonging to the Institute of Industrial

	Organic Chemistry, Branch Pszczyna. The soil texture showed it was a sandy loam.
Study design:	number of rates: 5 application rates + control; number of replicates: 4 replicates/rate for carrot, oats, onion and 7 replicates/rate for cabbage, pea, sunflower . The total number of plants per application rate – 20 for carrot, oats, onion and 21 for cabbage, pea, sunflower. test termination: 14 days after the emergence of 50% of the control seedlings
Application rates:	Control, 153.7; 384.0; 960.0; 2400.0 and 6000 mL/ha (i.e. 36.6 g of boscalid + 10.1 g of difenoconazole/ha, 91.4 g of boscalid + 25.2 g of difenoconazole/ha, 228.5 g of boscalid + 63 g of difenoconazole/ha, 571.2 g of boscalid + 157.4 g of difenoconazole/ha, 1428 g of boscalid + 393.6 g of difenoconazole/ha) Volume of distilled water used to prepare the highest rate: 400 L water/ha.
Test conditions:	temperature: 16.6 – 27.9°C, humidity: 45.5 – 94.9%, lighting: 16 h light : 8 h dark; light intensity: 87.2 – 127.0 μE/m^2/s; carbon dioxide concentration: 310 – 340 ppm
Statistical analysis:	ER_{10}, ER_{25}, ER_{50} – probit analysis, NOER – Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity, Williams Multiple Sequential t-test Procedure or Welch-t test for Inhomogeneous Variances with Bonferroni-Holm Adjustment, Fisher`s Exact Binomial Test with Bonferroni Correction (emergence of plants)
Endpoints:	ER_{10}, ER_{25}, ER_{50}, NOER

Results and Conclusions

1. The test item i.e. Boscalid 23.3% + Difenoconazole 6.6% SC had no impact on the growth and seedling emergence of pea, sunflower carrot and oats. The test item slightly impacted the growth of cabbage and onion.
2. Plants of all analyzed species emerged at all of analyzed concentrations.
   After the application of the test item at rate equal 153.7 mL/ha, carrot emergence was delayed by one day in comparison to the control. For the other species used in the experiment, the emergence of plants was not delayed in comparison to the control group, i.e. pea, sunflower, cabbage, onion and oats.
3. The death of plant was noticed at cultivation of pea and onion.
4. Only one phytotoxic symptom such as stunted growth of onion was observed during the experiment.
5. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length, it was proved that the test item did not inhibit the process of growth of pea, sunflower, cabbage, carrot and oats. The shoot length was slightly inhibited for onion.
6. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot dry weight, it was proved that the test item did not inhibit the process of growth of pea, sunflower, cabbage, carrot and oats. The shoot length was slightly inhibited for onion.
7. The following order of the test plant sensitivity was noticed:
   pea, oats, carrot > sunflower > cabbage > onion

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as ml of the test item/ ha for all test species are given below.

ER_{10} and NOER values (as g of test item/ha)

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
Shoot length (plants without roots)						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000

NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000
Plant dry weight (plants without roots)						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of boscalid / ha for all test species are given below.

ER_{10} and NOER values (as g of boscalid/ha)

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0
NOER	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0
Shoot length (plants without roots)						
ER_{50}	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0
NOER	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0
Plant dry weight (plants without roots)						
ER_{50}	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0
NOER	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of difenoconazole / ha for all test species are given below.

ER_{10} and NOER values (as g of difenoconazole/ha)

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6
NOER	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6
Shoot length (plants without roots)						
ER_{50}	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6
NOER	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6
Plant dry weight (plants without roots)						
ER_{50}	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6
NOER	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6

ZRMS comments:

The study is considered valid. All validity criteria were met.

- the seedling emergence (validity criterion: at least 70%) was as follows:

- 97.6 – 85.7% – pea,

- 97.6 – 85.7 % – sunflower,

- 100.0 – 88.1% – cabbage,

- 92.5 – 85.0% – carrot,

- 95.0 – 95.0% – oats,

- 82.5 – 97.5% – onion,

- the mean survival of the emerged control seedlings was 100% in case of all experimental species (validity criterion: at least 90%),
- the control seedlings did not exhibit any visible phytotoxic symptoms,

- environmental conditions for all plants belonging to the same species were identical.

None phytotoxic symptom such was observed during the experiment after 14 days.

Agreed endpoints:

ER_{10}, ER_{25}, ER_{50} and NOER values (as ml test item/ha)

	Pea Pisum sativum	**Sunflower** Helianthus annuus	**Cabbage** Brassica oleracea var. capitata	**Carrot** Daucus carota	**Onion** Allium cepa	**Oats** Avena sativa
Plant number at the end of the experiment						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	> 6000	> 6000	> 6000	> 6000	> 6000	≥ 6000
Shoot length (plants without roots)						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	153.7
Plant dry weight (plants without roots)						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000

Reference:	KCP 10.6.2-02
Report	"Boscalid 23.3% + Difenoconazole 6.6% SC. Terrestrial Plant Test: Vegetative Vigour Test". Paweł Pieczka, 2018, G/133/17. Institute of Industrial Organic Chemistry Branch Pszczyna
Guideline(s):	OECD Guideline No. 227 (2006)
Deviations:	According to OECD Guideline No. 227 (2006), the light intensity should be 350 ± 50μE/m^2/s. However, these values are recommended for tests conducted in greenhouses. The experiment was conducted in a test room, where only artificial lighting was used. The light intensity was between 83.1 – 137.4 lx. Good control plant vigour was observed. Therefore, it was concluded that the light intensity was suitable for plant growing. The study was finished in May and not in April as it was planned. The ER_{10} was determined additionally on the Sponsor's request.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Materials and methods

Test item:	Boscalid 23.3% + Difenoconazole 6.6% SC; Batch Number SCL – 20245; active substance: Boscalid 23.8% w/v, Difenoconazole 6.56% w/v
Test species:	pea (*Pisum sativum*), sunflower (*Helianthus annuus*), cabbage (*Brassica oleracea var. capitata*), carrot (*Daucus carota*), onion (*Allium cepa*), oats (*Avena sativa*)
Soil:	Agricultural soil collected from the place belonging to the Institute of Indus-trial Organic Chemistry, Branch Pszczyna. The soil texture showed it was a sandy loam.
Study design:	number of rates: 5 application rates + control; number of replicates: 4 replicates/rate for carrot, oats, onion and 7 replicates/rate for cabbage, pea, sunflower.

	The total number of plants per application rate – 20 for carrot, oats, onion and 21 for cabbage, pea, sunflower. test termination: 21 days after the spraying.
Application rates:	Control, 153.7; 384.0; 960.0; 2400.0 and 6000 mL/ha (i.e. 36.6 g of boscalid + 10.1 g of difenoconazole/ha, 91.4 g of boscalid + 25.2 g of difenoconazole/ha, 228.5 g of boscalid + 63 g of difenoconazole/ha, 571.2 g of boscalid + 157.4 g of difenoconazole/ha, 1428 g of boscalid + 393.6 g of difenoconazole/ha) Volume of deionised water used to prepare the highest rate: 400 L water/ha
Test conditions:	temperature: 16.6 – 27.9°C, humidity: 45.5 – 94.9%, controlled light – dark cycles (16h:8h), light intensity: 83.1 – 137.4 μE/m^2/s, carbon dioxide concentration: 310 – 340 ppm.
Statistical analysis:	ER_{10}, ER_{25}, ER_{50} – probit analysis NOER (final number of plants) – Fisher's Exact Binomial Test with Bonferroni Correction. NOER (shoot length and dry weight) - Shapiro-Wilk's Test on Normal Distribution, Levene's Test on Variance Homogeneity (with Residuals), Williams Multiple Sequential t-test Procedure.
Endpoints:	ER_{10}, ER_{25}, ER_{50}, NOER

Results and Conclusions

1. The test item, i.e. Boscalid 23.3% + Difenoconazole 6.6% SC applied at rates ranging from 153.7 to 6000 mL/ha had no impact on vegetative vigour of pea, sunflower, cabbage, carrot, onion and oats.
2. The test item did not cause mortality of pea, sunflower, cabbage, carrot and onion at rates ranging from 153.7 to 6000 mL/ha. The mortality of oats at rate equal to 384.0 mL/ha was observed but it was only 5% in comparison to the control.
3. ER_{50} values determined for all tested species on the basis of the plant number, shoot length and weight at the end of the experiment were above 6000 mL/ha.
4. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the shoot length it was proved that the test item did not inhibit the process of growth of pea, sunflower, cabbage, carrot and onion. A slight impact on the process of growth was observed for oats at the rates ranging from 384.0 to 6000 mL/ha.
5. On the basis of NOER, ER_{10}, ER_{25} and ER_{50} values determined from the dry shoot weight it was proved that the test item did not inhibited the process of growth of all test species.
6. There were any phytotoxic symptoms observed for all test species.
7. The following order of the test plant sensitivity was noticed:
   pea, sunflower, cabbage, carrot, onion> oats.

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as ml of the test item/ ha for all test species are given below.

ER_{10}, ER_{25}, ER_{50} and NOER values (as ml test item/ha)

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	> 6000	> 6000	> 6000	> 6000	> 6000	≥ 6000
Shoot length (plants without roots)						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	153.7
Plant dry weight (plants without roots)						
ER_{50}	> 6000	> 6000	> 6000	> 6000	> 6000	> 6000
NOER	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000	≥ 6000

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of boscalid / ha for all test species are given below.

ER_{10}, ER_{25}, ER_{50} and NOER values (as g of boscalid/ha)

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0
NOER	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0	≥ 1428.0
Shoot length (plants without roots)						
ER_{50}	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0
NOER	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	36.6
Plant dry weight (plants without roots)						
ER_{50}	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0	> 1428.0
NOER	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0	≥ 1428.0

The ER_{50} and NOER values determined on the basis of plants number at the end of the experiment, shoot length and shoot dry weight measurements expressed as g of difenoconazole / ha for all test species are given below.

ER_{10}, ER_{25}, ER_{50} and NOER values (as g of difenocolazole/ha)

	Pea *Pisum sativum*	**Sunflower** *Helianthus annuus*	**Cabbage** *Brassica oleracea var. capitata*	**Carrot** *Daucus carota*	**Onion** *Allium cepa*	**Oats** *Avena sativa*
Plant number at the end of the experiment						
ER_{50}	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6
NOER	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6	≥ 393.6
Shoot length (plants without roots)						
ER_{50}	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6
NOER	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	10.1
Plant dry weight (plants without roots)						
ER_{50}	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6	> 393.6
NOER	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6	≥ 393.6

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

A 2.8 KCP 10.8 Monitoring data