

# **FINAL** REGISTRATION REPORT

## **Part B**

### **Section 9**

#### **Ecotoxicology**

Detailed summary of the risk assessment

Product code: IN005B1570

Product name: ~~INDOFIL~~ Difenoconazole 250 G/L EC greener

Chemical active substance:

Difenoconazole, 250 g/L

Central Zone

Zonal Rapporteur Member State: Poland

#### **CORE ASSESSMENT**

Article 33: Application for authorisation

Applicant: Indofil Industries (Netherlands) B.V.

Submission date: Ferbruary 2022

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## Version history

When	What
February 2022	V0 – Original version from applicant Indofil Industries (Netherlands) B.V. for submission to z-RMS, Poland, in the frame of the PPP Authorization according to Article 33 of Regulation (EC) No. 1107/2009
August 2023	Initial evaluation by the zRMS
May 2024	Corrected version by zRMS after commenting
June 2024	Applicant update to include chronic laboratory studies with adult honey bees and honey bee larvae
August 2024	ZRMs made changes in RR in line to reviewed comments during 3 <sup>rd</sup> round

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## 9 Ecotoxicology (KCP 10)

This document reviews the eco-toxicological studies for the product IN005B1570, an emulsifiable concentrate formulation containing 250 g/L Difenoconazole for use on oilseed rape, pome fruits (apples and pears), carrot, cauliflower, broccoli and cabbage.

Difenoconazole was first included in Annex I to Directive 91/414/EEC by Commission Directive 2008/69/EC of 1 July 2008.

A full risk assessment according to Uniform Principles is provided which demonstrates that the product is safe for the environment.

Where appropriate this document refers to the conclusion of the EU review for Difenoconazole. This will be where:

- The active substance data are relied upon in the risk assessment of the formulation; or when
- The EU review concluded that additional data/information should be considered at national re-registration.

The EFSA Scientific report for Difenoconazole (EFSA Scientific Report, 2011;9(1):1967) is considered to provide the relevant review information or a reference to where such information can be found.

The Commission Implementation Regulation for Difenoconazole (540/2011) provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation.

For the implementation of the uniform principles as referred to in Article 29(6) of Regulation (EC) No. 1107/2009, the conclusions of the review report on Difenoconazole, and in particular Appendices I and II thereof, as finalised in the Standing Committee on the Food Chain and Animal Health on 22 January 2008 shall be taken into account.

In the overall assessment Member States must pay particular attention to:

- The operator safety in spray applications. Conditions of use shall include adequate protective measures.
- The protection of aquatic organisms. Risk mitigation measures such as buffer zones shall be applied, where appropriate.
- The protection of birds and small mammals. Risk mitigation measures shall be applied, where appropriate.

Conditions of use shall include risk mitigation measures, where appropriate.

Information on the detailed composition of IN005B1570 can be found in the confidential dossier of this submission (Registration Report – Part C)

## 9.1 Critical GAP and overall conclusions

**Table 9.1-1: Table of critical GAPs**

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15						
Use -No. *	Member state(s)	Crop and/or situation (crop destina- tion / purpose of crop)	F, Fn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. num- ber a) per use b) per crop/ season	Min. interval between applica- tions (days)	L product/ha a) max. rate per appl. b) max. total rate per crop/season	g as/ha a) max. rate per appl. b) max. total rate per crop/seaso n	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	Central EU: PL - DE, CZ ; BE, NL, AT, SI, IE	BRSNW Oilseed rape	F	LEPTMA (Lepto- sphaeria maculans) SCLECS (Sclero- tinia sclerotiorum), ALTEBI (Alter- naria brassicae)	foliar spray	Autumn and Spring applica- tions BBCH 14-18 and BBCH 30- 69	2 (1 in autumn and 1 in spring or 2 in autumn)	21 (but usual- ly longer in practice)	a) 0.5 b) 1	a) 125 b) 250	100-500	NA	Dose rate: 125 g a.i./ha 0,5 L/ha Formulat- ed Product (or 0,25 L/ha per single application if 2 applications in autumn)	A	A	R	A	A	A	A
2	Central EU: PL - DE, CZ ; BE, NL, AT, SI, IE	MABSD, PYUCO (Apples, Pears)	F	VENTIN, VENTPI(Venturia inaequalis, Venturia pyrina) PODOLE[Powdery mildew (Podosphaera leucotricha)]	foliar spray	BBCH 57-84	3	7 ( label: spray interval from 7 to 10 days )	a) 0.225 b) 0.675	a) 56.25 b) 168.75	100-1500	21	Proposed LWA rate 0.15 L/10000 m2 LWA with a maxi- mum of 0.225 L/ha in for DE, AT, BE, NL, (UK) and maximum of 0,2 L/ha in PL (CZ and SI to be confirmed if max 0,225 or 0,2 L/ha)  Dose rate Formu- lated Product:: Scab 0,015 L/hl / 0,225	A	A	R	A	A	A	A

													L/ha PL 0,2 L/ha FR 0,15 L/ha  Powdery mildew 0,2 - 0,225 L/ha all countries								
3	Central EU: PL - DE, CZ ; BE, NL, AT, SI, IE	DAUCS (Garden carrot)	F	Leaf blight of carrot - ALTEDA (Alternaria dauci) Black rot of carrot - ALTERA (Alternaria radici- na) Powdery mildew of carrot - ERY SHE (Erysiphe heraclei)	foliar spray	from BBCH 39- 40	3	14	a) 0.5 b) 1.5	a) 125 b) 375	200-1000	14	Dose rate range Formulated Product in label:: 0,4 - 0,5 L/ha 0,5 L/ha in FR, ES 0,4 L/ha in DE (0,3 L/ha UK)	A	A	R	A	A	A	A	A
4	Central EU: PL - DE, CZ ; BE, NL, AT, SI, IE	BRSOB (cauliflower)	F	ALTEBI (Alternaria brassicicola) MYCOBR (Mycosphaerella brassicicola)	foliar spray	BBCH 20-39	3	14	a) 0.5 b) 1.5	a) 125 b) 375	200-1000	21	Dose rate Formu- lated Product in label:: 0,5 L/ha but possible also a dose rate range 0,3 - 0,4 - 0,5 L/ha	A	A	R	A	A	C	A	A
5	Central EU: PL - DE, CZ ; BE, NL, AT, SI, IE	BR SOK (broccoli)	F	ALTEBI (Alternaria brassicicola) MYCOBR (Mycosphaerella brassicicola)	foliar spray	BBCH 20-39	3	7	a) 0.5 b) 1.5	a) 125 b) 375	200-1000	14	Dose rate Formu- lated Product in label:: 0,5 L/ha but possible also a dose rate range 0,3 - 0,4 - 0,5 L/ha	A	A	R	A	A	C	A	A
6	Central EU: PL - DE, CZ ; BE, NL, AT, SI, IE	BR SOL (cabbage)	F	ALTEBI (Alternaria brassicicola) MYCOBR (Mycosphaerella brassicicola)	foliar spray	BBCH 20-39	3	7	a) 0.5 b) 1.5	a) 125 b) 375	200-1000	r 21	Dose rate Formu- lated Product in label:: 0,5 L/ha but possible also a dose rate range 0,3 - 0,4 - 0,5 L/ha	A	A	R	A	A	C	A	A

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

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

Explanation for column 15 – 21 “Conclusion”



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

**Remarks table:**

- (1) Numeration necessary to allow references
- (2) Use official codes/nomenclatures of EU
- (3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
- (4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
- (5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (*e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
- (6) Method, *e.g.* high volume spraying, low volume spraying, spreading, dusting, drench  
Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
- (7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
- (8) The maximum number of application possible under practical conditions of use must be provided
- (9) Minimum interval (in days) between applications of the same product.
- (10) For specific uses other specifications might be possible, *e.g.*: g/m<sup>3</sup> 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

**zRMS comments:**

The dRR was prepared by applicant. All comments and conclusions of the zRMS are presented in grey. Minor changes are introduced directly in the text and highlighted in grey. Not agreed or not relevant information is struck through and shaded for transparency.

### 9.1.1 Overall conclusions

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

##### *Effects on birds*

The risk assessment for birds was carried out according to EFSA/2009/1438.

The screening  $TER_a$  values for difenoconazole and the metabolite CGA 131013 for all proposed uses of IN005B1570 are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to birds is acceptable following use according to the proposed use pattern for these crops.

The  $TER_{lt}$  values for difenoconazole and the metabolite for uses of IN005B1570 in the crop group Orchards are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to birds is acceptable following use according to the proposed use patterns for this crop and application scenario.

For all other uses a Tier 1 risk assessment is required. For almost all crop scenarios, the Tier 1  $TER_{lt}$  values are greater than the trigger of 5, indicating that long-term risk to birds is acceptable following use according to the proposed use patterns for these crops.

For the active substance difenoconazole, the  $TER_{lt}$  value for use of IN005B1570 in oilseed rape in the scenario for medium herbivorous / granivorous bird "pigeon" is below the trigger of 5, indicating need for further refinement. For the metabolite, the  $TER_{lt}$  value for use of IN005B1570 in oilseed rape in the scenario for large herbivorous bird "goose" and medium herbivorous / granivorous bird "pigeon", and in Leafy vegetables in the scenario for the small granivorous bird "finch", small omnivorous bird "lark", and small insectivorous bird "wagtail" are below the trigger of 5, indicating need for further refinement.

Based on refined assessment step of refined  $MAF_m$  and reproductive dietary risk assessment, the calculated TER values for the long-term risk resulting from the difenoconazole and metabolite exposure in all the evaluated scenarios achieve the acceptability criteria  $TER \geq 5$  for long-term effects, indicating that long-term risk to birds is acceptable following use according to the proposed use patterns for the intended crops.

Acceptable risk for birds due to drinking water exposure was demonstrated. In the risk assessment for earthworm-eating birds and fish-eating birds via secondary poisoning, the TER values are greater than the trigger of 5, indicating acceptable risk for birds and indicating therefore no need for further refinement.

##### *Effects on terrestrial vertebrates other than birds*

The risk assessment for mammals was carried out according to EFSA/2009/1438.

The screening  $TER_a$  values for difenoconazole and the metabolite CGA 131013 for all proposed uses of IN005B1570 are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following use according to the proposed use pattern for these crops.

The  $TER_{lt}$  values for difenoconazole for uses of IN005B1570 in all crop groups are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is

unacceptable following use according to the proposed use patterns for this crop and application scenario. For difenoconazole, therefore for all uses a Tier 1 risk assessment is required.

For the active substance difenoconazole, for almost all crop scenarios, the Tier 1 TER<sub>lt</sub> values are greater than the trigger of 5, indicating that long-term risk to birds is acceptable following use according to the proposed use patterns for these crops. However, for leafy vegetables, the TER<sub>lt</sub> value for use of IN005B1570 in the scenario for small herbivorous mammal "vole" is below the trigger of 5, indicating need for further refinement.

Based on the refined assessment step of reproductive dietary risk assessment, the calculated TER values for the long-term risk resulting from the difenoconazole exposure in the evaluated scenario did not achieve the acceptability criteria  $TER \geq 5$  for long-term effects.

Since the approach used for the focal species vole is very conservative, some MS, such as Germany, consider a reduced trigger value of 2 for the long-term risk assessment. Thus, since TER<sub>lt</sub> is greater than the trigger value of 2, acceptable risk could be shown in Tier 2. Therefore, long-term risk to mammals is acceptable following use according to the proposed use patterns for all intended crops.

Acceptable risk for mammals due to drinking water exposure was demonstrated. In the risk assessment for earthworm-eating mammals and fish-eating mammals via secondary poisoning, the TER values are greater than the trigger of 5, indicating acceptable risk for mammals and indicating therefore no need for further refinement.

#### 9.1.1.2 Effects on aquatic organisms (KCP 10.2)

No unacceptable risk to aquatic organisms is seen following step 1 for the metabolites CGA 71019 and CGA 205375 following application of the formulation IN005B1570 on all intended groups. For all proposed uses, an acceptable risk for the active substance is seen after FOCUS step 4, concluding an acceptable risk for the formulation IN005B1570 to aquatic organisms considering the following mitigation measures:

Crop	Mitigation measure
Oilseed rape	10 m no-spray buffer zone and 10m vegetative filter strip
Orchards	25 m no-spray buffer zone
Carrot	20 m no-spray buffer zone and 20 m vegetative filter strip
Leafy vegetables	20 m no-spray buffer zone and 20 m vegetative filter strip

#### 9.1.1.3 Effects on bees (KCP 10.3.1)

The risk assessment performed for both the active substance and the formulated product derived hazard quotients lower than 50, indicating that the active substance as well as the formulation IN005B1570 pose an acceptable risk to bees from oral and contact exposure according to the proposed use.

#### 9.1.1.4 Effects on arthropods other than bees (KCP 10.3.2)

In-field HQ values based on first tier laboratory studies with *Aphidius rhopalosiphi* and *Typhlodromus pyri* were below the trigger value of 2, and therefore an acceptable risk is concluded following use according to the proposed use pattern.

#### 9.1.1.5 Effects on non-target soil meso- and macrofauna (KCP 10.4), Effects on soil

## microbial activity (KCP 10.5)

### *Effects on non-target soil meso- and macrofauna*

The chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to difenoconazole, its relevant metabolite CGA 71019 and the formulation IN005B1570 are greater than the trigger of 5, indicating that the risk to earthworms and other non-target soil organisms (meso- and macrofauna) is acceptable following use of IN005B1570 according to the proposed use pattern.

### *Effects on soil microbial activity*

Effects at expected soil concentrations for proposed uses of IN005B1570 are below the Commission Regulation (EU) No. 546/2011 triggers of 25%, indicating that the risk to soil micro-organisms is acceptable following use of IN005B1570 according to the proposed use pattern.

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

The worst-case TER values are well greater than the trigger value of 5 and therefore it is considered that risks to non-target plants after IN005B1570 applications are acceptable.

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

No data available.

## 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). The risk envelope concept exploits the idea that uses with similar characteristics can be assessed groupwise and that the risk assessment for all use groups can be simplified by focusing on the group with worst-case characteristics as a representation for all other groups. At the beginning of each section, it is also detailed which crop group is used. The risk envelope approach allows the risk assessment to remain concise rather than document all 10 unique uses, which can be seen in the GAP and reduces the risk assessment significantly.

**Table 9.1-2: Critical use pattern of IN005B1570 grouped according to crop group and application pattern**

Grouping according to crop group and application pattern			
Group	Intended uses	relevant parameter or value for sorting	relevant use parameters for grouping
<b>Effects on birds and mammals (9.2 and 9.3)</b>			
Oilseed rape	Oilseed rape (BBCH 14-18 and BBCH 30-69)	Oilseed Rape Difenoconazole: 2 x 0.125 kg a.s./ha, interval 21 days	Crop group according to EFSA/2009/1438 and application pattern
Orchards	Pome fruits (BBCH 57-84)	Pome fruits Difenoconazole: 3 x 0.056 kg a.s./ha, interval 7 days	

Root and Stem Vegetables	Carrot (BBCH 39-40)	Carrot Difenoconazole: 3 x 0.125 kg a.s./ha, interval 14 days	
Leafy Vegetables	Cauliflower (BBCH 19)	Cauliflower Difenoconazole: 3 x 0.125 kg a.s./ha, interval 14 days	
	Broccoli (BBCH 19-21)	Broccoli Difenoconazole: 3 x 0.125 kg a.s./ha, interval 7 days	
	Cabbage (BBCH 19)	Cabbage Difenoconazole: 3 x 0.125 kg a.s./ha, interval 7 days	
Effects on aquatic organisms (9.5)			
All intended directed spray applications in field crops and orchards <sup>1</sup>	Oilseed rape  Pome fruits  Carrot  Cauliflower  Broccoli  Cabbage	All intended uses; Max. 0.375 kg a.s./ha/year	Crop group and max. annual application rate
Effects on bees (9.6)			
All intended uses up to a single application rate of 0.125 kg a.s./ha	All crops  Oilseed rape, Orchards, roots and stem vegetables and leafy vegetables.	Not crop specific; Max. 0.125 kg a.s./ha	Max. single application rate
Effects on non-target arthropods (9.7)			
All intended uses up to a maximum application rate of 3 x 0.125 kg a.s./ha covering all other intended uses with a maximum application rate of 0.375 kg/ha in any 12 month period across use categories	All crops  Oilseed rape, Orchards, roots and stem vegetables and leafy vegetables.	3 x 0.125 kg a.s./ha	Worst-case application pattern
Effects on non-target soil meso- and macrofauna (9.8)			
Effects on soil microbial activity (9.9)			
Annual crops	All crops  Oilseed rape, roots and stem vegetables and leafy vegetables.	Annual crop: Max: 0.375 kg a.s./ha	Annual / perennial crop
Perennial crops	Orchard crops	Perennial crop: Max: 0.169 kg a.s./ha	
Effects on non-target terrestrial plants (9.10)			
Oilseed rape	Oilseed rape	Max. 2 x 0.125 kg a.s./ha	Application pattern

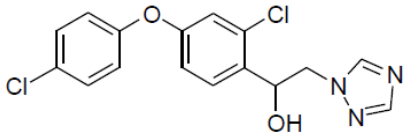
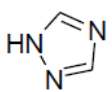
Orchards	Pome fruits	Max. 3 x 0.056 kg a.s./ha	
Root and Stem Vegetables	Carrot	Max. 3 x 0.125 kg a.s./ha	
Leafy Vegetables	Cauliflower Broccoli Cabbage	Max. 3 x 0.125 kg a.s./ha	

### 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 INDOFIL Difenconazole 250 EC is indicated in the tables.

**Table 9.1-3 Metabolites of Difenconazole**

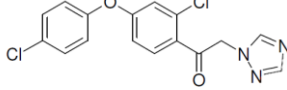
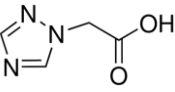
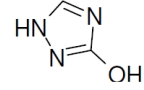
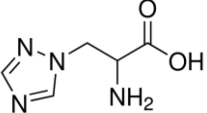
**Table 9.1-3.1: Metabolites of difenconazole potentially relevant for exposure assessment**

Metabolite	Molar mass (g/mole)	Chemical structure	Maximum observed occurrence in compartments	Risk assessment required?
CGA-205375 1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-1H-[1,2,4] triazol-yl]-ethanol	350		Soil: 9.7% Water/Sediment: 11.6%	Yes, soil and aquatic organisms
CGA 71019 (1,2,4-triazole)	69		Soil: 23.4% Water/Sediment: 9.6%	Yes, soil and aquatic organisms

**zRMS comments:**

Information regarding difenconazole metabolites provided in Table 9.1-3.1 is in line with data reported in EFSA Journal 2011;9(1):1967.

**Table 9.1-3.2: Other Metabolites of difenconazole**

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
CGA 205374 1-[2-[2-chloro-4-(4-chloro-phenoxy)-phenyl]-2-1H-[1,2,4] triazol-yl]-ethanone		-	Soil: - Water/Sediment: -	No, maximum occurrence does not exceed 10% in the EU Assessment
CGA 142856 1,2,4-triazol-1-yl-acetic acid 28711-29-7		127.10	Soil: - Water/Sediment: -	No, maximum occurrence does not exceed 10% in the EU Assessment
NOA 457654 1H-1,2,4-triazol-3-ol		85.065	Soil: - Water/Sediment: -	No, maximum occurrence does not exceed 10% in the EU Assessment
CGA 131013 2-amino-3-[1,2,4] triazol-1-yl-propanic acid 114419-45-3		156.14	Soil: - Water/Sediment: -	No, maximum occurrence does not exceed 10% in the EU Assessment

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

Avian toxicity studies have been carried out with Difenconazole and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on birds of INDOFIL Difeno 250 EC were not evaluated as part of the EU assessment of Difenconazole. However, the provision of new data on INDOFIL Difenconazole 250 EC is not considered essential.

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
Japanese quail	Difenconazole	Acute	<b>LD<sub>50</sub> &gt;2000 mg/kg bw</b>	EFSA Conclusion Report 2011; 9(1):1967
Mallard duck	Difenconazole	Short-term (5 day)	<b>LC<sub>50</sub> &gt;349 mg/kg bw/d</b> LC <sub>50</sub> >5000 mg/kg feed	EFSA Conclusion Report 2011; 9(1):1967

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Difenconazole	Long-term	NOEL = <b>9.71 mg/kg bw/d</b> NOEL = 100 mg/kg feed	EFSA Conclusion Report 2011; 9(1):1967
Japanese quail	CGA 131013	Acute	No data	EFSA Conclusion Report 2011; 9(1):1967
Mallard duck	CGA 131013	Short-term (5 day)	LC <sub>50</sub> > <b>1342 mg/kg bw/d</b> LC <sub>50</sub> >5000 mg/kg feed	EFSA Conclusion Report 2011; 9(1):1967
Bobwhite quail	CGA 131013	Long-term	No data	EFSA Conclusion Report 2011; 9(1):1967

**Bold** indicates endpoints used in risk assessment.

CGA 131013 was considered to be the only major metabolite. A total conversion of Difenconazole to CGA 131013 was assumed in the risk assessment.

#### **zRMS comments:**

zRMS accept the toxicity endpoints for difenconazole according to EFSA Conclusion Report 2011; 9(1):1967. The toxicity data for acute and long-term risk were agreed at the EU level. For acute risk assessment, the short-term dietary LDD<sub>50</sub> = 349 mg/kg bw/d was taken into account. In addition the metabolite, CGA131013, is formed in plants. Applicant provided a risk assessment for the metabolite - CGA131013. However, according to the RMS, it is not necessary. The risk assessment for the active substance difenconazole will cover the risk for the metabolite - CGA131013.

#### **Justification:**

The short-term avian LC<sub>50</sub> for CGA131013 is > 1342 mg/kg b.w./day, and therefore clearly has a lower dietary toxicity to birds compared to the parent compound difenconazole, for which a 5-day LC<sub>50</sub> value of >349 mg/kg bw/day has been determined. Since the acute and long-term risk assessments below demonstrate acceptable risk to the parent compound, the ecological risks from potential exposure to this metabolite are expected to be negligible, thus CGA131013 will not be considered further in the risk assessment, which is in line with the residue definition for difenconazole. Negligible exposure to this metabolite is expected when applied by foliar spray.

### **9.2.1.1 Justification for new endpoints**

Not relevant.

### **9.2.2 Risk assessment for spray applications**

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

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

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



screening/ first-tier assessment. The inputs for the calculation are described below:

Metabolite appl. rate = maximum transformation \* (metabolite molecular weight / parent molecular weight)

- Maximum transformation = parent application rate \* formation fraction
- Formation fraction = 1 (worst case assumption)
- CGA 131013 molecular weight = 69 g/mol
- Difenoconazole molecular weight = 406 g/mol

To achieve a concise risk assessment, the risk envelope approach is applied. Therefore, for each group listed in Table 9.1-2, the risk assessment only for the worst-case was evaluated, as it also covers the risk to birds from all other intended uses within the group. Table 9.2.2-1 summarises how the proposed uses were grouped.

**Table 9.2.2-1: Crop groups assessed at screening step**

Crop group		Uses covered		Nr of applications per season	Application rate (kg a.s / ha)
Orchards and ornamentals	Orchards	2	Apples, Pears	3	0.056
	<b>Risk envelope</b>			<b>3</b>	<b>0.056</b>
Bulbs and onions like crops, cereals, fruiting vegetables, leafy vegetables, potatoes etc	Oilseed rape	1	Oilseed rape	2	0.125
	Root and Stem Vegetables	3	Carrot	3	0.125
	Leafy Vegetables	4	Cauliflower	3	0.125
		5	Broccoli	3	0.125
		6	Cabbage	3	0.125
	<b>Risk envelope</b>			<b>3</b>	<b>0.125</b>

**zRMS comments:**

Agreed.

**Screening assessment**

The results of the acute and reproductive first-tier risk assessments are summarised in the following tables. Acute toxicity of LC<sub>50</sub> >349 mg/kg bw/d was used as the endpoint in the risk assessment since it is the most conservative scenario. Since no long-term data is available for the metabolite, metabolite toxicity was considered to be 10 times greater than the active substance as a very conservative approach.

**Table 9.2-2: Screening step of the acute risk for birds due to the use of IN005B1570**

Active substance / product		Difenoconazole/ IN005B1570				
Acute toxicity - LD <sub>50</sub> (mg/kg bw)						
Difenoconazole		> 349				
CGA 131013		> 1342				
TER Criterion		10				
Interval between application		7				
Crop group	Uses covered	Appl. rate (kg a.s / ha)*	SV <sub>90</sub>	MAF <sub>90</sub>	DDD (mg a.s / kg bw)	TER

Active substance: Difenconazole						
Orchards and ornamentals	2	0.056	46.8	1.6	4.21	82.86
Bulbs and onion like crops etc	1, 3-6	0.125	158.8	1.6	31.76	10.99
Metabolite: CGA 131013 *						
Orchards and ornamentals	2	0.010	46.8	1.6	0.72	1874.74
Bulbs and onion like crops etc	1, 3-6	0.021	158.8	1.6	5.40	249.92

\* Formation fraction = 1 (worst case assumption)

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:**

Agreed. The acute risk assessment for difenconazole for screening step have been accepted by zRMS.

**Table 9.2.2-3: Screening step of the long-term/reproductive risk for birds due to the use of IN005B1570**

Active substance / product		Difenconazole/ IN005B1570					
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)							
Difenconazole		9.71					
CGA 131013 **		0.971					
TER Criterion		5					
Interval between application		7					
Crop group	Uses covered	Appl. rate (kg a.s / ha)*	SV <sub>m</sub>	MAF <sub>m</sub>	TWA	DDD (mg a.s / kg bw)	TER
Active substance: Difenconazole							
Orchards and ornamentals	2	0.056	18.2	2	0.53	1.09	8.95
Bulbs and onion like crops etc	1, 3-6	0.125	64.8	2	0.53	8.59	<b>1.13</b>
Metabolite: CGA 131013 *							
Orchards and ornamentals	2	0.010	18.2	2	0.53	0.18	5.26
Bulbs and onion like crops etc	1, 3-6	0.021	64.8	2	0.53	1.46	<b>0.67</b>

\* Formation fraction = 1 (worst case assumption)

\*\* No long-term data available. Metabolite toxicity considered to be 10 times bigger than the a.s. (worst case)

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.

Given that the calculated acute TERs for all intended uses is above the trigger of 10, acceptable acute risks to birds have been demonstrated for all uses. Therefore, the acute risk to birds' exposure to both the active substance and metabolite is considered sufficiently addressed. For the reproductive toxicity, the TER criterion is surpassed for the proposed uses of IN005B1570 for bulbs and onion like crops etc group for both difenconazole and the metabolite. Therefore, a first-tier reproductive risk assessment for the active substance and metabolite is presented below.

**Tier 1 assessment for uses 1, 3-6**

Tier 1 risk assessment is conducted for those intended uses for which the calculated reproductive TER values were below the trigger of 5 in the screening assessment. The Tier 1 assessment results are summarised in Table 9.2.2-4 and 9.2.2-5.

**zRMS comments:**

Agreed. The long-term risk assessment for difenconazole for screening step have been accepted by zRMS.

**Table 9.2.2-4: First-tier assessment of the reproductive risk for birds for the active substance Difenconazole due to the use of IN005B1570**

Active substance / product		Difenconazole										
Reproductive toxicity - LD50 (mg/kg bw)		9.71										
TER Criterion		5										
Crop group		Uses covered		Growth stage	Nr of appl. per season	Appl. rate (kg a.s / ha)	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub>	TWA	DDD (mg a.s / kg bw)	TER
Bulbs and onions like crops, cereals, fruiting vegetables, leafy vegetables, etc	Oilseed rape	1	Oilseed rape	BBCH 14-18	2	0.125	Large herbivorous bird "goose"	15.9	1.4	0.53	1.47	6.58
							Small omnivorous bird "lark"	10.9	1.4	0.53	1.01	9.60
							medium herbivorous/ granivorous bird "pigeon"	22.7	1.4	0.53	2.11	4.61
							Small insectivorous bird "wagtail"	5.9	1.4	0.53	0.55	17.74
				BBCH 30-69	2	0.125	Small insectivorous bird "dunnock"	2.7	1.4	0.53	0.25	38.77
							Small omnivorous bird "lark"	3.3	1.4	0.53	0.31	31.72
							medium herbivorous/ granivorous bird "pigeon"	1.1	1.4	0.53	0.10	95.17
	Root and Stem Vegetables	3	Carrot	From BBCH 39-40	3	0.125	Small granivorous bird "finch"	3.4	1.5	0.53	0.34	28.74
							Small omnivorous bird "lark"	3.3	1.5	0.53	0.33	29.61
							Small insectivorous bird "wagtail"	9.7	1.5	0.53	0.96	10.07
	Leafy Vegetables	4 5 60	Cauliflower Broccoli Cabbage	from BBCH 19	3	0.125	Small granivorous bird "finch"	12.6	2	0.53	1.67	5.82
							Small omnivorous bird "lark"	10.9	2	0.53	1.44	6.72
							Small insectivorous bird "wagtail"	9.7	2	0.53	1.29	7.55

**Table 9.2.2-5: First tier assessment of the reproductive risk for birds for the metabolite CGA131013 due to the use of IN005B1570**

Active substance / product				CGA 131013								
Reproductive toxicity – LD <sub>50</sub> (mg/kg bw)				0.971								
TER Criterion				5								
Crop group		Uses covered		Growth stage	Nr of appl. per season	Appl. rate (kg a.s / ha)	Generic focal +	SV <sub>m</sub>	MAF <sub>m</sub>	TWA	DDD (mg a.s / kg bw)	TER
Bulbs and onions like crops, cereals, fruiting vegetables, leafy vegetables, etc	Oilseed rape	1	Oilseed rape	BBCH 14-18	2	0.021	Large herbivorous bird "goose"	15.9	1.4	0.53	0.25	3.87
							Small omnivorous bird "lark"	10.9	1.4	0.53	0.17	5.65
							medium herbivorous/ granivorous bird "pigeon"	22.7	1.4	0.53	0.36	2.71
							Small insectivorous bird "wagtail"	5.9	1.4	0.53	0.09	10.44
				BBCH 30-69	2	0.021	Small insectivorous bird "duncock"	2.7	1.4	0.53	0.04	22.81
							Small omnivorous bird "lark"	3.3	1.4	0.53	0.05	18.67
	Root and Stem Vegetables	3	Carrot	From BBCH 39-40	3	0.021	medium herbivorous/ granivorous bird "pigeon"	1.1	1.4	0.53	0.02	56.00
							Small granivorous bird "finch"	3.4	1.5	0.53	0.06	16.91
							Small omnivorous bird "lark"	3.3	1.5	0.53	0.06	17.42
	Leafy Vegetables	4 4 6	Cauliflower Broccoli Cabbage	from BBCH 19	3	0.021	Small insectivorous bird "wagtail"	9.7	1.5	0.53	0.16	5.93
							Small granivorous bird "finch"	12.6	2	0.53	0.28	3.42
							Small omnivorous bird "lark"	10.9	2	0.53	0.25	3.96
						Small insectivorous bird "wagtail"	9.7	2	0.53	0.22	4.45	

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.

Acceptable reproductive risks to birds have been demonstrated for the active substance for uses 3 – Carrot, and 4, 5, and 6 – Leafy vegetables. The risk is not fully resolved for the use 1 – Oilseed rape and further consideration of the risk to the medium herbivorous/ granivorous bird “pigeon” generic focal species is provided at the higher tier risk assessment.

~~For the metabolite CGA 131013, acceptable reproductive risks to birds have been demonstrated for the use 3 – Carrot. The risk is not fully resolved for the uses 1 – Oilseed rape, and 8, 9, and 10 – Leafy vegetables and further consideration for the generic focal species is provided at the higher tier risk assessment.~~

**zRMS comments:**

Agreed. The long-term risk assessment for difenconazole for 1-tier step have been accepted by zRMS. The  $TER_{lt}$  value for medium herbivorous/granivorous bird "pigeon" is below the trigger value of 5 and should be refinement.

### 9.2.2.2 Higher-tier risk assessment

The first-tier risk assessment after the application of IN005B1570 indicates a long-term risk of the active substance and the metabolite CGA 131013 for some generic species. Thus, a refined risk assessment for long-term exposure of birds must be performed. A refinement of the reproductive dietary risk assessment was considered. For this refinement, the calculation below is considered.

Toxicity Exposure Ratio (TER) = Toxicity endpoint/ Daily Dietary Dose (DDD)

DDD = (FIR/bw) x PT x PD x RUD x DF x Application rate x MAF x TWA

Where:

- FIR/bw= Food intake rate/body weight
- PT = Fraction of diet obtained in the treated area (related to foraging time)
- PD = Composition of diet obtained from the treated area
- RUD = Residue per unit dose
- DF = Deposition factor
- MAF = Multiple application factor if more than one application
- TWA = Time weighted average factor (only for reproductive risk assessment)

For the intended use 1 – Oilseed rape, the calculated MAF used in the previous risk assessment tiers was very conservative. For this use, the number of applications is 2 with an application interval of 21 days, but the MAF value calculated for 14 days was used. A more realistic approach is to use the MAF<sub>m</sub> value for the 21-day application interval calculated using the formula in Appendix H of EFSA/2009/1438, as described below.

$$MAF_m = (1 - e^{-nki}) / (1 - e^{-ki})$$

Whith:

$k = \ln(2)/DT_{50}$  (rate constant)

$n$  = number of applications

$i$  = application interval (d)

Considering a default DT<sub>50</sub> of 10 days on foliage, the calculated MAF<sub>m</sub> for oilseed rape is 1.23, that is for 2 applications with 21-day application interval.

The results of the higher tier risk assessment are presented in Tables 9.2.2-6 and 9.2.2-7.

**Table 9.2.2-6. Higher Tier assessment of the reproductive risk for birds for difenoconazole due the use of IN005B1570 in all crop uses**

Active substance / product				Difenoconazole										
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)				9.71										
TER Criterion				5										
Use(s)	Crop	Growth stage	Generic focal species	Nr. of appl. per season	Rate (kg a.s./ha)	FIR/bw	PT	PD	RUDm	DF*	MFA <sub>m</sub>	TWA	DDD (mg a.s./ kg bw/d)	TER
1	Oilseed rape	BBCH 14-18	Medium herbivorous / granivorous bird "pigeon"	2	0.125	0.79	1.0	1 100 % crop shoots	28.7	0.6	1.23	0.53	0.88	8.74

\* FOCUS, 2014 (ground water assessment)

**Table 9.2.2-6. Higher Tier assessment of the reproductive risk for birds for the metabolite CGA 131013 due the use of IN005B1570 in all crop uses**

Active substance / product				CGA 131013										
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)				0.971										
TER Criterion				5										
Use(s)	Crop	Growth stage	Generic focal species	Nr. of appl. per season	Rate (kg a.s./ha)	FIR/bw	PT	PD	RUDm	DF*	MFA <sub>m</sub>	TWA	DDD (mg a.s./ kg bw/d)	TER
1	Oilseed rape	BBCH 14-18	Large herbivorous bird "goose"	2	0.021	0.55	1.0	1.0 100 % crop shoots	28.7	0.6	1.23	0.53	0.07	7.38
			Medium herbivorous / granivorous bird "pigeon"			0.79	1.0	1.0 100 % crop shoots	28.7	0.6	1.23	0.53	0.15	5.14
4, 5, 6	Leafy Vegetables	from BBCH 19	Small granivorous bird "finch"	3	0.021	0.31	1.0	1.0 100 % seeds	40.2	0.6	2	0.53	0.05	5.77

Active substance / product				CGA 131013										
Reproductive toxicity – LD <sub>50</sub> (mg/kg bw)				0.971										
TER Criterion				5										
Use(s)	Crop	Growth stage	Generic focal species	Nr. of appl. per season	Rate (kg a.s./ha)	FIR/bw	PT	PD	RUDm	DF*	MFA <sub>m</sub>	TWA	DDD (mg a.s./kg bw/d)	TER
			Small omnivorous bird "lark"			0.52	1.0	1.0 25 % crop leaves 25 % weed seeds 50 % ground arthropods	21	0.6	2	0.53	0.08	6.58
			Small insectivorous bird "wagtail"			0.79	1.0	1.0 50 % ground arthropods 50 % foliar arthropods	12.3	0.6	2	0.53	0.10	7.40

FIR/bw: Food intake rate/body weight; PT: fraction of diet obtained in treated area; PD = Composition of diet obtained from the treated are; RUD = residue per unit dose; DF = Deposition factor; 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.



In the higher tier assessment of the reproductive risk for birds for difenoconazole and the metabolite CGA-131013 due the use of IN005B1570 in all crop uses, the resulting TER is above the trigger value of 5. Therefore, acceptable risks to the outstanding generic focal species are demonstrated for all intended uses.

#### zRMS comments:

zRMS considers unacceptable the use of a DP value of 0.6 in oilseed rape. According to EFSA (2009) estimation of residues on undergrowth vegetation using FOCUS interception factors becomes increasingly uncertain with decreasing soil cover of the crop and increasing height of weeds. Thus, reliable predictions are only deemed possible where the largest part of the soil surface is actually covered by the crop and the undergrowth vegetation is clearly smaller than the crop (such as plants orchards, vineyards or crop plants in the late growth stage).

#### Higher tier assessment was performed by zRMS

Based on Prosser, 2010 for winter oilseed rape at autumn (BBCH 14-18, September - November) and spring OSR (BBCH 14-18, March-May) the relevant PT values were taken into consideration. The PT = 0.29 and PT = 0.84 (90<sup>th</sup> percentile, consumers only) were used for winter OSR and spring OSR, respectively.

#### Winter OSR

Active substance / product			Difenoconazole									
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)			9.71									
TER Criterion			5									
Use(s)	Crop Growth stage	Generic focal species	Nr. of appl. per season	Rate (kg a.s./ha)	FIR/bw	PT	PD	RUDm	MFA <sub>m</sub>	TWA	DDD (mg a.s./ kg bw/d)	TER
1	Oilseed rape BBCH 14-18	Medium herbivorous / granivorous bird "pigeon"	2	0.125	0.79	0.29	1 100 % crop shoots	28.7	1.23	0.53	0.54	17.98

#### Spring OSR

Active substance / product			Difenoconazole									
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)			9.71									
TER Criterion			5									
Use(s)	Crop Growth stage	Generic focal species	Nr. of appl. per season	Rate (kg a.s./ha)	FIR/bw	PT	PD	RUDm	MFA <sub>m</sub>	TWA	DDD (mg a.s./ kg bw/d)	TER
1	Oilseed rape BBCH 14-18	Medium herbivorous / granivorous bird "pigeon"	2	0.125	0.79	0.84	1 100 % crop shoots	28.7	1.23	0.53	1.55	6.26

The application rate of 2 x 125 g a.s./ha confirm the safe use of IN005B1570 in winter and spring oilseed rape.

In the higher tier assessment of the reproductive risk for birds for difenoconazole due the use of IN005B1570 in all crop uses, the resulting TER is above the trigger value of 5. Therefore, acceptable risks to the outstanding generic focal species are demonstrated for all intended uses.

#### Updated April 2024

**According CZ comments:** For the completeness of the risk assessment, the following scenarios should be mentioned in first tier of reproductive risk for birds in leafy vegetables: BBCH 10-19 medium herbivorous/granivorous bird, BBCH 10-19 small insectivorous bird. From the risk assessment of medium herbivorous bird, the unacceptable risk can be concluded, therefore the higher tier is needed.

Intended use	Leafy crops				
Active substance/product	Difenoconazole 3 x 125 g s.a./ha				
Application rate (kg/ha)					
Reprod. toxicity (mg/kg bw/d)	9.71				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>
Leafy crops, BBCH 10-19	Medium herbivorous /granivorous bird “pigeon”	37.0	2 x 0.53	4.9	1.98
Leafy crops, BBCH 10-19	Small insectivorous birds “wagtail”	11.3	2 x 0.53	1.5	6.47

The refined risk assessment for medium herbivorous/granivorous bird in leafy vegetables scenario should be resolved. The focal species in the OSR and leafy vegetables scenario should be supported by scientific literature or a monitoring study. The Applicant not provided the data for further refinement. Therefore, in this case, as the application is intended only in BBCH from 19, it can reasonably be assumed that the risk assessment for medium herbivorous//granivorous birds is also accepted in leafy crops for BBCH from 20.

**The risk assessment is acceptable for birds in leafy crops in BBCH from 20.**

**According CZ comments:** For the completeness of the risk assessment, the following scenarios should be mentioned in first tier of reproductive risk for birds in oilseed rape: BBCH ≥ 40 small omnivorous bird and medium herbivorous bird.

Intended use	Oilseed rape				
Active substance/product	Difenoconazole 2 x 125 g s.a./ha				
Application rate (kg/ha)					
Reprod. toxicity (mg/kg bw/d)	9.71				
TER criterion	5				
Crop scenario Growth stage	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>
Oilseed rape, BBCH ≥40	Small omnivorous bird “lark”	2.7	1.4 x 0.53	0.25	38.84
Oilseed rape, BBCH ≥40	Medium herbivorous/granivorous bird “pigeon”	0.9	1.4 x 0.53	0.08	121.375

**The risk assessment is acceptable for birds in OSR.**

**According CZ comments:** For the completeness of the risk assessment, the following scenarios should be mentioned in first tier of reproductive risk for birds in root and stem vegetables: BBCH 10-39 small granivorous bird and BBCH 10-39 small omnivorous bird.

Intended use		Root and stem vegetables				
Active substance/product		Difenoconazole 3 x 125 g s.a./ha				
Application rate (kg/ha)						
Reprod. toxicity (mg/kg bw/d)		9.71				
TER criterion						
		5				
Crop scenario Growth stage	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>	
Root and stem vegetables BBCH 10-39	Small granivorous bird “finch”	11.4	1.5 x 0.53	1.13	8.59	
Root and stem vegetables 10-39	Small omnivorous bird “lark”	10.9	1.5 x 0.53	1.08	8.99	

**The risk assessment is acceptable for birds in carrots (root and stem vegetables).**

### 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

The ‘Leaf scenario’ is relevant for birds taking water that is collected in leaf whorls after application and applies to leafy vegetables forming heads or with a morphology that facilitates collection of rain/irrigation water sufficiently to attract birds, i.e. for the before named crops at BBCH  $\geq 41$ .

Since none of the proposed uses falls into these categories, the leaf scenario does not apply to the use of “INDOFIL Difenconazole 250 EC”.

#### zRMS comments:

Agreed

### Puddle scenario

The ‘Puddle scenario’ is relevant for birds taking water from puddles formed on the soil surface of a field when a (heavy) rainfall event follows the application of a pesticide to a crop or bare soil. This is relevant for all uses of “INDOFIL Difenconazole 250 EC” and should therefore be assessed.

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 ( $K_{oc} < 500$  L/kg) or 3000 in the case of more sorptive substances ( $K_{oc} \geq 500$  L/kg).

For Difenconazole, the ratio of highest application rate (400 g a.s./ha) to lowest relevant endpoint (NO-AEL = 9.71 mg a.s./kg bw/d) is 41.19. As the  $K_{f,oc}$  for Difenconazole is 3759.4 mL/g (EFSA Conclusion Report 2011; 9(1):1967), it is a more sorptive substance and, therefore, the risk can be considered acceptable without the need for further calculations.

**zRMS comments:**

Agreed.

#### 9.2.2.4 Effects of secondary poisoning

According to EFSA/2009/1438, substances with a  $\log P_{ow} \geq 3$  have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains. The  $\log P_{ow}$  of Difenoconazole amounts to 4.4 and that of the metabolite CGA 205375 amounts to 3.8, therefore both exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

#### Risk assessment for earthworm-eating birds via secondary poisoning

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, since the crop interception is considered, the assessment for the use group leafy vegetables also covers the risk for birds from all other intended uses in groups oilseed rape, orchards, root and stem vegetables.

**Table 9.2.2-6: Assessment of the risk for earthworm-eating birds due to exposure to Difenoconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in leafy vegetables**

Parameter	Difenoconazole	CGA 205375*	comments
PEC <sub>soil</sub> (twa = 21 d) (mg/kg soil)	0.358	0.044	Leafy vegetables is the worst-case PEC <sub>soil</sub> calculated for multiple application in all crops
$\log P_{ow}$	$4.36 \pm 0.02$ at 25°C, pH 8	3.8	
Pow	22908.68	6309.57	
Koc	2943	2661	geomean values used according to EFSA guidance
foc	0.02	0.02	Default
BCF <sub>worm</sub>	4.68	1.44	$BCF_{worm/soil} = (0.84 + 0.012 \times P_{ow}) / (foc \times Koc)$
PEC <sub>worm</sub>	1.68	0.06	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	1.76	0.07	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	9.71	0.971	
TER <sub>lt</sub>	5.51	14.61	>5, no further refinement required

\* No toxicity data available for metabolites. Therefore, assume 10 times more toxicity as a conservative approach. PEC<sub>soil</sub> from dRR Part B8, table 8.7-7 (multiple applications). TER values shown in bold fall below the relevant trigger.

Acceptable risk for earthworm-eating birds via secondary poisoning for difenoconazole and the metabolite CGA 205375 due the use of IN005B1570 is demonstrated, since the resulting TER is above the trigger value of 5. Therefore, the risk can be considered acceptable without the need for further calculations.

#### zRMS comments:

Agreed.

### 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, based on the regulatory acceptable concentration for aquatic organisms as a limit value for admissible concentrations of Difenconazole in water.

BCF is not available for the metabolite CGA 205375 in the EFSA conclusion (EFSA Journal 2011;9(1):1967). However, the metabolite has the same molecular structure as the parent compound (difenconazole) with an alcohol group. Since the log Kow from the metabolite is lower than the log Kow reported for the active substance, the BCF will be lower and can be considered within the bioaccumulation assessment of the active substance.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case Step 1 PEC<sub>sw</sub> calculated for multiple applications in leafy vegetables was used, since it also covers the risk for birds from all other intended uses in groups oilseed rape, orchards, root and stem vegetables.

**Table 9.2.2-7: Assessment of the risk for fish-eating birds due to exposure to Difenconazole via bioaccumulation in fish (secondary poisoning) for the intended use in leafy vegetables**

Parameter	Difenconazole	Comments
PEC <sub>sw</sub> - step 1 (mg/L)	0.029	Leafy vegetables is the worst-case Step 1 PEC <sub>sw</sub> calculated for multiple application in all crops
BCF <sub>fish</sub>	330	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	9.51	PEC <sub>fish</sub> = PEC <sub>water</sub> × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	1.51	DDD = PEC <sub>fish</sub> × 0.159
NOEL (mg/kg bw/d)	9.71	
TER <sub>lt</sub>	6.42	>5, no further refinement required

PEC<sub>soil</sub> from dRR Part B8, table 8.7-7 (multiple applications). TER values shown in bold fall below the relevant trigger.

Acceptable risk for fish-eating birds via secondary poisoning for difenconazole and the metabolite due the use of IN005B1570 is demonstrated since the resulting TER is above the trigger value of 5. Therefore, the risk can be considered acceptable without the need for further calculations.

#### zRMS comments:

Agreed

#### 9.2.2.5 Biomagnification in terrestrial food chains

Not relevant.

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

Not relevant.

#### 9.2.4 Overall conclusions

The risk assessment for birds was carried out according to EFSA/2009/1438.

The screening  $TER_a$  values for difenoconazole and the metabolite CGA 205375 for all proposed uses of IN005B1570 are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to birds is acceptable following use according to the proposed use pattern for these crops.

The  $TER_{lt}$  values for difenoconazole and the metabolite CGA 205375 for uses of IN005B1570 in the crop group Orchards are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to birds is acceptable following use according to the proposed use patterns for this crop and application scenario.

For all other uses a Tier 1 risk assessment is required. For almost all crop scenarios, the Tier 1  $TER_{lt}$  values are greater than the trigger of 5, indicating that long-term risk to birds is acceptable following use according to the proposed use patterns for these crops.

For the active substance difenoconazole, the  $TER_{lt}$  value for use of IN005B1570 in oilseed rape in the scenario for medium herbivorous / granivorous bird "pigeon" is below the trigger of 5, indicating need for further refinement. For the metabolite, the  $TER_{lt}$  value for use of IN005B1570 in oilseed rape in the scenario for large herbivorous bird "goose" and medium herbivorous / granivorous bird "pigeon", and in Leafy vegetables in the scenario for the small granivorous bird "finch", small omnivorous bird "lark", and small insectivorous bird "wagtail" are below the trigger of 5, indicating need for further refinement. In addition the metabolite, CGA131013, is formed in plants. Applicant provided a risk assessment for the metabolite - CGA131013. However, according to the RMS, it is not necessary. The risk assessment for the active substance difenoconazole will cover the risk for the metabolite - CGA131013. Based on refined assessment step of refined  $MAF_m$  and reproductive dietary risk assessment, the calculated TER values for the long-term risk resulting from the difenoconazole and metabolite exposure in all the evaluated scenarios achieve the acceptability criteria  $TER \geq 5$  for long-term effects, indicating that long-term risk to birds is acceptable following use according to the proposed use patterns for all intended crops.

Acceptable risk for birds due to drinking water exposure was demonstrated. In the risk assessment for earthworm- eating birds and fish-eating birds via secondary poisoning, the TER values are greater than the trigger of 5, indicating acceptable risk for birds and indicating therefore no need for further refinement.

The risk assessment for birds should be considered at MSs levels.



### 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 Difenconazole and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in Section 6 (Mammalian Toxicology) of this report.

Effects on mammals due to the use of IN005B1570 were not evaluated as part of the EU assessment of Difenconazole. However, the provision of further data on the formulation IN005B1570 is not considered essential.

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	Difenconazole	Oral Acute	LD <sub>50</sub> > 1453 mg/kg bw	EFSA Scientific Report (2011;9(1):1967)
Rat	Difenconazole	Long term	NOAEL = 17.3 mg/kg bw/d	EFSA Scientific Report (2011;9(1):1967)
Rat	Metabolite CGA 131013	Oral Acute	LD <sub>50</sub> = >5000 mg/kg bw	EFSA Scientific Report (2011;9(1):1967)
Rat	Metabolite CGA 131013	Long Term	LD <sub>50</sub> = 100 mg/kg bw	EFSA Scientific Report (2011;9(1):1967)

CGA 131013 was considered to be the only major metabolite. A total conversion of Difenconazole to CGA 131013 was assumed in the risk assessment.

#### **zRMS comments:**

zRMS accept the toxicity endpoints for difenconazole for mammals according to EFSA Conclusion Report 2011; 9(1):1967. In addition the metabolite, CGA131013, is formed in plants. However, according to the RMS, it is not necessary. The risk assessment for the active substance difenconazole will cover the risk for the metabolite - CGA131013. Studies in mammals have shown low toxicity from CGA131013, with acute LD<sub>50</sub> values in both rats and mice of >5 000 mg a.s./kg food and a lowest NOAEL of 100 mg a.s./kg bw/day in reproductive toxicity studies in rats. Toxicity of the metabolite is significantly lower than for the parent compound difenconazole which has an acute LD<sub>50</sub> of 1 453 mg a.s./kg bw/day and long-term NOAEL of 17.3 mg a.s./kg bw/day in rat.

#### 9.3.1.1 Justification for new endpoints

Not relevant.

#### 9.3.2 Risk assessment for spray applications

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

To achieve a concise risk assessment, the risk envelope approach is applied. Therefore, for each group listed in Table 9.1-2, the risk assessment only for the worst-case was evaluated, as it also covers the risk to mammals from all other intended uses within the group. Table 9.3-2 summarises how the proposed uses were grouped.

**Table 9.3-2: Crop groups assessed at screening step**

Crop group		Uses covered		Nr of appl. per season	Appl. rate (kg a.s / ha)
Cotton, leafy vegetables, orchards and ornamentals etc	Orchards	2	Apples, Pears	3	0.056
	Leafy Vegetables	4	Cauliflower	3	0.125
		5	Broccoli	3	0.125
		6	Cabbage	3	0.125
	Risk envelope			3	0.125
Bulbs and onion like crops, cereals, oilseed rape, root and stem vegetables etc	Oilseed rape	1	Oilseed rape	2	0.125
	Root and Stem Vegetables	3	Carrot	3	0.125
	Risk envelope			3	0.125

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

For the screening step risk assessment, it has been assumed that 100% of the parent becomes the metabolite. The application rate for the metabolite CGA 131013 was calculated as the respective maximum occurrence transformation, multiplying by a conversion factor (metabolite molecular weight / parent molecular weight) to correct for molecular weight. This calculation is explained in detail in part 9.2.2.1. This is a worst-case assumption and therefore appropriate as a screening/ first-tier approach.

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

**Table 9.3-3: Screening assessment of the acute risk for mammals due to the use of IN005B1570**

Active substance / product		Difenoconazole/ IN005B1570				
Acute toxicity - LD <sub>50</sub> (mg/kg bw)						
Difenoconazole		1453				
CGA 131013		5000				
TER Criterion		10				
Interval between appl. (worst-case)		7				
Crop group	Uses covered	Appl. rate (kg a.s / ha)*	SV <sub>90</sub>	MAF <sub>90</sub>	DDD (mg a.s / kg bw)	TER
Active substance: Difenoconazole						
Cotton, leafy vegetables, orchards and ornamentals etc	2, 4-6	0.125	136.4	1.6	27.28	53.26

<b>Active substance / product</b>		Difenoconazole/ IN005B1570				
<b>Acute toxicity - LD<sub>50</sub> (mg/kg bw)</b>						
<b>Difenoconazole</b>		1453				
<b>CGA 131013</b>		5000				
<b>TER Criterion</b>		10				
<b>Interval between appl. (worst-case)</b>		7				
Crop group	Uses covered	Appl. rate (kg a.s / ha)*	SV <sub>90</sub>	MAF <sub>90</sub>	DDD (mg a.s / kg bw)	TER
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables etc	1, 3	0.125	118.4	1.6	23.68	61.36
Metabolite: CGA 131013						
Cotton, leafy vegetables, orchards and ornamentals etc	2, 4-6	0.021	136.4	1.6	4.64	1078.46
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables etc	1, 3	0.021	118.4	1.6	4.02	1242.41

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:**

Agreed.

**Table 9.3-4: Screening assessment of the long-term/reproductive risk for mammals due to the use of IN005B1570**

<b>Active substance / product</b>		Difenoconazole/ IN005B1570					
<b>Reproductive toxicity - LD<sub>50</sub> (mg/kg bw)</b>							
<b>Difenoconazole</b>		17.3					
<b>CGA 131013</b>		100					
<b>TER Criterion</b>		5					
<b>Interval between appl. (worst-case)</b>		7					
<b>Crop group</b>	<b>Uses covered</b>	<b>Appl. rate (kg a.s / ha)</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub></b>	<b>TWA</b>	<b>DDD (mg a.s / kg bw)</b>	<b>TER</b>
Active substance: Difenoconazole							
Cotton, leafy vegetables, orchards and ornamentals etc	2, 4-6	0.125	72.3	2.0	0.53	9.58	<b>1.81</b>
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables etc	1, 3	0.125	48.3	2.0	0.53	6.40	<b>2.70</b>
Metabolite: CGA 131013							
Cotton, leafy vegetables, orchards and ornamentals etc	2, 4-6	0.021	72.3	2.0	0.53	1.63	61.42
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables etc	1, 3	0.021	48.3	2.0	0.53	1.09	91.94

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.

At screening assessment, the acute TER for all uses is greater than the trigger value of 10, therefore, no further risk assessment is required for all proposed uses for difenoconazole and its metabolites.

The TER for the reproductive risk of mammals' exposure to the metabolite after application of IN005B1570 in all intended uses is above the trigger value of 5 and, therefore, acceptable. In the evaluation of the reproductive toxicity for the active substance Difenoconazole, the reproductive TER for all uses is below the trigger value of 5. Therefore, a first-tier risk assessment for the active substance is presented below.

**zRMS comments:**

Agreed.

***Tier 1 reproductive assessment for all uses***

Tier 1 risk assessment is conducted for those intended uses for which the calculated reproductive TER values were below the trigger of 5 in the screening assessment. The Tier 1 assessment results are summarised in Table 9.3-5.

**Table 9.3-5: Tier 1 risk assessment of the long-term/reproductive risk for mammals due to the use of IN005B1570**

Active substance / product				Difenoconazole								
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)				17.3								
TER Criterion				5								
Crop group		Uses covered		Growth stage	Nr of appl. per season	Appl. rate (kg a.s / ha)	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub>	TWA	DDD (mg a.s / kg bw)	TER
Cotton, leafy vegetables, orchards and ornamentals etc	Orchards	2	Apples, Pears	BBCH 57-84	3	0.056	small herbivorous mammal "vole"	21.7	2.0	0.53	1.29	13.37
							Frugivorous mammal "dormouse"	22.7	2.0	0.53	1.35	12.78
							Large herbivorous mammal "lagomorph"	4.3	2.0	0.53	0.26	67.48
							Small omnivorous mammal "mouse"	2.3	2.0	0.53	0.14	126.15
	Leafy Vegetables	4 5 6	Cauliflower Broccoli Cabbage	from BBCH 19	3	0.125	Small insectivorous mammal "shrew"	4.2	2.0	0.53	0.56	31.09
							<b>small herbivorous mammal "vole" (BBCH 40-49)</b>	72.3	2.0	0.53	9.58	<b>1.81</b>
							Large herbivorous mammal "lagomorph"	14.3	2.0	0.53	1.89	9.13
							Small omnivorous mammal "mouse"	7.8	2.0	0.53	1.03	16.74
Bulbs and onion like crops, cereals, oilseed rape, root and stem vegetables etc	Oilseed rape	1	Oilseed rape	BBCH 14-18	2	0.125	Large insectivorous mammal "shrew"	4.2	1.4	0.53	0.39	44.41
							Small omnivorous mammal "mouse"	7.8	1.4	0.53	0.72	23.91
				BBCH 30-69	2	0.125	Small insectivorous mammal "shrew"	1.9	1.4	0.53	0.18	98.17
							small herbivorous mammal "vole"	18.1	1.4	0.53	1.68	10.31
							Small omnivorous mammal "mouse"	2.3	1.4	0.53	0.21	81.10

Active substance / product				Difenoconazole								
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)				17.3								
TER Criterion				5								
Crop group		Uses covered		Growth stage	Nr of appl. per season	Appl. rate (kg a.s / ha)	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub>	TWA	DDD (mg a.s / kg bw)	TER
					2	0.125	Large herbivorous mammal "lagomorph" (all season)	14.3	1.4	0.53	1.33	13.04
	Root and Stem Vegetables	3	Carrot	From BBCH 39-40	3	0.125	small herbivorous mammal "vole"	21.7	1.5	0.53	2.16	8.02
							Small omnivorous mammal "mouse"	2.3	1.5	0.53	0.23	75.69



**zRMS comments:**

Agreed.

Acceptable reproductive risks to mammals have been demonstrated for all uses, except for leafy vegetables. Since the risk is not fully resolved for the uses 4, 5 and 6 – Leafy vegetables, further consideration of the risk to the small herbivorous mammal "vole" generic focal species is provided at the higher tier risk assessment.

### 9.3.2.2 Higher-tier risk assessment

The first-tier risk assessment after the application of IN005B1570 indicates a long-term risk of difenconazole for the generic specie Common vole. Thus, a refined risk assessment for long-term exposure of mammals must be performed. A refinement of the reproductive dietary risk assessment was considered. For this refinement, the calculation below is considered.

Toxicity Exposure Ratio (TER) = Toxicity endpoint/ Daily Dietary Dose (DDD)

$$DDD = (FIR/bw) \times PT \times PD \times RUD \times DF \times \text{Application rate} \times MAF \times TWA$$

Where:

- FIR/bw= Food intake rate/body weight
- PT = Fraction of diet obtained in the treated area (related to foraging time)
- PD = Composition of diet obtained from the treated area
- RUD = Residue per unit dose
- DF = Deposition factor
- MAF = Multiple application factor if more than one application
- TWA = Time weighted average factor (only for reproductive risk assessment)

Refinement presented below is based on more realistic deposition/ interception values according to FO-CUS 2014 groundwater.

**Table 9.3-6. Higher Tier assessment of the reproductive risk for mammals for difenoconazole due the use of IN005B1570 in all crop uses**

Active substance / product				Difenoconazole										
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)				17.3										
TER Criterion				5										
Use(s)	Crop	Growth stage	Generic focal species	Nr. of appl. per season	Rate (kg a.s./ha)	FIR/bw	PT	PD	RUD <sub>m</sub>	DF*	MFA <sub>m</sub>	TWA	DDD (mg a.s./ kg bw/d)	TER
4, 5, 6	Leafy vegetables	BBCH > 40	Small herbivorous mammal "vole"	3	0.125	1.33	1.0	1 100 % grass	54.2	0.6* 0.3	1.2 2	0.53	4.71 2.87	4.90 6.03

FIR/bw: Food intake rate/body weight; PT: fraction of diet obtained in treated area; PD = Composition of diet obtained from the treated are; RUD = residue per unit dose; DF = Deposition factor; 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.

\* FOCUS, 2014 (ground water assessment)

\*zRMS corrected this value. However, according to EFSA Guidance Document to obtain DegT50 values (EFSA Journal 2014;12(5):3662), the interception for leafy vegetables BBCH 40-49 would be 70%, which would mean that 30% of the total applied product would end up in the grass. The corrected refined risk assessment was provided in the box.

Based on the higher risk assessment step, TER value for the long-term risk resulting from an exposure of small herbivorous mammal 'vole' to difenconazole is 4.90, that is slightly above the trigger of 5.

It is important to take into account that in leafy vegetable crops, common vole is only considered as a generic focal species from the crop growth stage BBCH 40, with worst case between BBCH 40-49. For the use of IN005B1570 for leafy vegetables, the scenario method is the application period from BBCH 19, when the common vole is not relevant yet.

Furthermore, according to Jacob *et al.* (2014<sup>1</sup>), the common vole is primarily a grassland species that is well adapted to steppe habitats. Primary habitats are meadows, set-aside land, flower strips, grassy field verges, alfalfa and clover fields. They prefer to inhabit undisturbed short vegetation and can be found in grass leys in forests after clear cuts and other grassy habitats. This habitat preference is of advantage for voles as survival of voles is greater in primary habitats where refuges are more abundant than in secondary habitats (agricultural areas). Additionally, it must also be considered that secondary habitats cannot maintain common vole populations for long periods given the seasonal nature of farming. Populations in secondary / agricultural habitats will be regularly disrupted by harvest and tilling.

Realistically, when considering benefits and damage caused by the common vole during periodic outbreaks, the associated crop losses and management cost suggest that this species is the most serious vertebrate pest in European agriculture.

The species' status as a crop pest, its high fecundity, resilience to disturbance and intermittent colonisation of crop habitats are important characteristics that should be reflected in risk assessment. Based on the information provided in the scientific literature, it seems justified to modify elements of the current risk assessment scheme for plant protection products including the use of realistic food intake rates, reduced assessment trigger values (already applied in certain EU member states e.g. Germany), or to consider the use of alternate focal rodent species in European risk assessment.

EFSA/2009/1438 indicates that a 25 g vole must consume 1.33 times its own bodyweight (the default food intake rate / bodyweight (FIR/bw)) to satisfy the theoretical daily energy expenditure (DEE). However, in laboratory studies, common voles have been found to only consume about a third of their body weight per day and values as low as 10% based on the uptake of dry matter have been reported. As shown in laboratory studies, even at low temperatures when food uptake is highest, an amount of food equivalent to about 50% of the body weight is eaten, although this was not verified under field conditions.

The common vole is a model species that exists in cropped areas and, given body weight and food intake rates, does represent a worst-case exposure model. It seems therefore reasonable to consider an adjustment in the trigger values to account for reduced uncertainty, associated with the evaluation of derived TER values from acute and reproduction dietary risk assessments (e.g. Germany already accepts trigger values of  $\geq 5$  in the acute and  $\geq 2$  in the long-term risk assessment)<sup>2</sup>.

The use of alternate focal species that exist alongside the common vole in the field such as the wood mouse or small mammals within the same feeding guild (e.g. field vole) is a pragmatic approach to risk assessment where common voles are not widely distributed (e.g. proposed for the Northern zone<sup>3</sup>). However, this position, although pragmatic, cannot be consistently applied across member states as often sufficient field data is not available. In these cases the pragmatic use of the wood mouse as the representative small mammal species is considered a viable alternate species, considered protective of small mammals in agricultural landscapes.

<sup>1</sup> Jacob, J., Manson, P., Barfknecht, R. Fredricks, T. (2014): Common voles (*Microtus arvalis*) ecology and management: implications for risk assessment of plant protection products. Pest Manag. Sci. 70(6): 869-878.

<sup>2</sup> Adoption of the EFSA-Guidance Document on Risk Assessment for Birds and Mammals. BVL notification no. 10/02/14 of 1 June 2010, German Federal Gazette ('Bundesanzeiger'), 2228-2229.

<sup>3</sup> Pesticide risk assessment for birds and mammals. Selection of relevant species and development of standard scenarios for higher tier risk assessment in the Northern Zone in accordance with Regulation EC 1107/2009. Version 1.2, May 2015.

Some of these pragmatic modifications to the approaches used in risk assessment are already applied in some EU Member States. Therefore, it is reasonable to consistently apply such pragmatic and realistic approaches in risk assessments for plant protection products across the EU.

When considering the modifications proposed by Jacob *et al.* (2014) for the presented long-term risk assessment for IN005B1570 the assessment results in the following conclusions:

- (i) When considering that a 25 g vole must consume 0.5 times its own bodyweight to satisfy the theoretical daily energy expenditure (DEE), the outcome of the higher tier risk assessment would be more favourable, as presented below:

Table 9.3-7. Refined risk assessment of the reproductive risk for mammals for difenoconazole due the use of IN005B1570

Active substance / product				Difenoconazole							
Reproductive toxicity - LD <sub>50</sub> (mg/kg bw)				17.3							
TER Criterion				5							
Application rate (kg a.s. / ha)				3 * 0.125							
Crop	Growth stage	Generic focal species	FIR/ bw	PT	PD	RUDm	DF*	MFA <sub>m</sub>	TWA	DDD (mg a.s./ kg bw/d)	TER
Leafy vegetables	BBCH > 40 (From BBCH 19)	small herbivorous mammal "vole"	0.5	1.0	1 100 % grass	54.2	0.6	1.2	0.53	1.29	<b>13.38</b>

\* FOCUS, 2014 (ground water assessment)

- (ii) When considering a reduced trigger value of 2 for the long-term risk assessment presented above, acceptable risk could be shown in Tier 2 (see Table 9.3-6).
- (iii) The above presented long-term risk assessment shows that for the other considered small mammalian species, the omnivorous wood mouse, safe uses could be demonstrated with a high margin of safety (see Table 9.3-5).

In conclusion, considering the above-mentioned, acceptable risk can be demonstrated for small herbivorous mammals and it therefore seems highly unlikely that population abundances of small herbivorous mammals such as the vole will be affected on a long-term scale.

**zRMS comments:**

Agreed. The refined risk assessment have been corrected by zRMS. Further refined risk assessment for vole is not necessary.

The TER<sub>lt</sub> for vole is lower than the trigger of 5 for leafy vegetables (BBCH 40-49). The Applicant used DF = 0.6. However, according to EFSA Guidance Document to obtain DegT<sub>50</sub> values (EFSA Journal 2014;12(5):3662), the interception for leafy vegetables BBCH 40-49 would be 70%, which would mean that 30% of the total applied product would end up in the grass.

<b>Active substance / product</b>				Difenoconazole							
<b>Reproductive toxicity - LD<sub>50</sub> (mg/kg bw)</b>				17.3							
<b>TER Criterion</b>				5							
<b>Application rate (kg a.s. / ha)</b>				3 * 0.125							
Crop	Growth stage	Generic focal species	FIR/ bw	PT	PD	RUDm	DF*	MFA <sub>m</sub>	TWA	DDD (mg a.s./ kg bw/d)	TER
Leafy vegetables	BBCH > 40 (From BBCH 19)	small herbivorous mammal "vole"	1.33	1.0	1 100 % grass	54.2	0.3	1.23	0.53	1.76 2.87	9.83 6.03

\* FOCUS, 2014 (ground water assessment)

The relevance of the “vole” for the scenario of small herbivorous mammals in higher tier risk assessment is highly discussed by the different Member States on EU level. Therefore, it is proposed to conclude on the overall relevance of the “vole” scenario for the higher tier risk assessment on Member State level.

**Acceptable reproductive risks to mammals have been demonstrated for all uses.**

**Updated April 2024**

**According NL comments:** zRMS still proposes to use a deposition factor of 0.3 for leafy vegetables. However, according to Appendix E of the EFSA guidance document birds & mammals (2009), the relevant BBCH growth stage for which a DF can be applied in leafy vegetables is BBCH ≥ 50. Hence, for leafy vegetables at BBCH 40-49 interception cannot be used in the risk assessment. Furthermore, NL does not accept a trigger value of 2.

Could you please take these considerations into account in the risk assessment for mammals?

According to the comment by NL DF of 0.3 can be applied in leafy vegetables is BBCH ≥ 50. Hence, for leafy vegetables at BBCH 40-49 interception cannot be used in the risk assessment. Therefore, the refined risk assessment for mammals presented is only sufficient for leafy crops only for BBCH ≥ 50.

However, a long-term risk for small herbivorous mammal "vole", leafy vegetables, application crop directed (BBCH 40-49) was observed. As the application is intended only in BBCH from 19, it can reasonably be assumed that the risk assessment for vole is also accepted in leafy crops for BBCH 19-39.

**Conclusion: Risk assessment for mammals for leafy crops is accepted only for BBCH 19-39 or BBCH ≥ 50.**

**The refinement risk assessment should be accepted by MSs level.**

**According CZ comments:** For the completeness of the risk assessment, the following scenarios

should be mentioned in first tier of reproductive risk for mammals in OSR: BBCH  $\geq 40$  small omnivorous mammals. However, the risk assessment for this species will be acceptable.

Intended use		Oilseed rape				
Active substance/product		Difenoconazole 2 x 125 g s.a./ha				
Application rate (kg/ha)						
Reprod. toxicity (mg/kg bw/d)		17.3				
TER criterion		5				
Crop scenario Growth stage	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>	
BBCH $\geq 40$	Small omnivorous mammal	1.9	1.4 x 0.53	0.18	96.11	

The risk assessment for oilseed rape is accepted.

**According to CZ comments:** For the completeness of the risk assessment, the following scenarios should be mentioned in first tier of reproductive risk for mammals in root and stem vegetables: BBCH  $\geq 20$  small insectivorous mammals and BBCH 10-39 small omnivorous mammals. However, the risk assessment for this species will be acceptable.

Intended use		Root and stem vegetables				
Active substance/product		Difenoconazole 3 x 125 g s.a./ha				
Application rate (kg/ha)						
Reprod. toxicity (mg/kg bw/d)		17.3				
TER criterion		5				
Crop scenario Growth stage	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>it</sub>	
BBCH $\geq 20$	small insectivorous mammals	1.9	1.5 x 0.53	0.19	91.05	
BBCH 10-39	small omnivorous mammals	7.8	1.5 x 0.53	0.78	22.18	

The risk assessment for carrots (root and stem vegetables) is accepted.

**According to CZ comments:** It is necessary to specify the BBCH for the leafy vegetables scenario. If it should cover BBCH from 19 onwards, it is necessary to take into account all scenarios corresponding to this BBCH. It is essential to include focal species for BBCH $\geq 20$  small insectivorous mammals and BBCH  $\geq 50$  small herbivorous and small omnivorous mammals. After consultation with the HBU evaluator at the national level of the Czech Republic, an application window of BBCH 19-39 is recommended for cauliflower, broccoli and cabbage. However, only focal species falling within this application window will be evaluated at the CZ national level.

Intended use		Leafy crops				
Active substance/product		Difenoconazole 3 x 125 g s.a./ha				
Application rate (kg/ha)						
Reprod. toxicity (mg/kg bw/d)		17.3				
TER criterion						
		5				
Crop scenario Growth stage	Generic focal species	SV <sub>m</sub>	MAF <sub>m</sub> × TWA	DDD <sub>m</sub> (mg/kg bw/d)	TER <sub>lt</sub>	
BBCH ≥20	small insectivorous mammal “shrew”	1.9	2 x 0.53	0.25	69.2	
BBCH ≥50	Small herbivorous mammal "vole”	21.7	2 x 0.53	2.88	6.0	
BBCH ≥50	Small omnivorous mammal “mouse”	2.3	2 x 0.53	0.3	57.6	
The risk assessment for leafy crops BBCH≥50 is accepted.						



### 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 ( $K_{oc} < 500$  L/kg) or 3000 in the case of more sorptive substances ( $K_{oc} \geq 500$  L/kg).

For Difenoconazole, the ratio of highest application rate (400 g a.s./ha) to lowest relevant endpoint (NO-AEL = 17.3 mg a.s./kg bw/d) is 23.12. As the  $K_{f,oc}$  for Difenoconazole is 3759.4 mL/g (EFSA Conclusion Report 2011; 9(1):1967), it is a more sorptive substance and, therefore, the risk can be considered acceptable without the need for further calculations.

#### zRMS comments:

Agreed.

### 9.3.2.4 Effects of secondary poisoning

According to EFSA/2009/1438, substances with a  $\log P_{ow} \geq 3$  have potential for bioaccumulation and should be assessed for the risk of biomagnification in aquatic and terrestrial food chains. The  $\log P_{ow}$  of Difenoconazole amounts to 4.4 and that of the metabolite CGA 205375 amounts to 3.8, therefore both exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is required.

#### Risk assessment for earthworm-eating mammals via secondary poisoning

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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, since crop interception is taken into account, the assessment for the use group leafy vegetables also covers the risk for mammals from all other intended uses in groups oilseed rape, orchards, root and stem vegetables.

**Table 9.3-7: Assessment of the risk for earthworm-eating mammals due to exposure to Difenoconazole via bioaccumulation in earthworms (secondary poisoning) for the intended use in leafy vegetables**

Parameter	Difenoconazole	CGA 205375	comments
PEC <sub>soil</sub> (twa = 21 d) (mg/kg soil)	0.358	0.044	Worst-case PEC <sub>soil</sub> calculated for multiple applications in all crops
$\log P_{ow} / P_{ow}$	4.36	3.8	
Pow	22908.68	6309.57	
Koc	2943	2661	geomean values used according to EFSA guidance
foc	0.02	0.02	Default
BCF <sub>worm</sub>	4.68	1.44	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw}) / (0.84 + 0.012 * Pow) / (foc * Koc)$
PEC <sub>worm</sub>	1.68	0.06	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	2.15	0.08	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	17.3	100	
TER <sub>It</sub>	8.06	1234.35	>5, no further refinement required

TER values shown in bold fall below the relevant trigger.

Acceptable risk for earthworm-eating mammals via secondary poisoning for difenoconazole and the metabolite CGA 205375 due to the use of IN005B1570 is demonstrated since the resulting TER is above the trigger value of 5. Therefore, the risk can be considered acceptable without the need for further calculations.

#### zRMS comments:

Agreed.

### Risk assessment for fish-eating mammals via secondary poisoning

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

BCF is not available for the metabolite CGA 205375 in the EFSA conclusion (EFSA Journal 2011;9(1):1967). However, the metabolite has the same molecular structure as the parent compound (difenoconazole) with an alcohol group. Since the log Kow from the metabolite is lower than the log Kow reported for the active substance, the BCF will be lower and can be considered within the bioaccumulation assessment of the active substance.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the worst-case Step 1 PEC<sub>sw</sub> calculated for multiple applications in leafy vegetables was used, since it also covers the risk for mammals from all other intended uses in groups oilseed rape, orchards, root and stem vegetables.

**Table 9.3-2: Assessment of the risk for fish-eating mammals due to exposure to Difenoconazole via bioaccumulation in fish (secondary poisoning) for the intended use in leafy vegetables**

Parameter	Difenoconazole	comments
PEC <sub>sw</sub> (twa = 21 d) (mg/L)	0.02883	Worst-case Step 1 PEC <sub>sw</sub> calculated for multiple application in leafy vegetables
BCF <sub>fish</sub>	330	
BMF	-	biomagnification factor (relevant for BCF ≥ 2000)
PEC <sub>fish</sub>	9.51	PEC <sub>fish</sub> = PEC <sub>water</sub> × BCF <sub>fish</sub>
Daily dietary dose (mg/kg bw/d)	1.35	DDD = PEC <sub>fish</sub> × 0.142
NOEL (mg/kg bw/d)	17.30	
TER <sub>lt</sub>	12.81	>5, no further refinement required

TER values shown in bold fall below the relevant trigger.

Acceptable risk for fish-eating mammals via secondary poisoning for difenoconazole and the metabolite due to the use of IN005B1570 is demonstrated since the resulting TER is above the trigger value of 5. Therefore, the risk can be considered acceptable without the need for further calculations.

#### zRMS comments:

Agreed.

#### **9.3.2.5 Biomagnification in terrestrial food chains**

Not relevant.

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

Not relevant.

#### **9.3.4 Overall conclusions**

The risk assessment for mammals was carried out according to EFSA/2009/1438.

The screening  $TER_a$  values for difenoconazole and the metabolite CGA 131013 for all proposed uses of IN005B1570 are greater than the Commission Regulation (EU) No. 546/2011 trigger of 10, indicating that acute risk to mammals is acceptable following use according to the proposed use pattern for these crops.

The  $TER_{lt}$  values for difenoconazole for uses of IN005B1570 in all crop groups are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that long-term risk to mammals is unacceptable following use according to the proposed use patterns for this crop and application scenario. For difenoconazole, therefore for all uses a Tier 1 risk assessment is required.

For the active substance difenoconazole, for almost all crop scenarios, the Tier 1  $TER_{lt}$  values are greater than the trigger of 5, indicating that long-term risk to birds is acceptable following use according to the proposed use patterns for these crops. However, for leafy vegetables, the  $TER_{lt}$  value for use of IN005B1570 in the scenario for small herbivorous mammal "vole" is below the trigger of 5, indicating need for further refinement.

Based on the refined assessment step of reproductive dietary risk assessment, the calculated TER values for the long-term risk resulting from the difenoconazole exposure in the evaluated scenario did not achieve the acceptability criteria  $TER \geq 5$  for long-term effects.

Since the approach used for the focal species vole is very conservative, some MS, such as Germany, consider a reduced trigger value of 2 for the long-term risk assessment. Thus, since  $TER_{lt}$  is greater than the trigger value of 2, acceptable risk could be shown in Tier 2. Therefore, long-term risk to mammals is acceptable following use according to the proposed use patterns for all intended crops.

Acceptable risk for mammals due to drinking water exposure was demonstrated. In the risk assessment for earthworm-eating mammals and fish-eating mammals via secondary poisoning, the TER values are greater than the trigger of 5, indicating acceptable risk for mammals and indicating therefore no need for further refinement.

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

No available information.

## 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 Difenconazole and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents, as well as in **Błąd! Nie można odnaleźć źródła odwołania.** of this document (new studies).

Effects of IN005B1570 on aquatic organisms were not evaluated as part of the EU assessment of Difenconazole. New data submitted with this application are listed in Appendix 1 and summarised in **Błąd! Nie można odnaleźć źródła odwołania.**

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 – Difenconazole and relevant metabolites**

Species	Substance	Exposure System	Results	Reference
<b>Fish</b>				
<i>Oncorhynchus mykiss</i> (Rainbow Trout)	Difenconazole	96 h, (flow through)	EC <sub>50</sub> = 1.1 (0.98 – 1.1) mg a.s./L	EFSA Scientific Report (2011; 9 (1) : 1967)
<i>Fathead minnow</i>	Difenconazole	34 d (flow through)	NOEC = 0.0076 mg a.s./L	
<i>Pimephales promelas</i>	Difenconazole	34d, f	NOEC = 0.0036 mg a.s./L*	
<i>Oncorhynchus mykiss</i> (Rainbow Trout)	CGA 71019	96 h (static)	EC <sub>50</sub> = 498 (378-657) mg a.s./L	
<i>Oncorhynchus mykiss</i> (Rainbow Trout)	CGA 205375	96 h (static)	EC <sub>50</sub> = 0.74 (0.58-0.95) mg a.s./L	
<i>Oncorhynchus mykiss</i> (Rainbow Trout)	CGA 71019	28 d (static renewal)	NOEC = 3.2 mg a.s./L	
Zebrafish ( <i>Brachydanio rerio</i> )	IN005B1570	96 h (static)	LC <sub>50</sub> > 0.89 a.s mg/L <sub>nom</sub>	KCP 10.2.1/03 Noe, F. (2021)
<b>Aquatic Invertebrates</b>				
<i>Daphnia Magna</i>	Difenconazole	48 h (static)	EC <sub>50</sub> = 0.77 (0.59 – 0.95 mg a.s./L	EFSA Scientific Report (2011; 9 (1) : 1967)
<i>Mysidopsis bahia</i>	Difenconazole	96 h (flow through)	EC <sub>50</sub> = 0.15 (0.11-0.22) mg a.s./L	
<i>Crassostrea virginica</i>	Difenconazole	96 h (flow through)	EC <sub>50</sub> >30 mg a.s./L	
<i>Daphnia Magna</i>	Difenconazole	21 d (flow through)	NOEC = 0.0036 mg a.s./L	EFSA supporting publication 2014:EN-680
<i>Daphnia magna</i>	Metabolite CGA 71019	48 h, (static)	EC <sub>50</sub> >100 mg a.s./L	EFSA Scientific Report (2011; 9 (1) : 1967)
<i>Daphnia magna</i>	CGA 205375	21 d, (static)	EC <sub>50</sub> = 1.4 (1.2-1.7) mg a.s./L	
<i>Daphnia magna</i>	IN005B1570	48 h (static)	EC <sub>50</sub> = 0.89 mg a.s./L <sub>nom</sub>	KCP 10.2.1/01 Noe, F (2021)
<b>Sediment Dwelling organisms</b>				
<i>Chironomus riparius</i>	Difenconazole	28 d (static)	NOEC = 0.015 mg a.s./L NOEC = 0.0525 mg a.s./kg**	EFSA Scientific Report (2011; 9 (1) : 1967)
<i>Chironomus riparius</i>	SCORE 250EC	28 d (static)	NOEC = 0.075	

Species	Substance	Exposure System	Results	Reference
			mg a.s./L	
<i>Chironomus riparius</i>	CGA 205375	28 d (static)	NOEC = 0.4 mg a.s./L	
<i>Chironomus riparius</i>	CGA 205375	28 d (static)	NOEC <sub>sediment</sub> = 10 mg a.s./kg dw	
Algae				
<i>Scenedesmus subspicatus</i>	Difenoconazole	72 h (static)	<b>E<sub>b</sub>C<sub>50</sub> = 0.032 (0.026 – 0.039) mg a.s./L</b>	EFSA Scientific Report (2011; 9 (1) : 1967)
<i>Selenastrum capricornutum</i>	CGA 71019	96 h (static)	E <sub>b</sub> C <sub>50</sub> = 8 mg a.s./L E <sub>r</sub> C <sub>50</sub> >31 mg a.s./L	
<i>Selenastrum capricornutum</i>	CGA 250375	72 h (static)	E <sub>b</sub> C <sub>50</sub> = 1.2 (1.2-1.3) mg a.s./L E <sub>r</sub> C <sub>50</sub> = 3.1 (3.0-3.2) mg a.s./L	
Green Algae ( <i>Pseudokirchneriella subcapitata</i> )	IN005B1570	72 h (static)	E <sub>r</sub> C <sub>50</sub> = 1.7 mg a.s./L <sub>nom</sub> E <sub>y</sub> C <sub>50</sub> = 0.48 mg a.s./L <sub>nom</sub>	KCP 10.2.1/02 <div></div>

nom: based on nominal concentrations; mm: based on mean measured concentrations. Endpoints in **Bold** are used in the assessment

\*0.0525 mg/kg sediment – estimated

it should be noted that the NOEC value was based on very conservative estimate of the concentration in the sediment (see Addendum B.9. October 2010). and no effects were seen at the highest test concentration. It should be considered by MSs level.

\*\* Endpoint updated in line with the outcome of the addendum for difenconazole following the evaluation of confirmatory data after Annex I inclusion (May 2014) which provided consideration of the fish full life-cycle test with Fathead Minnow (*Pimephales promelas*) by Gallagher et al. 2009

**zRMS comments:** zRMS point out that highlight that during Evaluation of confirmatory data after Annex 1 inclusion (2014), the endpoint derived from the study by Gallagher (2009) is considered as new relevant chronic endpoint for fish. Due to uncertainties related to the statistical power of the endocrine disruption parameters in the fish full life cycle test, the NOEC value should conservatively be set to 0.0036 mg/L based on effects on male body weight. This approach was agreed by different Member States (Outcome of the consultation on the pesticide risk assessment of confirmatory data for the active substance difenconazole, EFSA supporting publication 2014:EN-680). Risk assessment has been amended by zRMS consequently.

#### 9.5.1.1 Justification for new endpoints

Not relevant.

#### 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).

The relevant global maximum FOCUS Step 1, 2, and 3 PEC<sub>sw</sub> for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below. If an unacceptable risk is concluded at Step 3 calculations, mitigation measures (Step 4) are presented.

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC<sub>sw</sub>, PEC<sub>sed</sub>) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per all

intended uses for each FOCUS scenario and each organism group.

**Table 9.5-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Difenconazole for each organism group based on FOCUS Steps 1 - 4 calculations for the use of IN005B1570 in oilseed rape**

IN005B1570 (Oilseed rape)								
Group		Fish acute	Fish chronic	Inverteb. Acute (Daphnia)	Inverteb. Chronic (Daphnia)	Algae		Sed. dwell. prolonged
Time scale		Acute	Chronic	Acute	Chronic	Chronic		<i>Chironomus riparius</i>
Endpoint		LC <sub>50</sub>	NOEC	LC <sub>50</sub>	NOEC	E <sub>r</sub> C <sub>50</sub>		NOEC
(µg/L)		890	3.6	150	3.6	32		52.5
AF		100	10	100	10	10		10
RAC (µg/L)		8.9	0.36	1.5	0.36	3.2		5.25
FOCUS Scenarios	PEC <sub>SW</sub> <sub>max</sub> (µg/L)	PEC/RAC *					PEC <sub>sed-max</sub> (µg/kg)	
Step 1								
	19.22	2.160	53.389	12.813	53.389	6.006	498.1	94.876
Step 2								
N-Europe (Oct-Feb)	5.259	0.591	14.608	3.506	14.608	1.643	150	28.571
N-Europe (Mar-May)	2.435	0.274	6.764	1.623	6.764	0.761	67.1	12.781
Step 3								
D3/ditch	0.69	0.078	1.917	0.460	1.917	0.216	0.784	0.149
D4/pond	0.026	0.003	0.072	0.017	0.072	0.008	0.571	0.109
D4/steam	0.589	0.066	1.636	0.393	1.636	0.184	0.133	0.025
D5/pond	0.026	0.003	0.072	0.017	0.072	0.008	0.503	0.096
D5/stream	0.635	0.071	1.764	0.423	1.764	0.198	0.184	0.035
R1/pond	0.057	0.006	0.158	0.038	0.158	0.018	1.696	0.323
R1/stream	0.447	0.050	1.242	0.298	1.242	0.140	2.27	0.432
R3/stream	0.629	0.071	1.747	0.419	1.747	0.197	4.433	0.844
Step 4 - 5 m no spray buffer zone								
D3/ditch	0.179	0.020	0.497	0.119	0.497	0.056	0.209	0.040
D4/steam	0.208	0.023	0.578	0.139	0.578	0.065	0.085	0.016
D5/stream	0.224	0.025	0.622	0.149	0.622	0.070	0.065	0.012



R1/steram	0.317	0.036	0.881	0.211	0.881	0.099	2.259	0.430
R3/stream	0.386	0.043	1.072	0.257	1.072	0.121	4.4	0.838
<b>Step 4 - 10m no spray buffer zone   10 vegetative filter strip</b>								
R3/stream	0.174	0.020	0.483	0.116	0.483	0.054	0.857	0.163

**Table 9.5-3:** Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Difenconazole for each organism group based on FOCUS Steps 1 - 4 calculations for the use of IN005B1570 in Orchards

IN005B1570 (Orchards)								
Group		Fish acute	Fish chronic	Inverteb. Acute (Daphnia)	Inverteb. Chronic (Daphnia)	Algae		Sed. dwell. prolonged
Time scale		Acute	Chronic	Acute	Chronic	Chronic		<i>Chironomus riparius</i>
Endpoint		LC <sub>50</sub>	NOEC	LC <sub>50</sub>	NOEC	E <sub>r</sub> C <sub>50</sub>		NOEC
(µg/L)		890	3.6	150	3.6	32		52.5
AF		100	10	100	10	10		10
RAC (µg/L)		8.9	0.36	1.5	0.36	3.2		5.25
FOCUS Scenarios	PEC <sub>sw</sub> <sub>max</sub> (µg/L)	PEC/RAC					PEC <sub>sed</sub> - <sub>max</sub> (µg/kg)	
Step 1								
	27.97	3.143	77.694	18.647	77.694	8.741	435.3	82.914
Step 2								
N-Europe (Oct-Feb)	6.967	0.783	19.353	4.645	19.353	2.177	117.3	22.343
N-Europe (Mar-May)	6.967	0.783	19.353	4.645	19.353	2.177	101.5	19.333
Step 3								
D3/ditch	3.543	0.398	9.842	2.362	9.842	1.107	7.587	1.445
D4/pond	0.544	0.061	1.511	0.363	1.511	0.170	6.627	1.262
D4/stream	3.703	0.416	10.286	2.469	10.286	1.157	1.749	0.333
D5/pond	0.525	0.059	1.458	0.350	1.458	0.164	6.934	1.321
D5/stream	3.995	0.449	11.097	2.663	11.097	1.248	2.288	0.436
R1/pond	0.49	0.055	1.361	0.327	1.361	0.153	5.829	1.110
R1/stream	2.831	0.318	7.864	1.887	7.864	0.885	1.534	0.292

IN005B1570 (Orchards)								
Group		Fish acute	Fish chronic	Inverteb. Acute (Daphnia)	Inverteb. Chronic (Daphnia)	Algae		Sed. dwell. prolonged
Time scale		Acute	Chronic	Acute	Chronic	Chronic		<i>Chironomus riparius</i>
Endpoint		LC <sub>50</sub>	NOEC	LC <sub>50</sub>	NOEC	ErC <sub>50</sub>		NOEC
(µg/L)		890	3.6	150	3.6	32		52.5
AF		100	10	100	10	10		10
RAC (µg/L)		8.9	0.36	1.5	0.36	3.2		5.25
FOCUS Scenarios	PEC <sub>sw</sub> <sub>max</sub> (µg/L)	PEC/RAC					PEC <sub>sed</sub> <sub>max</sub> (µg/kg)	
R3/stream	3.992	0.449	11.089	2.661	11.089	1.248	1.959	0.373
R4/stream	2.831	0.318	7.864	1.887	7.864	0.885	0.998	0.190
Step 4 - 20 m no spray buffer zone								
D3/ditch	0.414	0.047	1.150	0.276	1.150	0.129	0.98	0.187
D4/pond	0.1	0.011	0.278	0.067	0.278	0.031	1.325	0.252
D4/stream	0.477	0.054	1.325	0.318	1.325	0.149	0.242	0.046
D5/pond	0.096	0.011	0.267	0.064	0.267	0.030	1.379	0.263
D5/stream	0.515	0.058	1.431	0.343	1.431	0.161	0.32	0.061
R1/pond	0.102	0.011	0.283	0.068	0.283	0.032	1.319	0.251
R1/stream	0.366	0.041	1.017	0.244	1.017	0.114	0.944	0.180
R3/stream	0.516	0.058	1.433	0.344	1.433	0.161	0.282	0.054
R4/stream	0.366	0.041	1.017	0.244	1.017	0.114	0.793	0.151
Step 4 - 25m no spray buffer zone								
D3/ditch	0.226	0.025	0.628	0.151	0.628	0.071	0.549	0.105
D4/stream	0.261	0.029	0.725	0.174	0.725	0.082	0.135	0.026
D5/stream	0.281	0.032	0.781	0.187	0.781	0.088	0.179	0.034
R1/stream	0.212	0.024	0.589	0.141	0.589	0.066	0.903	0.172
R3/stream	0.281	0.032	0.781	0.187	0.781	0.088	0.181	0.034
R4/stream	0.245	0.028	0.681	0.163	0.681	0.077	0.784	0.149

**Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Difenoconazole for each organism group based on FOCUS Steps 1 - 4 calculations for the use of IN005B1570 in Carrots**

IN005B1570 (Carrots)								
Group		Fish acute	Fish chronic	Inverteb. Acute (Daphnia)	Inverteb. Chronic (Daphnia)	Algae		Sed. dwell. prolonged
Time scale		Acute	Chronic	Acute	Chronic	Chronic		Chironomus riparius
Endpoint		LC <sub>50</sub>	EC10	LC <sub>50</sub>	NOEC	E <sub>r</sub> C <sub>50</sub>		NOEC
(µg/L)		890	3.6	150	3.6	32		52.5
AF		100	10	100	10	10		10
RAC (µg/L)		8.9	0.36	1.5	0.36	3.2		5.25
FOCUS Scenarios	PEC <sub>sw</sub> <sub>max</sub> (µg/L)	PEC/RAC					PEC <sub>sed</sub> -max (µg/kg)	
Step 1								
	28.83	3.239	80.083	19.220	80.083	9.009	747.1	142.305
Step 2								
N-Europe (Oct-Feb)	2.993	0.336	8.314	1.995	8.314	0.935	82.47	15.709
N-Europe (Mar-May)	2.068	0.232	5.744	1.379	5.744	0.646	55.33	10.539
Step 3								
D3/ditch	0.575	0.065	1.597	0.383	1.597	0.180	0.782	0.149
R1/pond	0.18	0.020	0.500	0.120	0.500	0.056	5.149	0.981
R1/stream	0.545	0.061	1.514	0.363	1.514	0.170	12.47	2.375
R3/stream	0.688	0.077	1.911	0.459	1.911	0.215	4.757	0.906
R4/stream	1.136	0.128	3.156	0.757	3.156	0.355	10.84	2.065
Step 4 - 10 m no spray buffer zone   10 m vegetative filter strip								
D3/ditch	0.08	0.009	0.222	0.053	0.222	0.025	0.118	0.022
R1/stream	0.244	0.027	0.678	0.163	0.678	0.076	2.156	0.411
R3/stream	0.314	0.035	0.872	0.209	0.872	0.098	1.01	0.192
R4/stream	0.516	0.058	1.433	0.344	1.433	0.161	2.418	0.461
Step 4 - 20 m no spray buffer zone   20 m vegetative filter strip								

R4/stream	0.27	0.030	0.750	0.180	0.750	0.084	1.048	0.200
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**Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Difenconazole for each organism group based on FOCUS Steps 1 - 4 calculations for the use of IN005B1570 in Leafy Vegetables**

IN005B1570 (Leafy vegetables)								
Group		Fish acute	Fish chronic	Inverteb. Acute (Daphnia)	Inverteb. Chronic (Daphnia)	Algae		Sed. dwell. prolonged
Time scale		Acute	Chronic	Acute	Chronic	Chronic		<i>Chironomus riparius</i>
Endpoint		LC <sub>50</sub>	NOEC	LC <sub>50</sub>	NOEC	E <sub>r</sub> C <sub>50</sub>		NOEC
(µg/L)		890	3.6	150	3.6	32		52.5
AF		100	10	100	10	10		10
RAC (µg/L)		8.9	0.36	1.5	0.36	3.2		5.25
FOCUS Scenarios	PEC <sub>sw</sub> <sub>max</sub> (µg/L)	PEC/RAC					PEC <sub>sed</sub> - <sub>max</sub> (µg/kg)	
Step 1								
	28.83	3.239	80.083	19.220	80.083	9.009	766	145.905
Step 2								
N-Europe (Oct-Feb)	4.283	0.481	11.897	2.855	11.897	1.338	120.3	22.914
N-Europe (Mar-May)	3.565	0.401	9.903	2.377	9.903	1.114	99.2	18.895
Step 3								
D3/ditch	0.575	0.065	1.597	0.383	1.597	0.180	0.877	0.167
D4/pond	0.042	0.005	0.117	0.028	0.117	0.013	0.765	0.146
D4/stream	0.436	0.049	1.211	0.291	1.211	0.136	0.143	0.027
R1/pond	0.318	0.036	0.883	0.212	0.883	0.099	8.377	1.596
R1/stream	0.554	0.062	1.539	0.369	1.539	0.173	55.76	10.621
R3/stream	0.704	0.079	1.956	0.469	1.956	0.220	44.54	8.484
R4/stream	1.097	0.123	3.047	0.731	3.047	0.343	11.52	2.194
Step 4 - 10 m no spray buffer zone   10 m vegetative filter strip								
D3/ditch	0.08	0.009	0.222	0.053	0.222	0.025	0.132	0.025

D4/stream	0.144	0.016	0.400	0.096	0.400	0.045	0.137	0.026
R1/pond	0.135	0.015	0.375	0.090	0.375	0.042	3.342	0.637
R1/stream	0.252	0.028	0.700	0.168	0.700	0.079	8.815	1.679
R3/stream	0.318	0.036	0.883	0.212	0.883	0.099	7.124	1.357
R4/stream	0.496	0.056	1.378	0.331	1.378	0.155	2.366	0.451
<b>Step 4 - 20 m no spray buffer zone   20 m vegetative filter strip</b>								
R1/stream	0.132	0.015	0.367	0.088	0.367	0.041	3.06	0.583
R3/stream	0.166	0.019	0.461	0.111	0.461	0.052	2.498	0.476
R4/stream	0.26	0.029	0.722	0.173	0.722	0.081	0.986	0.188

**Table 9.5-6 Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA 71019 for each organism group based on FOCUS Steps 1 calculations for the use of IN005B1570 in all intended crops**

CGA 71019								
Group		Fish acute	Fish chronic	Inverteb. Acute (Daphnia)	Inverteb. Chronic (Daphnia)	Algae		Sed. dwell. prolonged
Time scale		Acute	Chronic	Acute	Chronic	Chronic		<i>Chironomus riparius</i>
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	E <sub>b</sub> C <sub>50</sub>		NOEC
(µg/L)		498000	3200	100000	No data	8000		No data
AF		100	10	100	10	10		10
RAC (µg/L)		4980	320	1000	No data	800		No data
FOCUS Scenarios	PEC <sub>sw</sub> <sub>max</sub> (µg/L)	PEC/RAC					PEC <sub>sed</sub> - max (µg/kg)	
Oilseed rape								
Step 1								
Step 1	4.246 4.25	0.001 0.0008	0.013 0.013	0.004 0.00425	-	0.005 0.005	3.518	-
Orchards								
Step 1								
Step 1	3.122 1.81	0.001 0.00036	0.010 0.0057	0.003 0.00181	-	0.004 0.0023	2.567	-

Carrots								
Step 1								
Step 1	6.368 3.50	0.004 0.0007	0.020 0.01	0.006 0.0035	-	0.008 0.004	5.239	-
Leafy vegetables								
Step 1								
Step 1	6.368 3.50	0.004 0.0007	0.020 0.01	0.006 0.0035	-	0.008 0.004	5.277	-

**Table 9.5-7 Aquatic organisms: acceptability of risk (PEC/RAC < 1) for metabolite CGA 205375 for each organism group based on FOCUS Step 1 calculations for the use of IN005B1570 in all intended crops**

CGA 205375										
Group		Fish acute	Fish chronic	Inverteb. Acute (Daphnia)	Inverteb. Chronic (Daphnia)	Algae		Sed. dwell. prolonged		
Time scale		Acute	Chronic	Acute	Chronic	Chronic		<i>Chironomus riparius</i>		
Endpoint		LC <sub>50</sub>	NOEC	EC <sub>50</sub>	NOEC	E <sub>b</sub> C <sub>50</sub>		NOEC		
(µg/L)		740	No data	1400	No data	1200		10000	400	
AF		100	10	100	10	10		10	10	
RAC (µg/L)		7.4	No data	14	No data	120		1000	40	
FOCUS Scenarios	PEC <sub>sw</sub> <sub>max</sub> (µg/L)	PEC/RAC					PEC <sub>sed</sub> <sub>max</sub> (µg/kg)			
Oilseed rape										
Step 1	3.594	0.486	-	0.257	-	0.030	90.73	0.091	0.09	
Orchards										
Step 1	3.931	0.531	-	0.281	-	0.033	70.24	0.070	0.098	
Carrots										
Step 1	5.392	0.729	-	0.385	-	0.045	136.1	0.136	0.13	
Leafy vegetables										
Step 1	5.392	0.729	-	0.385	-	0.045	136.1	0.136	0.13	

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

Effects on aquatic organisms for IN005B1570 have not been evaluated as part of an EU reviews of difenoconazole. Data on IN005B1570 are evaluated here, and risk assessments for IN005B1570 with the proposed use pattern are provided here and are considered adequate.

The acute and long-term risk to aquatic organisms was assessed from risk quotients between predicted environmental concentrations (PEC) following applications according to the use pattern and regulatory acceptable concentrations (RAC), estimated from studies with difenoconazole, relevant difenoconazole metabolites and IN005B1570.

For the intended use Oilseed rape, calculated PEC/RAC ratios did not indicate a long-term acceptable risk for the most sensitive group of aquatic organisms at Step 3 calculations, that is Daphnia. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC<sub>sw</sub> considering reduced exposure of surface water bodies with a 5 m no-spray buffer zone, and a 10 m no spray buffer zone and 10 m vegetative filter strip. At Step 4 with 5m no spray buffer zone, calculated PEC/RAC ratios indicated an acceptable risk for all scenarios, except R3/stream. At Step 4 with a 10m no spray buffer zone and 10 vegetative filter strip, calculated PEC/RAC ratio indicated an acceptable risk for all aquatic organisms evaluated, so no further assessment is necessary.

For the intended use Orchards, calculated PEC/RAC ratios did not indicate an acute and long-term acceptable risk for the aquatic organisms Daphnia, for long term for fish, and for the specie *C. riparius* at Step 3 calculations. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC<sub>sw</sub> considering reduced exposure of surface water bodies with a 20 m and a 25 m no-spray buffer zones. Calculated PEC/RAC ratios indicated an acceptable risk for all aquatic organisms evaluated in the scenarios D4/pond, d5/pond and R1/pond at Step 4 with 20 m no spray zone. At Step 4 with 25 m no spray zone, calculated PEC/RAC ratios indicated an acceptable risk for all aquatic organisms evaluated for all scenarios, so no further assessment is necessary.

For the intended use Carrots, calculated PEC/RAC ratios did not indicate a long-term acceptable risk for the all aquatic organisms at Step 3 calculations. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC<sub>sw</sub> considering reduced exposure of surface water bodies with a 10 m no spray zone and 10 m vegetative filter strip, and 20 m no-spray buffer zone and 20 m vegetative filter strip. At Step 4, calculated PEC/RAC ratios indicated an acceptable risk for all aquatic organisms evaluated, so no further assessment is necessary.

For the intended use Leafy vegetables, calculated PEC/RAC ratios did not indicate a long-term acceptable risk for all aquatic organisms at Step 3 calculations. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC<sub>sw</sub> considering reduced exposure of surface water bodies with a 10 m no-spray buffer zone and 10 m vegetative filter strip, and a 20 m no spray buffer zone and 20 m vegetative filter strip. At Step 4, calculated PEC/RAC ratios indicated an acceptable risk for all aquatic organisms evaluated, so no further assessment is necessary.

The PEC/RAC ratios for difenoconazole metabolites CGA 71019 and 205375 using maximum initial FOCUS Step 1 PEC<sub>SW</sub> values in all uses are less than the trigger value of 1, indicating that the risk to aquatic organisms is acceptable following use of IN005B1570 according to the proposed use pattern.

### 9.5.3 Overall conclusions

No unacceptable risk to aquatic organisms is seen following step 1 for the metabolites CGA 71019 and CGA 205375 following application of the formulation IN005B1570 on all intended groups. For all pro-



posed uses, an acceptable risk for the active substance is seen after FOCUS step 4, concluding an acceptable risk for the formulation IN005B1570 to aquatic organisms.

For oilseed rape, a 10 m no-spray buffer zone and 10m vegetative filter strip guarantees protection for aquatic systems in all scenarios when IN005B1570 is applied. For orchards, the protection is achieved with a 25 m no-spray buffer zone; for carrots, it is with a 20 m no-spray buffer zone and 20 m vegetative filter strip; and for leafy vegetables, it is with a 20 m no-spray buffer zone and 20 m vegetative filter strip.

#### zRMS comments:

The evaluation of the risk for aquatic 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” (EFSA Journal 2013;11(7):3290).

The ratios between predicted environmental concentrations in surface water bodies (PEC<sub>SW</sub>, PEC<sub>SED</sub>) and regulatory acceptable concentrations (RAC) for a.s.- difenoconazole and difenoconazole metabolites CGA 71019 and 205375 based on the worst case for aquatic organisms were <1 indicating acceptable risk to aquatic organisms with applying:

1. For oilseed rape, a 10 m no-spray buffer zone and 10m vegetative filter strip.
2. For orchards, the protection is achieved with a 25 m no-spray buffer zone.
3. For carrots, it is with a 20 m no-spray buffer zone and 20 m vegetative filter strip.
4. For leafy vegetables, it is with a 20 m no-spray buffer zone and 20 m vegetative filter strip.

Final risk mitigation measures should be considered at MSs level.

The risk assessment for aquatic organisms for formulation was performed by zRMS.

zRMS agrees with PEC<sub>sw</sub> calculated at formulation (dRR part B8).

The PEC<sub>sw</sub> for the formulation IN005B1570 was calculated using the SWASH drift calculator.

The PEC<sub>sw</sub> values for the formulation are presented in the following table.

#### FOCUS Step 3 PEC<sub>sw</sub> values for IN005B1570 following application to field crops and pome fruit

Crop	Application rate (g FP/ha)*	Max. PEC <sub>sw</sub> (µg/L)		
		Ditch	Stream	Pond
Field crops	570.1	3.663	2.718	0.125
Pome fruit (early)	256.5	20.18	18.45	1.213

\* Based on formulation density of 1.1401 g/mL

In the following tables, the ratios between predicted environmental concentrations in surface water bodies (PEC<sub>SW</sub>, PEC<sub>SED</sub>) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

## POME FRUIT

**Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Difenoconazole 25% SC for each organism group based on drift calculations for pome fruit (early)**

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>B. rerio</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC <sub>50</sub> >3900	EC <sub>50</sub> 3900	E <sub>r</sub> C <sub>50</sub> 7500
AF		100	100	10
RAC (µg/L)		39	39	750
Distance (1m)	Max. PEC <sub>sw</sub> (µg/L)	PEC/RAC		
	20.18	0.52	0.52	0.03

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

## FIELD CROPS

**Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Difenoconazole 25% SC for each organism group based on drift calculations for pome fruit (early)**

Group		Fish acute	Inverteb. acute	Algae
Test species		<i>B. rerio</i>	<i>D. magna</i>	<i>P. subcapitata</i>
Endpoint (µg/L)		LC <sub>50</sub> >3900	EC <sub>50</sub> 3900	E <sub>r</sub> C <sub>50</sub> 7500
AF		100	100	10
RAC (µg/L)		39	39	750
Distance (1m)	Max. PEC <sub>sw</sub> (µg/L)	PEC/RAC		
	3.663	0.09	0.09	0.004884

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 risk assessment for aquatic organisms for **IN005B1570** was accepted.

### Conclusions:

The ratios between predicted environmental concentrations in surface water bodies (PEC<sub>SW</sub>, PEC<sub>SED</sub>) and regulatory acceptable concentrations (RAC) for a.s.- difenoconazole and difenoconazole metabolites CGA 71019 and 205375 based on the worst case for aquatic organisms were <1 indicating acceptable risk to aquatic organisms with applying:

1. For oilseed rape, a 10 m no-spray buffer zone and 10m vegetative filter strip.
2. For orchards, the protection is achieved with a 25 m no-spray buffer zone.
3. For carrots, it is with a 20 m no-spray buffer zone and 20 m vegetative filter strip.
4. For leafy vegetables, it is with a 20 m no-spray buffer zone and 20 m vegetative filter strip.

Final risk mitigation measures should be considered at MSs level.

The risk assessment for aquatic organisms for formulation was performed by zRMS.

**Updated April 2024**

According to the NL comments: The PEC<sub>sw</sub> values used in these tables are PEC<sub>sw</sub> for the formulation. However, this is not in accordance with the aquatic GD (EFSA, 2013). A PEC<sub>sw</sub> expressed as formulation is likely to underestimate exposure, since it is calculated based on drift values, and no input parameters for the formulation are available to calculate this for multiple applications. Ctgb is of the opinion that the formulation risk assessment should be performed with formulation endpoints expressed on a total a.s. basis, and PEC's for the a.s.

The risk assessment for formulation based on s.s. was performed by RMS:

IN005B1570				
Group	I	Fish acute <i>Brachydanio rerio</i>	<i>Daphnia magna</i>	Green algae
Time scale		Acute	Acute	Acute
Endpoint		LC <sub>50</sub>	EC <sub>50</sub>	E <sub>r</sub> C <sub>50</sub>
(µg/L)		>890	890	1700
AF		100	100	10
RAC (µg/L)		8.9	8.9	170
FOCUS Scenarios PEC <sub>sw</sub> <sub>max</sub> (µg/L)	PEC <sub>sw</sub> /RAC			
Oilseed rape				
Step 1	19.22	2.16	2.16	0.11
Step 2	5.259	0.59	0.59	0.03
Orchards				
Step 1	27.97	3.14	3.14	0.16
Step 2	6.967	0.78	0.78	0.04
Carrots				
Step 1	28.83	3.2	3.2	0.17
Step 2	2.993	0.34	0.34	0.017
Leafy vegetables				
Step 1	28.83	3.2	3.2	0.17
Step 2	4.283	0.48	0.48	0.025
The risk assessment for formulation IN005B1570 based s.a. was accepted by RMS (step 2).				

## 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 Difenconazole. Full details of these studies are provided in the respective EU DAR and related documents as well as in table of this document (new studies).

Effects on bees of IN005B1570 were not evaluated as part of the EU assessment of Difenconazole. New data submitted with this application are listed in Appendix 1 and summarised in table.

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
Honeybee	Difenoconazole	Oral	LD <sub>50</sub> = >177 µg/bee	EFSA Journal 2011; 9(1):1967
Honeybee	Difenoconazole	Contact	LD <sub>50</sub> = >100 µg/bee	
Higher tier studies (tunnel test, field studies) Acute honey bee laboratory data for the product IN005B1570				
Honeybee	IN005B1570	Oral	LD <sub>50</sub> > 99.99 µg/bee NOEL = 45.4 µg/bee  LD <sub>50</sub> > 35.1 µg/bee*	KCP 10.3.1.1.1, Ponti, B. (2021)
Honeybee	IN005B1570	Contact	LD <sub>50</sub> = 43.7 µg/bee NOEL = 20.75 µg/bee	KCP 10.3.1.1.1, Ponti, B. (2021)
Honeybee	IN005B1570	Chronic Oral	LDD50 = 14.39 µg a.s./bee/Day NOEDD= 7.99 µg a.s/bee/day	KCP 10.3.1.2, Ponti, B. (2023)
Honeybee	IN005B1570	Larval development	ED50=40.14 µg a.s./larva NOED=28.13 µg a.s./larva	KCP 10.3.1.3, Noè, F. (2023)

Endpoints in **Bold** are used in the assessment

\*According DE comment - the oral endpoint was additionally determined based on the actual consumed dose. DE is of the opinion, that the lower oral LD<sub>50</sub> of >35.1 µg/bee should be considered for the calculation of hazard quotients in Table 9.6-2 and in the risk assessment of the zRMS.

### 9.6.1.1 Justification for new endpoints

The results of studies conducted for the product IN005B1570 are more conservative than those presented in the EFSA Conclusion (EFSA Journal 2011;9(1):1967), and therefore were used in this study.

Per request from the cMS and to comply with the data requirements of Commission regulation (EU) No 284/2013, studies on the formulation IN005B1570 for chronic oral toxicity and for larval development were included in appendix I and summarised in appendix II.

## 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 (SAN-CO/10329/2002 rev.2 (final), October 17, 2002).

### 9.6.2.1 Hazard quotients for bees

A Hazard Quotient (HQ) approach has been defined by the EPPO risk assessment scheme to identify use patterns which pose a negligible acute risk to honeybees. The HQ is determined by calculating the ratio between the application rate (expressed in g a.s./ha) and the lowest laboratory contact and oral LD<sub>50</sub> (expressed in µg a.s./bee). HQ values higher than 50 indicate the need of higher tiered activities to clarify the actual risk to honeybees.

Application of pesticides can potentially result in exposure of bees either through direct over-spray, or by

contact with residues on plants whilst bees are foraging for food.

**Table 9.6-2: First-tier assessment of the risk for bees due to the use of IN005B1570 in all crops**

Use No.	Crop	Scenario   Toxicity	LD <sub>50</sub> (lab.) (µg/bee)	Single Appl. rate (g/ha)	Q <sub>HO</sub> , Q <sub>HC</sub> Criterion: QH < 50
Active substance: Difenoconazole					
01-10	All intended uses (risk envelope)	Oral acute	177	125	0.71
		Contact	100		1.25
Product: IN005B1570					
1	Oilseed rape	Oral acute	99.99 <b>&gt;35.1</b>	125	1.25 <b>3.56</b>
		Contact	43.7		2.86
2	Orchards (apples, pears)	Oral acute	99.99 <b>&gt;35.1</b>	56.25	0.56 <b>1.60</b>
		Contact	43.7		1.29
3	Carrots	Oral acute	99.99 <b>&gt;35.1</b>	125	1.25 <b>3.56</b>
		Contact	43.7		2.86
4, 6, 7	Leafy vegetables	Oral acute	99.99 <b>&gt;35.1</b>	125	1.25 <b>3.56</b>
		Contact	43.7		2.86

Q<sub>HO</sub>, Q<sub>HC</sub>: Hazard quotients for oral and contact exposure. Q<sub>H</sub> values shown in bold breach the relevant trigger.

All the hazard quotients for the active substances and formulation are less than 50, indicating that the risk to bees is acceptable following use of IN005B1570 according to the proposed use patterns.

**zRMS comments:**

The HQ values are lower than the trigger of 50, indicating low risk to bees from following application of **IN005B1570**.

According to Commission regulation (EU) No 284/2013, point 10.3.1. (Effects on bees) the Applicant should provide also the chronic study for adult bees and the chronic test for larvae for formulated product. The risk assessment based on this study should be considered when GD for Bees, 2013 is implemented at EU level. Thus, concerned Member States must decide on the consideration of data requirements and the risk assessment at national level.

**According DE comment:**

According to Commission regulation (EU) No 284/2013, chronic laboratory studies with adult honey bees and honey bee larvae are required for the authorisation of IN005B1570 regardless of the implementation of the EFSA Bee Guidance Document. Without such studies, DE cannot finalize its risk assessment of IN005B1570 for bees. The applicant should submit chronic studies during the authorisation process of IN005B1570. Thus, concerned Member States must decide on the consideration of data requirements and the risk assessment at national level.

**According to the AT comment:**

The data requirements to address chronic and larval toxicity to bees are not fulfilled. Please note that Austria will conduct the tier 1 risk assessment according to the EFSA Guidance Document on the risk as-

assessment of plant protection products on bees (2013). Thus, concerned Member States must decide on the consideration of data requirements and the risk assessment at national level.

#### Updated April 2024

To address the data requirements for chronic and larval toxicity to bees the applicant as shared with the zRMS the following studies, as well as the updated dRR Part B9, B5 and Part A:

- Chronic oral bee study (KCP 10.3.1.2)
  - Larvae bee studies (KCP 10.3.1.3)
  - Validation of the analytical method (KCP 5.1.2.16) Additionally, the applicant has shared the studies directly with the cMS that requested them. The chronic studies for bees were accepted by zRMS.
- 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 IN005B1570 are < 50, indicating an acceptable risk to adult bees.

The risk assessment based on studies for bees (adult and larvae) was performed in line with the EFSA 2013 guideline by zRMS.

### THE RISK ASSESSMENT BASED ON STUDIES FOR BEES (ADULT AND LARVAE) WAS PERFORMED IN LINE WITH THE EFSA 2013 GUIDELINE

#### Screening step

#### Screening step assessment of the chronic risk for bees due to the use of IN005B1570 in oilseed rape.

<b>Intended uses</b>	Oilseed rape			
<b>Product</b>	IN005B1570			
<b>Single application rate (g product/ha)</b>	125 g/ha			
<b>Test design</b>	<b>Endpoints (µg/bee/d or µg/larva)</b>	<b>Ef x SV</b>	<b>ETR</b>	<b>Trigger</b>
Acute oral toxicity	LD <sub>50</sub> > 35.1 µg/bee	7.6	0.027	0.2
Acute contact toxicity	LD <sub>50</sub> = 43.7 µg/bee	1.0	0.0029	42
Chronic oral toxicity	LDD <sub>50</sub> = 14.39 µg/bee/day	7.6	0.06	0.03
Larvae toxicity	NOED= 28.13 µg ai/larva	4.4	0.019	0.2

The screening step risk assessment above has indicated a potential chronic adult oral risk therefore a Tier 1 assessment has been provided by zRMS.

#### First-tier assessment of the chronic risk for bees due to the use of IN005B1570 in oilseed rape.

<b>Intended use</b>	Oilseed rape
<b>Product</b>	IN005B1570
<b>Application rate (g product/ha)</b>	125 g/ha

Crop Category	Application	BBCH	category	scenario	Ef	SV HB	SV BB	SV SB	TWA HB	TWA BB	TWA SB	ETR HB	trigger	Risk indicator	Ratio
Oilseed rape (rapeseed spray DW)	10 - 29	chronic	treated crop	1	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,036	0,03	!	1,2
Oilseed rape (rapeseed spray DW)	30 - 39	chronic	treated crop	1	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,036	0,03	!	1,2
Oilseed rape (rapeseed spray DW)	40 - 69	chronic	treated crop	1	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,036	0,03	!	1,2
Oilseed rape (rapeseed spray DW)	≥ 70	chronic	treated crop	1	0	0	0	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	10 - 29	chronic	weeds	1	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,018	0,03	OK	
Oilseed rape (rapeseed spray DW)	30 - 39	chronic	weeds	0,3	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,005	0,03	OK	
Oilseed rape (rapeseed spray DW)	40 - 69	chronic	weeds	0,25	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,005	0,03	OK	
Oilseed rape (rapeseed spray DW)	≥ 70	chronic	weeds	0,25	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,005	0,03	OK	
Oilseed rape (rapeseed spray DW)	10 - 29	chronic	field margin	0,0092	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	30 - 39	chronic	field margin	0,0092	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	40 - 69	chronic	field margin	0,0092	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	≥ 70	chronic	field margin	0,0092	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	10 - 29	chronic	adjacent crop	0,0033	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	30 - 39	chronic	adjacent crop	0,0033	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	40 - 69	chronic	adjacent crop	0,0033	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	≥ 70	chronic	adjacent crop	0,0033	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,000	0,03	OK	
Oilseed rape (rapeseed spray DW)	10 - 29	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	ch	0,003	0,03	OK	
Oilseed rape (rapeseed spray DW)	30 - 39	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	ch	0,003	0,03	OK	
Oilseed rape (rapeseed spray DW)	40 - 69	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	ch	0,003	0,03	OK	
Oilseed rape (rapeseed spray DW)	≥ 70	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	ch	0,003	0,03	OK	

The chronic adult oral ETR for **IN005B1570** is below the trigger for downward sprays, indicating no potential risk with the exception of exposure to the treated crop between BBCH 10-69. As the risk assessment based on the EFSA Guidance (2013) is not approved yet and certain parts are currently under revision, this approach and its consequence on overall conclusion of bee risk assessment should be considered at national level of particular MSs.

#### First-tier assessment of the chronic risk for bees due to the use of IN005B1570 in orchards.

<b>Intended use</b>	Orchards
<b>Product</b>	IN005B1570
<b>Application rate (g product/ha)</b>	56.25 g/ha

Crop Category	Application	BBCH	category	scenario	Ef	SV HB	SV BB	SV SB	TWA HB	TWA BB	TWA SB	ETR HB	trigger	Risk indicator	Ratio
Orchards 1: Almonds, spray SUW	40 - 69	chronic	treated crop	1	8,2	11,4	7,3	0,72	0,72	0,72	ch	0,023	0,03	OK	
Orchards 1: Almonds, spray SUW	≥ 70	chronic	treated crop	1	0	0	0	0,72	0,72	0,72	ch	0,000	0,03	OK	
Orchards 1: Almonds, spray SUW	40 - 69	chronic	weeds	0,3	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,002	0,03	OK	
Orchards 1: Almonds, spray SUW	≥ 70	chronic	weeds	0,3	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,002	0,03	OK	
Orchards 1: Almonds, spray SUW	40 - 69	chronic	field margin	0,097	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,001	0,03	OK	
Orchards 1: Almonds, spray SUW	≥ 70	chronic	field margin	0,097	2,9	5,9	2,3	0,72	0,72	0,72	ch	0,001	0,03	OK	
Orchards 1: Almonds, spray SUW	40 - 69	chronic	adjacent crop	0,066	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,001	0,03	OK	
Orchards 1: Almonds, spray SUW	≥ 70	chronic	adjacent crop	0,066	5,8	9,9	5,7	0,72	0,72	0,72	ch	0,001	0,03	OK	
Orchards 1: Almonds, spray SUW	40 - 69	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	ch	0,002	0,03	OK	
Orchards 1: Almonds, spray SUW	≥ 70	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	ch	0,002	0,03	OK	
Orchards 1: Almonds, granules	40 - 69	chronic	treated crop	0,3	5,8	9,9	5,7	1	1	1	ch	0,007	0,03	OK	
Orchards 1: Almonds, granules	≥ 70	chronic	treated crop	0,3	0	0	0	1	1	1	ch	0,000	0,03	OK	
Orchards 1: Almonds, granules	40 - 69	chronic	weeds	0,09	2,9	5,9	2,3	1	1	1	ch	0,001	0,03	OK	
Orchards 1: Almonds, granules	≥ 70	chronic	weeds	0,09	2,9	5,9	2,3	1	1	1	ch	0,001	0,03	OK	
Orchards 1: Almonds, granules	40 - 69	chronic	field margin	0,096	2,9	5,9	2,3	1	1	1	ch	0,001	0,03	OK	
Orchards 1: Almonds, granules	≥ 70	chronic	field margin	0,096	2,9	5,9	2,3	1	1	1	ch	0,001	0,03	OK	
Orchards 1: Almonds, granules	40 - 69	chronic	adjacent crop	0,045	5,8	9,9	5,7	1	1	1	ch	0,001	0,03	OK	
Orchards 1: Almonds, granules	≥ 70	chronic	adjacent crop	0,045	5,8	9,9	5,7	1	1	1	ch	0,001	0,03	OK	
Orchards 1: Almonds, granules	40 - 69	chronic	next crop	1	0,54	0,78	0,49	1	1	1	ch	0,002	0,03	OK	
Orchards 1: Almonds, granules	≥ 70	chronic	next crop	1	0,54	0,78	0,49	1	1	1	ch	0,002	0,03	OK	

The chronic adult oral ETR for **IN005B1570** is below the trigger for downward sprays, indicating no potential risk in orchards.

#### First-tier assessment of the chronic risk for bees due to the use of IN005B1570 in carrots.

<b>Intended use</b>	Carrots
<b>Product</b>	IN005B1570
<b>Application rate (g product/ha)</b>	125 g/ha



Crop Category	Application	BBCH	category	scenario	Ef	SV HB	SV BB	SV SB	TWA HB	TWA BB	TWA SB	ETR HB	trigger	Risk indicator	Ratio
Root vegetables: Anise spray DW	10 - 39	chronic	treated crop	1	5,8	9,9	5,7	0,72	0,72	0,72	Ch	0,036	0,03	!	1,2
Root vegetables: Anise spray DW	40 - 69	chronic	treated crop	1	5,8	9,9	5,7	0,72	0,72	0,72	Ch	0,036	0,03	!	1,2
Root vegetables: Anise spray DW	10 - 39	chronic	weeds	1	2,9	5,9	2,3	0,72	0,72	0,72	Ch	0,018	0,03	OK	
Root vegetables: Anise spray DW	40 - 69	chronic	weeds	0,3	2,9	5,9	2,3	0,72	0,72	0,72	Ch	0,005	0,03	OK	
Root vegetables: Anise spray DW	10 - 39	chronic	field margin	0,0092	2,9	5,9	2,3	0,72	0,72	0,72	Ch	0,000	0,03	OK	
Root vegetables: Anise spray DW	40 - 69	chronic	field margin	0,0092	2,9	5,9	2,3	0,72	0,72	0,72	Ch	0,000	0,03	OK	
Root vegetables: Anise spray DW	10 - 39	chronic	adjacent crop	0,0033	5,8	9,9	5,7	0,72	0,72	0,72	Ch	0,000	0,03	OK	
Root vegetables: Anise spray DW	40 - 69	chronic	adjacent crop	0,0033	5,8	9,9	5,7	0,72	0,72	0,72	Ch	0,000	0,03	OK	
Root vegetables: Anise spray DW	10 - 39	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	Ch	0,003	0,03	OK	
Root vegetables: Anise spray DW	40 - 69	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	Ch	0,003	0,03	OK	

The chronic adult oral ETR for **IN005B1570** is below the trigger for downward sprays, indicating no potential risk with the exception of exposure to the treated crop between BBCH 10-69. As the risk assessment based on the EFSA Guidance (2013) is not approved yet and certain parts are currently under revision, this approach and its consequence on overall conclusion of bee risk assessment should be considered at national level of particular MSs.

#### First-tier assessment of the chronic risk for bees due to the use of IN005B1570 in leafy crops.

<b>Intended use</b>	Leafy crops
<b>Product</b>	IN005B1570
<b>Application rate (g product/ha)</b>	125 g/ha

Crop Category	Application	BBCH	category	scenario	Ef	SV HB	SV BB	SV SB	TWA HB	TWA BB	TWA SB	ETR HB	trigger	Risk indicator	Ratio
Leafy vegetables: Artich spray DW	10 - 49	chronic	treated crop	1	5,8	9,9	5,7	0,72	0,72	0,72	Ch	0,036	0,03	!	1,2
Leafy vegetables: Artich spray DW	10 - 49	chronic	weeds	1	2,9	5,9	2,3	0,72	0,72	0,72	Ch	0,018	0,03	OK	
Leafy vegetables: Artich spray DW	10 - 49	chronic	field margin	0,0092	2,9	5,9	2,3	0,72	0,72	0,72	Ch	0,000	0,03	OK	
Leafy vegetables: Artich spray DW	10 - 49	chronic	adjacent crop	0,0033	5,8	9,9	5,7	0,72	0,72	0,72	Ch	0,000	0,03	OK	
Leafy vegetables: Artich spray DW	10 - 49	chronic	next crop	1	0,54	0,78	0,49	0,72	0,72	0,72	Ch	0,003	0,03	OK	

The chronic adult oral ETR for **IN005B1570** is below the trigger for downward sprays, indicating no potential risk with the exception of exposure to the treated crop between BBCH 10-49. As the risk assessment based on the EFSA Guidance (2013) is not approved yet and certain parts are currently under revision, this approach and its consequence on overall conclusion of bee risk assessment should be considered at national level of particular MSs.

#### Updated July 2024

**Comments zRMS:** The risk to honeybees from exposure to IN005B1570 following its application in oilseed rape and orchards, carrots and leafy crops was assessed according to the SANCO/10329/2002 guidance. HQ values for oral and contact exposure are below the relevant trigger. Therefore, it can be assumed that the intended uses of IN005B1570 represent low acute and contact risk exposure to honeybees.

Furthermore, the screening and first-tier risk assessment, performed according to EFSA (2013) was performed by zRMS. All the ETRs and HQs for IN005B1570 indicate an acceptable acute and chronic risks to honeybees with exception of chronic bees (adult) risk for the application in oilseed rape for the exposure to the treated crop (BBCH 10-69), in carrots for the exposure to the treated crop (BBCH 10-69) and leafy crops for the exposure to the treated crop (BBCH 10-49).

The risk assessment based on the EFSA Guidance (2013) is not approved yet and certain parts are currently under revision. Therefore, this approach and its consequence on overall conclusion of bee risk assessment should be considered at national level of particular MSs.

The chronic risk assessment for bees should be considered by 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

No available information.

### 9.6.4 Effects on solitary bees

No available information.

### 9.6.5 Overall conclusions

The risk assessment performed for both the active substance and the formulated product derived hazard quotients lower than 50, indicating that the active substance as well as the formulation IN005B1570 pose an acceptable risk to bees from oral and contact exposure according to the proposed use.

## 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 Difenconazole. Full details of these studies are provided in the respective EU DAR and related documents as well as in table of this document (new studies).

Effects of IN005B1570 on non-target arthropods were not evaluated as part of the EU assessment of Difenconazole. New data submitted with this application are listed in Appendix 1 and summarised in **Błąd! Nie można odnaleźć źródła odwołania.**

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**

Species	Substance	Exposure System	Results (g as/ ha)	Reference
<i>Typhlodromus pyri</i> (adults)	IN005B1570	Laboratory test	LR <sub>50</sub> = 299 g/ha ER <sub>50</sub> = 181.3 g/ha	KCP10.3.2.1/02, Dini, R. (2021)
<i>Aphidius rhopalosiphi</i> (adults)	IN005B1570	Laboratory test	LR <sub>50</sub> = 326.7 g/ha ER <sub>50</sub> = 181.3 g/ha	KCP 10.3.2.1/01, Dini, R. (2021)

#### 9.7.1.1 Justification for new endpoints

Studies with IN005B1570 were not included in the EU review and have been conducted to characterise the risk from the current formulation. The studies were conducted with the two most sensitive indicator species, *A. rhopalosiphi* and *T. pyri* in the EU data set.

## 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

The potential risk of IN005B1570 to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ = exposure/toxicity) with the predicted environmental rate (PER) and the lowest lethal rate (LR50) values according to the following formula:

$$PER_{in-field} = \text{Application rate} \times \text{MAF}$$

The results of the risk assessment are summarized below.

**Table 9.7-2: First-tier assessment of the in-field risk for non-target arthropods due to the use of IN005B1570 in all crops**

Uses covered		Species	Results LR <sub>50</sub> (g as/ ha)	Nr. of application	Appl. Rate (g as/ha)	MAF	In-field HQ criterion: HQ ≤ 2
1	Oilseed rape	<i>T. pyri</i>	299	2	125	1.7	0.711
		<i>A. rhopalosiphi</i>	326.7	2	125	1.7	0.650
2	Apples, Pears;	<i>T. pyri</i>	299	3	56.25	2.3	0.433
		<i>A. rhopalosiphi</i>	326.7	3	56.25	2.3	0.396
3 - 6	Carrot Cauliflower Broccoli Cabbage	<i>T. pyri</i>	299	3	125	2.3	0.962
		<i>A. rhopalosiphi</i>	326.7	3	125	2.3	0.880

MAF: Multiple application factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

The in-field HQ value for exposure to maximum application rate for the representative species *T. pyri*, and *A. rhopalosiphi* is below the trigger value, therefore, no further consideration is required, and an acceptable risk is concluded following use according to the proposed use pattern.

#### **zRMS comment:**

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. The calculations of the risk assessment for in – field for two indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi* based on laboratory studies were corrected by zRMS. zRMS used the lowest toxicity endpoints from studies based on reproduction parameter (the worst case). Finally, the risk in - field for NTA is considered acceptable (HQ values were below 2 for these species).

**First-tier assessment of the in-field risk for non-target arthropods due to the use of IN005B1570 in all crops**

Uses covered		Species	Results LR <sub>50</sub> (g as/ha)	Nr. of application	Appl. Rate (g as/ha)	MAF	In-field HQ criterion: HQ ≤ 2
1	Oilseed rape	<i>T. pyri</i>	181.3	2	125	1.7	1.17
		<i>A. rhopalosiphi</i>	181.3	2	125	1.7	1.17
2	Apples, Pears;	<i>T. pyri</i>	181.3	3	56.25	2.3	0.71
		<i>A. rhopalosiphi</i>	181.3	3	56.25	2.3	0.71
3 - 6	Carrot	<i>T. pyri</i>	181.3	3	125	2.3	1.59
	Broccoli Cabbage	<i>A. rhopalosiphi</i>	181.3	3	125	2.3	1.59

MAF: Multiple application factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

Finally, the risk in - field for NTA is considered acceptable (HQ values were below 2 for these species).

### 9.7.2.2 Risk assessment for off-field exposure

Not relevant.

**zRMS comment:**

Risk assessment for off-field exposure was not performed by Applicant. The risk assessment for off-field exposure was performed by zRMS.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group “field crops” also covers the risk for non-target arthropods from intended uses in groups “oilseed rape”, “cauliflower”, “carrots”, “cabbage” and “broccoli”. The assessment for the use group “fruit crop” also covers the risk in groups “apple, pear”.

First-tier assessment of the off-field risk for non-target arthropods due to the use of IN005B1570

Extended laboratory test

<b>Intended use</b>		Field crops (oilseed rape)			
<b>Active substance/product</b>		IN005B1570			
<b>Application rate (g/ha)</b>		2 × 125			
<b>MAF</b>		1.7 (foliar)			
<b>vdf</b>		10 (2D) and 5 (2D)**, n.a. (3D)***			
<b>Test species Tier I</b>	<b>LR<sub>50</sub> (lab.) (g/ha)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub> (g/ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i> (2-D)	181.3	2.38%	1.0115	5	0.0279
<i>Aphidius rhopalosiphii</i> Mortality test (2-D) Reproduction test (3-D)	181.3		5.0575		0.1395
<b>Intended use</b>		Field crops (carrot, cauliflower, broccoli, cabbage)			
<b>Active substance/product</b>		IN005B1570			
<b>Application rate (g/ha)</b>		3 × 125			
<b>MAF</b>		2.3 (foliar)			
<b>vdf</b>		10 (2D) and 5 (2D)**, n.a. (3D)***			
<b>Test species Tier I</b>	<b>LR<sub>50</sub> (lab.) (g/ha)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub> (g/ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i> (2-D)	181.3	2.01%	1.15575	5	0.001275
<i>Aphidius rhopalosiphii</i> Mortality test (2-D) Reproduction test (3-D)	181.3		5.7775		0.15937
<b>Intended use</b>		Fruiting vegetables (apples, pears) BBCH 57-84			
<b>Active substance/product</b>		IN005B1570			
<b>Application rate (g/ha)</b>		3 × 56.25			
<b>MAF</b>		2.3 (foliar)			
<b>vdf</b>		10 (2D) and 5 (2D)**, n.a. (3D)***			
<b>Test species Tier I</b>	<b>LR<sub>50</sub> (lab.) (g/ha)</b>	<b>Drift rate</b>	<b>PER<sub>off-field</sub> (g/ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i> (2-D)	181.3	11.01	2.8488	5	0.07857
<i>Aphidius rhopalosiphii</i> Mortality test (2-D) Reproduction test (3-D)	181.3		14.2442		0.39283

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<sub>50</sub> or ER<sub>50</sub> from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

\*\*According to Working document on Risk Assessment of Plant Protection Products in the Central Zone (CZSC, May 2021) VDF of 5 should be used for all the tiers of the assessment as an interim solution until the revision of the current risk assessment scheme.

\*\*\* not applicable

The 1-tier studies for the indicator species *A.rhopalosiphii* and *T.pyri* was provided by Applicant.

The risk off-field for NTA for laboratory test for *A.rhopalosiphii* and *T.pyri* is considered acceptable (HQ values were below 2 for these species). To achieve a complete risk assessment, the risk envelope approach have been applied.

In addition, no unacceptable in and off-field risk were obtained after risk assessment with IN005B1570 formulation.

### 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

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

Effects on non-target arthropods other than bees for IN005B1570 have not been evaluated as part of an EU review. Data on IN005B1570 are evaluated here, and risk assessments for IN005B1570 with the proposed use pattern are provided here and are considered adequate.

In-field HQ values based on first tier laboratory studies with *Aphidius rhopalosiphii* and *Typhlodromus pyri* were below the trigger value of 2, and therefore, an acceptable risk is concluded following use according to the proposed use pattern.

### 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 difenoconazole, its relevant metabolite CGA 71019 and IN005B1570. Full details of these studies are provided in the respective EU DAR and related documents, as well as in table of this document (new studies).

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of IN005B1570 were not evaluated as part of the EU assessment of difenoconazole. Data on IN005B1570 are evaluated here. New data submitted with this application are listed in Appendix 1 and summarised in table.

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	Time scale	Results (mg a.s./ kg dw soil)	Reference
Earthworms				
<i>Eisenia foetida</i>	difenoconazole	Acute 14 days	LC <sub>50</sub> > 610 mg a.s./ kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967
<i>Eisenia foetida</i>	difenoconazole	Chronic	No reliable data	EFSA Scientific Report, 2011; 9 (1):1967
<i>Eisenia foetida</i>	IN005B1570	Acute 14 days	LC <sub>50</sub> > 227.2 mg a.s./ kg dw soil	KCP 10.4.1.1, Dini, R. 2021
<i>Eisenia foetida</i>	IN005B1570	Chronic	NOEC <sub>corr</sub> = 75.75 mg a.s./ kg dw NOEC = 151.5 mg a.s./ kg dw	KCP 10.4.1.1, Dini, R. 2021
<i>Eisenia foetida</i>	Metabolite CGA 71019	Acute	LC <sub>50</sub> > 1000 mg a.s./ kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967
<i>Eisenia foetida</i>	Metabolite CGA 71019	Chronic	NOEC = 1.0 mg a.s./ kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967

Species	Substance	Time scale	Results (mg a.s./ kg dw soil)	Reference
<i>Eisenia foetida</i>	Metabolite CGA 205375	Acute	LC <sub>50</sub> = 312 mg a.s./ kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967
<i>Eisenia fetida</i>	CGA205375	Mixed into substrate 56 d, chronic	NOEC = 9.6 mg/kg dw NOEC <sub>corr</sub> = 4.8 mg/kg dw*	Confirmatory data Difenconazole, EFSA (2014)
<b>Other soil macro-organisms</b>				
<i>Folsomia candida</i>	difenconazole	Chronic 28 days	NOEC = 500 mg a.s./ kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967
<i>Folsomia candida</i>	CGA205375	Mixed into substrate 56 d, chronic	NOEC = 2.4 mg/kg dw NOEC <sub>corr</sub> = 1.2 mg/kg dw*	Confirmatory data Difenconazole, EFSA (2014)
<i>Folsomia candida</i>	Metabolite CGA 71019	Chronic 28 days	NOEC = 1.8 mg a.s./ kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967
<i>Folsomia candida</i>	IN005B1570	Reproduction 28 days	NOEC <sub>corr</sub> = <b>5.05 mg a.s./ kg dw</b> NOEC = 10.1 mg a.s./ kg dw	KCP 10.4.2.1/01, Dini, R. (2021)
<i>Hypoaspis aculeifer</i>	IN005B1570	Reproduction 14 days	NOEC <sub>corr</sub> = <b>33.65 mg a.s./ kg dw</b> NOEC = 67.3 mg a.s./ kg dw	KCP 10.4.2.1/02, Dini, R. (2021)
<b>Single species tests</b>				
<i>Marasmius oraede</i>	difenconazole	6 days	NOEC 1.64 mg as/kg	EFSA Scientific Report, 2011; 9 (1):1967
<i>Mucor circinelloides</i>	difenconazole	3 days	NOEC 4.9 mg as/kg	EFSA Scientific Report, 2011; 9 (1):1967
<i>Paecilomyces marquandii</i>	difenconazole	17 days	NOEC 16.4 mg as/kg	EFSA Scientific Report, 2011; 9 (1):1967
<i>Phytophthora nicotianae</i>	difenconazole	17 days	NOEC 16.4 mg as/kg	EFSA Scientific Report, 2011; 9 (1):1967
<b>Field studies</b>				
<b>Litter bag test</b>				

Endpoints in **bold** are the ones used in the risk assessment

### 9.8.1.1 Justification for new endpoints

The earthworm study with IN005B1570 was not included in the EU review and has been conducted to characterise the risk from the current formulation. Tests with the formulation on *Folsomia* and *Hypoaspis* were also conducted.

### 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).

As stated in Commission Regulation EU No 284/2013 of 1 March 2013, “For plant protection products applied as a foliar spray, data on the relevant two non-target arthropod species might be taken into ac-

count for a preliminary risk assessment. If effects do occur on either species, testing on *Folsomia candida* and *Hypoaspis aculeifer* shall be required (see point 10.4.2.1)."

The formulated product IN005B1570 is applied as a foliar spray treatment. As demonstrated above, acceptable risks are expected towards the non-target arthropod species (*Typhlodromus pyri* and *Aphidius rhopalosiphi*). Nevertheless, a more conservative approach was considered and the risk with the two aforementioned species was assessed.

### 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, Tables 8.7-3 to 8.7-10. According to the assessment of environmental-fate data, multi-annual accumulation in soil does not need to be considered for Difenconazole.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group leafy vegetables also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses (see 9.1.2). Only a chronic risk assessment was carried out, in line with current data requirements. The results of the risk assessment for difenconazole, its metabolite and the formulation IN005B1570 are summarized below.

**Table 9.8-3: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) of the metabolites due to the use of IN005B1570 in leafy vegetables**

Intended use		Leafy vegetables		
Chronic effects on earthworms				
Product/active substance	Specie	NOEC (mg a.s./kg dw)	PEC <sub>soil</sub> initial Maximum PEC <sub>s</sub> (mg a.s./kg dw)	TER <sub>It</sub> (criterion TER ≥ 5)
Difenoconazole	E. foetida	No reliable data	0.428	-
CGA 71019		1	0.017 0.370 <sup>1</sup>	58.82 2.7
CGA205375		NOEC <sub>corr</sub> = 4.8	0.044	109.1
IN005B1570		75.75	0.428	176.99
Chronic effects on other soil macro- and mesofauna				
Product/active substance	Specie	NOEC (mg/kg dw)	PEC <sub>soil</sub> initial (mg/kg dw)	TER <sub>It</sub> (criterion TER ≥ 5)
Difenoconazole	F. candida	500	0.428	1168.22
CGA 71019		1.8	0.017 0.370 <sup>1</sup>	105.88 4.86
CGA205375		NOEC <sub>corr</sub> = 1.2	0.044	27.27
IN005B1570		5.05	0.428	11.80
IN005B1570	H. aculeifer	33.65	0.428	78.62

TER values shown in bold fall below the relevant trigger.

<sup>1</sup> based on  $DT_{50} = 346.6$  d (CRD, 2014; EFSA 2018)

**Table 9.8-3a: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) of the metabolites due to the use of IN005B1570 in carrots**

Intended use	Carrots
Chronic effects on earthworms	

Product/active substance	Specie	NOEC (mg a.s./kg dw)	PEC <sub>soil</sub> initial Maximum PEC <sub>s</sub> (mg a.s./kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Difenoconazole	E. foetida	No reliable data	0.225	-
CGA 71019		1	0.197 <sup>1</sup>	5.07
IN005B1570		75.75	0.225	336.6
CGA205375		NOEC <sub>corr</sub> = 4.8	0.023	208.7
Chronic effects on other soil macro- and mesofauna				
Product/active substance	Specie	NOEC (mg/kg dw)	PEC <sub>soil</sub> initial (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Difenoconazole	F. candida	500	0.225	2222.22
CGA 71019		1.8	0.197 <sup>1</sup>	9.14
CGA205375		NOEC <sub>corr</sub> = 1.2	0.023	52.2
IN005B1570		5.05	0.225	22.44
IN005B1570	H. aculeifer	33.65	0.225	149.56

TER values shown in bold fall below the relevant trigger.

<sup>1</sup> based on DT<sub>50</sub> = 346.6 d (CRD, 2014; EFSA 2018)

**First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) of the metabolites due to the use of IN005B1570 in pome/stone fruit**

Intended use		Carrots		
Chronic effects on earthworms				
Product/active substance	Specie	NOEC (mg a.s./kg dw)	PEC <sub>soil</sub> initial Maximum PEC <sub>s</sub> (mg a.s./kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Difenoconazole	E. foetida	No reliable data	0.145	-
CGA 71019		1	0.125 <sup>1</sup>	8
IN005B1570		75.75	0.145	522.41
CGA205375		NOEC <sub>corr</sub> = 4.8	0.015	320
Chronic effects on other soil macro- and mesofauna				
Product/active substance	Specie	NOEC (mg/kg dw)	PEC <sub>soil</sub> initial (mg/kg dw)	TER <sub>it</sub> (criterion TER ≥ 5)
Difenoconazole	F. candida	500	0.145	3448.28
CGA 71019		1.8	0.125 <sup>1</sup>	14.4
CGA205375		NOEC <sub>corr</sub> = 1.2	0.015	80
IN005B1570		5.05	0.145	34.83
IN005B1570	H. aculeifer	33.65	0.145	232.07

TER values shown in bold fall below the relevant trigger.

<sup>1</sup> based on DT<sub>50</sub> = 346.6 d (CRD, 2014; EFSA 2018)

**First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) of the metabolites due to the use of IN005B1570 in winter oilseed rape**

Intended use		Winter oilseed rape		
Chronic effects on earthworms				
Product/active substance	Specie	NOEC	PEC <sub>soil</sub> initial Maximum PEC <sub>s</sub>	TER <sub>it</sub>
		(mg a.s./kg dw)	(mg a.s./kg dw)	(criterion TER ≥ 5)
Difenoconazole	<i>E. foetida</i>	No reliable data	0.227	-
CGA 71019		1	0.196 <sup>1</sup>	5.10



CGA205375		NOEC <sub>corr</sub> = 4.8	0.023	208.69
IN005B1570		75.75	0.227	333.7
<b>Chronic effects on other soil macro- and mesofauna</b>				
Product/active substance	Specie	NOEC (mg/kg dw)	PEC <sub>soil</sub> initial (mg/kg dw)	TER <sub>t</sub> (criterion TER ≥ 5)
Difenconazole	<i>F. candida</i>	500	0.227	2202.64
CGA 71019		1.8	0.196 <sup>1</sup>	9.18
CGA205375		NOEC <sub>corr</sub> = 1.2	0.023	52.17
IN005B1570		5.05	0.227	22.25
IN005B1570	<i>H. aculeifer</i>	33.65	0.227	148.24

TER values shown in bold fall below the relevant trigger.

<sup>1</sup> based on DT<sub>50</sub> = 346.6 d (CRD, 2014; EFSA 2018)

The chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to difenconazole, its relevant metabolite CGA 71019 and the formulation IN005B1570 are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that the risk to earthworms and other non-target soil organisms (meso- and macrofauna) is acceptable following use of IN005B1570 according to the proposed use pattern.

**zRMS comment:** The risk assessment provided by Applicant was accepted by RMS. The long-term TER value exceed the Annex VI long-term trigger value of 5 indicating that **INDOFIL Difenconazole 250 EC** was not poses long-term risk to earthworms and other non-target soil organisms (meso- and macrofauna) when applied according to the proposed use rates.

According to the AT comment - Study KCP 10.4.1.1/01, Dini, R. (2021) - NOEC should be 296.3 mg product/kg soil dw.

RMS comments: Bodyweight changes should be treated with caution in replicates where mortality occurred, as it is not known whether the individual(s) that died were relatively heavy or light. No behavioral and pathological signs were observed in the surviving worms after the period of exposure. On the other hand bodyweight changes was high in the negative control (22%). There is also no clear dose-response relationship in terms of the bodyweight changes parameter and no statistically significant changes in body weight were recorded at the tested concentrations. In our opinion in this case NOEC should be 666.7 mg product/kg soil dw. On the other hand TER with high margin of safety was achieved for formulation (TER = 176.99). In our opinion, the conclusion of the risk assessment for earthworms should not be changed. The risk assessment for earthworms should be considered by MSs level.

PECs for the metabolite CGA 71019 was corrected according to dRR Part B8 based on DT<sub>50</sub> = 346.6 d (CRD, 2014; EFSA 2018). For the metabolite CGA 71019 the risk assessment for earthworms and *F.candida* did not meet the trigger value of 5 for leafy crops. The risk for leafy crops in terms of soil organisms such as earthworms and *F.candida* is unacceptable. Refinement risk assessment for soil organisms such as earthworms and *F.candida* for leafy crops is required.

Risk assessment for earthworms and other soil macroorganisms such as *F.candida* as well as *H. aculeifer* for CGA 71019 for winter oilseed rape, orchards and carrots is acceptable.

Risk assessment for earthworms and other soil macroorganisms such as *F.candida* for metabolite CGA-20535 was performed by zRMS. The risk assessment for earthworms and other soil macroorganisms such as *F.candida* for metabolite CGA-20535 is low.

**Risk assessment for soil organisms should be considered by MSs level.**

### 9.8.2.2 Higher-tier risk assessment

Not relevant.

### 9.8.3 Overall conclusions

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

The chronic TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exposed to difenoconazole, its relevant metabolite CGA 71019 and the formulation IN005B1570 are greater than the Commission Regulation (EU) No. 546/2011 trigger of 5, indicating that the risk to earthworms and other non-target soil organisms (meso- and macrofauna) is acceptable following use of IN005B1570 according to the proposed use pattern.

**zRMS comment:** ~~Agreed.~~ The risk for leafy crops in terms of soil organisms such as earthworms and *F.candida* is unacceptable. Refinement risk assessment for soil organisms such as earthworms and *F.candida* for leafy crops is required.

Risk assessment for earthworms and other soil macroorganisms such as *F.candida* as well as *H. aculeifer* for winter oilseed rape, orchards and carrots is acceptable.

## 9.9 Effects on soil microbial activity (KCP 10.5)

### 9.9.1 Toxicity data

Studies on effects on soil microorganisms have been carried out with Difenoconazole and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents as well as in of this document (new studies).

Effects on soil microorganisms of IN005B1570 were not evaluated as part of the EU assessment of Difenoconazole. New data submitted with this application are listed in Appendix 1 and summarised in **Błąd! Nie można odnaleźć źródła odwołania..**

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

Species	Substance	Time scale	Results (mg a.s./ kg dw soil)	Reference
<b>Soil micro-organisms</b>				
N- mineralisation	Difenoconazole	28 days	<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 Scientific Report, 2011; 9 (1):1967
N- mineralisation	Metabolite CGA 71019	28 days	<25% effect at day 28 at 0.035 and 0.353 mg a.s./kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967

Species	Substance	Time scale	Results (mg a.s./ kg dw soil)	Reference
N- mineralisation	Metabolite CGA 205375	28 days	<25% effect at day 28 at 0.09 and 0.22 mg a.s./kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967
N- mineralisation	IN005B1570	28 days	< 25 % effect at day 28 at 0.46 and 2.3 mg/kg d.w., expressed as formulation IN005B1570	KCP 10.5, Tediosi, E. (2021)
<del>C- mineralisation</del>	<del>Difenconazole</del>	<del>28 days</del>	<del>&lt;25% effect at day 28 at 1.67 and 16.7 mg a.s./kg dw soil</del>	<del>EFSA Scientific Report, 2011; 9 (1):1967</del>
<del>C- mineralisation</del>	<del>Metabolite CGA 71019</del>	<del>28 days</del>	<del>&lt;25% effect at day 28 at 0.035 and 0.353 mg a.s./kg dw soil</del>	<del>EFSA Scientific Report, 2011; 9 (1):1967</del>
<del>C- mineralisation</del>	<del>Metabolite CGA 205375</del>	<del>28 days</del>	<del>&lt;25% effect at day 28 at 0.09 and 0.22 mg a.s./kg dw soil</del>	<del>EFSA Scientific Report, 2011; 9 (1):1967</del>

### 9.9.1.1 Justification for new endpoints

The N-mineralisation study with IN005B1570 was not included in the EU review and has been conducted to characterise the risk from the current 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).

In the study conducted with the formulation, on the 28th day the difference (mean value) between nitrogen transformation rate in untreated soil and treated soils was lower than 25%. Therefore, effects at expected soil concentrations for proposed uses of IN005B1570 are below the Commission Regulation (EU) No. 546/2011 trigger of 25%, indicating that the risk to soil micro-organisms is acceptable following use of IN005B1570 according to the proposed use pattern.

### 9.9.3 Overall conclusions

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

Effects at expected soil concentrations for proposed uses of IN005B1570 are below the Commission Regulation (EU) No. 546/2011 trigger of 25%, indicating that the risk to soil micro-organisms is acceptable following use of IN005B1570 according to the proposed use pattern.

**zRMS comment:** The risk assessment was accepted by RMS. Study on effects of formulation INDOFIL Difenconazole 250 EC on soil nitrogen turnover was evaluated by the zRMS and considered acceptable. For details of evaluation, please refer to Appendix 2. Provided above endpoints are confirmed to be correct. Information regarding effects of difenconazole and metabolite CGA 71019 and CGA 205375 on nitrogen mineralisation is in line with the EU agreed data reported in EFSA Scientific Report, 2011; 9

(1):1967). Information regarding effects on carbon mineralisation is no longer a data requirement and for this reason it was struck through in Table 9.9-1.

**Updated April 2024**

### **Risk assessment for microorganisms for leafy crops**

Intended use	Leafy crops (covers intended uses on oilseed rape and orchards, carrots) 3 x 125 g s.a.ha		
N-mineralisation			
Product	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable?
Difenoconazole	16.7 (at 28 d)	0.428	Yes
CGA71019	0.353 (at 28 d)	0.370	Yes*
CGA205375	0.22 (at 28 d)	0.044	Yes
IN005B1570	2.3 (at 28 d)	0.570	Yes

PECs for metabolite CGA71019 is a little bit higher than max. conc. with effects ≤ 25 % for leafy crops. However, the above difference is small (only 4.6% compared to the PECs) and since PECs is based on the worst case based on DT<sub>50</sub> = 346.6 d (CRD, 2014; EFSA 2018) in opinion of RMS, the risk assessment for metabolite CGA71019 to soil microorganisms in this case should be accepted. It should be considered by MSs level.

### **Risk assessment for microorganisms for carrots**

Intended use	Carrots 3 x 125 g s.a.ha		
N-mineralisation			
Product	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable?
Difenoconazole	16.7 (at 28 d)	0.225	Yes
CGA71019	0.353 (at 28 d)	0.197	Yes
CGA205375	0.22 (at 28 d)	0.023	Yes
IN005B1570	2.3 (at 28 d)	0.304	Yes

### **Risk assessment for microorganisms for pome/stone fruit**

Intended use	Pome/stone fruit (3 x 56.25 g/ha)		
N-mineralisation			
Product	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable?
Difenoconazole	16.7 (at 28 d)	0.145	Yes
CGA71019	0.353 (at 28 d)	0.125	Yes
CGA205375	0.22 (at 28 d)	0.015	Yes
IN005B1570	2.3 (at 28 d)	0.137	Yes

### **Risk assessment for microorganisms for winter oilseed rape**

Intended use	Oilseed rape (2 x 125 g/ha)		
N-mineralisation			
Product	Max. conc. with effects ≤ 25 % (mg/kg dw)	PEC <sub>soil</sub> (mg/kg dw)	Risk acceptable?
Difenoconazole	16.7 (at 28 d)	0.227	Yes
CGA71019	0.353 (at 28 d)	0.196	Yes
CGA205375	0.22 (at 28 d)	0.023	Yes
IN005B1570	2.3 (at 28 d)	0.456	Yes
Effects at expected soil concentrations for proposed uses of IN005B1570 are below the Commission Regulation (EU) No. 546/2011 trigger of 25%, indicating that the risk to soil micro-organisms is acceptable following use of IN005B1570 according to the proposed use pattern.			

## 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 IN005B1570. Full details of these studies are provided in of this document.

Effects on non-target terrestrial plants of IN005B1570 were not evaluated as part of the EU assessment of Difenoconazole. New data submitted with this application are listed in Appendix 1 summarised. 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	ER <sub>50</sub> (g a.i./ha)	Reference
<i>Avena sativa</i> <i>Brassica napus</i> <i>Glycine maxima</i>	Difenoconazole	Emergence	> 10 mg a.s./ kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967
<i>Avena sativa</i> <i>Brassica napus</i> <i>Glycine maxima</i>	Difenoconazole	Vegetative vigour	> 10 mg a.s./ kg dw soil	EFSA Scientific Report, 2011; 9 (1):1967
<i>Avena sativa</i> <i>Brassica napus</i> <i>Allium cepa</i> <i>Lolium perenne</i> <i>Raphanus sativus</i> <i>Glycine max</i>	IN005B1570	Seedling	> 1562.5 g a.s./ ha	KCP 10.6.2/02, Noe, F. (2021)
<i>Avena sativa</i> <i>Brassica napus</i> <i>Allium cepa</i>	IN005B1570	Vegetative vigour	> 1562.5 g a.s./ ha	KCP 10.6.2/01, Noe, F. (2021)

Species	Substance	Exposure System	ER <sub>50</sub> (g a.i./ha)	Reference
<i>Lolium perenne</i> <i>Raphanus sativus</i> <i>Glycine max</i>				

### 9.10.1.1 Justification for new endpoints

The seedling emergence and vegetative vigour test studies with IN005B1570 were not included in the EU review and have been conducted to characterise the risk from the current 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”, (SAN-CO/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.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group leafy vegetables also covers the risk for non-target terrestrial plants from all other intended uses

**Table 9.10-2: Assessment of the risk for non-target plants due to the use of IN005B1570 in leafy vegetables**

<b>Intended use</b> <b>Active substance/product</b> <b>Application rate (g/ha)</b> <b>MAF</b>	Leafy vegetables Difenoconazole/ IN005B1570 3 × 125 g a.s./ha 2.3			
<b>Test species</b>	<b>ER<sub>50</sub></b> (g a.s./ ha)	<b>Drift rate</b> (%)	<b>PER<sub>off-field</sub></b> (g/ha)	<b>TER</b> <b>criterion:</b> <b>TER ≥ 5</b>
<i>Avena sativa</i> <i>Brassica napus</i> <i>Allium cepa</i> <i>Lolium perenne</i> <i>Raphanus sativus</i> <i>Glycine max</i>	1562.5	2.77	7.96	196.20

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

<b>Intended use</b>	Leafy vegetables
<b>Active substance/product</b>	Difenoconazole/ IN005B1570

<b>Application rate (g/ha)</b> <b>MAF</b>	3 × 125 g a.s./ha 1			
<b>Test species</b>	<b>ER<sub>50</sub></b> <b>(g a.s./ ha)</b>	<b>Drift rate</b> <b>(%)</b>	<b>PER<sub>off-field</sub></b> <b>(g/ha)</b>	<b>TER</b> <b>criterion:</b> <b>TER ≥ 5</b>
<i>Avena sativa</i> <i>Brassica napus</i> <i>Allium cepa</i> <i>Lolium perenne</i> <i>Raphanus sativus</i> <i>Glycine max</i>	1562.5	2.77	3.46	451.6

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

The TER value is above the trigger value of 5, therefore, acceptable risks are expected due to application of IN005B1570 in the intended uses.

**zRMS comment:** Agreed. Calculations in terms of risk assessment for non-target plants are accepted by RMS. No additional calculations are needed. Acceptable risks are expected due to application of IN005B1570 in the intended uses. No buffer zone is required.

### 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

The worst-case TER values are well greater than the trigger value of 5 and therefore it is considered that risks to non-target plants after IN005B1570 applications are acceptable.

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

No available data.

## 9.12 Monitoring data (KCP 10.8)

No available data.

## 9.13 Classification and Labelling

According to the criteria given in Regulation (EC) No 1272/2008 of the European Parliament and of the

Council of 16 December 2008, the following classification and labelling with regard to ecotoxicological data is proposed for the preparation:

**Table 9.13-1: Justified proposals for classification and labelling for INDOFIL Difenconazole 250 g/L EC (IN005B1570) according to Regulation (EC) No 1272/2008**

Hazard class(es), categories	Acute Toxicity (Category 4) Eye irritation (Category 2) <del>Aquatic Acute Category 1</del> Aquatic Chronic Category 1
Hazard pictograms or Code(s) for hazard pictogram(s)	GHS07 GHS09
Signal word	Warning
Hazard statement(s)	H302 H319 <del>H400</del> H410
Precautionary statement(s)	P270, P273, P280, P301+P312, P305+P351+P338, P337+P313, P391, P501
Additional labelling phrases	To avoid risks to man and the environment, comply with the instructions for use. [EUH401]

**Justification:**

The formulation IN005B1570 should not be classified as Aquatic Acute 1 (H400), since the acute toxicity endpoints determined in the available studies with IN005B1570 for all the 3 trophic levels are higher than 1 mg/L (lowest EC<sub>50</sub> = 3.9 mg IN005B1570/L for *Daphnia magna*). The zRMS agrees with the proposed chronic classification as Aquatic Chronic 1 (H410), achieved using the summation method and based on the harmonized classification of the active substance difenconazole.



## Appendix 1 Lists of data considered in support of the evaluation

### List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	Noe, F.	2021	Difenoconazole 250 g/L EC greener – IN005B1570: Acute Toxicity to <i>Daphnia magna</i> in a 48-hour Immobilization Test under Semi-Static Exposure ChemService Study No. CH – 0249/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.2.1/02	Noe, F.	2021	Difenoconazole 250 g/L EC greener – IN005B1570: Toxicity to Green Algae <i>Pseudokirchneriella subcapitata</i> in a Growth Inhibition Study ChemService Study No. CH – 0250/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.2.1/03	■	2021	Difenoconazole 250 g/L EC greener – IN005B1570: ■ GLP Unpublished	Y	Indofil Industries (Netherlands) B.V.
KCP 10.3.1.1.1	Ponti, B.	2021	Difenoconazole 250 g/L EC greener – IN005B1570.: Acute Contact and Oral Toxicity to adult worker honeybees <i>Apis mellifera</i> L. ChemService Study No. CH – 0251/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.3.1.2	Ponti, B.	2023	Difenoconazole 250 g/L EC greener – IN005B1570.: Chronic Oral Toxicity to adult worker honeybees <i>Apis mellifera</i> L. (10-day feeding) ChemService Study No. CH – 0103/2023 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.3.1.3	Noe, F.	2023	Difenoconazole 250 g/L EC greener – IN005B1570.: honeybees <i>Apis mellifera</i> L Larval Toxicity Test with Repeated Exposure ChemService Study No. CH – 0104/2023 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.3.2.1/01	Dini, R	2021	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on the Parasitoid <i>Aphidius rhopalosiphii</i> De Stefani Perez (Hymenoptera, Braconidae) under laboratory conditions ChemService Study No. CH – 0253/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.3.2.1/02	Dini, R	2021	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on the predatory mite <i>Typhlodromus pyri</i>	N	Indofil Industries

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
			<i>Scheuten</i> (Acari: Phytoseiidae) under laboratory conditions ChemService Study No. CH – 0252/2021 GLP Unpublished		(Netherlands) B.V.
KCP 10.4.1.1	Dini, R	2021	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on Reproduction of Earthworm <i>Eisenia fetida</i> in an Artificial Soil Study ChemService Study No. CH – 0254/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.4.2.1/01	Dini, R	2021	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on Collembolan Reproduction in an Artificial Soil Study ChemService Study No. CH – 0255/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.4.2.1/02	Dini, R	2021	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on <i>Hypoaspis (Geolaelaps) aculeifer</i> Reproduction in an Artificial Soil Study ChemService Study No. CH – 0256/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.5	Tediosi, E	2022	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on Soil Microorganisms - Nitrogen Transformation Test ChemService Study No. CH – 0770/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.6.2/01	Noe, F.	2022	Difenoconazole 250 g/L EC greener – IN005B1570: Vegetative Vigour Test of Terrestrial Plants ChemService Study No. CH – 0772/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.
KCP 10.6.2/02	Noe, F.	2022	Difenoconazole 250 g/L EC greener – IN005B1570: Seedling Emergence and Seedling Growth Test of Terrestrial Plants ChemService Study No. CH – 0771/2021 GLP Unpublished	N	Indofil Industries (Netherlands) B.V.

**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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	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
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

## Appendix 2 Detailed evaluation of the new studies

### A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

#### A 2.1.1 KCP 10.1.1 Effects on birds

##### A 2.1.1.1 KCP 10.1.1.1 Acute oral toxicity

##### A 2.1.1.2 KCP 10.1.1.2 Higher tier data on birds

#### A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

##### A 2.1.2.1 KCP 10.1.2.1 Acute oral toxicity to mammals

##### A 2.1.2.2 KCP 10.1.2.2 Higher tier data on mammals

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

### A 2.2 KCP 10.2 Effects on aquatic organisms

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

##### A 2.2.1.1 KCP 10.2.1/01 Study 1

Comments of zRMS:	Study was carried out according to appropriate OECD 202 and all validity criteria were met.	
	<p>The validity criteria:</p> <p>The test is not accepted if more than 10% of the control daphnids are immobilized during the 48-hour test period or more than 10% shows other signs of disease or stress as, for example, trapping at surface of water. The oxygen concentration at the end of the test should be <math>\geq 3</math> mg/L in control and in the test vessels.</p> <p>In the study:</p>	
	Control Immobilisation Rate:	In the negative control, 0% immobilization was observed and no daphnid was trapped on the test water surface or showed other signs of disease or stress, thus the validity criterion was met.
	Dissolved Oxygen Con-	Was $\geq 5.78$ mg O <sub>2</sub> /L in in all treatment groups at

	centration:		the end of the test; thus validity criterion was met.		
	Agreed endpoints:				
	Time (h)	EC <sub>0</sub> (mg/L)	EC <sub>50</sub> (mg/L)	EC <sub>100</sub> (mg/L)	NOEC (mg/L)
	Nominal test item concentration				
	24	2.1 (N/A – N/A) (*)	4.9 (4.1 – 5.8) (*)	10.0 (N/A – N/A) (*)	2.1
	48	0.94 (N/A – N/A) (*)	3.9 (3.2 – 4.7) (*)	10.0 (N/A – N/A) (*)	2.1
	Nominal concentration of the active ingredient				
	24	0.47 (N/A – N/A) (*)	1.1 (0.93 – 1.3) (*)	2.3 (N/A – N/A) (*)	0.47
	48	0.21 (N/A – N/A) (*)	0.89 (0.73 – 1.1) (*)	2.3 (N/A – N/A) (*)	0.47
	(*) 95% confidence limits N/A = not applicable				
The study is considered acceptable.					

Reference:	KCP 10.2.1/01, Noe, F. (2021)
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Acute Toxicity to <i>Daphnia magna</i> in a 48-hour Immobilization Test under Semi-Static Exposure. Noe, F., 2021, ChemService Study No. CH – 0249/2021
Guideline(s):	Yes <ul style="list-style-type: none"> <li>- OECD Guideline for Testing of Chemicals No. 202. “Daphnia sp., Acute Immobilization Test”, April 2004.</li> <li>- OECD Series on Testing and Assessment No. 23 - Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures ENV/JM/MONO(2000)6/REV1 (2019).</li> </ul>
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Objective

The purpose of this study was to determine the influence of the test item Difenoconazole 250 g/L EC greener – IN005B1570 on the mobility of *Daphnia magna*. For this purpose, young daphnids (< 24 hours old) were exposed in a semi-static test at six different nominal concentrations in a geometric series, differing by a constant factor (of 2.2): 0.19, 0.43, 0.94, 2.1, 4.5 and 10 mg/L. These test item concentrations correspond to 0.044, 0.10, 0.21, 0.47, 1.0 and 2.3 mg Difenoconazole/L. The effects in the mobility of the daphnids were recorded after 24 and 48 hours. The test method of application and the test system are recommended by the test guidelines and *Daphnia magna* is the recommended test species. The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium.

## Materials and methods

Test Item: Difenoconazole 250 g/L EC greener – IN005B1570; batch no.: ID\_F021\_0321\_1; content of a.i.: Difenoconazole: 250 g/L(nominal);

259 g/L (analytical) according to certificate of analysis

Test Species:	<p>The study was performed on the freshwater crustacean <i>Daphnia magna</i> Straus. At the test start the organisms used were less than 24 hours old and were not first brood progeny.</p> <p>Source: A strain of this species was supplied by MicroBioTests Inc., Belgium in July 2017 (batch number: DM060417). Since that time, the clone has been bred in the laboratories of the Test Facility under conditions identical to those of the test, with regard to temperature, light and water quality, so that no acclimation was needed prior to testing.</p>
Test Design:	<p>The test was performed in glass beakers filled with 40 mL of test medium. The daphnids were randomly distributed into the test vessels at the test initiation. For the test concentration and for the negative control, group of 20 daphnids were used divided into four replicates of five daphnids each. The daphnids were randomly distributed to the test vessels at test initiation. The daphnids loading rate was more than 2 mL per one daphnid. The mobility of the daphnids was determined in a semi-static 48-hour test by visual observation after 24 and 48 hours.</p>
Endpoints:	Number of immobile organisms after 24 and 48 hours
Test Concentrations:	Six different nominal concentrations in a geometric series, differing by a constant factor (of 2.2): 0.19, 0.43, 0.94, 2.1, 4.5 and 10 mg test item/ L, which correspond to 0.044, 0.10, 0.21, 0.47, 1.0 and 2.3 mg Difenconazole/L
Test Conditions:	<p>Incubation temperature: 19.96 – 20.04°C with a mean value of 20.0°C and a standard deviation of 0.02°C. Daily photoperiod: 16 hours light and 8 hours darkness. Light intensity: the measured values were in the range 1098 – 1121 Lux, according to the provided range (1000-1500 Lux). pH: 7.27 – 7.92. Dissolved oxygen concentration: &gt; 5.0 mg/L (range: 5.90 – 7.74 mg/L).</p>
Statistical methods	<p>CETIS v. 1.8.7.7 software was used to carry out the statistical analysis. The EC<sub>0</sub> and EC<sub>100</sub> was determined directly from the Raw Data. The EC<sub>50</sub> values at 24 and 48 hours were determined by statistical analysis with Spearman-Kärber method. The NOEC values at 24 and 48 hours were determined by statistical analysis with Fisher Exact/Bonferroni-Holm Test. As input data, nominal test item concentrations were used.</p>

## Results and discussions

A full dose-response test was performed to evaluate the effect of the test item on *Daphnia magna* under static conditions.

The daphnids were observed for immobility after 24 and 48 hours of exposure. The observed immobilization in the test item concentrations is reported in the following table.

**Table 1. Summary of Biological Results**

Nominal test item concentration (mg/L)	% of immobilized <i>Daphnia</i>	
	24 h	48 h

0.0 (negative control)	0	0
0.19	0	0
0.43	0	0
0.94	0	0
2.1	0	10
4.5	40	60
10	100	100

The obtained experimental results allowed calculation of the EC<sub>0</sub>, EC<sub>100</sub>, EC<sub>50</sub> values and the NOEC at 24 and 48 hours. Results, expressed in terms of nominal test item concentrations and nominal active ingredient concentrations, are reported in the following table.

**Table 2. Summary of Biological Results**

Time (h)	EC <sub>0</sub> (mg/L)	EC <sub>50</sub> (mg/L)	EC <sub>100</sub> (mg/L)	NOEC (mg/L)
Nominal test item concentration				
24	2.1 (N/A – N/A) (*)	4.9 (4.1 – 5.8) (*)	10.0 (N/A – N/A) (*)	2.1
48	0.94 (N/A – N/A) (*)	3.9 (3.2 – 4.7) (*)	10.0 (N/A – N/A) (*)	2.1
Nominal concentration of the active ingredient				
24	0.47 (N/A – N/A) (*)	1.1 (0.93 – 1.3) (*)	2.3 (N/A – N/A) (*)	0.47
48	0.21 (N/A – N/A) (*)	0.89 (0.73 – 1.1) (*)	2.3 (N/A – N/A) (*)	0.47

(\*) 95% confidence limits

N/A = not applicable

#### Analytical test results

The quantification of the active ingredient Difenoconazole of the test item Difenoconazole 250 g/L EC greener – IN005B1570 in the test samples was performed using liquid chromatography with MS/MRM detection.

In fresh solutions, prepared at time 0 and 24 hours, the analytical recoveries of Difenoconazole were in the range 101.5% - 119.9% and 82.9% - 120.2% of the nominal values, respectively.

In spent solutions (after 24 and 48 hours of exposure), the analytical recoveries of Difenoconazole were in the range 114.6% - 118.9% and 117.2% - 120.0% of the nominal values.

The analytical recoveries were referred to Difenoconazole nominal content calculated from the nominal content in g/L and the density declared on the Certificate of Analysis supplied by the Sponsor (resulting in 22.7 % w/w).

Since all analytical recoveries were in the range 80 % - 120 % of the nominal concentrations, the biological results were referred to the test item nominal concentrations and to the nominal active ingredient concentrations, as recommended by OECD Guidance Document on testing of difficult substances No. 23, 2019.

#### Conclusion

The EC<sub>0</sub>, the EC<sub>100</sub>, the EC<sub>50</sub> value and the NOEC at 24 and 48 hours under semi-static conditions were assessed.

The 24- hour EC<sub>0</sub>, EC<sub>50</sub>, EC<sub>100</sub> and NOEC were found to be respectively 2.1, 4.9, 10, and 2.1 mg/L of the test item Difenoconazole 250 g/L EC greener – IN005B1570 with 95 % confidence, which is equivalent to 0.47, 1.1, 2.3, and 0.47 mg/ L of the active ingredient Difenoconazole, respectively.

The 48- hour EC<sub>0</sub>, EC<sub>50</sub>, EC<sub>100</sub> and NOEC were found to be respectively 0.94, 3.9, 10, and 2.1 mg/L of

the test item Difenoconazole 250 g/L EC greener – IN005B1570 with 95 % confidence, which is equivalent to 0.21, 0.89, 2.3, and 0.47 mg/ L of the active ingredient Difenoconazole, respectively.

#### Validity criteria

Control Immobilisation Rate:	In the negative control, 0% immobilization was observed and no daphnid was trapped on the test water surface or showed other signs of disease or stress, thus the validity criterion was met.
Dissolved Oxygen Concentration:	Was $\geq 5.78$ mg O <sub>2</sub> /L in in all treatment groups at the end of the test; thus validity criterion was met.

#### A 2.2.1.2 KCP 10.2.1/02 Study 2

Comments of zRMS:	Study was carried out according to appropriate OECD 201 (2006) and all validity criteria were met.					
	The validity criteria: After 72h:					
	Cell Density Increase in Control Cultures:			At the test end the cell density in the negative control had increased on average by a factor of 652, corresponding to a daily specific growth rate equal to 2.16. This value complies with the validity criterion of the test		
	Coefficient of Variation of Sectional (Daily) Growth Rates in Control Cultures:			16.0 % and thus, the validity criterion was met.		
	Coefficient of Variation of Average Growth between Control Replicates:			0.4 % and thus, the validity criterion was met.		
	<i>Pseudokirchneriella subcapitata</i>					
	<b>Agreed endpoints*:</b>					
	Time (h)	E <sub>r</sub> C <sub>10</sub> (mg a.i./L)	E <sub>r</sub> C <sub>20</sub> (mg a.i./L)	E <sub>r</sub> C <sub>50</sub> (mg a.i./L)	NOEC (mg a.i./L)	LOEC (mg a.i./L)
	<b>Nominal test item concentration</b> Geometric mean of a.i. difenoconazole measured concentrations					
	0 - 24	2.3 (N/A – 4.1)	4.5 (2.6 – 5.7)	18.9 (14.2– 22.2)	0.90	2.9
0 - 48	2.8 (1.8 – 3.5)	5.0 (4.1 – 5.9)	14.5 (13.1 – 15.8)	0.90	2.9	
0 - 72	1.7 (1.4 – 2.0)	3.2 (3.0 – 3.4)	7.5 (6.9 – 8.0)	0.30	0.90	
<b>Nominal concentration of the active ingredient</b> Geometric mean of a.i. difenoconazole measured concentrations						
0 - 24	0.52 (N/A – 0.93)	1.0 (0.59 – 1.3)	4.3 (3.2 – 5.0)	0.20	0.66	
0 - 48	0.64 (0.41 – 0.79)	1.1 (0.93 – 1.3)	3.3 (3.0 – 3.6)	0.20	0.66	
0 – 72	0.39 (0.32 – 0.45)	0.73 (0.68 – 0.77)	1.7 (1.6 - 1.8)	0.068	0.20	
* Since not all the analytical recoveries were in the range 80%-120% of the nominal concentrations during the test period, the biological results were referred to geometric mean of a.i. Difenoconazole measured concentrations, as recommended by OECD Guideline No. 201.						
<b>The study is considered acceptable.</b>						



Reference:	KCP 10.2.1/02, Noe, F. (2021)
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Toxicity to Green Algae <i>Pseudokirchneriella subcapitata</i> in a Growth Inhibition Study. Noe, F., 2021, ChemService Study No. CH – 0250/2021
Guideline(s):	Yes <ul style="list-style-type: none"> <li>- OECD Guideline for Testing of Chemicals No. 201, “Freshwater alga and cyanobacteria, growth inhibition test”, 2011.</li> <li>- OECD Series on Testing and Assessment No 23 - Guidance Document On Aquatic Toxicity Testing Of Difficult Substances And Mixtures ENV/JM/MONO(2019).</li> </ul>
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Objective

The purpose of this study is to determine the toxicity of the test item to fresh-water green algae *Pseudokirchneriella subcapitata*. For this purpose, an exponentially growing algal population was exposed for 72 hours to an aqueous medium containing the test item at five different nominal concentrations. The inhibition of growth in relation to control cultures was determined over a test period of 72 hours, and thus over several algal generations. The test method of application and the test system are recommended by the test guidelines and *Pseudokirchneriella subcapitata* is one of the recommended test species. The purpose of the analytical part of this study was to verify the concentrations of the test item in the test medium.

## Materials and methods

Test Item:	Difenoconazole 250 g/L EC greener – IN005B1570; batch no.: ID_F021_0321_1; content of a.i.: Difenoconazole:250 g/L(nominal); 259 g/L (analytical) according to certificate of analysis
Test Species:	The study was performed with the unicellular, fresh-water green algae <i>Pseudokirchneriella subcapitata</i> strain SAG 61.81 (also known as <i>Raphidocelis subcapitata</i> ; formerly known as <i>Selenastrum capricornutum</i> ), cultured in the laboratories of the Test Facility and originally purchased from the Institute of Plant Physiology of the University of Göttingen, Germany.
Test Design:	<p>Three replicates for each test concentrations and six for negative control were prepared. At test start, 50 mL of the relevant test solution was poured into each test flask and inoculated with algae to obtain a starting cell density of about 104 cells/mL.</p> <p>The inoculum was taken from a pre-culture started 3 days before the test beginning (containing approximately 106 cells/mL), in order to ensure that the assay was performed with an exponentially growing population. The test flasks were incubated in a controlled environment chamber, under continuous illumination and constant shaking. The flasks were randomly distributed under the light and moved every day during the test.</p> <p>Algal cell density was measured every 24 hours by taking aliquots from each test concentration replicate and the negative control, dilut-</p>

ing them in a NaCl 9 g/L solution and reading with an electronic particle counter. The measured values were used to calculate the percentage inhibition of cell growth as yield and as growth rate in comparison to the solvent control.

**Endpoints:** The percent inhibition of end-points growth rate and yield at 24, 48 and 72 hours of exposure compared to the negative control.

**Test Concentrations:** Negative control (algal medium without test item) and 0.30, 0.90, 2.9, 9.4 and 30 mg test item/L.; corresponding to 0.068, 0.20, 0.66, 2.1 and 6.8 mg/L as active ingredient Difenconazole (calculated considering test item active ingredient content and density reported in the CoA, resulting in a purity of 22.7% w/w).

**Test Conditions:** Test environment: controlled environmental test chamber. Incubation temperature: 23.89 – 24.26°C. Light regime: continuous illumination. Light intensity: 6581 - 7208 Lux. pH: 7.88 - 7.96 at the test start; 7.78 – 8.18 at the test end (mean values)

**Statistical methods** The CETIS v.1.8.7.7 software was used to carry out the statistical analysis. The ErC<sub>x</sub> and ErC<sub>y</sub> values were determined by Linear interpolation (ICPIN) method for growth rate and yield endpoint. The NOEC and LOEC values were calculated by Bonferroni Adj t Test, after verifying data variances and data distribution by Bartlett Equality of Variance (equal variance) and by Shapiro Wilk W Normality test (normal distribution), respectively. As input data the nominal test item concentrations and geometric mean of measured active ingredient Difenconazole were used.

## Results and discussions

A full dose-response test was performed to evaluate the effect of the test item Difenconazole 250 g/L EC greener – IN005B1570 on *Pseudokirchneriella subcapitata*.

The ErC<sub>10</sub>, the ErC<sub>20</sub> and the ErC<sub>50</sub> values, the NOEC and LOEC values together with their 95% confidence limits (LCL - Lower Confidence Limit and UCL - Upper Confidence Limit), expressed in terms of nominal test item concentrations, are reported in the following table.

**Table 1. Summary of Biological Results**

Time (h)	ErC <sub>10</sub> (mg a.i./L)	ErC <sub>20</sub> (mg a.i./L)	ErC <sub>50</sub> (mg a.i./L)	NOEC (mg a.i./L)	LOEC (mg a.i./L)
Nominal test item concentration					
0 - 24	2.3 (N/A – 4.1)	4.5 (2.6 – 5.7)	18.9 (14.2 – 22.2)	0.90	2.9
0 - 48	2.8 (1.8 – 3.5)	5.0 (4.1 – 5.9)	14.5 (13.1 – 15.8)	0.90	2.9
0 - 72	1.7 (1.4 – 2.0)	3.2 (3.0 – 3.4)	7.5 (6.9 – 8.0)	0.30	0.90
Nominal concentration of the active ingredient					
0 - 24	0.52 (N/A – 0.93)	1.0 (0.59 – 1.3)	4.3 (3.2 – 5.0)	0.20	0.66
0 - 48	0.64 (0.41 – 0.79)	1.1 (0.93 – 1.3)	3.3 (3.0 – 3.6)	0.20	0.66

0 – 72	0.39 (0.32 – 0.45)	0.73 (0.68 – 0.77)	1.7 (1.6 – 1.8)	0.068	0.20
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#### Analytical Results:

The quantification of the active ingredient Difenconazole of the test item Difenconazole 250 g/L EC greener – IN005B1570 in the test samples was performed using liquid chromatography MS/MRM detection. In fresh solutions, prepared at time 0, the analytical recoveries of the concentrations of active ingredient Difenconazole were in the range 95.5 % - 119.6 % of the nominal concentration.

After 72 hours of exposure the analytical recoveries were in the range 51.4 % - 117.1 % of the nominal concentration.

The analytical recoveries were referred to Difenconazole nominal content calculated considering the concentration expressed in g/L and the density on the Certificate of Analysis supplied by the Sponsor (22.7% w/w).

Since not all the analytical recoveries were in the range 80 % - 120 % of the nominal concentrations during the test period, the biological results were referred to geometric mean of a.i. Difenconazole measured concentrations, as recommended by OECD Guideline No. 201.

#### Conclusion

The influence of IN005B1570 on the growth of the freshwater green algae *Pseudokirchneriella subcapitata* was assessed in a static concentration-response test. The 72-hour EyC50 was calculated to be 0.48 mg a.i./L, and the 72-hour ErC50 value was calculated to be 1.843 mg a.i./L. The 72-hour NOEyC was determined to be 0.068 mg a.i./L and the associated 72-hour LOEyC was 0.20 mg a.i./L. The 72-hour NOErC was determined to be 0.048 mg a.i./L and the associated 72-hour LOErC was 0.17 mg a.i./L.

The initial concentrations and the maintenance of the exposure concentrations during the test were verified in the analytical part. All reported results refer to geometric mean concentrations.

#### Validity criteria

After 72h:

Cell Density Increase in Control Cultures:	At the test end the cell density in the negative control had increased on average by a factor of 652, corresponding to a daily specific growth rate equal to 2.16. This value complies with the validity criterion of the test
Coefficient of Variation of Sectional (Daily) Growth Rates in Control Cultures:	16.0 % and thus, the validity criterion was met.
Coefficient of Variation of Average Growth between Control Replicates:	0.4 % and thus, the validity criterion was met.

#### A 2.2.1.3 KCP 10.2.1/03 Study 3

Comments of zRMS:	Study was carried out according to appropriate OECD 203 (2019) and all validity criteria were met.	
	The validity criteria:	
	Fish mortality in the negative control	no mortality was observed during the

	medium	test period, validity criterion was met.	
	Agreed endpoints:		
	Time (day)	LC <sub>50</sub> (mg/L) Nominal test item concentration	LC <sub>50</sub> (mg a.i./L) Nominal Difenoconazole concentration
	1 (24 hours)	> 3.9	> 0.89
	2 (48 hours)	> 3.9	> 0.89
	3 (72 hours)	> 3.9	> 0.89
	4 (96 hours)	> 3.9	> 0.89
	The study is considered acceptable.		

Reference:	KCP 10.2.1/03, [REDACTED]
Report	Difenoconazole 250 g/L EC greener [REDACTED]
Guideline(s):	<p>Yes</p> <ul style="list-style-type: none"> <li>- OECD Guideline for Testing of Chemicals, No. 203. “Fish, acute toxicity test”, 2019.</li> <li>- OECD Series on Testing and Assessment No 23 - Guidance Document On Aquatic Toxicity Testing Of Difficult Substances And Mixtures ENV/JM/MONO (2019).</li> <li>- OECD Environment, Health and Safety Publications Series on Testing and Assessment No. 126 Short Guidance on the Threshold Approach for Acute Fish Toxicity (2010).</li> </ul> <p>The use of fish was regulated by “DIRECTIVE 2010/63/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 22 September 2010 on the protection of animals used for scientific purposes.</p>
Deviations:	<p>Yes</p> <p>The study was performed with zebrafish obtained from Model Organism Facility Department of Cellular, Computational and Integrative Biology - CIBIO University of Trento instead of Research Foundation “Edmund Mach” (S. Michele all’Adige - Italy). The reason of change was the availability of organisms. The change did not impact the study.</p>
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Objective

The purpose of this study was to determine the acute toxicity of the test item Difenoconazole 250 g/L EC greener – IN005B1570 on zebrafish (*Brachydanio rerio*) in a 96-hour semi-static test.

## Materials and methods

Test Item: The test item was Difenoconazole 250 g/L EC greener – IN005B1570, supplied by Indofil, batch number IND\_F021\_0321\_1.

Test Species: Zebra fish (*Brachydanio rerio*). Fish were approximately the same age

and size. The mean body length was 1.25 cm while the mean body weight was 0.0117 g.

Test Design:	<p>This study encompassed 6 treatment groups (5 dose rates of the test item and a control) with three replicates per test concentration and control.</p> <p>At test start 12 fronds were introduced in each replicate and incubated for 7 days under semi-static conditions. The frond numbers were determined on day 3, 5 and 7. The dry weight of each replicate was determined at test termination.</p>
Endpoints:	Mortality at 24, 48, 72 and 96 hours
Test Concentrations:	The fish were exposed to aqueous test media containing the test item at only one nominal concentration of 3.9 mg/L, corresponding to the lower EC <sub>50</sub> value assessed in the algae test (KCP 10.2.1/02) or <i>Daphnia magna</i> test (KCP 10.2.1/01).
Test Conditions:	The test was performed in temperature-controlled aquaria and incubated in a temperature-controlled room. The test method and the test species are recommended by international test guidelines.
Statistical methods	The CETIS v.1.8.7.7 software was used to carry out the statistical analysis, it automatically chooses the most appropriate analysis for the pool of data. The LC <sub>50</sub> values were determined by statistical analysis with Linear Interpolation (ICPIN).

## Results and discussions

A limit test under semi-static conditions was performed to evaluate the effect of test item on zebra fish (*Brachydanio rerio*). As reported in the table, in fresh solution, prepared at time 0, 24, 48 and 72 hours, the analytical recovery of Difenconazole was 100.6 %, 89.3%, 99.3% and 112.4% of the nominal value. In spent solution after 24, 48, 72 and 96 hours of exposure, the analytical recovery of a.i. was 100.0%, 107.6%, 97.2% and 101.1%.

In the negative control, no mortality was observed during the test period. This value complies with the validity criterion reported in the guidelines (maximum mortality of one fish, if seven fish are used, in the negative control medium at the end of the test).

In addition, sub-lethal effects (visible anomalies) were also assessed after 24, 48, 72 hours of exposure and at the end of the test. No remarkable observations were made concerning the appearance of fish in the negative control. In the test solution all fish showed hypoactivity at all observation times. In the afternoon of day 2 all fish showed abnormal bottom distribution.

The obtained experimental results allowed calculation of the LC<sub>50</sub> values at 24, 48, 72 and 96 hours. LC<sub>50</sub> values, calculated in terms of nominal test item concentration, that is Difenconazole 250 g/L EC greener – IN005B1570, and nominal active ingredient Difenconazole concentration are reported in the following table.

Table 1. Summary of Biological Results

Time (day)	LC <sub>50</sub> (mg/L) Nominal test item concentration	LC <sub>50</sub> (mg a.i./L) Nominal Difenconazole concentration
1 (24 hours)	> 3.9	> 0.89

2 (48 hours)	> 3.9	> 0.89
3 (72 hours)	> 3.9	> 0.89
4 (96 hours)	> 3.9	> 0.89

On the definitive test, the percent mortalities up to 96 h post treatment were 0% at the tested concentration of 100.0 mg a.i./L.

#### Summary of analytical results

Time (hours)	Test samples Nominal concentrations (mg test item/L)	Nominal Difenoconazole Concentrations (µg a.i./L) (1)	Actual Difenoconazole Concentration (µg a.i./L)	Analytical recovery (%)
0	Control	0.0	n.d.	-
	3.9	885.3	890.4	100.6
24 spent	Control	0.0	n.d.	-
	3.9	885.3	885.7	100.0
24 fresh	Control	0.0	n.d.	-
	3.9	885.3	790.9	89.3
48 spent	Control	0.0	n.d.	-
	3.9	885.3	953.0	107.6
48 fresh	Control	0.0	n.d.	-
	3.9	885.3	878.7	99.3
72 spent	Control	0.0	n.d.	-
	3.9	885.3	860.5	97.2
72 fresh	Control	0.0	n.d.	-
	3.9	885.3	995	112.4
96 spent	Control	0.0	n.d.	-
	3.9	885.3	895	101.1

(1) Nominal concentrations expressed as Difenoconazole (calculated considering test item active ingredient content and density reported in the CoA, resulting in a purity of 22.7% w/w).  
n.d. not detected, lower than L.O.D. (1.9 µg/L)

## Conclusion

The 96-hour LC<sub>50</sub> to zebrafish under semi-static conditions was found to be > 3.9 mg/L of Difenoconazole 250 g/L EC greener – IN005B1570, which is equivalent to 0.89 mg/L of the active ingredient Difenoconazole.

## Validity criteria

Fish mortality in the negative control medium	no mortality was observed during the test period, validity criterion was met.
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**A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms**

**A 2.2.3 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**

**KCP 10.3.1.1.2 Acute contact toxicity to bees**

Comments of zRMS:	<p>The study is acceptable. This study was evaluated according to OECD 213. The study met the relevant validity criteria. The following endpoints are considered valid for use in the risk assessment:</p> <p>1. Oral exposure:</p> <p>LD<sub>50</sub> &gt; 440 µg product/bee; NOEL = 200 µg product/bee</p> <p>LD<sub>50</sub> &gt; 99.9 µg s.a./bee; NOEL = 45.4 µg s.a./bee</p> <p>LD<sub>50</sub> &gt;35.1 µg s.a./bee on basis of actually consumed test item doses</p> <table><tr><th colspan="5">Mortality of test item and reference item to honeybees</th></tr><tr><th rowspan="2">Test item doses (µg/bee)</th><th rowspan="2">No. of tested bees</th><th colspan="3">No. of dead bees</th></tr><tr><th>6 hours</th><th>24 hours</th><th>48 hours</th></tr><tr><td>Negative control</td><td>30</td><td>0</td><td>2</td><td>2</td></tr><tr><td>8.6</td><td>30</td><td>1</td><td>1</td><td>2</td></tr><tr><td>18.8</td><td>30</td><td>0</td><td>2</td><td>2</td></tr><tr><td>41.4</td><td>30</td><td>2</td><td>2</td><td>2</td></tr><tr><td>91.0</td><td>30</td><td>1</td><td>3</td><td>3</td></tr><tr><td>200.0</td><td>30</td><td>3</td><td>6</td><td>7</td></tr><tr><td>440.0</td><td>30</td><td>5</td><td>14</td><td>14</td></tr><tr><td>Reference item doses (µg/bee)</td><td>No. of tested bees</td><td>6 hours</td><td>24 hours</td><td>48 hours</td></tr><tr><td>0.05</td><td>30</td><td>2</td><td>2</td><td>NA</td></tr><tr><td>0.10</td><td>30</td><td>1</td><td>13</td><td>NA</td></tr><tr><td>0.22</td><td>30</td><td>16</td><td>29</td><td>NA</td></tr></table> <p>*NA not applicable (data required for reference item is LD<sub>50</sub> 24 hours)</p> <p>2. Contact exposure:</p> <p>LD<sub>50</sub> = 192.3 µg product/bee; NOEL = 91 µg product/bee</p> <p>LD<sub>50</sub> = 43.7 µg s.a./bee; NOEL = 20.7 µg s.a./bee</p> <p>Special attention should be paid for the mortality rates of the reference substance group in the contact test in the original study report. Here, the mortality in the 0.15 µg/bee treatment is incorrectly given as 36.7%. It should be 63.3%.</p>	Mortality of test item and reference item to honeybees					Test item doses (µg/bee)	No. of tested bees	No. of dead bees			6 hours	24 hours	48 hours	Negative control	30	0	2	2	8.6	30	1	1	2	18.8	30	0	2	2	41.4	30	2	2	2	91.0	30	1	3	3	200.0	30	3	6	7	440.0	30	5	14	14	Reference item doses (µg/bee)	No. of tested bees	6 hours	24 hours	48 hours	0.05	30	2	2	NA	0.10	30	1	13	NA	0.22	30	16	29	NA
Mortality of test item and reference item to honeybees																																																																					
Test item doses (µg/bee)	No. of tested bees	No. of dead bees																																																																			
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Negative control	30	0	2	2																																																																	
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0.10	30	1	13	NA																																																																	
0.22	30	16	29	NA																																																																	

Mortality of test item and reference item to honeybees						
Test item doses (µg/bee)	No. of tested bees	No. of dead bees				
		4 hours	24 hours	48 hours	72 hours	96 hours
Negative control	30	0	0	1	1	1
8.6	30	0	1	1	1	1
18.8	30	0	1	4	5	5
41.4	30	1	1	3	6	6
91.0	30	0	1	5	5	5
200.0	30	3	14	14	15	16
440.0	30	3	23	30	30	30
Reference item doses (µg/bee)	No. of tested bees	4 hours	24 hours	48 hours	72 hours	96 hours
0.07	30	1	3	NA	NA	NA
0.15	30	4	19	NA	NA	NA
0.33	30	1	30	NA	NA	NA

\*NA not applicable (data required for reference item is LD<sub>50</sub> 24 hours)

Reference:	KCP 10.3.1.1, Ponti, B. (2021)
Report	Difenoconazole 250 g/L EC greener – IN005B1570.: Acute Contact and Oral Toxicity to adult worker honeybees <i>Apis mellifera</i> L. ChemService Study No. CH – 0251/2021
Guideline(s):	Yes <ul style="list-style-type: none"> <li>- OECD, Guideline for the Test of chemicals No. 213: “Honeybees, acute oral toxicity test”, adopted on 1998.</li> <li>- OECD, Guideline for the Test of chemicals No. 214: “Honeybees, acute contact toxicity test”, adopted on 1998.</li> </ul>
Deviations:	Yes, described below
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

#### Deviation No. 1

Change No. 1	During the contact toxicity test, one punctual measurement of humidity was outside the acceptable range.
Reason of change:	Handling the animals required a prolonged opening of the climatic chamber.

Change No. 2	During the oral toxicity test, one punctual measurement of humidity was outside the acceptable range.
Reason of change:	Handling the animals required a prolonged opening of the climatic chamber.



Impact on the study:	None, since the validity criteria for the control were met.
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Change No. 3	The first observation for mortality in the oral toxicity test was done after 6 hours, instead that after 4 hours as reported in the study plan.
Reason of change:	Since not all the food was consumed after 4 hours, the feeding solution with the test item were supplied for a maximum of 6 hours.
Impact on the study:	None.

## Objective

The purpose of this study is to assess the acute oral and contact effects of the test item Difenoconazole 250 g/L EC greener – IN005B1570 to adult worker honeybees of *Apis mellifera* L. Mortality of the bees was used as the toxic endpoint. Sublethal effects, such as changes in behaviour, were also assessed.

## Materials and methods

Test Item:	Difenoconazole 250 g/L EC greener – IN005B1570, Batch Code: IND_F021_0321_1, content: Difenoconazole: 259 g/L (analytical), 250 g/L (nominal), according to certificate of analysis.
Test Species:	Honey bee ( <i>Apis mellifera</i> L.); randomly collected from an adequately fed, healthy diseases-free and queen right beehive treated with no pesticides. The bees were collected ad hoc from a commercial supplier (Apicoltura Costenaro G&C SNC).
Test Design:	For acute oral test, young adult worker honeybees were exposed to six doses of the test item dispersed in a 50% (w/v) aqueous sucrose solution, for about 6 hours; mortality was recorded several times until 48 hours and compared with the control value. For acute contact test, young adult worker honeybees were exposed to six doses of the test item dispersed in water by direct application to the thorax (droplets). A negative control with uncontaminated sucrose solution and a reference item (dimethoate – Batch number: BCBS9338 at 0.10 g/L) were also tested. The results were analyzed at 24 and 48 hours for the acute oral test and at 24, 48, 72 and 96 hours for the acute contact test. 3 replicates per treatment, each consisting of 10 bees per test cage. The bees were randomly distributed to the test cages at test initiation.
Endpoints:	Daily assessment of mortality and behavioural abnormalities up to 48 hours for the acute oral test and to 96 hours for the acute contact test. Endpoints: LC <sub>50</sub> , LDD <sub>50</sub> , NOEC, NOEDD.
Test Concentrations:	Oral test dose: Negative control (aqueous sucrose solution without test item) and 8.6, 18.8, 41.4, 91.0, 200.0 and 440 µg test item/bee, corresponding to 2.0, 4.3, 9.4, 20.7, 45.4 and 99.9 µg a.i./bee. Additionally, reference item dimethoate was tested in three different doses: 0.05, 0.10, 0.22 µg/bee. Contact test dose: Negative control (deionized water), and 8.6, 18.8, 41.4, 91.0, 200.0 and 440 µg test item/bee corresponding to 2.0, 4.3, 9.4, 20.7, 45.4 and 99.9 µg a.i./bee. Additionally, reference item dimethoate was tested in three different doses: 0.07, 0.15, 0.33 µg/bee.

Test Conditions: Temperature and relative humidity (mean value): 25.0 °C and 59.8 % for oral test, 25.0 °C and 60.2 % for contact test. Photoperiod: constant darkness, except during assessments

## Results and discussions

In the negative control 6.7 % of mortality was observed and no bees showed other signs of disease or stress (Table 6). These values comply with the validity criteria of the test, that provides a maximum mortality of 10% in the negative control at the end of the test.

The obtained experimental results allowed to calculate, for the test item, the LD50 values and the NOEL at 24 and 48 hours, as shown in the following table:

### ORAL TOXICITY TEST: RESULTS

#### Mortality of test item and reference item to honeybees

Test item doses (µg/bee)	No. of tested bees	No. of dead bees		
		6 hours	24 hours	48 hours
Negative control	30	0	2	2
8.6	30	1	1	2
18.8	30	0	2	2
41.4	30	2	2	2
91.0	30	1	3	3
200.0	30	3	6	7
440.0	30	5	14	14
Reference item doses (µg/bee)	No. of tested bees	6 hours	24 hours	48 hours
0.05	30	2	2	NA
0.10	30	1	13	NA
0.22	30	16	29	NA

\*NA not applicable (data required for reference item is LD<sub>50</sub> 24 hours)

### Bees behaviour and appearance.

Test item doses (µg/bee)	Bees behaviour		
	6 hours	24 hours	48 hours
Negative control	NE	NE	NE
8.6	NE	3 F	NE
18.8	NE	NE	NE
41.4	NE	NE	NE
91.0	1 M	NE	NE
200.0	2 M	1 P	5 F
440.0	NE	NE	NE
Reference item doses (µg/bee)	4 hours	24 hours	48 hours
0.05	NE	NE	NA
0.10	NE	NE	NA
0.22	NE	NE	NA

NE: No effect

F: Freeze

M: Moribund

P: Paralysis

n.d. no detectable (100 % mortality)

\*NA not applicable (data required for reference item is LD<sub>50</sub> 24 hours)

**Table 1. Summary of mortality during oral test**

Endpoint	Mortality (Oral test )			
	on the basis of nominal test item and a.i. doses		on basis of actually consumed test item doses	
	24 hours	48 hours	24 hours	48 hours
LD <sub>50</sub> (µg test item/ bee)	>440	>440	>154.6	>154.6
NOEL (µg test item/ bee)	200	200	147.5	147.5
LD <sub>50</sub> (µg a.i./ bee)	>99.9	>99.9	>35.1	>35.1
NOEL (µg a.i./ bee)	45.4	45.4	33.5	33.5

In the negative control and in the solvent control 3.3 % of mortality was observed and no bees showed other signs of disease or stress. These values comply with the validity criteria of the test, that provides a maximum mortality of 10% in the negative control at the end of the test.

The obtained experimental results allowed to calculate, for the test item, the LD<sub>50</sub> values and the NOEL at 24, 48, 72 and 96 hours. The results, calculated in terms of nominal test doses, were as follows:

**Table 2. Summary of mortality during contact test**

Endpoint	Mortality (Contact test ) on basis of nominal test item doses			
	24 hours	48 hours	72 hours	96 hours
LD <sub>50</sub> (µg test item/ bee)	252	215.4	205.3	192.3
NOEL (µg test item/ bee)	91	91	91	91

LD <sub>50</sub> (µg a.i./ bee)	57.2	48.9	46.6	43.7
NOEL (µg a.i./ bee)	20.7	20.7	20.7	20.7

## CONTACT TOXICITY TEST: RESULTS

### Mortality of test item and reference item to honeybees

Test item doses (µg/bee)	No. of tested bees	No. of dead bees				
		4 hours	24 hours	48 hours	72 hours	96 hours
Negative control	30	0	0	1	1	1
8.6	30	0	1	1	1	1
18.8	30	0	1	4	5	5
41.4	30	1	1	3	6	6
91.0	30	0	1	5	5	5
200.0	30	3	14	14	15	16
440.0	30	3	23	30	30	30
Reference item doses (µg/bee)	No. of tested bees	4 hours	24 hours	48 hours	72 hours	96 hours
0.07	30	1	3	NA	NA	NA
0.15	30	4	19	NA	NA	NA
0.33	30	1	30	NA	NA	NA

\*NA not applicable (data required for reference item is LD<sub>50</sub> 24 hours)

### Bees behaviour and appearance.

Test item doses (µg/bee)	Bees behaviour				
	4 hours	24 hours	48 hours	72 hours	96 hours
Negative control	NE	NE	NE	NE	NE
8.6	1 A	NE	2 F	1 F 4 S	1F 4 S
18.8	1 A	NE	26 SI	25 SI	25 SI
41.4	NE	NE	NE	24 SI	24 SI
91.0	2 M	NE	NE	NE	NE
200.0	1 S 1 M	1 S	4 S	15 SI	14 SI
440.0	16 S	2 Ap 2 F	n.d	n.d	n.d
Reference item doses (µg/bee)	4 hours	24 hours	48 hours	72 hours	96 hours
0.07	1 A	NE	NA	NA	NA
0.15	2 SI	NE	NA	NA	NA
0.33	1 Ap 1 M 1 Ag	n.d	NA	NA	NA

NE No effect

A: Affected

SI: slowed down

Ap: apathetic

M: Moribund

Ag: agitated

F: Freeze

S: Spasm

n.d. no detectable (100 % mortality)

\*NA not applicable (data required for reference item is LD<sub>50</sub> 24 hours)

### Conclusion

The LD<sub>50</sub> value and the NOEL at 24 and 48 hours for the acute oral toxicity test and LD<sub>50</sub> value and the NOEL at 24, 48, 72 and 96 hours for the acute contact toxicity test were assessed.

For oral acute toxicity, the LD<sub>50</sub> value (48 hours) of IN005B1570 was 99.9 µg a.i./ bee and the NOEL was 45.4 µg a.i./ bee.

For oral contact toxicity, the LD<sub>50</sub> value (96 hours) of IN005B1570 was 43.7 µg a.i./ bee and the NOEL was 20.7 µg a.i./ bee.

### Validity criteria

Control Mortality:	Mortality in the negative controls was < 10%
Validity of the Test:	The LD <sub>50</sub> of the toxic standard meets the specified range (LD <sub>50</sub> -24h is in the range 0.10-0.35 µg a.i./bee as reported in the OECD 213 (oral test) and in the range 0.10-0.30 µg a.i./bee as reported in the OECD 214 (contact test)).

### A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 245 (217) of the test were met.			
	The following validity criteria were met during the test:			
	Negative control mortality:	In the negative control 6.7 % mortality was observed. No other signs of toxicity or stress. These values comply with the validity criteria of the test, that requires a maximum mortality of 10% in the negative control at the end of the test.		
	Positive control mortality:	In the positive control (0.8 mg Dimethoate/kg feed) 100% mortality was observed at the end of the test. This meets the validity criteria of the test, that requires mortality ≥ 90%.		
	Deviation: none Agreed toxicity endpoints:			

Critical concentration (g test item/L feeding solution)	LC <sub>10</sub>	LC <sub>20</sub>	LC <sub>50</sub>	NOEC
10 days	2.3 (1.4 – 3.0) (*)	3.0 (2.0 – 3.8) (*)	4.9 (4.0 – 6.0) (*)	2.5
Critical dose (µg test item/bee/day)	LDD <sub>10</sub>	LDD <sub>20</sub>	LDD <sub>50</sub>	NOEDD
10 days	58.09 (n.d. – 61.67) (*)	60.20 (n.d. – 63.33) (*)	63.97 (61.83 – 66.19) (*)	34.53

(\*) 95% confidence limits  
n.d. non detectable

The LC<sub>10,20,50</sub>/LDD<sub>10,20,50</sub> with 95 % confidence limits and the NOEC/NOEDD values at the end of the exposure (10 days), expressed as nominal Difenconazole concentration / mean daily Difenconazole uptake were assessed as follows.

Critical concentration (g a.i./L feeding solution)	LC <sub>10</sub>	LC <sub>20</sub>	LC <sub>50</sub>	NOEC
10 days	0.52 (0.31 – 0.68) (*)	0.68 (0.45 – 0.85) (*)	1.11 (0.90 – 1.35) (*)	0.56
Critical dose (µg a.i./bee/day)	LDD <sub>10</sub>	LDD <sub>20</sub>	LDD <sub>50</sub>	NOEDD
10 days	13.07 (n.d. – 13.88) (*)	13.55 (n.d. – 14.25) (*)	14.39 (13.91 – 14.89) (*)	7.77

(\*) 95% confidence limits  
n.d. non detectable

Reference:	KCP 10.3.1.2, Ponti, B. (2023)
Report	Difenconazole 250 g/L EC greener – IN005B1570: Chronic Oral Toxicity to adult worker honeybees <i>Apis mellifera</i> L.(10-day feeding) ChemService Study No. CH – 0103/2023

Guideline(s):	Yes, OECD, Guideline for the Test of chemicals No. 245: “Honey bee ( <i>Apis mellifera</i> L.), chronic oral toxicity test (10-day feeding)”, October 2017.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

## Objective

The purpose of this study was to assess the chronic oral effects of the test item Difenoconazole 250 g/L EC greener – IN005B1570 to adult worker honey bees of *Apis mellifera* L.

For this purpose, young adult worker honey bees (max. 2 days old at test start) were exposed to five doses of the test item dispersed in a 50 % w/v aqueous sucrose solution by continuous and *ad libitum* feeding over a period of 10 days.

Mortality and behavioural abnormalities were recorded daily during the 10-day test period.

The test was carried out according to OECD guideline 245 (2017) and in compliance with the Principles of Good Laboratory Practice (GLP).

## Materials and methods

**Test Item:** Difenoconazole 250 g/L EC greener – IN005B1570, Batch Code: IND\_F021\_0123\_1, content: Difenoconazole: 252 g/L (analytical), 250 g/L (nominal), according to certificate of analysis.

**Test Species:** Honey bee (*Apis mellifera* L.); Young adult bees (max. 2 days old) reared from brood combs taken from queen-right colonies that have no symptoms of diseases and that have a known maintenance and physiological status history were used for the test. The bees were collected from a commercial supplier (Apicoltura Costenaro G&C SNC).

**Test Design:** Young adult worker honeybees were exposed to five doses of the test item dispersed in a 50% (w/v) aqueous sucrose solution. The contaminated feeding solution was provided *ad libitum* to the bees inside a syringe inserted in the top of the cage and changed every 24 hours. The test was performed in autoclavable and well-ventilated stainless-steel cages of 4.5 x 8.5 x 6.5 cm in constant temperature and relative humidity conditions. The number of dead honey bees per cage was recorded daily. Additionally, any behavioural difference among the honey bees of the control group and those of the test item groups were recorded.

**Endpoints:** Daily assessment of mortality and behavioural abnormalities. Endpoints: LC<sub>10</sub>, LC<sub>20</sub>, LC<sub>50</sub>, LDD<sub>10</sub>, LDD<sub>20</sub>, LDD<sub>50</sub>, NOEC, NOEDD.

**Test Concentrations:** Five concentrations in a geometric series, using a spacing factor of 2., were tested: 0.625, 1.25, 2.50, 5.0, 10.0 g test item/L feeding solution corresponding to 0.521, 1.04, 2.1, 4.2 and 8.3 g test item/kg (according to the aqueous sucrose solution density of 1.20 g/mL).

Additionally, a negative control (sucrose solution without test item), and

positive control (dimethoate) were tested.

#### Test Conditions:

Temperature values measured during the test period were in the range 32.6°C to 33.1 °C (mean value 33.0°C), which are within the values required by the OECD Test Guideline (33 ± 2°C).

Humidity values measured during the test period were in the range 55.2 % to 67.8 % (mean value 60.1%), which are within the values required by the OECD Test Guideline (60 ± 10 %).

Short deviations for temperature and humidity (<2 hours per day) from the stated conditions, e.g. during assessments, was not considered a guideline deviation.

#### Results and discussions

Analytical recovery of Difenoconazole residues were in the range 99.3% – 115.2 % of the nominal concentrations, as reported in the following table.

**Table 1. Difenoconazole measured concentrations and analytical recovery (fresh)**

Nominal test item concentration (g test item/L feeding solution)	Difenoconazole nominal concentrations (mg a.i./L sucrose solution) <sup>(1)</sup>	Difenoconazole measured conc. (mg a.i./L sucrose solution)	Recovery (%)
0.625	140.63	139.69	99.3
10.0	2250.0	2591.44	115.2

<sup>(1)</sup> considering the active ingredient content reported in the CoA (22.5 %)

The analytical results show that the analytical recoveries for the two concentrations were in the range of 80% - 120% of the nominal concentration. Therefore, endpoints were based on nominal concentrations. No active ingredient was detected in the control samples.

The bees were observed for mortality each 24 hours during the 10-day test period. The observed mortality in the negative control and test item concentrations are reported in the following table.

**Table 2. Summary of mortality during test**

Treatment group	Concentration (g test item/L food)	Food uptake (mg feeding solution/bee/day) (1)	Daily dosage		Mortality after 10 days	
			Test item (µg test item/bee/day)	a.i. (µg a.s./bee/day)	absolute (%)	corrected (%) <sup>(2)</sup>
Negative control	0.0	40.79	!	!	6.7	!
Test item	0.625	34.15	17.79	4.00	0	-7.1



Treatment group	Concentration	Food uptake (mg feeding solu-	Daily dosage		Mortality after 10 days	
	1.25	33.20	34.53	7.75	0	-7.1
	2.50	23.35	49.03	11.03	20.0	14.3
	5.00	15.05	63.23	14.23	46.7*	42.9
	10.00	8.65	69.96	15.49	90.0*	89.3
Positive control	0.8 (Dimethoate)	23.40	18.27 (Dimethoate)		86.7	85.7

(1) adjusted for evaporation from the feeders. (2) corrected according to Abbot's formula. Negative values are to be considered equal to 0. \* statistically significant difference in pairwise comparison between treatment and untreated control (Fisher Exact/Bonferroni Hommel Test).

### Conclusion

The effects of the test item Difenoconazole 250 g/L EC greener – IN005B1570 on adult worker honey bees (*Apis mellifera* L.) were assessed in a chronic oral toxicity test (10-days feeding).

The  $LC_{10,20,50}/LDD_{10,20,50}$  with 95 % confidence limits and the NOEC/NOEDD values at the end of the exposure (10 days), expressed as nominal test item concentration / mean daily test item uptake were assessed as follows.

**Table 3. Summary of the endpoints (g test item/L)**

Critical concentration (g test item/L feeding solution)	LC <sub>10</sub>	LC <sub>20</sub>	LC <sub>50</sub>	NOEC
10 days	2.3 (1.4 – 3.0) (*)	3.0 (2.0 – 3.8) (*)	4.9 (4.0 – 6.0) (*)	2.5
Critical dose (µg test item/bee/day)	LDD <sub>10</sub>	LDD <sub>20</sub>	LDD <sub>50</sub>	NOEDD
10 days	58.09 (n.d. – 61.67) (*)	60.20 (n.d. – 63.33) (*)	63.97 (61.83 – 66.19) (*)	34.53

(\*) 95% confidence limits.n.d. non detectable

The  $LC_{10,20,50}/LDD_{10,20,50}$  with 95 % confidence limits and the NOEC/NOEDD values at the end of the exposure (10 days), expressed as nominal Cymoxanil concentration / mean daily Cymoxanil uptake were assessed as follows.

**Table 4. Summary of the endpoints (g a.s/L)**

Critical concentration (g a.s./L feeding solution)	LC <sub>10</sub>	LC <sub>20</sub>	LC <sub>50</sub>	NOEC
10 days	0.52 (0.31 – 0.68) (*)	0.68 (0.45 – 0.85) (*)	1.11 (0.90 – 1.35) (*)	0.56

Critical dose (µg a.s./bee/day)	LDD <sub>10</sub>	LDD <sub>20</sub>	LDD <sub>50</sub>	NOEDD
10 days	13.07 (n.d. – 13.88) (*)	13.55 (n.d. – 14.25) (*)	14.39 (13.91 – 14.89) (*)	7.77

(\*) 95% confidence limits . n.d. non detectable

#### Validity criteria

Negative control mortality:	In the negative control 6.7 % mortality was observed and no bees showed other signs of toxicity or stress. These values comply with the validity criteria of the test, that requires a maximum mortality of 15 % in the negative control at the end of the test.
Positive control mortality:	In the positive control (0.8 mg Dimethoate/kg feeding solution) 85.7 % mortality was observed at the end of the test. This value complies with the validity criteria of the test, that requires mortality ≥ 50 % at the end of the test.

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

Comments of zRMS:	The study is acceptable. The validity criteria according OECD 239 (2021) of the test were met.	
	The following validity criteria were met during the test:	
	Negative control:	Cumulative mortality of the larvae from D3 to D8 for the negative control: 8.3% (acceptable if $\leq 15\%$ ); Emergency rate of adults at D22 for the negative control: 75.0% (acceptable if $\geq 70\%$ ).
	Reference sub-stance:	Cumulative mortality in the reference substance treated group (48.0 mg a.i./kg diet) at D8: 91.7 % (acceptable if $\geq 50\%$ ).
	Deviation: During the test period, some punctual measurement of humidity were outside the acceptable range.	
	Reason: Handling the animals required an opening of the climatic chamber. This deviation does not affect the validity of the study. The validity criteria for the control were met.	
	Agreed toxicity endpoints:	

<b>Emergence: results assessed on the basis of nominal test item concentrations/doses</b>				
End points (µg test item/larva)	ED <sub>10</sub>	ED <sub>20</sub>	ED <sub>50</sub>	NOED
22 days	107.8 (N/A – 163.3) (*)	134.0 (85.8 – 160.6) (*)	178.4 (154.3 – 205.3) (*)	125
End points (mg test item/kg diet)	EC <sub>10</sub>	EC <sub>20</sub>	EC <sub>50</sub>	NOEC
22 days	699.7 (N/A – 1054.0) (*)	870.0 (513.4 – 1032.0) (*)	1158.0 (971.0 – 1301.0) (*)	811.69
(*) 95% confidence limits; N/A not applicable				
<b>Emergence: results assessed on the basis of nominal active ingredient concentrations/doses</b>				
End points (µg active ingredient/larva)	ED <sub>10</sub>	ED <sub>20</sub>	ED <sub>50</sub>	NOED
22 days	24.26 (N/A – 36.74) (*)	30.15 (19.31 – 36.14) (*)	40.14 (34.72 – 46.19) (*)	28.13
End points (mg active ingredient/kg diet)	EC <sub>10</sub>	EC <sub>20</sub>	EC <sub>50</sub>	NOEC
22 days	157.43 (N/A – 237.15) (*)	195.75 (115.52 – 232.20) (*)	260.55 (218.48 – 292.73) (*)	182.63
(*) 95% confidence limits; N/A not applicable				

Reference:	KCP 10.3.1.3, Noè, F. (2023)
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Honeybees ( <i>Apis mellifera</i> L) Larval Toxicity Test with Repeated Exposure ChemService Study No. CH – 0104/2023
Guideline(s):	Yes OECD, Guidance Document on Honey Bee ( <i>Apis mellifera</i> ) Larval Toxicity Test, Repeated Exposure. Series on Testing and Assessment No. 239 (2021).
Deviations:	Yes, described below
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

Deviation No. 1

Change No. 1

During the contact toxicity test, one punctual measurement of humidity was outside the acceptable range.

Reason of change:

Handling the animals required a prolonged opening of the climatic chamber.

Impact on the study:

None, since the validity criteria for the control were met.

Impact on the study:

None.

## Objective

The aim of this study was to assess the effects on larval development and adult emergence of the test item Difenoconazole 250 g/L EC greener – IN005B1570 to honey bee larvae (*Apis mellifera* L.) in a laboratory test over a period of 22 days with repeated exposure.

## Materials and methods

**Test Item:** Difenoconazole 250 g/L EC greener – IN005B1570, Batch Code: IND\_F021\_0123\_1, content: Difenoconazole: 252 g/L (analytical), 250 g/L (nominal), according to certificate of analysis.

**Test Species:** The aim of this study was to assess the effects on larval development and adult emergence of the test item Difenoconazole 250 g/L EC greener – IN005B1570 to honey bee larvae (*Apis mellifera* L.) in a laboratory test over a period of 22 days with repeated exposure. The bees were collected from a commercial supplier (Apicoltura Costenaro G&C SNC).

**Test Design:** *Apis mellifera* L. honey bees larvae were collected from three different colonies. The test is conducted during the egg laying period of the queen. Three days before the test start (D-3), in order to ensure the production of larvae from three colonies, the queens of a minimum of three colonies were confined in their own colony in an exclusion cage containing an empty comb or a comb with emerging worker brood and empty cells. Two days before the test start (D-2) (maximum 30 hours after encaging) the queen was released from the cage, after checking the presence of eggs. All larvae were fed once a day from D1 to D6, except on D2. Larval mortality was recorded from Day 3 to Day 8; pupae mortality from D8 to D15. On D22 the number of emerged bees and non-emerged bees (pupal mortality) were counted. Other observations, such as the appearance and size of the larvae, behaviour, morphological differences and any other adverse effects after emergence, were qualitatively recorded.

**Endpoints:** Daily assessment of mortality and behavioural abnormalities. Endpoints: LC<sub>10</sub>, LC<sub>20</sub>, LC<sub>50</sub>, LDD<sub>10</sub>, LDD<sub>20</sub>, LDD<sub>50</sub>, NOEC, NOEDD.

**Test Concentrations:** Negative control (larvae not exposed to test item) and 62.5, 125, 250, 500, 1000 µg product/larva (14.06, 28.13, 56.25, 112.50 and 225.0 µg active ingredient Difenoconazole/larva). These doses corresponding to 405.84, 811.69, 1623.38, 3246.76 and 6493.51 mg test item/kg diet.

**Test Conditions:** Temperature: 33.60 – 34.70 °C (34.37 °C as mean value), Relative humidity: range: 72.9 – 98.3 % (97.9 % as mean value) (D1 - D8), range: 59.9 – 83.1 % (77.3 % as mean value) (D8 – D15), range: 49.6 – 70.7 % (65.8 % as mean value) (D15 – D22), Photoperiod: constant darkness, except during assessments

## Results and discussions

A full test was performed to evaluate the effect of the test item on honey bee larvae (*Apis mellifera* L.) exposed to five concentrations of the test item, with repeated exposure, in a test of 22 days duration (i.e. to adult emergence).

In the following table a summary of larvae mortality at day 8, on pupae mortality at day 15, pupae mortality from day 8 to 22 and on total mortality (larval and pupal) and % of emergence at day 22 during the test period are reported.

**Table 1. Summary of mortality during test**

Nominal doses (µg test item/ larvae)	Mortality (D3-8) (%)	Mortality (D8-15) (%)	Pupal Mortality (22 days) (%)	22-Day Larval + Pupal Mortality (22days) (%)	% emer- gence at day 22
Negative control	8.3	12.1	18.2	25.0	75.0
62.5	8.3	9.1	15.2	22.2	77.8
125	11.1	15.6	25.0	33.3	66.7
250	30.6	68.0	84.0	88.9	11.1
500	86.1	100.0	100.0	100.0	0
1000	100.0	100.0	100.0	100.0	0

#### Analytical results

Samples of test solutions used to make up diets (lowest and highest concentration of serial dilution) were taken daily after preparation from Day 3 to Day 6. The analytical results are summarized in the following table:

**Table 2. Summary of the analytical results**

Samples (g/L)	Difenoconazole Nominal Conc. (mg/L) <sup>(1)</sup>	Difenoconazole measured conc. (mg/L)				Analytical Recovery (%)			
		D3	D4	D5	D6	D3	D4	D5	D6
TS1 (3.05)	1003.50	1158.67	1111.29	999.63	1054.26	115.5	110.7	99.6	105.1
TS5 (71.43)	16071.75	16479.73	17174.86	18040.26	18845.19	102.5	106.9	112.2	117.3

<sup>(1)</sup> calculated considering content 22.5 % w/w

The mean recoveries of difenoconazole were 99.6-117.3% in the samples of the feeding test solutions. Therefore, endpoints were based on nominal concentrations. No active ingredient was detected in the control samples.

#### Conclusion

The effects of test item Difenoconazole 250 g/L EC greener – IN005B1570 on honey bee larvae (*Apis mellifera* L), with repeated exposure, were assessed in a test of 22 days duration (i.e. to adult emergence).

The EC<sub>10</sub>, EC<sub>20</sub>, EC<sub>50</sub> and the ED<sub>10</sub>, ED<sub>20</sub>, ED<sub>50</sub> and the NOEC/NOED values at the end of the test (22 days) were assessed.

Results are expressed as nominal test item concentrations/doses and nominal Difenoconazole concentrations/doses.

**Table 2. Summary of endpoints (µg test item/larva)**

End points (µg test item/larva)	ED <sub>10</sub>	ED <sub>20</sub>	ED <sub>50</sub>	NOED
22 days	107.8 (N/A – 163.3) (*)	134.0 (85.8 – 160.6) (*)	178.4 (154.3 – 205.3) (*)	125
End points (mg test item/kg diet)	EC <sub>10</sub>	EC <sub>20</sub>	EC <sub>50</sub>	NOEC
22 days	699.7 (N/A – 1054.0) (*)	870.0 (513.4 – 1032.0) (*)	1158.0 (971.0 – 1301.0) (*)	811.69

(\*) 95% confidence limits; N/A not applicable

**Table 3. Summary of endpoints (µg active ingredient/larva)**

End points (µg active ingredient/larva)	ED <sub>10</sub>	ED <sub>20</sub>	ED <sub>50</sub>	NOED
22 days	24.26 (N/A – 36.74) (*)	30.15 (19.31 – 36.14) (*)	40.14 (34.72 – 46.19) (*)	28.13
End points (mg active ingredient/kg diet)	EC <sub>10</sub>	EC <sub>20</sub>	EC <sub>50</sub>	NOEC
22 days	157.43 (N/A – 237.15) (*)	195.75 (115.52 – 232.20) (*)	260.55 (218.48 – 292.73) (*)	182.63

(\*) 95% confidence limits; N/A not applicable

#### Validity criteria

Negative control:	Cumulative mortality of the larvae from D3 to D8 for the negative control: 8.3% (acceptable if ≤ 15 %); Emergency rate of adults at D22 for the negative control: 75.0% (acceptable if ≥ 70%).
Reference substance:	Cumulative mortality in the reference substance treated group (48.0 mg a.i./kg diet) at D8: 91.7 % (acceptable if ≥ 50 %).

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.1.7 KCP 10.3.2.1 Effects on arthropods other than bees

A 2.3.1.8 KCP 10.3.2.1/01 Study 1

Study Comments: KCP 10.3.2.1/01	<b>zRMS comments:</b> The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met. <b>Validity criteria</b> <table border="1" data-bbox="448 389 1441 607"> <tr> <td data-bbox="448 389 759 450">Control Mortality:</td><td data-bbox="759 389 1441 450">Control mortality was 0.0% and so this validity criterion was met.</td></tr> <tr> <td data-bbox="448 450 759 517">Reproduction of Control:</td><td data-bbox="759 450 1441 517">The mean control number of mummies/females was 20.9 and so this validity criterion was met.</td></tr> <tr> <td data-bbox="448 517 759 607">The level of Mortality in the Toxic Reference Treatment</td><td data-bbox="759 517 1441 607">Was 85% and so this validity criterion was met.</td></tr> </table>	Control Mortality:	Control mortality was 0.0% and so this validity criterion was met.	Reproduction of Control:	The mean control number of mummies/females was 20.9 and so this validity criterion was met.	The level of Mortality in the Toxic Reference Treatment	Was 85% and so this validity criterion was met.
Control Mortality:	Control mortality was 0.0% and so this validity criterion was met.						
Reproduction of Control:	The mean control number of mummies/females was 20.9 and so this validity criterion was met.						
The level of Mortality in the Toxic Reference Treatment	Was 85% and so this validity criterion was met.						
Agreed endpoints:	<u>Laboratory test</u> LR <sub>50</sub> = 1438 g product/ha based on mortality LR <sub>50</sub> = 326.7 g s.a./ha based on mortality  LR <sub>50</sub> > 798 g product/ha based on reproduction LR <sub>50</sub> > 181.3 g s.a./ha based on reproduction						

<b>Reference:</b>	<b>KCP 10.3.2.1/01, Dini, R. (2021)</b>
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> De Stefani Perez (Hymenoptera, Braconidae) under laboratory conditions. Dini, R 2021. ChemService Study No. 0253/2021
Guideline(s):	Yes - IOBC/WPRS Guidelines to evaluate side-effects of plant protection products to non-target arthropods. Blumel S. et al., 2000 - ESCORT I Guidance Document on Regulatory Testing Procedures for Pesticides with Non– Target Arthropods. (Barrett K.L. et al., eds.1994) - ESCORT II Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non–Target Arthropods. (Candolfi M.P. et al. 2001) - ESCORT III Linking Non-Target Arthropod Testing and Risk Assessment with Protection Goals. (Anne A. A. et al 2012)
Deviations:	Yes, deviation in Test temperatures during test period. Reason of change: The test temperatures were slightly higher then recommended range (0.3°C). No impact on the study, since all validity criteria were meet.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

### Objective

The purpose of this study is to determine the effects of Difenoconazole 250 g/L EC greener – IN005B1570 on the survival and the reproduction of the Parasitoid *Aphidius rhopalosiphi* in a 14-day exposure test under laboratory conditions. Toxicant effects of the test item on *Aphidius rhopalosiphi* were determined based on assessments of adult mortality after 2, 24 and 48 hours of exposure and reproduction after 10-12 days.

### Materials and methods

Test Item:	Difenconazole 250g/L EC greener – IN005B1570
Test Species:	Parasitoid <i>Aphidius rhopalosiphi</i> . The parasitoid was purchased from a commercial supplier as mummies. After 3-4 days the mummies began hatching and the tests were carried out on adults (less than 48 hours old).
Test Design:	14 day test under laboratory conditions. For the test item rates as well as for the controls (negative and positive), 10 wasps per replicate, with at least 5 females, were used. Four replicates for controls and for each of the rates were prepared. Each test unit was labelled with study number, treatment group and replicate number. Assessment of adult mortality was carried out after 2, 24 and 48 hours in the negative and positive controls. Assessment of the reproductive output was carried out after 10-12 days by counting the mummies.
Endpoints:	Adult mortality and reproductive output.
Reference Item:	Dimethoate 400 EC (400g/L dimethoate). Batch number: 10238284A.
Test Concentrations:	The test was performed at three different application rates 0.35, 0.7, and 1.4 L product/ha corresponding to 399, 798 and 1596 g product/ha (calculated based on test item density 1.14 g/mL). A positive control with the reference item, Rogor® (Dimethoate 400 EC) at the application rate of 0.3 g/ha was tested (corresponding to 0.3 mL formulated/ha). A negative control without test item was tested.
Test Conditions:	For the assessment of mortality; 2 treated glass plates (10x10 cm) held apart by an untreated aluminium frame and with ventilation holes. Temperature within the range of 19.3°C to 22.3°C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 1503 lux to 1512 lux. For the assessment of reproduction; : glass cylinders with a 9-11 cm of diameter and 10-12 cm high, containing aphid-infested cereals plants and covered with a net. Temperature within the range of 19.8°C to 20.1°C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 10953 lux to 11573 lux.
Statistics:	Dunnett Multiple Comparison Test (reproduction), Trimmed Spearman-Kärber (mortality endpoint), Shapiro-Wilk W normality test - normal distribution (reproduction), d Bartlett Equality of Variance - equal variances (reproduction), Linear Interpolation - ICPIN (reproductive endpoint).

## Results and discussions

All study validity criteria were met.

The highest observed mortality after 48 hours of exposure in the tested concentrations was at 1596 g test item/ha with 57.5% of *Aphidius rhopalosiphi* dead. 0.0% of the *Aphidius rhopalosiphi* died in the negative control after 48 hours.

The reproductive negative control had 20.9 mummies/females after 10 - 12 days. The feeding activity in all the treated groups was comparable to the control. All test groups were fed a 1:3 solution honey and water.



The data on mortality and reproductive output were statistically analysed and the following results obtained.

Table 12. Effect of Difenconazole 250g/L EC greener – IN005B1570 on the survival of the parasitoid *Aphidius rhopalosiphi* in a 14-day exposure study

Survival   48 hours	NOER (g/ha)	LOER (g/ha)	LR <sub>50</sub> (g/ha)
g product/ha	798	1596	1438 (1195 – 1731) (*)
g/ha active substance	181.3	362.6	326.7 (271.5 – 393.3) (*)

NOER/LOER and LR<sub>50</sub> value (\*) with 95% confidence limits), in terms of nominal rate.

Table 2. Effect of Difenconazole 250g/L EC greener – IN005B1570 on the reproduction of the parasitoid *Aphidius rhopalosiphi* in a 14-day exposure study

Survival   48 hours	NOER (g/ha)	LOER (g/ha)	LR <sub>50</sub> (g/ha)
g product/ha	798	>798	>798
g/ha active substance	181.3	>181.3	>181.3

NOER/LOER and LR<sub>50</sub> value (with 95% confidence limits), in terms of nominal rate.

## Conclusion

In a parasitoid *Aphidius rhopalosiphi* survival and the reproduction study with Difenconazole 250g/L EC greener – IN005B1570 the NOER for survival after 48 hours was determined to be 798 g test item/ha, i.e. the highest concentration tested. The LOER for survival after 48 hours was estimated to be 1596 g test item/ha.

The LC<sub>50</sub> was estimated to be 1438 (1195 – 1731)(95% confidence limits) g test item/ha. The NOER for reproduction was determined to be 798 g test item/ha. The LOER for reproduction was determined to be >798g test item/ha. The EC<sub>50</sub> was estimated to be >798 g test item/ha.

## Reference Item Test:

In the reference item group 85.0% mortality was observed after 24 hours. The level of mortality in the toxic reference treatment was in the expected range.

## Validity criteria

Control Mortality:	Control mortality was 0.0% and so this validity criterion was met.
Reproduction of Control:	The mean control number of mummies/females was 20.9 and so this validity criterion was met.
The level of Mortality in the Toxic Reference Treatment	Was 85% and so this validity criterion was met.

## A 2.3.1.9 KCP 10.3.2.1/02 Study 2

Study Comments:	zRMS comments:
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KCP 10.3.2.1/02	The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met.	
	<b>Validity criteria</b>	
	Control Mortality:	Control mortality was 15.0% and so this validity criterion was met.
	Reproduction of Control:	The mean control number of eggs/female was 4.70 and so this validity criterion was met.
	The level of Mortality in the Toxic Reference Treatment	Was 83.5% and so this validity criterion was met.
Agreed endpoints:	<u>Laboratory test</u>  LR <sub>50</sub> = 1316 g product/ha based on mortality LR <sub>50</sub> = 299 g s.a./ha based on mortality  LR <sub>50</sub> > 798 g product/ha based on reproduction LR <sub>50</sub> > 181.3 g s.a./ha based on reproduction	

<b>Reference:</b>	<b>KCP 10.3.2.1/02, Dini, R. (2021)</b>
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on the predatory mite <i>Typhlodromus pyri</i> Scheuten (Acari: Phytoseiidae) under laboratory conditions. Dini, R 2021. ChemService Study No. 0252/2021
Guideline(s):	Yes - IOBC/WPRS Guidelines to evaluate side-effects of plant protection products to non-target arthropods. Blumel S. et al., 2000 - ESCORT I Guidance Document on Regulatory Testing Procedures for Pesticides with Non– Target Arthropods. (Barrett K.L. et al., eds.1994) - ESCORT II Guidance Document on Regulatory Testing and Risk Assessment Procedures for Plant Protection Products with Non–Target Arthropods. (Candolfi M.P. et al. 2001) - ESCORT III Linking Non-Target Arthropod Testing and Risk Assessment with Protection Goals. (Anne A. A. et al 2012)
Deviations:	Yes, in Test temperatures during test period. The test temperatures were recorded one day in late respect the start of the biological phase (on 4th of May, instead of on 3rd of May). No impact on the study, since all validity criteria were achieved.
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

### Objective

The purpose of this study is to determine the effects of Difenoconazole 250 g/L EC greener – IN005B1570 on the survival and the reproduction of the predatory mite *Typhlodromus pyri* in a 14-days exposure test under laboratory conditions. The toxicant effects of the test item on *Typhlodromus pyri* were determined based on assessments of adult mortality after 7 days and reproduction after 14 days of exposure.

### Materials and methods

Test Item: Difenoconazole 250g/L EC greener – IN005B1570

Test Species:	Predatory mite <i>Typhlodromus pyri</i> . The predatory mite was purchased from a commercial supplier as eggs. After 3 days the eggs began hatching and the tests were carried out on the protonymphs (24 hours old).
Test Design:	14 day test in a controlled environmental test chamber. For the test item rates as well as for the controls (negative and positive), 20 protonymphs per replicate were used. Five replicates for controls and for each of the rates were prepared. Each test unit was labelled with study number, treatment group and replicate number. Assessment of mortality was carried out after 7 days in the negative and positive controls. Assessment of the reproductive output was carried out after 10-14 days by counting the eggs, larvae and females from day 7 to 14 included.
Endpoints:	Adult mortality and reproductive output.
Reference Item:	Dimethoate 400 EC (400g/L dimethoate). Batch number: 10238284A.
Test Concentrations:	The test was performed at three different application rates 0.35, 0.7, and 1.4 L product/ha corresponding to 399, 798 and 1596 g product/ha (calculated based on test item density 1.14 g/mL). A positive control with the reference item, Rogor® (Dimethoate 400 EC) at the application rate of 15 g/ha was tested (corresponding to 15 mL formulated/ha). A negative control without test item was tested.
Test Conditions:	For the assessment of mortality and reproduction; Open method (Louis & Ufer, 1995) for mortality and reproduction assessment. Temperature within the range of 24.8°C to 25.1°C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 1122 lux to 1210 lux.
Statistics:	Dunnett Multiple Comparison Test (NOER/LOER and reproduction endpoint), Trimmed Spearman-Kärber (mortality endpoint), Linear Interpolation (ICPIN) (reproduction endpoint).

## Results and discussions

All study validity criteria were met.

The highest observed mortality after 7 days (1 week) of exposure in the tested concentrations was in the reference item group, with 86.0% of *Typhlodromus pyri* dead. 15.0% of the *Typhlodromus pyri* died in the negative control after 48 hours.

The reproductive negative control had a mean number of 4.70 after 14 days of eggs/female. The reproductive output was evaluated after 14 days by counting eggs, larvae and females from day 7 to 14 included. The feeding activity in all the treated groups was comparable to the control. Pollen was supplied regularly.

The data on mortality and reproductive output were statistically analysed and the following results obtained.

Table 13. Effect of Difenconazole 250g/L EC greener – IN005B1570 on the survival of the parasitoid *Typhlodromus pyri* in a 14-day exposure study

Survival   48 hours	NOER (g/ha)	LOER (g/ha)	LR <sub>50</sub> (g/ha)
g product/ha	399	798	1316 (1117 – 1551) (*)
g/ha active substance	90.7	181.3	299.0 (253.8 – 325.4) (*)

NOER/LOER and LR50 value (\* with 95% confidence limits), in terms of nominal rate.

Table 2. Effect of Difenconazole 250g/L EC greener – IN005B1570 on the reproduction of the parasitoid *Typhlodromus pyri* in a 14-day exposure study

Survival   48 hours	NOER (g/ha)	LOER (g/ha)	ER <sub>50</sub> (g/ha)
g product/ha	798	>798	>798
g/ha active substance	181.3	>181.3	>181.3

NOER/LOER and LR50 value (with 95% confidence limits), in terms of nominal rate.

## Conclusion

In a parasitoid *Typhlodromus pyri* survival and the reproduction study with Difenconazole 250g/L EC greener – IN005B1570 the NOER for survival after 7 days of exposure was determined to be 399 g test item/ha.

The LOER for survival after 7 days was estimated to be 798 g test item/ha. The LC<sub>50</sub> was estimated to be 1316 (1117 – 1551)(95% confidence limits) g test item/ha. The NOER for reproduction was determined to be 798 g test item/ha. The LOER for reproduction was determined to be >798g test item/ha. The EC<sub>50</sub> was estimated to be >798 g test item/ha.

## Reference Item Test:

In the reference item group, there was 83.5% mortality observed after 7 days. This is compliant with the validity criterion of the test which states that the arithmetic mean mortality of this group is between 50% and 100%.

## Validity criteria

Control Mortality:	Control mortality was 15.0% and so this validity criterion was met.
Reproduction of Control:	The mean control number of eggs/female was 4.70 and so this validity criterion was met.
The level of Mortality in the Toxic Reference Treatment	Was 83.5% and so this validity criterion was met.

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

### A 2.4.1 KCP 10.4.1 Earthworms

# **A 2.4.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects**

## **A 2.4.1.1.1 KCP 10.4.1.1/01 Study 1**

Study Comments: KCP 10.4.1.1/01	<b>zRMS comments:</b> The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met.																							
	<b>Validity criteria</b>																							
	Control Mortality:		Control mortality was 10% and so this validity criterion was met.																					
	Reproduction of Control:		The mean number of juvenile earthworms per replicate was 321 and so this validity criterion was met.																					
	Coefficient of Variation of Reproduction in Control:		Was 5.9% and so this validity criterion was met.																					
Agreed endpoints:	NOEC, EC <sub>10</sub> , EC <sub>20</sub> and EC <sub>50</sub> values (with 95% confidence limits), expressed as nominal concentrations of test item, were as follows.																							
	<table><tr><th>Reproduction</th><th>EC<sub>10</sub> (LCL – UCL)</th><th>EC<sub>20</sub> (LCL – UCL)</th><th>EC<sub>50</sub> (LCL – UCL)</th><th>NOEC</th><th>LOEC</th></tr><tr><td>mg test item/kg d.w.</td><td>731.7 (475.4 – 812.4)</td><td>899.8 (736.7 – N/A)</td><td>&gt; 1000.0 (N/A – N/A)</td><td>666.7</td><td>1000.0</td></tr><tr><td>mg active substance /kg d.w.</td><td>166.2 (108.0 – 184.6)</td><td>204.4 (167.4 – N/A)</td><td>&gt; 227.2 (N/A – N/A)</td><td>151.5</td><td>227.2</td></tr></table>						Reproduction	EC <sub>10</sub> (LCL – UCL)	EC <sub>20</sub> (LCL – UCL)	EC <sub>50</sub> (LCL – UCL)	NOEC	LOEC	mg test item/kg d.w.	731.7 (475.4 – 812.4)	899.8 (736.7 – N/A)	> 1000.0 (N/A – N/A)	666.7	1000.0	mg active substance /kg d.w.	166.2 (108.0 – 184.6)	204.4 (167.4 – N/A)	> 227.2 (N/A – N/A)	151.5	227.2
Reproduction	EC <sub>10</sub> (LCL – UCL)	EC <sub>20</sub> (LCL – UCL)	EC <sub>50</sub> (LCL – UCL)	NOEC	LOEC																			
mg test item/kg d.w.	731.7 (475.4 – 812.4)	899.8 (736.7 – N/A)	> 1000.0 (N/A – N/A)	666.7	1000.0																			
mg active substance /kg d.w.	166.2 (108.0 – 184.6)	204.4 (167.4 – N/A)	> 227.2 (N/A – N/A)	151.5	227.2																			
	(*) 95% confidence limits																							

<b>Reference:</b>	<b>KCP 10.4.1.1/01, Dini, R. (2021)</b>
Report	Difenoconazole 250g/L EC greener – IN005B1570: Effects on reproduction of Earthworms <i>Eisenia fetida</i> in an artificial soil. Dini, R., 2021, ChemService Study No. 0254/2021
Guideline(s):	Yes - OECD, Guideline for the testing of chemicals No. 222, Earthworm, Reproduction Test (adopted July 29, 2016)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

### **Objective**

The purpose of this study was to investigate the effects of Difenoconazole 250g/L EC greener – IN005B1570 on the reproductive output and survival of the adult earthworm *Eisenia fetida*, in an eight week exposure test. The toxicant effects of the test item on the earthworm *Eisenia fetida* were determined based on assessments of adult mortality, growth and reproductive output. The reproductive output was

assessed after eight weeks of exposure by counting the juveniles produced. The adult mortality and adult weight change were recorded after four weeks of exposure.

## Materials and methods

Test Item:	Difenoconazole 250g/L EC greener – IN005B1570
Test Species:	Earthworm ( <i>Eisenia fetida</i> ), adult earthworms.
Test Design:	<p>8 week test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, 1 negative control); 4 replicates for the test item treatments and 8 replicates for the negative control with 10 earthworms each.</p> <p>Assessment of adult earthworm mortality and body weight was carried out after 28 days exposure of adult earthworms in treated artificial soil. Reproduction rate (number of offspring) was assessed after additional 28 days (assessed 56 days after application).</p>
Endpoints:	Adult mortality, growth and reproductive output.
Reference Item:	Carbendazim 98.6% purity. Batch number: BCCB6831.
Test Concentrations:	Control, 58.5, 87.8, 131.7, 197.5, 296.3, 444.4, 666.7 and 1000.0 mg/kg d.w. Eight different stock solutions of test item were prepared by direct weighing into 250 or 500 mL of deionised water, The solutions were poured into artificial soil (2.5 kg dry weight per treatment), and a further 540 or 290 mL of deionised water was added and finally the soil was thoroughly mixed.
Test Conditions:	Artificial soil according to OECD 222; initial pH 6.29 in the negative control, initial pH 6.02 – 6.47 in treated soil. Day 56 pH 7.92 in the negative control, day 56 pH 7.39 – 8.11 in treated soil. Initial soil moisture 30.6% of soil dry weight (corresponding to 40% of maximum water holding capacity (WHC) of the soil (78.9%) in accordance with OECD 222, which requires soil moisture content at 40-60% of the WHC); day 56 soil moisture 31% of soil dry weight. Temperature within the range of 19.6°C to 20.2°C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 487 lux to 751 lux.
Statistics:	Linear Interpolation (ICPIN) (mortality and reproduction end points). Bonferroni adj t Test (NOEC), Shapiro-Wilk W normality test (normal distribution), Bartlett equality of variance test (equal variances).

## Results and discussions

All study validity criteria were met.

The highest observed mortality after 28 days of exposure in the tested concentrations was at 58.5 mg test item/kg d.w, with 12.5% of earthworms dead. 10% of earthworms died in the negative control after 28

days. The body weight changes of the earthworms after 4 weeks exposure to Difenoconazole 250g/L EC greener – IN005B1570 varied above and much below the negative control.

The negative control had a mean weight change of 22.3% after 28 days of exposure. The test concentration at 58.5mg test item/kg d.w had a mean weight change of 39.5%. The lowest mean weight change was 5.4% at test concentration 1000.0 mg/kg d.w.

The negative control had a mean reproduction value of 345.5 juveniles and all replicates had at least 321 juveniles. At the test concentration of 1000.0 mg test item/kg d.w, the reproduction was statistically significantly reduced compared to the control. The feeding activity in all the treated groups was comparable to the control (see Table 1).

**Table 14. Effect of Difenoconazole 250g/L EC greener – IN005B1570 on earthworms (*Eisenia fetida*) in a 56-day reproduction study.**

Difenoconazole 250g/L EC greener – IN005B1570 [mg test item/ kg soil]	Control	58.5	87.8	131.7	197.5	296.3	444.4	666.7	1000.0
Mortality (day 28) [%]	10.0	12.5	2.5	0	0	5.0	2.5	0	5.0
Statistical Significance <sup>1)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.			
Body weight change (day 28) [%]	22.3	39.5	26.4	16.3	21.4	14.6	6.4	10.1	5.4
Statistical Significance <sup>2)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.			
Mean No. of juveniles (day 56)	345.5	344.5	337.3	336.5	330.8	333.3	330.5	326.5	258.8
Statistical Significance <sup>3)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
Reproduction inhibition [%] compared to control (day 56)	-	0.3	2.4	2.6	4.3	3.5	4.3	5.5	25.1
Food consumption [g]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Endpoints [mg test item/kg soil]									
Difenoconazole 250g/L EC greener – IN005B1570 [mg test item/ kg soil]	Control	58.5	87.8	131.7	197.5	296.3	444.4	666.7	1000.0
LC <sub>50</sub> <sup>4)</sup>	>1000.0								
NOEC (day 56 reproduction)	666.7								
LOEC (day 56 reproduction)	151.5								
EC <sub>50</sub> <sup>4)</sup>	>1000.0								

All concentrations are indicated per kg soil dry weight.

n.s. = not significantly different compared to the control \* = significantly different compared to the control

## Conclusion

In an earthworm reproduction and mortality study with Difenoconazole 250g/L EC greener – IN005B1570 the No Observed Effect Concentration (NOEC) for reproduction of the earthworm *Eisenia fetida* was determined to be 666.7 mg test item/kg soil, i.e. the highest concentration tested. The LOEC for reproduction changes was estimated to be 1000.0 mg test item/kg soil. The LC<sub>50</sub> was estimated to be >1000.0 mg test item/kg soil. The EC<sub>50</sub> was estimated to be >1000.0 mg test item/kg soil.

## Reference Item Test:

In the most recent test with the reference item Carbendazim, purity 98.6%. In accordance with OECD guideline 222, significant effects should be observed between 1 and 5 mg carbendazim/kg dry mass. In internal study 16/09/2020, the NOEC value was found to be < 1.0, the LOEC value 1.0 and the EC50 confidence interval 0.92 (0.71 – 1.4). This is in accordance with the OECD 222.

## Validity criteria



Control Mortality:	Control mortality was 10% and so this validity criterion was met.
Reproduction of Control:	The mean number of juvenile earthworms per replicate was 321 and so this validity criterion was met.
Coefficient of Variation of Reproduction in Control:	Was 5.9% and so this validity criterion was met.

#### 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 2.4.3 KCP 10.4.2.1/01 Study 1

Study Comments: KCP 10.4.2.1/01	<p><b>zRMS comments:</b> The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met.</p> <p><b>Validity criteria</b></p> <table> <tr> <td>Control Mortality:</td><td>Control mortality was 5.0% and so this validity criterion was met.</td></tr> <tr> <td>Reproduction of Control:</td><td>In the negative control a mean value of 283.8 juveniles were found and so this validity criterion was met.</td></tr> <tr> <td>Coefficient of Variation of Reproduction in Control:</td><td>Was 17.5% and so this validity criterion was met.</td></tr> </table>	Control Mortality:	Control mortality was 5.0% and so this validity criterion was met.	Reproduction of Control:	In the negative control a mean value of 283.8 juveniles were found and so this validity criterion was met.	Coefficient of Variation of Reproduction in Control:	Was 17.5% and so this validity criterion was met.		
Control Mortality:	Control mortality was 5.0% and so this validity criterion was met.								
Reproduction of Control:	In the negative control a mean value of 283.8 juveniles were found and so this validity criterion was met.								
Coefficient of Variation of Reproduction in Control:	Was 17.5% and so this validity criterion was met.								
Agreed endpoints:	<p><b>Survival</b></p> <p>LC<sub>50</sub> value (with 95% confidence limits), in terms of nominal test item concentration.</p> <table> <tr> <th>Survival</th><th>LC<sub>50</sub> (mg/kg soil d.w.)</th></tr> <tr> <td>mg test item/kg d.w.</td><td>60.0 (56.1 – 64.3) (*)</td></tr> </table> <p>(*) 95% confidence limits      N/A = not applicable</p> <p>LC<sub>50</sub> value (with 95% confidence limits), in terms of nominal test item concentration.</p> <table> <tr> <th>Survival</th><th>LC<sub>50</sub> (mg/kg soil d.w.)</th></tr> <tr> <td>mg active substance/kg d.w.</td><td>13.6 (12.7 – 14.6) (*)</td></tr> </table> <p>N/A = not applicable</p>	Survival	LC <sub>50</sub> (mg/kg soil d.w.)	mg test item/kg d.w.	60.0 (56.1 – 64.3) (*)	Survival	LC <sub>50</sub> (mg/kg soil d.w.)	mg active substance/kg d.w.	13.6 (12.7 – 14.6) (*)
Survival	LC <sub>50</sub> (mg/kg soil d.w.)								
mg test item/kg d.w.	60.0 (56.1 – 64.3) (*)								
Survival	LC <sub>50</sub> (mg/kg soil d.w.)								
mg active substance/kg d.w.	13.6 (12.7 – 14.6) (*)								



<b>Reproduction</b>				
EC <sub>10</sub> , EC <sub>50</sub> values (with 95% confidence limits) and NOEC in terms of nominal test item concentrations.				
<b>Reproduction</b>	<b>EC<sub>10</sub></b> (LCL – UCL)	<b>EC<sub>50</sub></b> (LCL – UCL)	<b>NOEC</b>	<b>LOEC</b>
mg test item/kg d.w.	34.3 (N/A – 53.0) (*)	71.2 (54.5 – 78.6) (*)	44.4	66.7
(*) 95% confidence limits		N/A = not applicable		
EC <sub>10</sub> , EC <sub>50</sub> values (with 95% confidence limits) and NOEC in terms of nominal active substance concentrations.				
<b>Reproduction</b>	<b>EC<sub>10</sub></b> (LCL – UCL)	<b>EC<sub>50</sub></b> (LCL – UCL)	<b>NOEC</b>	<b>LOEC</b>
mg active substance /kg d.w.	7.8 (N/A – 12.0) (*)	16.2 (12.4 – 17.9) (*)	10.1	15.1
(*) 95% confidence limits		N/A = not applicable		

<b>Reference:</b>	<b>KCP 10.4.2.1/01, Dini, R. (2021)</b>
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on Collembolan Reproduction in an Artificial Soil Study, Dini, R 2021. ChemService Study No. 0255/2021
Guideline(s):	Yes - OECD Guideline for Testing of Chemicals, N. 232, “Collembolan reproduction test in soil”, 29 July 2016.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

### Objective

The purpose of this study was to investigate the effects of Difenoconazole 250g/L EC greener – IN005B1570 on the reproductive output and survival of the Collembola (springtail) species *Folsomia candida* in a four week artificial soil study. The toxicant effects of the test item on *Folsomia candida* were determined based on assessments of adult mortality and reproductive output after 28 days of exposure.

### Materials and methods

Test Item: Difenoconazole 250g/L EC greener – IN005B1570

Test Species: Collembola (springtail) species (*Folsomia candida*). On arrival, from 9 to 12 day old synchronized juvenile organisms were alive and in good condition (normal behavior).

Test Design: 4 week test in treated artificial soil prepared according to OECD 323; different

concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, 1 negative control); 4 replicates for the test item treatments and 8 replicates for the negative control. Assessment of adult *Collembola* (springtail) survival and reproduction was carried out after 28 days. About 5 mg of granulated dried baker's yeast was added to each test vessels at the start of the test. After 2 weeks the vessels were checked, the uneaten food was removed and an amount of about 10 mg of fresh food was replaced.

Endpoints: Adult mortality and reproductive output.

Reference Item: Boric acid purity 100.1%. Batch number: AM1193865.

**Test Concentrations:** Control, 5.9, 8.8, 13.2, 19.8, 29.6, 44.4, 66.7 and 100.0 mg/kg d.w. A 500 mg/L stock solution was prepared by direct weighing 0.0500 g of the test item into 100 mL deionized water.

**Test Conditions:** Artificial soil according to OECD 323; initial pH 6.87 – 7.03 in treated soil, pH at experimental end 6.93 – 7.12 in treated soil. Initial pH 6.83 in the negative control, pH at experimental end 6.91; Initial soil moisture 29.4% of soil dry weight (corresponding to 40% of maximum water holding capacity (WHC) of the soil (78.9%) in accordance with OECD 323, which requires soil moisture content at 40-60% of the WHC); experimental end soil moisture 29.4% of soil dry weight. Temperature within the range of 19.9°C to 20.1°C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 659 lux to 689 lux.

Statistics: Linear Interpolation (ICPIN) (reproduction ( $EC_{50}$ )). the Equal Variance t Two-Sample Test ( $p < 0.05$ ) ( NOEC and LOEC values). Spearman-Kärber method (mortality ( $LC_{50}$ )).

## Results and discussions

All study validity criteria were met.

The highest observed mortality after 28 days of exposure in the tested concentrations was at 100.0 mg test item/kg d.w, with 100% of *Collembola* (springtail) dead. 5.0% of *Collembola* (springtail) died in the negative control after 28 days.

The negative control had a mean reproduction value of 283.8 juveniles after 14 days. At the test concentration of 100.0 mg test item/kg d.w, the reproduction was significantly reduced compared to the control.

Table 15. Effect of Difenoconazole 250g/L EC greener – IN005B1570 on Collembolan (*Folsomia candida*) in a 28-day reproduction study

<b>Difenoconazole 250g/L EC greener – IN005B1570 [mg test item/ kg soil]</b>	<b>Control</b>	<b>5.9</b>	<b>8.8</b>	<b>13.2</b>	<b>19.8</b>	<b>29.6</b>	<b>44.4</b>	<b>66.7</b>	<b>100.0</b>
Mortality (day 28) [%]	5.0	10.0	0	2.5	7.5	7.5	5.0	70.0	100.0
Statistical Significance <sup>1)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s	n.s	n.s

Difenoconazole 250g/L EC greener – IN005B1570 [mg test item/ kg soil]	Control	5.9	8.8	13.2	19.8	29.6	44.4	66.7	100.0
Mean No. of juveniles (day 14)	283.8	348.0	253.3	277.8	374.8	313.8	248.5	188.5	0
Statistical Significance <sup>3)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Reproduction inhibition [%] compared to control (day 14)	-	-22.6	10.7	2.1	-32.1	-10.6	12.4	33.6	100.0
Food consumption [g] (start of test)	5mg	5mg	5mg	5m5	5mg	5mg	5mg	5mg	5mg
<b>Endpoints [mg test item/kg soil]</b>									
LC <sub>50</sub> <sup>4)</sup>	60.0 (56.1 – 64.3)								
NOEC (day 14 reproduction)	44.4								
LOEC (day 14 reproduction)	66.7								
EC <sub>50</sub> <sup>4)</sup>	71.2 (54.5 – 78.6)								

In table 1 the statistical significance seems to be erroneously indicated. The NOEC is agreed, however.

### Conclusion

In a Collembola (springtail) survival and reproduction study with Difenoconazole 250g/L EC greener – IN005B1570 the No Observed Effect Concentration (NOEC) for reproduction of the Collembola (spring-tail) *Folsomia candida* determined to be 44.4 mg test item/kg soil, *i.e.* the highest concentration tested. The LOEC for reproduction changes was estimated to be 66.7 mg test item/kg soil. The LC<sub>50</sub> was estimated to be 60.0 mg test item/kg soil. The EC<sub>50</sub> was estimated to be 71.2 mg test item/kg soil.

### Reference Item Test:

In the most recent test with the reference item Boric Acid, purity 100.1%. In accordance with OECD 232, EC<sub>50</sub> should be observed at about 100 mg/kg of Boric Acid. In internal study 23/06/2020\_C with Boric Acid, the EC<sub>50</sub> confidence interval was 99.15 (62.27 – 141.7).

### Validity criteria

Control Mortality:	Control mortality was 5.0% and so this validity criterion was met.
Reproduction of Control:	In the negative control a mean value of 283.8 juveniles were found and so this validity criterion was met.
Coefficient of Variation of Reproduction in Control:	Was 17.5% and so this validity criterion was met.

### A 2.4.1 KCP 10.4.2.1/02 Study 2

Study Comments: KCP 10.4.2.1/04	<b>zRMS comments:</b> The study is considered valid. It is GLP, performed according to relevant guideline and all validity criteria are met.	
	<b>Validity criteria</b>	
	Control Mortality:	Control mortality was 1.3% and so this validity criterion was met.
	Reproduction of Control:	The number of juvenile earthworms per replicate was 143 to 185 and so this validity criterion was met.
	Coefficient of Variation of Reproduction in Control:	Was 8.0% and so this validity criterion was met.

Agreed endpoints:

**Survival**

LC<sub>50</sub> value (with 95% confidence limits), in terms of nominal test item concentration.

Survival	LC <sub>50</sub> (mg/kg soil d.w.)
mg test item/kg d.w.	> 1000.0 (N/A – N/A) (*)

N/A = not applicable

LC<sub>50</sub> value (with 95% confidence limits), in terms of nominal test item concentration.

Survival	LC <sub>50</sub> (mg/kg soil d.w.)
mg active substance/kg d.w.	> 227.2 (N/A – N/A) (*)

N/A = not applicable

**Reproduction**

EC<sub>10</sub>, EC<sub>50</sub> values (with 95% confidence limits) and NOEC in terms of nominal test item concentrations.

Reproduction	EC <sub>10</sub> (LCL – UCL)	EC <sub>50</sub> (LCL – UCL)	NOEC	LOEC
mg test item/kg d.w.	390.0 (60.3 – 632.3) (*)	> 1000.0 (N/A – N/A) (*)	296.3	444.4

(\*) 95% confidence limits

N/A = not applicable

EC<sub>10</sub>, EC<sub>50</sub> values (with 95% confidence limits) and NOEC in terms of nominal active substance concentrations.

Reproduction	EC <sub>10</sub> (LCL – UCL)	EC <sub>50</sub> (LCL – UCL)	NOEC	LOEC
mg active substance /kg d.w.	88.6 (13.7 – 143.7) (*)	> 227.2 (N/A – N/A) (*)	67.3	101.0

(\*) 95% confidence limits

N/A = not applicable

<b>Reference:</b>	<b>KCP 10.4.2.1/02, Dini, R. (2021)</b>
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on <i>Hypoaspis (Geolaelaps) aculeifer</i> Reproduction in an Artificial Soil Study. Dini, R 2021. ChemService Study No. 0256/2021
Guideline(s):	Yes - OECD Guideline for Testing of Chemicals, N. 226, “Predatory mite ( <i>Hypoaspis (Geolaelaps) aculeifer</i> ) reproduction test in soil”, 29 July 2016.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

### Objective

The purpose of this study was to investigate the effects of Difenoconazole 250g/L EC greener – on the

survival and reproduction of the soil mite species *Hypoaspis (Geolaelaps) aculeifer* in a two-week artificial soil study. Toxicant effects of the test item on *Hypoaspis (Geolaelaps) aculeifer* were determined based on assessments of adult mortality and reproductive output after 14 days of exposure.

## Materials and methods

Test Item:	Difenconazole 250g/L EC greener – IN005B1570
Test Species:	Soil mite species <i>Hypoaspis (Geolaelaps) aculeifer</i> . Petri dishes were sent containing the organisms, the supplier reported the period within the organisms must be used (between 24 <sup>th</sup> May and 31 <sup>st</sup> May 2021). Only females, with an age between 28 and 35 days after the first spawning, were used for the test.
Test Design:	2 week test in treated artificial soil prepared according to OECD 222; different concentrations of the test item were incorporated into the soil; 9 treatment groups (8 test item concentrations, 1 negative control); 4 replicates for the test item treatments and 8 replicates for the negative control. Assessment of adult soil mite <i>Hypoaspis (Geolaelaps) aculeifer</i> was carried out after 14 days.
Endpoints:	Adult mortality and reproductive output.
Reference Item:	Boric acid purity 100.1%. Batch number: AM1193865.
Test Concentrations:	Control, 58.5, 87.8, 131.7, 197.5, 296.3, 444.4, 666.7 and 1000.0 mg/kg d.w. For each tested concentration, test item was added to the artificial soil by preparing eight different stock solutions of test item prepared by direct weighing the test item into deionized water.
Test Conditions:	Artificial soil according to OECD 222; initial pH 6.41 – 6.47 in treated soil, pH at experimental end 6.46 – 6.55 in treated soil. Initial pH 6.39 in the negative control, pH at experimental end 6.51; Initial soil moisture 29.6% of soil dry weight (corresponding to 40% of maximum water holding capacity (WHC) of the soil (78.9%) in accordance with OECD 222, which requires soil moisture content at 40-60% of the WHC); experimental end soil moisture 28.1% of soil dry weight. Temperature within the range of 19.9°C to 20.1°C; photoperiod: 16 h light : 8 h dark, light intensity: within the range of 689 lux to 694 lux.
Statistics:	Linear Interpolation (ICPIN) (mortality (LC <sub>50</sub> )), Bonferroni Adj t test (NOEC and LOEC values).

## Results and discussions

All study validity criteria were met.

The highest observed mortality after 14 days of exposure in the tested concentrations was at 58.5, 197.5 and 444.4 mg test item/kg d.w, with 17.5% of *Hypoaspis (Geolaelaps) aculeifer* dead. 12.5% of *Hypoaspis (Geolaelaps) aculeifer* died in the negative control after 14 days.

The negative control had a mean reproduction value of 65.3 juveniles after 14 days. At the test concentration of 444.4, 666.7 and 1000.0 mg test item/kg d.w, the reproduction was statistically significantly reduced compared to the control. The feeding activity in all the treated groups was comparable to the control. A juvenile organism of *Folsomia candida* were added to each container at the start of the test and on day 2, 5, 8 and 12 of the test duration.

**Table 16. Effect of Difenoconazole 250g/L EC greener – IN005B1570 on Hypoaspis (*Geolaelaps*) *aculeifer* in a 14-day reproduction study**

Difenoconazole 250g/L EC greener – IN005B1570 [mg test item/ kg soil]	Control	58.5	87.8	131.7	197.5	296.3	444.4	666.7	1000.0
Mortality (day 14) [%]	12.5	17.5	12.5	12.5	17.5	12.5	17.5	15.0	12.5
Statistical Significance <sup>1)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 14)	65.3	65.3	63.8	64.0	62.8	63.0	56.8	55.5	43.8
Statistical Significance <sup>3)</sup>	-	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*
Reproduction inhibition [%] compared to control (day 14)	-	0.0	2.3	1.9	3.8	3.4	13.0	14.9	33.0
<b>Endpoints [mg test item/kg soil]</b>									
LC <sub>50</sub> <sup>4)</sup>	>1000.0								
NOEC (day 14 reproduction)	296.3								
LOEC (day 14 reproduction)	444.4								
EC <sub>50</sub> <sup>4)</sup>	>1000.0								

#### Conclusion

In a *Hypoaspis* (*Geolaelaps*) *aculeifer* survival and reproduction study with Difenoconazole 250g/L EC greener – IN005B1570 the No Observed Effect Concentration (NOEC) for reproduction of the Collembola *Hypoaspis* (*Geolaelaps*) *aculeifer* was determined to be 296.3 mg test item/kg soil, *i.e.* the highest concentration tested.

The LOEC for reproduction changes was estimated to be 444.4 mg test item/kg soil. The LC<sub>50</sub> was estimated to be >1000.0 mg test item/kg soil. The EC<sub>50</sub> was estimated to be >1000.0 mg test item/kg soil.

#### Reference Item Test:

The reference item used was Boric acid with a purity of 100.1%. Batch number AM1193865. In accordance with OECD 226, the EC<sub>50</sub> based on the number of juveniles should fall in the range between 100 and 500 mg/kg d.w soil of Boric Acid. In internal study 23/06/2020 with Boric Acid as reference item, the EC<sub>50</sub> confidence interval was found to be 194.4 (146.1 – 229.5).

#### Validity criteria

Control Mortality:	Control mortality was 1.3% and so this validity criterion was met.
Reproduction of Control:	The number of juvenile earthworms per replicate was 143 to 185 and so this validity criterion was met.
Coefficient of Variation of Reproduction in Control:	Was 8.0% and so this validity criterion was met.

**A 2.4.1.1 KCP 10.4.2.1 Species level testing**

**A 2.4.1.2 KCP 10.4.2.2 Higher tier testing**

**A 2.5 KCP 10.5 Effects on soil nitrogen transformation**

### A 2.5.1 KCP 10.5 Study 1

<p>Study Comments: KCP 10.5</p>	<p><b>RMS comments:</b></p> <p>The study was performed according to the OECD 216 guidelines (“Soil Microorganisms: Nitrogen Transformation Test”, 2000).</p> <p><b>RMS comments:</b></p> <p>The study was performed according to the OECD 216 guidelines. The variation between the control samples is less than <math>\pm 15\%</math>. In the present test the CV was in the range 1.9%–7.7% from test start until 28th day it. Therefore, the study can be considered <b>valid</b>.</p> <p>No deviations were issued in the study.</p> <p><b>Conclusion:</b></p> <p>The effects of Difenoconazole 250 g/L EC greener – IN005B1570 on nitrogen transformation were determined based on nitrogen transformation rates in treated soil compared to untreated soil.</p> <ul style="list-style-type: none"> <li>☑ At maximum tested concentration [1.75 L Gf-224/ha, equivalent to 2.723 mg GF-224/kg dry soil] effects on soil nitrate concentration were <math>&lt; 25\%</math>.</li> <li>☑ At maximum tested concentration [1.75 L GF-224/ha, equivalent to 2.723 mg GF-224/kg dry soil] effects on soil microbial respiration were <math>&lt; 25\%</math>.</li> </ul> <p><b>Updated April 2024</b></p> <p><b>According NL comments:</b> Ctgb notes that in the summary of the nitrification study results for the interval 14-28 days are missing. In addition, Ctgb notes that during the interval 0-7 days an effect <math>&gt; 25\%</math> was observed. Ctgb thinks that a deviation in any interval should be taken into account as an effect, not only after 28 days. Otherwise, sampling at day 7 and 14 would be meaningless, since comparing the nitrogen formation rate only for the interval day 0-28 will yield exactly the same results as comparing total nitrogen. Therefore, Ctgb thinks that an effect <math>&gt; 25\%</math> is observed at both treatments and this should be taken into account in the risk assessment for soil microbial.</p> <p><b>The result data from the microorganisms study was added by zRMS. Results in terms of interval rate (day 0-7, day -14, day 14-28): For both treatments, on the 28<sup>th</sup> day the difference (mean value) between nitrogen transformation rate in untreated soil and treated soil was lower than 25%.</b></p>
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Interval rate - Mean nitrate formation rate and percentage deviation from control in untreated and treated soil after 7, 14 and 28 days				
Interval rate (mg nitrate/kg soil d.w./day)				
	Time Replicate	0 – 7	7 - 14	14 - 28
Control (0.0 mg/kg soil d.w.)	A	1.93	3.85	3.34
	B	1.50	3.15	3.01
	C	2.69	2.64	3.17
	D	0.79	3.13	2.72
	Mean	1.73	3.19	3.06
0.46 mg/kg soil d.w. (2)	A	2.44	2.53	3.35
	B	2.72	3.74	2.91
	C	2.06	2.74	3.11
	D	2.88	2.56	3.10
	Mean	2.53	2.89	3.12 (ns)
	Deviation from Control % (1)	-46.2	9.4	-2.0
2.3 mg/kg soil d.w. (2)	A	3.42	3.79	3.86
	B	2.97	2.47	3.61
	C	2.82	4.28	3.32
	D	2.89	1.93	3.06
	Mean	3.03	3.12	3.46 (ns)
	Deviation from Control % (1)	-75.2	2.5	-13.2
(1) a positive percentage indicates that nitrate formation rate in the treated soil is lower than in the control soil. A negative percentage indicates that nitrate formation rate in the treated soil is higher than in the control soil. (2) concentrations expressed as test item.				
Agreed endpoints:	The overall mean rate of deviation for both test item concentrations (0.46 and 2.3 mg/kg soil d.w.) were -5.6% and -18.4% respectively. The interval mean rate of deviation for both test item concentrations (0.46 and 2.3 mg/kg soil d.w.) were - 2.0% and -13.2%. After assessment of these results, the test item can be evaluated as having no long-term influence on nitrogen transformation in the soil.			
Conclusion:				



Results in terms of <b>overall rate</b> :	For both treatments, on the 28 <sup>th</sup> day the difference (mean value) between nitrogen transformation rate in untreated soil and treated soils was lower than 25%, therefore the incubation was stopped.				
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Overall Rate (mg / kg soil dry weight per day) <sup>1</sup>					
	Control	0.46 mg test item/kg soil d.w.		2.3 mg test item/kg soil d.w.	
Interval	Nitrate-N Formation rate mg/kg/day	Nitrate-N Formation rate mg/kg/day	Deviation <sup>2</sup>	Nitrate-N Formation rate mg/kg/day	Deviation <sup>2</sup>
Day 0 - 7	1.73	2.53	-46.2	3.03	-75.2
Day 0 - 14	2.46	2.71	-10.1	3.07	-24.8
Day 0 - 28	2.76	2.91 (n.s.)	-5.6	3.27 (n.s.)	-18.4
<sup>1</sup> = related to intervals from time zero to the current sampling <sup>2</sup> = % deviation from control positive values = inhibitory effect; negative values = stimulatory effect ns: statistically not significantly different from control ( $\alpha = 0.05$ )					

Results in terms of <b>interval rate</b> :	For both treatments, on the 28 <sup>th</sup> day the difference (mean value) between nitrogen transformation rate in untreated soil and treated soils was lower than 25%, therefore the incubation was stopped.				
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Interval Rate (mg / kg soil dry weight per day) <sup>1</sup>					
	Control	0.46 mg test item/kg soil d.w.		2.3 mg test item/kg soil d.w.	
Interval	Nitrate-N Formation rate mg/kg/day	Nitrate-N Formation rate mg/kg/day	Deviation <sup>2</sup>	Nitrate-N Formation rate mg/kg/day	Deviation <sup>2</sup>
Day 0 - 7	1.73	2.53	-46.2	3.03	-75.2
Day 7 - 14	3.19	2.89	9.4	3.12	2.5
Day 14 - 28	3.06	3.12 (n.s.)	-2.0	3.46 (n.s.)	-13.2
<sup>1</sup> = related to successive intervals between samplings <sup>2</sup> = % deviation from control positive values = inhibitory effect; negative values = stimulatory effect ns: statistically not significantly different from control ( $\alpha = 0.05$ )					

According to OECD Guideline No. 216, the following effects of Difenoconazole 250 g/L EC greener – IN005B1570 on soil microbial nitrogen transformation were assessed.			
Test item concentration [mg/kg soil d.w.]	Overall rate - Deviation from control % (1)		
	0-7	0-14	0-28
0.46	-46.2	-10.1	-5.6
2.3	-75.2	-24.8	-18.4
(1) a negative percentage indicates that nitrate formation rate in the treated soil was higher than in control soil; a positive percentage indicates that nitrate formation rate in the treated soil was lower than in control soil.			
Test item concentration [mg/kg soil d.w.]	Interval rate - Deviation from control % (1)		
	0-7	7-14	14-28
0.46	-46.2	9.4	-2.0
2.3	-75.2	2.5	-13.2
(1) a negative percentage indicates that nitrate formation rate in the treated soil was higher than in control soil; a positive percentage indicates that nitrate formation rate in the treated soil was lower than in control soil.			
According to these results, the test item can be evaluated as having no long-term influence on nitrogen transformation in soil.			

Reference:	KCP 10.5, Tediosi, E. (2022)
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Effects on Soil Microorganisms - Nitrogen Transformation Test. Tediosi, E 2022. ChemService Study No. 0770/2021
Guideline(s):	Yes - OECD Guideline No. 216, “Soil Microorganisms: Nitrogen Transformation Test”, 2000.
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

### Objective

The purpose of this study was to investigate adverse effects of Difenoconazole 250 g/L EC greener – IN005B1570 on the process of nitrogen transformation of aerobic soil microorganisms. The effects of Difenoconazole 250 g/L EC greener – IN005B1570 on nitrogen transformation were determined based on nitrogen transformation rates in treated soil compared to untreated soil.

### Materials and methods

Test Item: Difenoconazole 250g/L EC greener – IN005B1570

Test Design: 4 week test in treated soil prepared according to OECD 216; different concentrations of the test item were incorporated into the soil; 3 treatment groups (2

test item concentrations, 1 negative control); 4 replicates for the test item treatments and 4 replicates for the negative control. Assessment of the soils nitrate concentration was carried out on day 0, 7, 14 and 28.

Endpoints: Nitrate concentration in treated and untreated soil.

Reference Item: Nitrate, standard for IC. Purity  $1000 \pm 4$  µg/mL. Batch number: BCCC1546.

Test Concentrations: 0.46 mg test item/kg d.w. and 2.3 mg test item/kg d.w. For each tested concentration, test item was added to the soil using water as a carrier. Control samples were treated with an equivalent amount of water only. One stock solution at 500.0mg/L was prepared by weighing 0.0503 g of test item in 100 ml of demineralized water. Two further stock solutions were prepared by dilution in demineralized water; 1.6 mL and 8.1 mL of Stock solution.

Test Conditions: Soil according to OECD 216; initial pH 6.01 – 5.98 in treated soils, pH at experimental end 5.93 – 5.95 in treated soils. Initial pH 5.93 in the negative control, pH at experimental end 5.96; Initial soil moisture 14.4% of soil dry weight (corresponding to 40.3% of maximum water holding capacity (WHC) in accordance with OECD 216, which requires soil moisture content at 40-60% of the WHC); experimental end soil moisture 14.3% of soil dry weight (corresponding to 40.1% of maximum water holding capacity (WHC) of the soil (78.9%) in accordance with OECD 216, which requires soil moisture content at 40-60% of the WHC); Temperature within the range of 19.96°C to 20.06°C; photoperiod: 24 h dark.

Statistics: Dunnett Multiple Comparison Test (statistically significant difference), Shapiro-Wilk W Normality test (data distribution), Bartlett Equality of Variance test (equality of variance).

### Results and discussions

All study validity criteria were met.

According to OECD Guideline no. 216, the following effects of Difenoconazole 250 g/L EC greener – IN005B1570 on soil microbial nitrogen transformation were assessed. The highest observed deviation from the control was observed after 7 days in the 2.3 mg nitrate/kg soil d.w with a mean of -75.2%. A negative percentage indicates that the nitrate formation rate in the treated soil is higher than in the control soil. After 7 days the control has a mean deviation of 1.73%.

The interval rate was also assessed over the duration of the study. The mean interval rate for the 2+

Table 17. Effect of Difenoconazole 250g/L EC greener – IN005B1570 on mean nitrate formation rate and percentage deviation from control in untreated and treated soil after 7, 14 and 28 days (Overall rate).

3;

Difenoconazole 250g/L EC greener – IN005B1570 [mg test item/ kg soil]	Control	0.46	2.3
Day 0 -7 [mean %]	1.73	2.53	3.03

3;

Difenoconazole 250g/L EC greener – IN005B1570 [mg test item/ kg soil]	Control	0.46	2.3
Day 7 - 14 [mean %]	2.46	2.71	3.07
Day 0 - 28 [mean %]	2.76	2.91 (ns)	3.27 (ns)
Deviation day 0 – 7 [%]	-	- 46.2	- 75.2
Deviation day 7 – 14 [%]	-	- 10.1	- 24.8
Deviation day 0 – 28 [%]	-	- 5.6	- 18.4

ns = Difference from control statistically not significant (P<0.05); assessed by Dunnett Multiple Comparison Test.

Table 2. Effect of Difenoconazole 250g/L EC greener – IN005B1570 on mean nitrate formation rate and percentage deviation from control in untreated and treated soil after 7, 14 and 28 days (Interval rate).

Difenoconazole 250g/L EC greener – IN005B1570 [mg test item/ kg soil]	Control	0.46	2.3
Day 0 -7 [mean %]	1.73	2.53	3.03
Day 7 - 14 [mean %]	3.19	2.89	3.12
Day 0 - 28 [mean %]	3.06	3.12 (ns)	3.46 (ns)
Deviation day 0 – 7 [%]	-	- 46.2	- 75.2
Deviation day 7 – 14 [%]	-	9.4	2.5
Deviation day 0 – 28 [%]	-	-2.0	-13.2
14-28 [%]			

ns = Difference from control statistically not significant (P<0.05); assessed by Dunnett Multiple Comparison Test.

## Conclusion

The overall mean rate of deviation for both test item concentrations (0.46 and 2.3 mg/kg soil d.w.) were -5.6% and -18.4% respectively. The interval mean rate of deviation for both test item concentrations (0.46 and 2.3 mg/kg soil d.w.) were -2.0% and -13.2%. After assessment of these results, the test item can be evaluated as having no long-term influence on nitrogen transformation in the soil.

## Validity criteria

The Variation in Nitrate Concentration between replicate control samples and coefficient of variation (CV):	The CV of nitrate concentration between replicates ranges from 1.9% to 7.7% and so this validity criterion was met.
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## 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

### A 2.6.3

### KCP 10.6.2/01 Study 1

#### ***zRMS comments:***

The study is based on test guidelines OECD 227 adopted 19 July 2006. A valid test requires that:

<b>Validity of the test :</b>	<b>Obtained :</b>
<input checked="" type="checkbox"/> The seedling emergence is at least 70%;	Fullfield.
<input checked="" type="checkbox"/> The plants in the control do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations). Plants exhibit only normal variation in growth and morphology for that particular species;	Fullfield
<input checked="" type="checkbox"/> The mean plant survival in the control is at least 90 % for the duration of the study;	Fullfield
<input checked="" type="checkbox"/> Environmental conditions for a particular species in the control are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source.	Yes. Conditions were identical for each species.

*Thus the validity criteria are met.*

#### **Following deviations from OECD 227 were noted:**

No deviation were issued.

#### **The phytotoxicity effect**

- The chlorosis, necrosis and other effects evaluation (wilting and deformation) were expressed as percentage of affected leaf area for each replicate. The range of percentage was classified in to 0-5 scale of values as reported in Section "Evaluations".
- The effects on plant and leaves were observed after 7, 14 and 21 days from the treatment.
- In the following table are presented the mean values of scale calculated among replicates for each dose rate at the end of test period.

At the end of the test no phytotoxic effects were shown in all tested species.

#### **Conclusion:**

		<b>Mortality (based on nominal test item application rates)</b>																																															
		<table><tr><th>Species (common name)</th><th>NOER (g a.s./ha)</th><th>LOER (g a.s./ha)</th><th>ER<sub>10</sub> (g a.s./ha)</th><th>ER<sub>20</sub> (g a.s./ha)</th><th>ER<sub>50</sub> (g a.s./ha)</th></tr><tr><td>Oat</td><td>1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td></tr><tr><td>Oilseed rape</td><td>1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td></tr><tr><td>Onion</td><td>1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td></tr><tr><td>Perennial ryegrass</td><td>1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td></tr><tr><td>Radish</td><td>1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td></tr><tr><td>Soybean</td><td>1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td></tr></table>	Species (common name)	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)	Oat	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5	Oilseed rape	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5	Onion	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5	Perennial ryegrass	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5	Radish	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5	Soybean	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5					
Species (common name)	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)																																												
Oat	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5																																												
Oilseed rape	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5																																												
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Perennial ryegrass	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5																																												
Radish	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5																																												
Soybean	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5																																												
		<b>Biomass (fresh weight) (based on nominal test item application rates)</b>																																															
		<table><tr><th>Species (common name)</th><th>NOER (g a.s./ha)</th><th>LOER (g a.s./ha)</th><th>ER<sub>10</sub> (g a.s./ha) with 95% conf. limits</th><th>ER<sub>20</sub> (g a.s./ha) with 95% conf. limits</th><th>ER<sub>50</sub> (g a.s./ha) with 95% conf. limits</th></tr><tr><td>Oat</td><td>40</td><td>100</td><td>50.5 (N/A – 263.4)</td><td>440.4 (N/A – N/A)</td><td>&gt; 1562.5</td></tr><tr><td>Oilseed rape</td><td>1562.5</td><td>&gt; 1562.5</td><td>592.1 (9.8 – N/A)</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td></tr><tr><td>Onion</td><td>625</td><td>1562.5</td><td>233.8 (N/A – 1263.0)</td><td>949.7 (N/A – N/A)</td><td>&gt; 1562.5</td></tr><tr><td>Perennial ryegrass</td><td>1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td><td>&gt; 1562.5</td></tr><tr><td>Radish</td><td>40</td><td>100</td><td>13.1 (3.7 – 225.9)</td><td>242.7 (23.7 – 1071.0)</td><td>&gt; 1562.5</td></tr><tr><td>Soybean</td><td>16</td><td>40</td><td>42.1 (9.3 – 306.4)</td><td>1169.0 (451.4 – N/A)</td><td>&gt; 1562.5</td></tr></table>	Species (common name)	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha) with 95% conf. limits	ER <sub>20</sub> (g a.s./ha) with 95% conf. limits	ER <sub>50</sub> (g a.s./ha) with 95% conf. limits	Oat	40	100	50.5 (N/A – 263.4)	440.4 (N/A – N/A)	> 1562.5	Oilseed rape	1562.5	> 1562.5	592.1 (9.8 – N/A)	> 1562.5	> 1562.5	Onion	625	1562.5	233.8 (N/A – 1263.0)	949.7 (N/A – N/A)	> 1562.5	Perennial ryegrass	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5	Radish	40	100	13.1 (3.7 – 225.9)	242.7 (23.7 – 1071.0)	> 1562.5	Soybean	16	40	42.1 (9.3 – 306.4)	1169.0 (451.4 – N/A)	> 1562.5					
Species (common name)	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha) with 95% conf. limits	ER <sub>20</sub> (g a.s./ha) with 95% conf. limits	ER <sub>50</sub> (g a.s./ha) with 95% conf. limits																																												
Oat	40	100	50.5 (N/A – 263.4)	440.4 (N/A – N/A)	> 1562.5																																												
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Perennial ryegrass	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5																																												
Radish	40	100	13.1 (3.7 – 225.9)	242.7 (23.7 – 1071.0)	> 1562.5																																												
Soybean	16	40	42.1 (9.3 – 306.4)	1169.0 (451.4 – N/A)	> 1562.5																																												
		N/A: not available																																															
		At the end of the test no phytotoxic effects were shown in all tested species.																																															
Agreed end-points:		The ER <sub>50</sub> values for mortality and biomass were > 1562.5 g a.s./ha for all six plant species tested.																																															

<b>Reference:</b>	<b>KCP 10.6.2/01, Noè, F. (2022)</b>
Report	Difenoconazole 250 g/L EC greener – IN005B1570: Vegetative Vigour Test of Terrestrial Plants. Noè, F, 2022. ChemService Study No. 0772/2021
Guideline(s):	Yes <ul style="list-style-type: none"> <li>- OECD Guideline No. 227, “Terrestrial Plant Test: Vegetative Vigour Test”, 2006.</li> <li>- EPPO bulletin 1/135 (4), Phytotoxicity assessment, 2014.</li> </ul>
Deviations:	No
GLP:	Yes
Acceptability:	Yes

Duplication (if vertebrate study)	No
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### Objective:

The purpose of this study was to investigate potential adverse effects of Difenconazole 250 g/L EC greener – IN005B1570 following its application on the leaves and above-ground portions of different plant species. The effects of Difenconazole 250 g/L EC greener – IN005B1570 was assessed by using six application rates on six different plant species (oilseed rape, radish, soybean, perennial ryegrass, oat and onion). The leaves and above ground portions of each plant were assessed after 21 days.

### Materials and methods

Test Item:	Difenconazole 250g/L EC greener – IN005B1570
Test Design:	3 week test (after plants reached 2-4 true leaf stage) prepared according to OECD 227; different concentrations of the test item were applied to the above ground portions of the plants and leaves surface; 7 treatment groups (6 test item concentrations, 1 negative control); The test item solutions were sprayed when plants had reached 2 to 4 true leaf stage, the sprayer is designed to mimic spray application in field. The test was replicated for each plant species. Plant mortality, plant shoot height, shoot fresh weight and visual response were determined for each replicate on day 21 after the treatment.
Endpoints:	Number of emerged plants, biomass (fresh weight) and the number of plants surviving.
Test Concentrations:	16, 40, 100, 250, 625, 1562.5 g a.s. difenconazole/ha (corresponding to 70.5, 176.2, 440.5, 1101.3, 2753.3 and 6883.3 g test item/ha). A negative control (test soil without test item) was also tested.
Test Conditions:	Soil according to OECD 227; initial pH 7.29 in soils, loamy sand standard soil (soil type 5M) with an organic carbon percentage of 0.88% ( $\pm$ 0.18), sieved with a mesh of 2 mm. Initial soil moisture 8.4% of soil dry weight. Temperature within the range of; day 25.70°C to 27.40°C; night 21.40°C to 22.50°C; photoperiod: 16 h light; 8 h dark, average wavelength: 400 – 700nm. Light conditions; in the range 12800 lux – 11950 lux. Relative humidity; 57.88% - 81.18%. Watering was made by hand in order to verify the humidity condition of soil before supplying water.
Statistics:	Dunnett Multiple Comparison Test (NOER and LOER for biomass), Dunnett Multiple Comparison Test or Steel Many-One Rank Sum test (NOER and LOER for mortality). Linear Interpolation (ICPIN) (biomass and mortality end-points).

### Results and discussions

All study validity criteria were met.

According to OECD Guideline no. 227, the effects of Difenconazole 250 g/L EC greener – IN005B1570 on plant mortality, and biomass (fresh weight) were assessed. 21 days after the test plants had reached 2-4 true leaf stage. When the test plants had reached the 2-4 true leaf stage the emergence of each species was



verified and recorded. Each plant species at each test item concentration had an emergence above 85% after 3 weeks. There was 0% mortality across all test groups at all test item concentrations after 21 days.

Table 1. Effect of nominal Difenconazole 250g/L EC greener – IN005B1570 application rates on mortality endpoints for the six non-target species in a 21 day study.

Species	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)
Oat	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Oilseed rape	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Onion	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Perennial ryegrass	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Radish	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Soybean	1562.5	>1562.5	>1562.5	>1562.5	>1562.5

Table 2. Effect of nominal Difenconazole 250g/L EC greener – IN005B1570 application rates on biomass (fresh weight) endpoints for the six non-target species in a 21 day study.

Species	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)
Oat	40	100	50.5	440.4	>1562.5
Oilseed rape	1562.5	>1562.5	592.1	>1562.5	>1562.5
Onion	1562.5	>1562.5	233.8	949.7	>1562.5
Perennial ryegrass	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Radish	40	100	13.1	242.7	>1562.5
Soybean	16	40	42.1	1169.0	>1562.5

## Conclusion

Difenconazole 250g/L EC greener – IN005B1570 was tested for effects on seedling emergence, mortality and biomass (fresh weight) of six different plant species. All plant species showed a NOER in terms of mortality of 1562.5 g a.s./ha. At the end of the 21 day test, no phytotoxic effects were present in any tested species. The analytical recoveries of the test concentrations of difenconazole in the stock solutions were in the range from 106.6% to 119.3% of the nominal values.

## Validity criteria

Seedling Emergence:	The seedling emergence in the negative control group was above 70% and so this validity criterion was met.
Phytotoxic effects:	There was no visible phytotoxic effects and so this validity criterion was met.

## A 2.6.4 KCP 10.6.2/02 Study 1

Study Comments: KCP 10.6.2/02	<b><i>zRMS comments:</i></b>	
	The validation criteria are met:	
	OECD 208:	Obtained:
	The seedlings emergence in the controls is at least 70%.	Seedlings emergence was at least 70% in the controls.
	The seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilt-	No phytotoxic effects were reported for any of the species in the control.



ing, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species.	
The mean survival of emerged control seedlings is at least 90% for the duration of the study.	There was no mortality of the controls during the study.
Environmental conditions for a particular species are identical and growing media contain the same amount of soil matrix, support media, or substrate from the same source for the control and treatment groups.	Conditions were identical for each species.

**Following deviations from OECD 208 were noted:**

No deviations were issued.

**The phytotoxicity effect**

The chlorosis, necrosis and other effects evaluation (wilting and deformation) were expressed as percentage of affected leaf area for each replicate. At the end of the test no phytotoxic effects were shown in all tested species.

**Conclusion:** The endpoints listed above are considered to be reliable and appropriate for use in regulatory risk assessment. It was compliant with GLP, and it was reported appropriately. The study has been fully considered in the risk assessment section.

**Agreed endpoints:**

**Emergence (based on nominal test item application rates)**

Species (common name)	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha) with 95% conf. limits	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)
Oat	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Oilseed rape	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Onion	1562.5	> 1562.5	228.2 (N/A – N/A)	> 1562.5	> 1562.5
Perennial ryegrass	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Radish	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Soybean	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5

N/A: not available

**Mortality (based on nominal test item application rates)**

Species (common name)	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)
Oat	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Oilseed rape	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Onion	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Perennial ryegrass	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Radish	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Soybean	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5

**Biomass (fresh weight) (based on nominal test item application rates)**

Species (common name)	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha) with 95% conf. limits	ER <sub>20</sub> (g a.s./ha) with 95% conf. limits	ER <sub>50</sub> (g a.s./ha)
Oat	1562.5	> 1562.5	413.2 (N/A – N/A)	> 1562.5	> 1562.5
Oilseed rape	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Onion	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Perennial ryegrass	100	250	29.1 (N/A – 155.3)	92.3 (N/A – 1109.0)	> 1562.5
Radish	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5
Soybean	1562.5	> 1562.5	> 1562.5	> 1562.5	> 1562.5

N/A: not available

<i>Avena sativa</i> <i>Brassica napus</i> <i>Allium cepa</i> <i>Lolium perenne</i> <i>Raphanus sativus</i> <i>Glycine max</i>	IN005B1570	Vegetative vig- our	> 1562.5 g a.s./ ha	KCP 10.6.2/01, Noe, F. (2021)
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*RMS pointed out that ER<sub>50</sub> based on emergence, mortality, biomass was above 1562.5 g a.s./ha for all tested species.*

Agreed endpoints: *ER<sub>50</sub> = 1562.5 g a.s./ha for all tested species.*

<b>Reference:</b>	<b>KCP 10.6.2/02, Noè, F. (2022)</b>
Report	Difenconazole 250 g/L EC greener – IN005B1570: Seedling Emergence and Seedling Growth Test of Terrestrial Plants. Noè, F. (2022)

	ChemService Study No. 0771/2021
Guideline(s):	Yes <ul style="list-style-type: none"> <li>- OECD Guideline No. 208, “Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test”, 2006.</li> <li>- EPPO bulletin 1/135 (4), Phytotoxicity assessment, 2014.</li> </ul>
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	No

### Objective:

The purpose of this study was to investigate adverse phytotoxic effects of Difenoconazole 250 g/L EC greener – IN005B1570 on seedling emergence and early growth of terrestrial plants following the application of test item into the standard soil. The effects of Difenoconazole 250 g/L EC greener – IN005B1570 was assessed by using six different plant species (oilseed rape, radish, soybean, perennial ryegrass, oat and onion).

### Materials and methods

Test Item: Difenoconazole 250g/L EC greener – IN005B1570

Test Design: 3 week test (after 50% emergence of negative control) prepared according to OECD 208; different concentrations of the test item were incorporated into the soil; 7 treatment groups (6 test item concentrations, 1 negative control); The test item was applied to the soil surface immediately after the seeds were planted by spraying the test item solutions on the soil surface by using a sprayer, this is to mimic spray application in field.  
The test was replicated for each plant species. Once 50% of plants had emerged, assessment of the number of emerged plants, the number of plants surviving and visual rating of plant conditions was recorded on days 7, 14 and 21. Additionally, plant shoot height and biomass (fresh weight) of surviving plants for all species was recorded at conclusion of test (day 21).

Endpoints: Number of emerged plants, biomass (fresh weight) and the number of plants surviving.

Test Concentrations: 16, 40, 100, 250, 625, 1562.5 g a.s. difenoconazole/ha (corresponding to 70.5, 176.2, 440.5, 1101.3, 2753.3 and 6883.3 g test item/ha). A negative control (test soil without test item) was also tested.

Test Conditions: Soil according to OECD 208; initial pH 7.31 in soils, loamy sand standard soil (soil type 5M) with an organic carbon percentage of 0.88% ( $\pm$  0.18), sieved with a mesh of 2 mm. Initial soil moisture 8.6% of soil dry weight.  
Temperature within the range of; day 25.90°C to 27.40°C; night 21.10°C to 22.0°C; photoperiod: 16 h light; 8 h dark, average wavelength: 400 – 700nm. Light conditions; in the range 10150 lux – 11950 lux. Relative humidity;

60.71% - 80.91%. Watering was made by hand in order to verify the humidity condition of soil before supplying water.

Statistics: Dunnett Multiple Comparison Test (NOER and LOER for biomass), Dunnett Multiple Comparison Test or Steel Many-One Rank Sum test (NOER and LOER for emergence and mortality). Linear Interpolation (ICPIN) (biomass, emergence and mortality end-points).

## Results and discussions

All study validity criteria were met.

According to OECD Guideline no. 208, the effects of Difenoconazole 250 g/L EC greener – IN005B1570 on plant emergence, visual rating of plant condition and plant survival were assessed. 21 days after 50% emergence of the seedlings, the emergence and mortality were assessed. Each plant species at each test item concentration had an emergence above 85% after 3 weeks. There was 0% mortality across all test groups at all test item concentrations after 21 days.

Table 18. Effect of nominal Difenoconazole 250g/L EC greener – IN005B1570 application rates on emergence endpoints for the six non-target species in a 21 day study.

Species	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)
Oat	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Oilseed rape	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Onion	1562.5	>1562.5	228.2	>1562.5	>1562.5
Perennial ryegrass	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Radish	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Soybean	1562.5	>1562.5	>1562.5	>1562.5	>1562.5

Table 2. Effect of nominal Difenoconazole 250g/L EC greener – IN005B1570 application rates on mortality endpoints for the six non-target species in a 21 day study.

Species	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)
Oat	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Oilseed rape	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Onion	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Perennial ryegrass	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Radish	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Soybean	1562.5	>1562.5	>1562.5	>1562.5	>1562.5

Table 3. Effect of nominal Difenoconazole 250g/L EC greener – IN005B1570 application rates on biomass (fresh weight) endpoints for the six non-target species in a 21 day study.

Species	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)
Oat	1562.5	>1562.5	413.2	>1562.5	>1562.5
Oilseed rape	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Onion	1562.5	>1562.5	228.2	>1562.5	>1562.5

Species	NOER (g a.s./ha)	LOER (g a.s./ha)	ER <sub>10</sub> (g a.s./ha)	ER <sub>20</sub> (g a.s./ha)	ER <sub>50</sub> (g a.s./ha)
Perennial ryegrass	100	250	29.1	92.3	>1562.5
Radish	1562.5	>1562.5	>1562.5	>1562.5	>1562.5
Soybean	1562.5	>1562.5	>1562.5	>1562.5	>1562.5

### Conclusion

Difenconazole 250g/L EC greener – IN005B1570 was tested for effects on seedling emergence, mortality and biomass (fresh weight) of six different plant species. All plant species showed a NOER in terms of emergence, mortality and biomass (fresh weight) of 1562.5 g a.s./ha. At the end of the 21 day test, no phytotoxic effects were shown in all tested species. The analytical recoveries of the test concentrations of difenconazole in the stock solutions were in the range from 90.5% to 100.5% of the nominal values.

### Validity criteria

Seedling Emergence:	The seedling emergence in the negative control group was above 70% and so this validity criterion was met.
Phytotoxic effects:	There was no visible phytotoxic effects and so this validity criterion was met.
The Mean Survival:	Was above 90% and so this validity criterion was met.

**A 2.6.5 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**