

# **FINAL REGISTRATION REPORT**

## **Part B**

### **Section 9**

#### **Ecotoxicology**

Detailed summary of the risk assessment

Product code: FGG01

Product name(s): Lozzare Pro, Miller Pro, Palator Pro

Chemical active substance:

Boscalid, 500 g/kg

Central Zone

Zonal Rapporteur Member State: Poland

#### **CORE ASSESSMENT**

(Article 33 application for a new product registration)

Applicant: UPL Holdings Coöperatief U.A.

Submission date: 08/05/2024

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## Version history

When	What
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November 2024	Assessment by ZRMS.
March 2025	Changes introduced following comments from MS

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## 9 Ecotoxicology (KCP 10)

This document reviews the Ecotoxicology studies and risk assessment for the first authorisation of the product LOZZARE PRO (FGG01) in the central zone member states Austria, Belgium, Czech Republic, Hungary, Netherlands, Poland (zRMS), Romania, Slovakia and Slovenia according to Regulation (EC) No 1107/2009.

The product is a water dispersible granule (WG) formulated fungicide containing 500 g/kg of the active substance boscalid. It is intended to be used as fungicide to control different pests in various crops.

The review report for boscalid (SANCO/3919/2007 – rev. 5, dated 21 January 2008) is considered to provide the relevant review information or a reference to where such information can be found.

The Annex I Inclusion Directive for boscalid (2008/44/EC) 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 of Annex VI, the conclusions of the review report on boscalid, and in particular Appendices I and II thereof, shall be taken into account. In this overall assessment Member States must pay particular attention to:

- the long-term risk to birds and soil organisms,
- the risk of accumulation in soil if the substance is used in perennial crops or in succeeding crops in crop rotation.

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

These concerns have been addressed within the current submission.

Appendix 1 of this document contains the list of references included in this document for support of the evaluation.

Appendix 2 of this document contains the detailed information of the new studies.

Information on the detailed composition of the formulation 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	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I**	Pests or Group of pests controlled (additionally: developmental stages of the pest or pest group)	Application				Application rate			PHI (days)	Remarks: e.g. g safener/ synergist per ha	Conclusion						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthropods	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1	AT, BE, CZ, SI	Grapevine, wine & table	F	<i>Botrytis cinerea</i> (BOTRI)	Spraying overall	BBCH 60-85	a) 1 per use b) 1 per crop / season	-	a) 1.0 kg/ha b) 1.0 kg/ha	a) 500 g/ha b) 500 g/ha	100-1000	21	0.72 kg product / 10000 m <sup>2</sup> LWA (optional)							
2	AT, BE, CZ, SI, PL	Grapevine, wine & table	F	<i>Uncinula necator</i> , Powdery mildew (UNCINE)	Spraying overall	BBCH 15-81	a) 3 per use b) 3 per crop / season	10-14	a) 0.2 kg/ha b) 1.0 kg/ha	a) 100 g/ha b) 300 g/ha	100-1000	21	0.14 kg product / 10000 m <sup>2</sup> LWA 0.02 kg/100 L (optional)							
3	AT, BE, CZ, HU, NL, PL, RO, SK	Oilseed rape (winter and spring)	F	<i>Sclerotinia sclerotiorum</i> (SCLESC)	Spraying overall	BBCH 57-69	a) 1 per use b) 1 per crop / season	-	a) 0.5 kg/ha b) 0.5 kg/ha	a) 250 g/ha b) 250 g/ha	100-300	35	-							
4	AT, BE, CZ, NL, SK, HU, RO	Oilseed rape (winter and spring)	F	<i>Alternaria</i> species (ALTESP)	Spraying overall	BBCH 57-69	a) 1 per use b) 1 per crop / season	-	a) 0.5 kg/ha b) 0.5 kg/ha	a) 250 g/ha b) 250 g/ha	100-300	35	-							



1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
5	HU, PL, RO, SK, AT, CZ	Oilseed rape (winter and spring)	F	<i>Leptosperia maculans</i> (LEPTMA)	Spraying overall	BBCH 13-57	a) 1 per use b) 1 per crop / season	-	a) 0.5 kg/ha b) 0.5 kg/ha	a) 250 g/ha b) 250 g/ha	100-300	35	-							
6	AT, BE, CZ, NL, PL	Beans and peas (fresh)	F	<i>Botrytis</i> (BOTRSP)	Spraying overall	BBCH 60-69	a) 2 per use b) 2 per crop / season	7	a) 1.0 kg/ha b) 2.0 kg/ha	a) 500 g/ha b) 1000 g/ha	150-600	7	-							
7	AT, BE, CZ	Beans and peas (fresh)	F	<i>Sclerotinia</i> (SCLESP)	Spraying overall	BBCH 60-69	a) 2 per use b) 2 per crop / season	7	a) 1.0 kg/ha b) 2.0 kg/ha	a) 500 g/ha b) 1000 g/ha	150-600	7	-							
<b>Minor uses according to Article 51 (zonal uses)</b>																				
8	PL	spring rape gold of pleasure, winter turnip rape, mustard, sunflower, poppy linseed, flax, hemp, borage	F	<i>Alternaria</i> species (ALTESP) <i>Sclerotinia sclerotiorum</i> (SCLESC)	Spraying overall	BBCH 57-69	a) 1 per use b) 1 per crop / season	-	a) 0.5 kg/ha b) 0.5 kg/ha	a) 250 g/ha b) 250 g/ha	100-300	35	-							
9	PL	spring rape gold of pleasure, winter turnip rape, mustard, poppy linseed, flax, hemp, borage	F	<i>Leptosperia maculans</i> (LEPTMA)	Spraying overall	BBCH 13-57	a) 1 per use b) 1 per crop / season	-	a) 0.5 kg/ha b) 0.5 kg/ha	a) 250 g/ha b) 250 g/ha	100-300	35	-							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
10	PL	Grapevine, wine & table	F	<i>Botrytis cinerea</i> (BOTRI)	Spraying overall	BBCH 60- 85	a) 1 per use  b) 1 per crop / season	-	a) 1.0 kg/ha  b) 1.0 kg/ha	a) 500 g/ha  b) 500 g/ha	100-1000	21	0.72 kg product / 10000 m <sup>2</sup> LWA (op- tional)							
11	PL	Beans for fresh seeds, Broad bean French beans, Peas for fresh seeds, edible podded peas	F	<i>Sclerotinia</i> (SCLESP) <i>Botrytis cinerea</i> (BOTRI)	Spraying overall	BBCH 60- 69	a) 2 per use  b) 2 per crop / season	7	a) 1.0 kg/ha  b) 2.0 kg/ha	a) 500 g/ha  b) 1000 g/ha	150-600	7	-							

\* 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

## 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), Overall conclusion~~

### 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)

~~9.1.1.2 Acute and long-term/reproductive TER values at the screening step and/or Tier 1 exceed the relevant trigger values; thus, risk for mammals can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.~~

~~The risk for mammals due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGG01.~~

~~The log Pow of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.~~

### ~~9.1.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)~~

#### Birds

Acute and long-term/reproductive TER values at the screening step and/or Tier 1 exceed the relevant trigger values; thus, risk for birds can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

The risk for birds due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGG01.

The log  $P_{ow}$  of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

#### Terrestrial vertebrates other than birds

Acute and long-term/reproductive TER values at the screening step and/or Tier 1 exceed the relevant trigger values; thus, risk for mammals can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

The risk for mammals due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGG01.

The log  $P_{ow}$  of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

#### Reptiles and amphibians

Appropriate test guidelines and guidance documents are currently not available to address the risk to reptiles and amphibians. However, due to the acceptable risk of Boscalid to terrestrial vertebrates and fish,

adverse effects following the intended uses are considered unlikely.

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

For all intended uses, no risk is indicated for sediment-dwelling organism at FOCUS step 1.

For the intended uses in oilseed rape (use 3 to 5), calculated PEC/RAC ratios for Boscalid at Tier 1 indicate acceptable risk for all groups of aquatic organisms in all FOCUS step 1 and/or 2 scenarios.

For the intended uses in vines (uses 1 & 2) and beans and peas (uses 6 & 7), calculated PEC/RAC ratios for Boscalid at Tier 1 indicate acceptable risk for all groups of aquatic organisms up to FOCUS step 3.

Therefore, further PEC/RAC ratios based on FOCUS Step 4  $PEC_{sw}$  are not required.

~~For all intended uses, no risk is indicated for sediment dwelling organism at FOCUS step 1.~~

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

Based on the acute Tier-1 risk assessment (honeybees and bumble bees) according to SANCO/10329/2002 rev.2 (final), October 17, 2002, and the chronic Tier-1 risk assessment (honeybees) according to the modified EPPO (2010)/ECPA (2017) scheme, respectively, acceptable risk to bees following the intended uses of FGG01 is indicated.

There is currently no validated methodology for the assessment of toxicity to bumble bees (chronic) and solitary bees (both acute and chronic). Consequently, such studies are not considered necessary for the time being, and no risk assessment is required. The risk assessment for honeybees (acute and chronic) and bumble bees (acute) indicates no risk to other pollinators with certain margin of safety.

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

For all intended uses, Tier-1 in-field and off-field HQ values are below the relevant trigger value for both indicator species. Thus, higher-Tier risk assessment and/or risk mitigation measures are not required.

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

#### Non-target soil meso- and macrofauna

All Tier-1 TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exceed the relevant trigger values indicating no risk for all intended uses. Therefore, higher-Tier risk assessment is not required.

#### Effects on soil microbial activity

The maximum concentration of Boscalid with effects on micro-organisms  $\leq 25\%$  exceed the respective maximum  $PEC_{soil}$  value indicating no risk for all intended uses.

~~9.1.1.6 The maximum concentration of Boscalid with effects on micro-organisms  $\leq 25\%$  exceed the respective maximum  $PEC_{soil}$  value indicating no risk for~~

~~all intended uses.~~

#### 9.1.1.7

##### 9.1.1.8 zRMS comments:

The risk envelope approach has been properly applied. The assessment for the use group grapevine covers the risk for soil microorganisms from all intended uses including minor ones, in terms of maximum soil loading.

Only new endpoint from the study N-mineralisation with FGG01 is used in the risk assessment since the formulation should be considered most relevant for the intended GAP.

The maximum concentration of Boscalid with effects on micro-organisms  $\leq 25\%$  exceed the respective maximum  $PEC_{soil}$  value indicating no risk for all intended uses, including minor ones, of FGG01 (Lozzare Pro) according to proposed use pattern.

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

Risk is considered acceptable since data on seedling emergence and vegetative vigour from six species do not indicate phytotoxic effects  $> 50\%$  at the maximum intended application rate. Therefore, a Tier-2 risk assessment based on dose-response data is not required.

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

Not available and not required.

#### 9.1.2 Grouping of intended uses for risk assessment

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

**Table 9.1-2: Critical use pattern of FGG01 grouped according to relevant criteria**

Group	Intended uses	relevant use parameters for grouping	relevant parameter or value for sorting
Birds and mammals, dietary risk assessment			
Vineyard	1, 2	Crop scenario	Application rate, number of applications: 1 × 500 g a.s./ha
Oilseed rape	3, 4, 5		Application rate, number of applications: 1 × 250 g a.s./ha
Pulses	6, 7		Application rate, number of applications: 2 × 500 g a.s./ha
Birds and mammals, drinking water assessment			
Pulses	All	Effective application rate	Application rate, number of applications: 2 × 500 g a.s./ha
Aquatic organisms			
Vines, 1 × 500 g a.s./ha, early application	1	PEC <sub>sw</sub> based on crop, application rate, number of applications, application timing (refer to efate)	Application rate, number of applications, application timing: 1 × 500 g a.s./ha, early application
Vines, 3 × 100 g a.s./ha, early application	2		Application rate, number of applications, application timing: 3 × 100 g a.s./ha, early application
Vines, 1 × 500 g a.s./ha, late application	1		Application rate, number of applications, application timing: 1 × 500 g a.s./ha, late application
Vines, 3 × 100 g a.s./ha, late application	2		Application rate, number of applications, application timing: 3 × 100 g a.s./ha, late application
Spring & winter oilseed rape, 1 × 250 g a.s./ha	3, 4, 5		n.a.
Fresh beans and peas, 2 × 500 g a.s./ha	6, 7		n.a.
Bees			
Grapevine	All	Single application rate	Maximum single application rate: 500 g a.s./ha
Non-target arthropods, in-field			
Beans and peas	All	PER <sub>in-field</sub>	Maximum PER <sub>in-field</sub> : 850 g a.s./ha foliage dwellers) / 950 g a.s./ha (soil dwellers)
Non-target arthropods, off-field			
Vineyard	1, 2	PER <sub>off-field</sub>	PER <sub>off-field</sub> : 4.010 g a.s./ha
Oilseed rape	3, 4, 5		PER <sub>off-field</sub> : 0.6925 g a.s./ha
Pulses	6, 7		PER <sub>off-field</sub> : 6.146 g a.s./ha
Soil organisms			
Grapevine	All	PEC <sub>soil</sub> (refer to efate)	Maximum PEC <sub>soil</sub> : 0.509 mg a.s./ha / 0.900 mg a.s./ha

**zRMS comments:**

zRMS agrees with proposal regarding grouping of intended uses (1-7) for risk assessments. At the same time, it believes, that way of grouping is in line with SANCO/11244/2011 and is adequate for risk assessment.

The assessment for replacement plants from part of table for zonal uses (1-7) can be applied for risk assessment for minor uses (8-11). The extrapolation result is shown in the table below.

1	2	3	4	5	6	7	8	9	10	11
Use- No. *	Crop and/or situation (crop destination / purpose of crop)	Pests or Group of pests con- trolled (additionally: developmental stages of the pest or pest group)	Application	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. inter- val between applications (days)	Application rate	g or kg as/ha  a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max	PHI (days)
			Method / Kind				kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season			
1	Grapevine, wine & table	<i>Botrytis ciner- ea</i> (BOTRI)	Spraying overall	BBCH 60-85	a) 1 per use  b) 1 per crop / season	-	a) 1.0 kg/ha  b) 1.0 kg/ha	a) 500 g/ha  b) 500 g/ha	100-1000	21
10	Grapevine, wine & table	<i>Botrytis ciner- ea</i> (BOTRI)	Spraying overall	BBCH 60-85	a) 1 per use  b) 1 per crop / season	-	a) 1.0 kg/ha  b) 1.0 kg/ha	a) 500 g/ha  b) 500 g/ha	100-1000	21
3	Oilseed rape (win- ter and spring)	<i>Sclerotinia sclerotiorum</i> (SCLESC)	Spraying overall	BBCH 57-69	a) 1 per use  b) 1 per crop / season	-	a) 0.5 kg/ha  b) 0.5 kg/ha	a) 250 g/ha  b) 250 g/ha	100-300	35
8	spring rape gold of pleasure, winter turnip rape, mustard, sunflower, poppy linseed, flax, hemp, borage	<i>Sclerotinia sclerotiorum</i> (SCLESC)	Spraying overall	BBCH 57-69	a) 1 per use  b) 1 per crop / season	-	a) 0.5 kg/ha  b) 0.5 kg/ha	a) 250 g/ha  b) 250 g/ha	100-300	35
4	Oilseed rape (win- ter and spring)	<i>Alternaria species</i> (ALTESP)	Spraying overall	BBCH 57-69	a) 1 per use  b) 1 per crop / season	-	a) 0.5 kg/ha  b) 0.5 kg/ha	a) 250 g/ha  b) 250 g/ha	100-300	35
8	spring rape gold of pleasure, winter turnip rape, mustard, sunflower, poppy linseed, flax, hemp, borage	<i>Alternaria species</i> (ALTESP)	Spraying overall	BBCH 57-69	a) 1 per use  b) 1 per crop / season	-	a) 0.5 kg/ha  b) 0.5 kg/ha	a) 250 g/ha  b) 250 g/ha	100-300	35
5	Oilseed rape (winter and spring)	<i>Leptosperia maculans</i> (LEPTMA)	Spraying overall	BBCH 13-57	a) 1 per use  b) 1 per crop / season	-	a) 0.5 kg/ha  b) 0.5 kg/ha	a) 250 g/ha  b) 250 g/ha	100-300	35
9	spring rape gold of pleasure, winter turnip rape, mustard, poppy	<i>Leptosperia maculans</i> (LEPTMA)	Spraying overall	BBCH 13-57	a) 1 per use  b) 1 per crop / season	-	a) 0.5 kg/ha  b) 0.5 kg/ha	a) 250 g/ha  b) 250 g/ha	100-300	35



	linseed, flax, hemp, borage									
6	Beans and peas (fresh)	<i>Botrytis</i> (BOTRSP)	Spraying overall	BBCH 60-69	a) 2 per use  b) 2 per crop / season	7	a) 1.0 kg/ha  b) 2.0 kg/ha	a) 500 g/ha  b) 1000 g/ha	150-600	7
11	Beans for fresh seeds, Broad bean French beans, Peas for fresh seeds, edible podded peas	<i>Botrytis cinerea</i> (BOTRI)	Spraying overall	BBCH 60-69	a) 2 per use  b) 2 per crop / season	7	a) 1.0 kg/ha  b) 2.0 kg/ha	a) 500 g/ha  b) 1000 g/ha	150-600	7
7	Beans and peas (fresh)	<i>Sclerotinia</i> (SCLESP)	Spraying overall	BBCH 60-69	a) 2 per use  b) 2 per crop / season	7	a) 1.0 kg/ha  b) 2.0 kg/ha	a) 500 g/ha  b) 1000 g/ha	150-600	7
11	Beans for fresh seeds, Broad bean French beans, Peas for fresh seeds, edible podded peas	<i>Sclerotinia</i> (SCLESP)	Spraying overall	BBCH 60-69	a) 2 per use  b) 2 per crop / season	7	a) 1.0 kg/ha  b) 2.0 kg/ha	a) 500 g/ha  b) 1000 g/ha	150-600	7

Major use – in yellow

Extrapolated minor use – in green

In case of spring rape, gold of pleasure, winter turnip rape, mustard, poppy, linseed, flax, hemp, borage, a basis for their inclusion in one group is how they are used for seed production, as in the case of oil seed rape.

### 9.1.3 Consideration of metabolites

Metabolite M510F64 occurred at amounts > 5% in 2 sequential measurements in the higher-tier outdoor water/sediment study presented for the original approval of Boscalid. Metabolite M510F64 reached maximum 9.4% TAR after 30 days in the water phase. Additionally, this compound is of no toxicological and ecotoxicological relevance. Following the approaches taken for the currently authorised products containing Boscalid, no exposure assessment was presented for metabolite M510F64.

No other metabolites potentially relevant for exposure assessment have been observed.

#### zRMS comment:

zRMS accepted approach; M510F64 - the metabolite Boscalid does not need to be evaluated because, according to the EU DAR (2002); it is non-relevant metabolite considering its toxicological and ecotoxicological properties.

## 9.2 Effects on birds (KCP 10.1.1)

### 9.2.1 Toxicity data

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

Effects on birds of FGG01 were not evaluated as part of the EU assessment of Boscalid.

However, the provision of further data on the formulation FGG01 is not considered essential, because the risk can be addressed based on active substance data. Boscalid is of low toxicity to birds and mammals. FGG01 does not contain any toxicologically classified co-formulants (refer to Part C of the present dossier); thus, the formulation is not expected to be more toxic than the active substance.

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
<i>Colinus virginianus</i>	Boscalid	Oral 1 d Acute	<b>LD<sub>50</sub> &gt; 2000</b> mg/kg bw <del>Extrapolated LD<sub>50</sub> =</del> <del>3776 mg/kg bw<sup>a</sup></del>	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008
<i>Colinus virginianus</i>	Boscalid	Dietary 5 d Short-term	LC <sub>50</sub> > 5000 mg/kg feed	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008
<i>Anas platyrhynchos</i>	Boscalid	Dietary 5 d Short-term	LC <sub>50</sub> > 5000 mg/kg feed	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008
<i>Colinus virginianus</i>	Boscalid	Dietary Reproductive toxicity	NOEC = 300 mg/kg feed <b>NOAED = 24.1</b> <b>mg/kg bw/d</b>	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008
<i>Anas platyrhynchos</i>	Boscalid	Dietary Reproductive toxicity	NOEC = 1000 mg/kg feed	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008

~~a — An extrapolation factor of 1.888 is applied according to EFSA/2009/1438 since 10 animals were tested at each dose level and no effects were observed.~~

**zRMS comments:**

Avian toxicity data for Boscalid are in line with EU agreed endpoints reported in Review report, SAN-CO/3919 /2007-rev. 5 (2008). However in case of acute toxicity the factor of 1.888 for extrapolating LD<sub>50</sub> values from limit dose tests for birds has been applied. This factor is in line with EFSA/2009/1438. No mortality and clear signs of toxicity were observed in limit dose of 2000 mg/kg b.w. in the surviving individuals. In effect the extrapolation factor of 1.8888 has been correctly applied for risk assessment.

**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).

Here, the assessment for the use group grapevine covers the risk for birds from all intended uses in grapevine (uses 1 and 2). The assessment for the use group oilseed rape covers the risk for birds from all intended uses in oilseed rape (uses 3 to 5). The assessment for the use group beans and peas covers the risk for birds from all intended uses in beans and peas (uses 6 and 7; see 9.1.2). Please note that not all growth states are relevant for all uses within a certain crop group.

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

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

**Table 9.2-2: Screening/First-Tier assessment of the acute and long-term/reproductive risk for birds due to the use of FGG01 in vineyard (uses 1 and 2)**

Intended use	Vineyard				
Active substance/product	Boscalid				
Application rate (kg/ha)	1 × 0.5				
Acute toxicity (mg/kg bw)	3776 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Vineyard	Small omnivorous bird	95.3	1.0	47.65	79.2 42.0
Reprod. toxicity (mg/kg bw/d)	24.1				
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>tt</sub>
Vineyard BBCH 10 - 19	Small granivorous bird “Finch”	6.9	1.0 × 0.53	1.83	13.2
Vineyard BBCH 10 - 19	Small insectivorous species “Redstart”	11.5		3.05	7.9
Vineyard BBCH 10 - 19	Small omnivorous bird “lark”	6.5		1.72	14.0
Vineyard BBCH ≥ 20	Small insectivorous species “Redstart”	9.9		2.62	9.2
Vineyard BBCH 20 - 39	Small granivorous bird “Finch”	5.7		1.51	16.0
Vineyard BBCH 20 - 39	Small omnivorous bird “lark”	5.4		1.43	16.8
Vineyard BBCH ≥ 40	Small granivorous bird “Finch”	3.4		0.90	26.7
Vineyard BBCH ≥ 40	Small omnivorous bird “lark”	3.3		0.87	27.6
Vineyard Ripening	Frugivorous bird “Trush/starling”	14.4		3.82	6.3

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

**Table 9.2-3: Screening/First-Tier assessment of the acute and long-term/reproductive risk for birds due to the use of FGG01 in oilseed rape (uses 3 to 5)**

Intended use	Oilseed rape				
Active substance/product	Boscalid				
Application rate (kg/ha)	1 × 0.25				
Acute toxicity (mg/kg bw)	3776-2000				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Bulbs and onion-like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird Large herbivorous bird	158.8	1.0	39.70	95.1 50.3
Reprod. toxicity (mg/kg bw/d)	24.1				
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>tt</sub>
Oilseed rape early (shoots) (BBCH 10-19)	Large herbivorous bird "goose"	15.9	1.0 × 0.53	2.11	11.4
Oilseed rape BBCH 10 – 19	medium herbivorous/granivorous bird "pigeon"	22.7		3.01	8.0
Oilseed rape BBCH 10 – 19	Small insectivorous bird "wagtail"	5.9		0.78	30.8
Oilseed rape BBCH 20 – 29	medium herbivorous/granivorous bird "pigeon"	3.5		0.46	52.0
Oilseed rape BBCH 20 – 29	Small insectivorous bird "wagtail"	2.8		0.37	65.0
Oilseed rape BBCH 30 – 39	medium herbivorous/granivorous bird "pigeon"	1.1		0.15	165.4
Oilseed rape BBCH 30 – 39	Small omnivorous bird "lark"	3.3		0.44	55.1
Oilseed rape late – late (with seeds) (BBCH 30-99)	Small insectivorous bird "dunnock"	2.7		0.36	67.4
Oilseed rape BBCH ≥ 40	medium herbivorous/granivorous bird "pigeon"	0.9		0.12	202.1
Oilseed rape BBCH ≥ 40	Small omnivorous bird "lark"	2.7		0.36	67.4

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

**Table 9.2-4: Screening/First-Tier assessment of the acute and long-term/reproductive risk for birds due to the use of FGG01 in pulses (uses 6 and 7)**

Intended use	Pulses				
Active substance/product	Boscalid				
Application rate (kg/ha)	2 × 0.5				
Acute toxicity (mg/kg bw)	3776 2000				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Bulbs and onion like crops, cereals, fruiting vegetables, leafy vegetables, legume forage, maize, oilseed rape, potatoes, pulses, root and stem vegetables, strawberries, sugar beet, and sunflower	Small omnivorous bird	158.8	1.4	111.2	34.0 18.0
Reprod. toxicity (mg/kg bw/d)	24.1				
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>
Pulses BBCH ≥ 10	Small insectivorous bird “wagtail”	9.7	1.6 × 0.53	4.11	5.9
Pulses BBCH ≥ 50	Small granivorous bird “finch”	3.4		1.44	16.7
Pulses BBCH ≥ 50	Small omnivorous bird “lark”	3.3		1.40	17.2

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

Acute and long-term/reproductive TER values at the screening step and/or Tier 1 exceed the relevant trigger values; thus, risk for birds can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

#### **zRMS comments**

##### First step in the risk assessment

The first step risk assessment for active substance boscalid performed in vineyard and oilseed rape and pulses is agreed by zRMS.

TER<sub>A</sub> and TER<sub>LT</sub> values for the exposure to the active substance when **FGG01\_CEU** is applied in a vineyard and oilseed rape and pulses is agreed by zRMS are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for birds.

Overall, acceptable acute and long-term risk may be concluded for birds exposed to **FGG01\_CEU**.

#### **9.2.2.2 Higher-tier risk assessment**

Not required.

### 9.2.2.3 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (cf. Appendix K of EFSA/2009/1438).

#### Leaf scenario

Since FGG01 is not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

#### Puddle scenario

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

With a  $K(f)_{oc}$  of 743 771 L/kg (geometric mean), Boscalid belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group pulses also covers the risk for birds from all other intended uses in terms of maximum effective application rate (see 9.1.2).

Effective application rate (g/ha)=	1000 <sup>a</sup>		
Acute toxicity (mg/kg bw) =	3776 2000	quotient =	9.26 0.5
Reprod. toxicity (mg/kg bw/d) =	24.1	quotient =	41.5

a MAF = 2, based on soil DT<sub>50</sub> of 139 d (refer to Environmental Fate Section) and interval of 7 d between applications

The ratios of effective application rate (in g/ha) to relevant endpoints (in mg/kg bw/d) do not exceed the relevant trigger values with high margin of safety. Thus, risk to birds from exposure to drinking water following the intended uses of FGG01 can be excluded.

#### zRMS comments:

The risk to birds from drinking water from puddles is acceptable when is used in all intended crops FGG01 at the recommended rates.

### 9.2.2.4 Effects of secondary poisoning

The log  $P_{ow}$  of Boscalid amounts to 2.96 (Review report, SANCO/3919 /2007-rev. 5, 21 January 2008) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

#### zRMS comments:

A risk assessment for birds for effects due to secondary poisoning is not required since the log  $P_{ow}$  of boscalid amounts to 2.96 and it does not exceed the trigger value of 3 from Review report, SANCO/3919/2007-rev. 5, 21 January 2008.

#### 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

Acute and long-term/reproductive TER values at the screening step and/or Tier 1 exceed the relevant trigger values; thus, risk for birds can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

The risk for birds due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGG01.

The log  $P_{ow}$  of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

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

The presented assessment covers the risk for birds from all intended uses, including minor ones, in terms of maximum effective application rate.

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

#### 9.3.1 Toxicity data

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

Effects on mammals of FGG01 were not evaluated as part of the EU assessment of Boscalid. However, the provision of further data on the formulation FGG01 is not considered essential, because the risk can be addressed based on active substance data. Boscalid is of low toxicity to birds and mammals. FGG01 does not contain any toxicologically classified co-formulants (refer to Part C of the present dossier); thus, the formulation is not expected to be more toxic than the active substance.

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

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

Species	Substance	Exposure System	Results	Reference
Rat	Boscalid	Oral 1 d Acute	<b>LD<sub>50</sub> &gt; 5000 mg/kg bw</b>	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)



Species	Substance	Exposure System	Results	Reference
Rat	Boscalid	Oral Reproductive toxicity	<b>NOEAEDD = 67 mg/kg bw/d</b>	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008 and DAR (2002)

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

**zRMS comments:**

Mammalian toxicity data for boscalid in Table 9.3-1 are in line with EU agreed endpoints reported in SANCO/3919/2007-rev. 5, January 2008.

### 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).

Here, the assessment for the use group grapevine covers the risk for mammals from all intended uses in grapevine (uses 1 and 2). The assessment for the use group oilseed rape covers the risk for mammals from all intended uses in oilseed rape (uses 3 to 5). The assessment for the use group beans and peas covers the risk for mammals from all intended uses in beans and peas (uses 6 and 7; see 9.1.2). Please note that not all growth states are relevant for all uses within a certain crop group.

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

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

**Table 9.3-2: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FGG01 in vineyard**

Intended use	Vineyard				
Active substance/product	Boscalid				
Application rate (kg/ha)	1 × 0.5				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Vineyard	Small herbivorous mammal	136.4	1.0	68.20	> 73.3
Reprod. toxicity (mg/kg bw/d)	67				
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>tt</sub>
Vineyard Application crop directed BBCH 10 - 19	Small herbivorous mammal "vole"	43.4	1.0 × 0.53	11.50	5.8
Vineyard Application crop directed BBCH 10 - 19	Small omnivorous mammal "mouse"	4.7		1.25	53.8
Vineyard BBCH 10 - 19	Small insectivorous mammal "shrew"	4.2		1.11	60.2
Vineyard BBCH 10 - 19	Large herbivorous mammal "lagomorph"	6.7		1.78	37.7
Vineyard Application crop directed BBCH 20 - 39	Small herbivorous mammal "vole"	36.1		9.57	7.0
Vineyard Application crop directed BBCH 20 - 39	Small omnivorous mammal "mouse"	3.9		1.03	64.8
Vineyard BBCH 20 - 39	Large herbivorous mammal "lagomorph"	5.5		1.46	46.0
Vineyard Application crop directed BBCH ≥ 40	Small herbivorous mammal "vole"	21.7		5.75	11.7
Vineyard Application crop directed BBCH ≥ 40	Small omnivorous mammal "mouse"	2.3		0.61	109.9
Vineyard Application crop directed BBCH ≥ 40	Small herbivorous mammal "vole"	21.7		5.75	11.7
Vineyard Application crop directed BBCH ≥ 40	Small omnivorous mammal "mouse"	2.3		0.61	109.9

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

**Table 9.3-3: Screening assessment of the acute and long-term/reproductive risk for mammals due to the use of FGG01 in oilseed rape (uses 3 to 5)**

<b>Intended use</b>	<b>Oilseed rape</b>				
<b>Active substance/product</b>	Boscalid				
<b>Application rate (kg/ha)</b>	1 × 0.25				
<b>Acute toxicity (mg/kg bw)</b>	> 5000				
<b>TER criterion</b>	10				
<b>Crop scenario Growth stage</b>	<b>Indicator species</b>	<b>SV<sub>90</sub></b>	<b>MAF<sub>90</sub></b>	<b>DDD<sub>90</sub> (mg/kg bw/d)</b>	<b>TER<sub>a</sub></b>
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	118.4	1.0	29.60	> 168.9
<b>Reprod. toxicity (mg/kg bw/d)</b>	67				
<b>TER criterion</b>	5				
<b>Crop scenario Growth stage</b>	<b>Indicator species</b>	<b>SV<sub>m</sub></b>	<b>MAF<sub>m</sub> × TWA</b>	<b>DDD<sub>m</sub> (mg/kg bw/d)</b>	<b>TER<sub>lt</sub></b>
Bulbs and onion like crops, cereals, oilseed rape, potatoes, root and stem vegetables, strawberries, sugar beet, and sunflower	Small herbivorous mammal	48.3	1.0 × 0.53	6.40	10.47

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

**Table 9.3-4: Screening/First-Tier assessment of the acute and long-term/reproductive risk for mammals due to the use of FGG01 in pulses (uses 6 and 7)**

Intended use	Pulses				
Active substance/product	Boscalid				
Application rate (kg/ha)	2 × 0.5				
Acute toxicity (mg/kg bw)	> 5000				
TER criterion	10				
Crop scenario Growth stage	Indicator species	SV <sub>90</sub>	MAF <sub>90</sub>	DDD <sub>90</sub> (mg/kg bw/d)	TER <sub>a</sub>
Cotton, fruiting vegetables, grassland, leafy vegetables, legume forage, maize, orchards, ornamentals/nursery, pulses, and vineyard	Small herbivorous mammal	136.4	1.4	95.48	> 52.4
Reprod. toxicity (mg/kg bw/d)	67				
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>
Pulses BBCH ≥ 20	Small insectivorous mammal “shrew”	1.9	1.6 × 0.53	0.81	83.2
Pulses BBCH ≥ 50	Large herbivorous mammal “lagomorph”	4.3		1.82	36.7
Pulses BBCH ≥ 50	Small herbivorous mammal “vole”	21.7		9.20	7.3
Pulses BBCH ≥ 50	Small omnivorous mammal “mouse”	2.3		0.98	68.7

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

Acute and long-term/reproductive TER values at the screening step and/or Tier 1 exceed the relevant trigger values; thus, risk for mammals can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

#### **zRMS comment:**

##### Screening step in the risk assessment

The screening step risk assessment for active substance boscalid performed in the Table 9.3-2 and Table 9.3-3 is validated by zRMS. TER<sub>A</sub> and TER<sub>LT</sub> values for the exposure to the active substance when FGG01 is applied in vineyard and oilseed rape and pulses are above the trigger of 10 and 5 for acute and long-term exposure, indicating acceptable risk for mammals.

Overall, acceptable acute and long-term risk may be concluded for mammals exposed to FGG01.

### **9.3.2.2 Higher-tier risk assessment**

Not required.

### 9.3.2.3 Drinking water exposure

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

#### Puddle scenario

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

With a  $K(f)_{oc}$  of 743 771 L/kg (geometric mean), Boscalid belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group pulses also covers the risk for mammals from all other intended uses in terms of maximum effective application rate (see 9.1.2).

Effective application rate (g/ha) =	1000 <sup>a</sup>			
Acute toxicity (mg/kg bw) >	5000	quotient	<	0.20
Reprod. toxicity (mg/kg bw/d) =	67	quotient	=	14.9

a MAF = 2, based on soil DT<sub>50</sub> of 139 d (refer to Environmental Fate Section) and interval of 7 d between applications

The ratios of effective application rate (in g/ha) to relevant endpoints (in mg/kg bw/d) do not exceed the relevant trigger values with high margin of safety. Thus, risk to mammals from exposure to drinking water following the intended uses of FGG01 can be excluded.

#### zRMS comments:

The risk to mammals from drinking water from puddles is acceptable when is used in all intended crops FGG01 at the recommended rates.

### 9.3.2.4 Effects of secondary poisoning

The log  $P_{ow}$  of Boscalid amounts to 2.96 (Review report, SANCO/3919 /2007-rev. 5, 21 January 2008) and thus does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

#### zRMS comments:

A risk assessment for terrestrial vertebrates other than birds for effects due to secondary poisoning is not required since the log  $P_{ow}$  of Boscalid amounts to 2.96 and it does not exceed the trigger value of 3 from Revie report, SANCO/3919 /2007-rev. 5, 21 January 2008.

### 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**

Acute and long-term/reproductive TER values at the screening step and/or Tier 1 exceed the relevant trigger values; thus, risk for mammals can be excluded for all intended uses. Therefore, a higher-Tier risk assessment is not required.

The risk for mammals due to uptake of contaminated drinking water from puddles is acceptable for all intended uses of FGG01.

The log  $P_{ow}$  of Boscalid does not exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is not required.

#### **zRMS comment:**

zRMS agrees with overall conclusions listed under point 9.3.4.

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

Appropriate test guidelines and guidance documents are currently not available to address the risk to reptiles and amphibians. However, due to the acceptable risk of Boscalid to terrestrial vertebrates and fish, adverse effects following the intended uses are considered unlikely.

### **9.5 Effects on aquatic organisms (KCP 10.2)**

#### **9.5.1 Toxicity data**

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

Effects on aquatic organisms of FGG01 were not evaluated as part of the EU assessment of Boscalid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
Rainbow trout	Boscalid	96 h, s	<b>LC<sub>50</sub> = 2.7 mg a.s./L<sub>mm</sub></b>	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
Rainbow trout	Boscalid	97 d (ELS), f	<b>NOEC = 0.125 mg a.s./L<sub>nom</sub></b>	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Daphnia magna</i>	Boscalid	48 h, s	<b>EC<sub>50</sub> = 5.33 mg a.s./L<sub>mm</sub></b>	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Daphnia magna</i>	Boscalid	21 d, ss	<b>NOEC = 1.31 mg a.s./L<sub>mm</sub></b>	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Chironomus riparius</i>	Boscalid	28 d, spiked water, s	<b>NOEC = 1.0 mg a.s./L<sub>nom</sub></b>	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Chironomus riparius</i>	Boscalid	28 d, spiked sediment, s	<b>NOEC = 23.26 mg a.s./kg sed. im</b>	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Pseudokirchneriella subcapitata</i>	Boscalid	96 h, s	E <sub>r</sub> C <sub>50</sub> = 3.75 mg a.s./L <sub>mm</sub> E <sub>y</sub> C <sub>50</sub> = 1.34 mg a.s./L <sub>mm</sub>	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<b>Higher-tier studies</b>				
Not relevant.				

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

**Table 9.5-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – FGG01**

Species	Substance	Exposure System	Results	Reference
<i>Daphnia magna</i>	Boscalid 500 g/kg WG (51.33 % a.s. w/w)	48 h, s	EC <sub>50</sub> = 75.683 mg/L <sub>nom</sub> corresponding to 38.848 mg a.s./L <sub>nom</sub>	New endpoint Rana (2023a) Report No. 502-3-07-31231, UPL/2022/21324 EFSA-2022-00011650 KCP 10.2.1/01
<i>Pseudokirchneriella subcapitata</i>	Boscalid 500 g/kg WG (51.33 % a.s. w/w)	96 h, s	ErC <sub>50</sub> = 3.819 mg/L <sub>nom</sub> corresponding to <b>1.960 mg a.s./L<sub>nom</sub></b>	New endpoint Rana (2023b) Report No. 501-3-07-31230, UPL/2022/21323 EFSA-2022-00011649 KCP 10.2.1/02
<b>Higher-tier studies (micro- or mesocosm studies)</b>				
Not relevant.				

s: static, ss: semi static; nom: based on nominal concentrations; twa: time-weighted average

Above toxicity studies with the formulation do not indicate higher toxicity of the product compared to the active substance. For fish, no product study with FGG01 was conducted to account for animal welfare and is not deemed required for the following reasons: Based on active substance data, fish is slightly more sensitive compared to aquatic invertebrates and algae. However, endpoints are still in the same range (factor 1.5 to 2). Unprotected data on the representative product BAS 510 01 F from the RAR indicates even lower sensitivity of fish compared to other organism groups. Both products are comparable in terms of formulation type (WG) and active substance content (500 g/kg Boscalid). In addition, FGG01 does not contain any (eco-)toxicologically classified co-formulants (refer to Part C of the present dossier); thus, the formulation is not expected to be significantly more toxic than the active substance. Therefore, it is reasonable and sufficiently worst case to extrapolate from a.s. data.

Toxicity studies with the formulation do not indicate higher toxicity of the product compared to the active substance. For fish, no product study with FGG01 was conducted to account for animal welfare and is not deemed required for the following reasons: Based on active substance data, fish is slightly more sensitive compared to aquatic invertebrates and algae. However, endpoints are still in the same range (factor 1.5 to 2). Unprotected data on the representative product BAS 510 01 F from the RAR indicates even lower sensitivity of fish compared to other organism groups. Both products are comparable in terms of formulation type (WG) and active substance content (500 g/kg Boscalid). In addition, FGG01 does not contain any (eco-)toxicologically classified co-formulants (refer to Part C of the present dossier); thus, the formulation is not expected to be significantly more toxic than the active substance. Therefore, it is reasonable and sufficiently worst case to extrapolate from a.s. data.

**zRMS comments:**

zRMS agrees, that risk for some aquatic organisms (*Daphnia magna*, *Pseudokirchneriella subcapitata*) can be addressed is based on active substance data. FGG01 (Lozzare Pro) does not contain any eco-toxicologically classified co-formulants. In effect the formulation is not expected to be more toxic to aquatic organisms than the active substance. However, in part C the applicant should include a compositions comparison of the two formulations in order to be able to use unprotected studies to clarify the risk to fish.



### 9.5.1.1 Justification for new endpoints

In a worst-case approach, lowest aquatic toxicity values from both active substance and product studies are used for Tier-1 risk assessment. For algae, the lowest  $E_rC_{50}$  from the product study is used for risk assessment since this is the regulatory relevant endpoint.

### 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  $PEC_{SW}$  for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the tables below:

- **Table 9.5-3:**  $1 \times 500$  g a.s./ha in vines (BBCH 60-85; use 1), early application
- **Table 9.5-4:**  $3 \times 100$  g a.s./ha in vines (BBCH 15-81; use 2), early application
- **Table 9.5-5:**  $1 \times 500$  g a.s./ha in vines (BBCH 60-85; use 1), late application
- **Table 9.5-6:**  $3 \times 100$  g a.s./ha in vines (BBCH 15-81; use 2), late application
- **Table 9.5-7:**  $1 \times 250$  g a.s./ha in spring & winter oilseed rape (BBCH 57-69; uses 3 & 4)
- **Table 9.5-8:**  $1 \times 250$  g a.s./ha in spring and winter oilseed rape (BBCH 13-57; use 5)
- **Table 9.5-9:**  $2 \times 500$  g a.s./ha in fresh beans and peas (legumes, (BBCH 60-69; uses 6 & 7)

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.

**Table 9.5-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Boscalid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FGG01 at 1 × 500 g a.s./ha in vines (BBCH 60-85; use 1), early application**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 1</b>									
	97.104	<b>3.60</b>	<b>7.77</b>	<b>1.82</b>	0.741	0.495	0.971	666.854	0.287
<b>Step 2</b>									
Northern Europe, March-May	14.440	0.535	<b>1.16</b>	0.271	0.110	0.074	0.144	97.657	0.042
Northern Europe, June-September	14.440	0.535	<b>1.16</b>	0.271	0.110	0.074	0.144	97.657	0.042
Northern Europe, October-February	24.289	0.900	<b>1.94</b>	0.456	0.185	0.124	0.243	170.781	0.073
Southern Europe, March-May	21.006	0.778	<b>1.68</b>	0.394	0.160	0.107	0.210	146.406	0.063
Southern Europe, June-September	17.723	0.656	<b>1.42</b>	0.333	0.135	0.090	0.177	122.032	0.052
Southern Europe, October-February	21.006	0.778	<b>1.68</b>	0.394	0.160	0.107	0.210	146.406	0.063

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro- longed	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 3</b>									
D3 <sup>a</sup> / ditch	8.531		0.682						
D6 / ditch	8.522		0.682						
R1 / pond	0.353		0.028						
R1 / stream <sup>b</sup>	6.269		0.502						
R2 / stream	8.429		0.674						
R3 / stream	8.828		0.706						
R4 / stream	6.287		0.503						

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

a Crop not defined, a surrogate crop “pome/stone fruit, early applications” was used, with the mean deposition mass in TOXSWA corrected for crop “vines, late application” (FPS Health, Food Chain Safety and Environment, 2021).

b Also covering D4 scenario since drift is the dominant entry route (AGES, 2022).

**Table 9.5-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Boscalid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FGG01 at 1 × 500 g a.s./ha in vines (BBCH 60-85; use 1), late application**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 1</b>									
	97.104	<b>3.60</b>	<b>7.77</b>	<b>1.82</b>	0.741	0.495	0.971	666.854	0.287
<b>Step 2</b>									
Northern Europe, March-May	14.440	0.535	<b>1.16</b>	0.271	0.110	0.074	0.144	97.657	0.042
Northern Europe, June-September	14.440	0.535	<b>1.16</b>	0.271	0.110	0.074	0.144	97.657	0.042
Northern Europe, October-February	24.289	0.900	<b>1.94</b>	0.456	0.185	0.124	0.243	170.781	0.073
Southern Europe, March-May	21.006	0.778	<b>1.68</b>	0.394	0.160	0.107	0.210	146.406	0.063
Southern Europe, June-September	17.723	0.656	<b>1.42</b>	0.333	0.135	0.090	0.177	122.032	0.052
Southern Europe, October-February	21.006	0.778	<b>1.68</b>	0.394	0.160	0.107	0.210	146.406	0.063

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro- longed	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 3</b>									
D3 <sup>a</sup> / ditch	8.537		0.683						
D6 / ditch	8.574		0.686						
R1 / pond	0.305		0.024						
R1 / stream <sup>b</sup>	6.288		0.503						
R2 / stream	8.429		0.674						
R3 / stream	8.863		0.709						
R4 / stream	6.287		0.503						

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

a Crop not defined, a surrogate crop “pome/stone fruit, early applications” was used, with the mean deposition mass in TOXSWA corrected for crop “vines, late application” (FPS Health, Food Chain Safety and Environment, 2021).

b Also covering D4 scenario since drift is the dominant entry route (AGES, 2022).

**Table 9.5-5: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Boscalid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FGG01 at 3 × 100 g a.s./ha in vines (BBCH 15-81; use 2), early application**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 1</b>									
	52.933	<b>1.96</b>	<b>4.23</b>	0.993	0.404	0.270	0.529	380.375	0.164
<b>Step 2</b>									
Northern Europe, March-May	7.054	0.261	0.564	0.132	0.054	0.036	0.071	50.643	0.022
Northern Europe, June-September	7.618	0.282	0.609	0.143	0.058	0.039	0.076	51.876	0.022
Northern Europe, October-February	15.493	0.574	<b>1.24</b>	0.291	0.118	0.079	0.155	113.304	0.049
Southern Europe, March-May	12.680	0.470	<b>1.01</b>	0.238	0.097	0.065	0.127	92.417	0.040
Southern Europe, June-September	9.494	0.352	0.760	0.178	0.072	0.048	0.095	65.801	0.028
Southern Europe, October-February	12.680	0.470	<b>1.01</b>	0.238	0.097	0.065	0.127	92.417	0.040

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro- longed	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 3</b>									
D3 <sup>a</sup> / ditch <sup>c</sup>	0.565		0.045						
D6 / ditch	0.870		0.070						
R1 / pond	0.046		0.004						
R1 / stream <sup>b</sup>	1.086		0.087						
R2 / stream	0.587		0.047						
R3 / stream <sup>c</sup>	0.582		0.047						
R4 / stream	2.199		0.176						

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

a Crop not defined, a surrogate crop “pome/stone fruit, early applications” was used, with the mean deposition mass in TOXSWA corrected for crop “vines, early application” (FPS Health, Food Chain Safety and Environment, 2021).

b Covering D4 scenario (AGES, 2022) even when dominant entry route is runoff, as this would indicate that emissions via drift would be even lower.

c PEC from single application

**Table 9.5-6: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Boscalid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FGG01 at 3 × 100 g a.s./ha in vines (BBCH 15-81; use 2), late application**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 1</b>									
	52.933	<b>1.96</b>	<b>4.23</b>	0.993	0.404	0.270	0.529	380.375	0.164
<b>Step 2</b>									
Northern Europe, March-May	7.054	0.261	0.564	0.132	0.054	0.036	0.071	50.643	0.022
Northern Europe, June-September	7.618	0.282	0.609	0.143	0.058	0.039	0.076	51.876	0.022
Northern Europe, October-February	15.493	0.574	<b>1.24</b>	0.291	0.118	0.079	0.155	113.304	0.049
Southern Europe, March-May	12.680	0.470	<b>1.01</b>	0.238	0.097	0.065	0.127	92.417	0.040
Southern Europe, June-September	9.494	0.352	0.760	0.178	0.072	0.048	0.095	65.801	0.028
Southern Europe, October-February	12.680	0.470	<b>1.01</b>	0.238	0.097	0.065	0.127	92.417	0.040



Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. pro- longed	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 3</b>									
D3 <sup>a</sup> / ditch <sup>c</sup>	1.707		0.137						
D6 / ditch	1.849		0.148						
R1 / pond	0.123		0.010						
R1 / stream <sup>b, c</sup>	1.257		0.101						
R2 / stream <sup>c</sup>	1.685		0.135						
R3 / stream <sup>c</sup>	1.772		0.142						
R4 / stream	1.587		0.127						

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

a Crop not defined, a surrogate crop “pome/stone fruit, early applications” was used, with the mean deposition mass in TOXSWA corrected for crop “vines, late application” (FPS Health, Food Chain Safety and Environment, 2021).

b Also covering D4 scenario since drift is the dominant entry route (AGES, 2022).

c PEC from single application

**Table 9.5-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Boscalid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FGG01 at 1 × 250 g a.s./ha in spring & winter oilseed rape (BBCH 57-69; uses 3 & 4)**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 1</b>									
	44.161	<b>1.64</b>	<b>3.53</b>	0.829	0.337	0.225	0.442	317.164	0.136
<b>Step 2</b>									
Northern Europe, March-May	3.405	0.126	0.272	0.064	0.026	0.017	0.034	23.638	0.010
Northern Europe, June-September	3.405	0.126	0.272	0.064	0.026	0.017	0.034	23.638	0.010
Southern Europe, March-May	5.457	0.202	0.437	0.102	0.042	0.028	0.055	38.872	0.017
Southern Europe, June-September	4.431	0.164	0.354	0.083	0.034	0.023	0.044	31.255	0.013

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

**Table 9.5-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Boscalid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FGG01 at 1 × 250 g a.s./ha in spring and winter oilseed rape (BBCH 13-57; use 5)**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 1</b>									
	44.161	<b>1.64</b>	<b>3.53</b>	0.829	0.337	0.225	0.442	317.164	0.136
<b>Step 2</b>									
Northern Europe, March-May	6.277	0.232	0.502	0.118	0.048	0.032	0.063	44.966	0.019
Northern Europe, June-September	3.405	0.126	0.272	0.064	0.026	0.017	0.034	23.638	0.010
Southern Europe, March-May	11.202	0.415	0.896	0.210	0.086	0.057	0.112	81.528	0.035
Southern Europe, June-September	4.431	0.164	0.354	0.083	0.034	0.023	0.044	31.255	0.013

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

**Table 9.5-9: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Boscalid for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of FGG01 at 2 × 500 g a.s./ha in fresh beans and peas (legumes, (BBCH 60-69; uses 6 & 7)**

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 1</b>									
	176.645	<b>6.54</b>	<b>14.1</b>	<b>3.31</b>	<b>1.35</b>	0.901	<b>1.77</b>	1,270	0.546
<b>Step 2</b>									
Northern Europe, March-May	14.381	0.533	<b>1.15</b>	0.270	0.110	0.073	0.144	101.073	0.043
Northern Europe, June-September	14.381	0.533	<b>1.15</b>	0.270	0.110	0.073	0.144	101.073	0.043
Southern Europe, March-May	24.060	0.891	<b>1.92</b>	0.451	0.184	0.123	0.241	172.943	0.074
Southern Europe, June-September	19.220	0.712	<b>1.54</b>	0.361	0.147	0.098	0.192	137.008	0.059

Group		Fish acute	Fish prolonged	Inverteb. acute	Inverteb. prolonged	Algae	Sed. dwell. prolonged		Sed. dwell. prolonged
Test species		<i>O. mykiss</i>	<i>O. mykiss</i>	<i>D. magna</i>	<i>D. magna</i>	<i>P. subcapitata</i>	<i>C. riparius</i>		<i>C. riparius</i>
Endpoint (µg/L)		LC <sub>50</sub> 2700	NOEC 125	EC <sub>50</sub> 5330	NOEC 1310	ErC <sub>50</sub> 1960	NOEC 1000		NOEC 23260
AF		100	10	100	10	10	10		10
RAC (µg/L)		27	12.5	53.3	131	196	100		2326
FOCUS Scenario	PEC <sub>gl-max</sub> (µg/L)							PEC <sub>gl-max</sub> (µg/kg)	
<b>Step 3</b>									
D3 / ditch <sup>a</sup>	2.619		0.210						
D4 / pond	2.841		0.227						
D4 / stream	3.556		0.284						
D5 / pond	0.969		0.078						
D5 / stream <sup>a</sup>	2.550		0.204						
D6 / ditch	5.984		0.479						
R1 / pond	0.704		0.056						
R1 / stream	5.926		0.474						
R2 / stream	2.117		0.169						
R3 / stream	4.652		0.372						
R4 / stream	9.504		0.760						

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

<sup>a</sup> single application

~~For all intended uses, no risk is indicated for sediment dwelling organism at FOCUS step 1.~~

~~For the intended uses in oilseed rape (use 3 to 5), calculated PEC/RAC ratios for Boscalid at Tier 1 indicate acceptable risk for all groups of aquatic organisms in all FOCUS step 1 and/or 2 scenarios.~~

~~For the intended uses in vines (uses 1 & 2) and beans and peas (uses 6 & 7), calculated PEC/RAC ratios for Boscalid at Tier 1 indicate acceptable risk for all groups of aquatic organisms up to FOCUS step 3.~~

~~Therefore, further PEC/RAC ratios based on FOCUS Step 4  $PEC_{SW}$  are not required.~~

### 9.5.3 Overall conclusions

For all intended uses, no risk is indicated for sediment-dwelling organism at FOCUS step 1.

For the intended uses in oilseed rape (use 3 to 5), calculated PEC/RAC ratios for Boscalid at Tier 1 indicate acceptable risk for all groups of aquatic organisms in all FOCUS step 1 and/or 2 scenarios.

For the intended uses in vines (uses 1 & 2) and beans and peas (uses 6 & 7), calculated PEC/RAC ratios for Boscalid at Tier 1 indicate acceptable risk for all groups of aquatic organisms up to FOCUS step 3.

Therefore, further PEC/RAC ratios based on FOCUS Step 4  $PEC_{SW}$  are not required.

#### **zRMS comments:**

zRMS agrees with overall conclusions listed under point 9.5.3.

## 9.6 Effects on bees (KCP 10.3.1)

### 9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with Boscalid and the formulated product for Annex 1 inclusion. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on bees of FGG01 were not evaluated as part of the EU assessment of active substance 1. New data submitted with this application are listed in **Błąd! Nie można odnaleźć źródła odwołania.** Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
<i>Apis mellifera</i>	Boscalid	Oral	LD <sub>50</sub> > 100 µg a.s./bee	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008 and DAR (2002)
<i>Apis mellifera</i>	Boscalid	Contact	LD <sub>50</sub> > 100 µg a.s./bee	
<i>Apis mellifera</i>	Boscalid (formulated)	Oral	<b>LD<sub>50</sub> &gt; 166 µg a.s./bee</b>	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008 and DAR (2002)
		Contact	<b>LD<sub>50</sub> &gt; 200 µg a.s./bee</b>	
<i>Apis mellifera</i>	Boscalid 500 WG = FGG01 (51.65 % a.s. w/w)	Oral	<b>LD<sub>50</sub> &gt; 200.7 µg a.s./bee</b>	New endpoint Knautz (2023) Report No. 165091035, UPL/2022/0605 EFSA-2022-00009374 KCP 10.3.1.1.1/01
		Contact	<b>LD<sub>50</sub> &gt; 249.3 µg a.s./bee</b>	
<i>Bombus terrestris</i>	Boscalid 500 WG = FGG01 (51.65 % a.s. w/w)	Oral	<b>LD<sub>50</sub> &gt; 544.4 µg a.s./bee</b>	New endpoint Knautz & Kowalczyk (2023a) Report No. 165091136, UPL/2022/2874 EFSA-2022-00012189 KCP 10.3.1.1.1/02
		Contact	<b>LD<sub>50</sub> &gt; 497 µg a.s./bee</b>	
<i>Apis mellifera</i>	Boscalid 500 WG = FGG01 (51.65 % a.s. w/w)	Oral Adult 10 d	LDD <sub>50</sub> > 50.8 µg a.s./bee/d NOEDD = 41.9 µg a.s./bee/d <b>LDD<sub>10</sub> = 38.2 µg a.s./bee/d</b>	New endpoint Knautz & Kowalczyk (2023b) Report No. 165091105, UPL/2022/2873 EFSA-2022-00012055 KCP 10.3.1.2/01
<i>Apis mellifera</i>	Boscalid 500 WG = FGG01 (51.36 % a.s. w/w)	Oral Larvae 22 d	<b>NOED = 31.84 µg a.s./larva</b> ED <sub>10</sub> = 42.54 µg a.s./larva	New endpoint Colli (2022) Report No. BT115/22, UPL/2022/2796 EFSA-2022-00011781 KCP 10.3.1.3/01
<b>Higher-tier studies (tunnel test, field studies)</b>				
Not relevant.				

### 9.6.1.1 Justification for new endpoints

Acute, chronic adult to bees and larvae toxicity studies as well as acute bumble bee studies with FGG01

are submitted with the present dossier to cover the data requirements for the product.

### 9.6.2 Risk assessment

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). In the absence of any implemented guidance, for chronic risk assessments The Nordic calculator tool for chronic bee risk assessment<sup>1</sup> following EPPO (2010)<sup>2</sup> and EPPO as modified by ECPA (2017)<sup>3</sup> schemes were used. This approach is deemed reasonable since both EFSA (2013)<sup>4</sup> and EFSA (2023)<sup>5</sup> have not yet been taken note of in the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF). There is no final decision on protection goals for bumble/solitary bees and future guidance for risk assessment is still open.

#### zRMS comments:

The evaluation of the risk for bees was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002). The required study on oral and contact toxicity of the formulated product Lozzare Pro to honeybees was conducted and considered to be valid. The endpoints as proposed by the applicant are considered acceptable and are used in the risk assessment. All hazard quotients for acute oral and acute contact exposure were below 50, the Commission Regulation (EU) No. 546/2011 criterion, indicating low risk to honey bees. The chronic studies with formulation Lozzare Pro were submitted. The studies were accepted by zRMS. The risk assessment based on these studies should be considered when GD for Bees, 2013 is implemented at EU level. Final decision should be taken into account at MSs level.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group grapevine covers the risk for bees from all intended uses in terms of maximum single application rate (see 9.1.2).

#### zRMS comments:

The assessment for the use group grapevine covers the risk for bees from all intended uses in terms of maximum single application rate.

<sup>1</sup> [https://edit.mst.dk/media/cpjhqzhz/nordic-calculator-tool-for-bees-acc-nz-gd-2021\\_ver3-3.xlsx](https://edit.mst.dk/media/cpjhqzhz/nordic-calculator-tool-for-bees-acc-nz-gd-2021_ver3-3.xlsx)

<sup>2</sup> EPPO (2010): Side effects for honeybees. OEPP/EPPO Bulletin 40, 323–331.

<sup>3</sup> ECPA (2017): Proposal for a protective and workable regulatory European bee risk assessment scheme based on the EFSA bee guidance and other new data and available approaches. POS/17/LO/28028. 09 June 2017.

<sup>4</sup> European Food Safety Authority, 2013. EFSA Guidance Document on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2013;11(7):3295, 268 pp., doi:10.2903/j.efsa.2013.3295

<sup>5</sup> EFSA (European Food Safety Authority), Adriaanse P, Arce A, Focks A, Ingels B, Jölli D, Lambin S, Rundlöf M, Süßenbach D, Del Aguila M, Ercolano V, Ferilli F, Ippolito A, Szentes Cs, Neri FM, Padovani L, Rortais A, Wassenberg J and Auteri D, 2023. Revised guidance on the risk assessment of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees). EFSA Journal 2023;21(5):7989, 133 pp. <https://doi.org/10.2903/j.efsa.2023.7989>



### 9.6.2.1 Hazard quotients for bees

*Tier-1 acute bee risk assessment according to SANCO/10329/2002 rev.2 (final), October 17, 2002*

**Table 9.6-2: First-tier assessment of the risk for bees due to the use of Boscalid 400 g/L SC 500 g/L WG in grapevine**

Intended use	Grapevine		
Active substance	Boscalid		
Application rate (g a.s./ha)	<del>2 × 500</del> 4 × 500		
Honeybees			
Test design	LD <sub>50</sub> (lab.) (µg/bee)	Single application rate (g/ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	> 166	500	< 3.0
Contact toxicity	> 200		< 2.5
Product	FGG01		
Application rate (g a.s./ha)	<del>2 × 500</del> 4 × 500		
Test design	LD <sub>50</sub> (lab.) (µg a.s./bee)	Single application rate (g a.s./ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	> 200.7	500	< 2.5
Contact toxicity	> 249.3		< 2.0
Bumble bees			
Product	FGG01		
Application rate (g a.s./ha)	<del>2 × 500</del> 4 × 500		
Test design	LD <sub>50</sub> (lab.) (µg a.s./bee)	Single application rate (g a.s./ha)	Q <sub>HO</sub> , Q <sub>HC</sub> criterion: Q <sub>H</sub> ≤ 50
Oral toxicity	> 544.4	500	< 0.92
Contact toxicity	> 497		< 1.0

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.

The acute contact and oral HQ values for honeybees and bumble bees are below the relevant trigger values indicating acceptable risk to bees following the intended uses of FGG01. Further refined risk assessment is therefore not required.

#### **zRMS comment:**

zRMS agrees that the acute contact and oral HQ values for honeybees and bumble bees are below the relevant trigger values indicating acceptable risk to bees following the intended uses of FGG01 (Lozzare Pro) including minor ones.

***Tier-1 chronic honeybee risk assessment for larvae according to EPPO (2010)***

**Table 9.6-3: First-tier assessment of the chronic risk for bee larvae due to the post-emergence use of FGG01 (covering all intended uses)**

Intended use	All uses		
Active substance	Boscalid		
Test design	NOED (µg a.s./larva/d)	Daily dose <sup>a</sup> (µg a.s./larva/d)	TER criterion: TER > 1
Oral toxicity, larvae, 22 d	31.84	0.0151	2109

TER: Toxicity exposure ratio. TER values shown in bold breach the relevant trigger.

a Daily dose: generic worst-case exposure of 0.0151 µg a.s./larva/day based on a worst-case residue value of 1 mg a.s./kg plant matrix and the worst-case sugar intake of drone larvae of 15.1 mg sugar/larva/day (according to Rortais et al., 2005). The sugar content of nectar and product specific application rate is not included in the risk assessment.

The chronic oral TER value for honeybees larvae exceeds the relevant trigger value indicating acceptable chronic risk to honeybee larvae following the intended uses of FGG01. Further refined risk assessment is therefore not required.

**zRMS comments:**

zRMS agrees, that the chronic oral TER value for honeybees larvae exceeds the relevant trigger value indicating acceptable chronic risk to honeybee larvae following the intended uses of FGG01 (Lozzare Pro) including minor ones.

***Tier-1 chronic honeybee risk assessment for adults and larvae according to EPPO (2010)/ECPA (2017)***

**Table 9.6-4: First-tier assessment of the chronic risk for bee adults and larvae due to the post-emergence use of FGG01 in grapevine (covering all intended uses)**

Intended use	Grapevine		
Active substance	Boscalid		
Application rate (g/ha)	2 × 500		
Test design	Endpoint	Daily dose (µg a.s./bee/d)	TER criterion: TER > 1
Oral toxicity, adults, 10 d	LDD <sub>10</sub> : 38.2 µg a.s./bee/d	Nectar: 0.6187 <sup>a</sup>	61.75
Oral toxicity, larvae, 22 d	NOED: 31.84 µg a.s./larva/d	Nectar: 0.2871 Pollen: 0.00610 Sum: 0.293 <sup>b</sup>	108.6

TER: Toxicity exposure ratio. TER values shown in bold breach the relevant trigger.

a Daily dose for adults:  $A.R. \times (0.128 \text{ g}/(1000 \times 0.3)) \times RUD \times 1000$ ; based on the worst-case sugar need of 128 mg/bee/day (according to Rortais et al., 2005) of a foraging bee feeding exclusively of nectar containing a more representative 30% sugar (according to EFSA, 2013); RUD for nectar 2.9 mg a.s./kg (foliar spray; according to EFSA, 2013)

b Daily dose for larvae  $A.R. \times (0.0594 \text{ g}/(1000 \times 0.3)) \times RUD \times 1000$  for nectar: based on the worst-case sugar need of 59.4 mg/larvae/d.p., nectar sugar content of 30% and RUD for nectar of 2.9 mg a.s./kg (all according to EFSA, 2013); Daily dose for larvae  $A.R. \times RUD \times 2$  for pollen: based on the pollen consumption of 2 mg/5 days and RUD for pollen of 6.1 mg a.s./kg (obtained from EFSA 2013)

The chronic oral TER values for adult honeybee and honeybee larvae, respectively, exceed the relevant trigger values indicating acceptable chronic risk to adult and larval honeybees following the intended uses of FGG01. Further refined risk assessment is therefore not required.

**zRMS comment:**

zRMS agrees, that the chronic oral TER values for adult honeybee and honeybee larvae, respectively, exceed the relevant trigger values indicating acceptable chronic risk to adult and larval honeybees following the intended uses of FGG01 (Lozzare Pro), including minor ones.

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

Not relevant.

#### **9.6.3 Effects on bumble bees**

Acute oral and contact toxicity data on bumble bees are available for FGG01 and considered in the above risk assessment. No risk is indicated following the intended uses.

There is currently no validated methodology for the assessment of chronic toxicity to bumble bees. Consequently, such studies are not considered necessary for the time being, and no chronic risk assessment for bumble bees is required. The risk assessment for honeybees (acute and chronic) and bumble bees (acute) indicates no risk to other pollinators with certain margin of safety.

**zRMS comments:**

Acute oral and contact toxicity data on bumble bees indicate no risk is following the intended uses of FGG01 (Lozzare Pro), including minor ones.

The risk assessment for honeybees (acute and chronic) and bumble bees (acute) may indicate no risk to other pollinators including bumble bees (chronic) following the intended use of FGG01 (Lozzare Pro), including minor ones.

#### **9.6.4 Effects on solitary bees**

There is currently no validated methodology for the assessment of toxicity to solitary bees. Consequently, such studies are not considered necessary for the time being, and no risk assessment for solitary bees is required. The risk assessment for honeybees (acute and chronic) and bumble bees (acute) indicates no risk to other pollinators with certain margin of safety.

**zRMS comment:**

The risk assessment for honeybees (acute and chronic) and bumble bees (acute) may indicate no risk to other pollinators, including solitary bees, following the intended use of FGG01 (Lozzare Pro) including minor ones.

#### **9.6.5 Overall conclusions**

Based on the acute Tier-1 risk assessment (honeybees and bumble bees) according to SANCO/10329/2002 rev.2 (final), October 17, 2002, and the chronic Tier-1 risk assessment (honeybees) according to the modified EPPO (2010)/ECPPA (2017) scheme, respectively, acceptable risk to bees following the intended uses of FGG01 is indicated.

There is currently no validated methodology for the assessment of toxicity to bumble bees (chronic) and solitary bees (both acute and chronic). Consequently, such studies are not considered necessary for the time being, and no risk assessment is required. The risk assessment for honeybees (acute and chronic) and bumble bees (acute) indicates no risk to other pollinators with certain margin of safety.

**zRMS comments:**

Acceptable risk to bees following the intended uses of FGG01 (Lozzare Pro), including minor ones is indicated.

The risk assessment for honeybees (acute and chronic) and bumble bees (acute) may indicate no risk to other pollinators (bumble bees (chronic) and solitary bees (acute and chronic) following the intended use of FGG01 (Lozzare Pro) including minor ones.

However, risk assessment for bees in accordance with the EFSA 2013 guideline should be presented.

## **9.7 Effects on arthropods other than bees (KCP 10.3.2)**

### **9.7.1 Toxicity data**

Studies on the toxicity to non-target arthropods have been carried out with the formulated product for Annex 1 inclusion. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target arthropods of FGG01 were not evaluated as part of the EU assessment of Boscalid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
<i>Aphidius rhopalosiphi</i> (adults)	Boscalid (formulated)	Laboratory test glass plates (2D)	LR <sub>50</sub> / ER <sub>50</sub> > 1800 g a.s./ha	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Typhlodromus pyri</i> (protonymphs)	Boscalid (formulated)	Laboratory test glass plates (2D)	LR <sub>50</sub> / ER <sub>50</sub> > 1800 g a.s./ha	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Chrysoperla carnea</i> (larvae)	Boscalid (formulated)	Laboratory test glass plates (2D)	LR <sub>50</sub> / ER <sub>50</sub> > 2400 g a.s./ha	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Pardosa sp.</i> (adults)	Boscalid (formulated)	Laboratory test glass plates (2D)	LR <sub>50</sub> / ER <sub>50</sub> > 2400 g a.s./ha	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Poecilus cupreus</i> (imagines)	Boscalid (formulated)	Laboratory test glass plates (2D)	LR <sub>50</sub> / ER <sub>50</sub> > 2400 g a.s./ha	Review report, SANCO/3919 /2007-rev. 5, 21 January 2008 and DAR (2002)
<i>Aphidius rhopalosiphi</i> (adults)	Boscalid 500 WG = FGG01 (51.36 % a.s. w/w)	Laboratory test glass plates (2D)	<b>LR<sub>50</sub> / ER<sub>50</sub> &gt; 8000 g/ha corresponding to 4108.4 g a.s./ha</b>	New endpoint Leopold (2023a) Report No. 165091001, UPL/2022/0591 EFSA-2022-00009224 KCP 10.3.2.1/01
<i>Typhlodromus pyri</i> (protonymphs)	Boscalid 500 WG = FGG01 (51.36 % a.s. w/w)	Laboratory test glass plates (2D)	LR <sub>50</sub> > 1662.1 g/ha corresponding to 853.7 g a.s./ha <b>ER<sub>50</sub> &gt; 1000 g/ha corresponding to 513.6 g a.s./ha</b>	New endpoint Leopold (2023b) Report No. 165091063, UPL/2022/0590 EFSA-2022-00009222 KCP 10.3.2.1/02
<b>Field or semi-field tests</b>				
Not relevant.				

### 9.7.1.1 Justification for new endpoints

Endpoints from studies with FGG01 are used for risk assessment since these are considered most relevant for the proposed GAP.

## 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

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group beans and peas covers the risk for non-target arthropods from all intended uses in terms of maximum PER (see 9.1.2).

**Table 9.7-2: First-tier assessment of the in-field risk for non-target arthropods due to the use of FGG01 in beans and peas – foliar-dwelling arthropods**

<b>Intended use</b>	Beans and peas		
<b>Active substance/product</b>	Boscalid in FGG01		
<b>Application rate (g a.s./ha)</b>	2 × 500		
<b>MAF</b>	1.7		
<b>Test species Tier I</b>	<b>ER<sub>50</sub> (lab.) (g a.s./ha)</b>	<b>PER<sub>in-field</sub> (g a.s./ha)</b>	<b>HQ<sub>in-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	> 4108.4	850	< 0.21
<i>Aphidius rhopalosiphi</i>	> 513.6		< 1.65

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

**Table 9.7-3: First-tier assessment of the in-field risk for non-target arthropods due to the use of FGG01 in beans and peas – soil-dwelling arthropods**

<b>Intended use</b>	Beans and peas		
<b>Active substance/product</b>	Boscalid in FGG01		
<b>Application rate (g a.s./ha)</b>	2 × 500		
<b>MAF</b>	1.9		
<b>Test species Tier I</b>	<b>ER<sub>50</sub> (lab.) (g a.s./ha)</b>	<b>PER<sub>in-field</sub> (g a.s./ha)</b>	<b>HQ<sub>in-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	> 4108.4	950	< 0.23
<i>Aphidius rhopalosiphi</i>	> 513.6		< 1.85

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

For all intended uses, Tier-1 in-field HQ values are below the relevant trigger value for both indicator species.

**zRMS comments:**

The risk envelope approach is properly applied. The assessment for the use group beans and peas covers the risk for non-target arthropods from all intended uses in terms of maximum PER.

For all intended uses, including minor ones, Tier-1 in-field HQ values are below the relevant trigger value for both indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi*. It may indicate for acceptable risk for non-target arthropods other than bee.

### 9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group grapevine covers the risk for non-target arthropods from all intended uses in grapevine (uses 1 and 2). The assessment for the use group oilseed rape covers the risk for non-target arthropods from all intended uses in oilseed rape (uses 3 to 5). The assessment for the use group beans and peas covers the risk for non-target arthropods from all intended uses in beans and peas (uses 6 and 7; see 9.1.2).

**Table 9.7-4: First-tier assessment of the off-field risk for non-target arthropods due to the use of FGG01 in grapevine (uses 1 and 2)**

<b>Intended use</b>	Grapevine				
<b>Active substance/product</b>	Boscalid in FGG01				
<b>Application rate (g/ha)</b>	1 × 500				
<b>MAF</b>	1				
<b>Vdf</b>	10				
<b>Test species Tier I</b>	<b>LR<sub>50</sub> / ER<sub>50</sub> (lab.) (g a.s./ha)</b>	<b>Drift factor</b>	<b>PER<sub>off-field</sub> (g a.s./ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	> 8000	0.0802	4.010	10	< 0.010
<i>Aphidius rhopalosiphi</i>	> 513.6				< 0.078

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.

**Table 9.7-5: First-tier assessment of the off-field risk for non-target arthropods due to the use of FGG01 in oilseed rape (uses 3 to 5)**

<b>Intended use</b>	Oilseed rape				
<b>Active substance/product</b>	Boscalid in FGG01				
<b>Application rate (g/ha)</b>	1 × 250				
<b>MAF</b>	1				
<b>Vdf</b>	10				
<b>Test species Tier I</b>	<b>LR<sub>50</sub> / ER<sub>50</sub> (lab.) (g a.s./ha)</b>	<b>Drift factor</b>	<b>PER<sub>off-field</sub> (g a.s./ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	> 8000	0.0277	0.6925	10	< 0.002
<i>Aphidius rhopalosiphi</i>	> 513.6				< 0.013

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.

**Table 9.7-6: First-tier assessment of the off-field risk for non-target arthropods due to the use of FGG01 in beans and peas (uses 6 and 7)**

<b>Intended use</b>	Beans and peas				
<b>Active substance/product</b>	Boscalid in FGG01				
<b>Application rate (g/ha)</b>	2 × 500				
<b>MAF</b>	1.7				
<b>Vdf</b>	10 <sup>a</sup>				
<b>Test species Tier I</b>	<b>LR<sub>50</sub> / ER<sub>50</sub> (lab.) (g a.s./ha)</b>	<b>Drift factor</b>	<b>PER<sub>off-field</sub> (g a.s./ha)</b>	<b>CF</b>	<b>HQ<sub>off-field</sub> criterion: HQ ≤ 2</b>
<i>Typhlodromus pyri</i>	> 8000	0.0723	6.146	10	< 0.015
<i>Aphidius rhopalosiphi</i>	> 513.6				< 0.12

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.

For all intended uses, Tier-1 off-field HQ values are below the relevant trigger value for both indicator species. Thus, higher-Tier risk assessment and/or risk mitigation measures are not required.

**zRMS comments:**

The risk envelope approach is properly applied. The assessment for the use group beans and peas covers the risk for non-target arthropods from all intended uses in terms of maximum PER.

For all intended uses including minor ones, Tier-1 in-field HQ values are below the relevant trigger value for both indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi*. It may indicate for acceptable risk for non-target arthropods other than bees following the intended use of FGG01 (Lozzare Pro) including minor ones..

### 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

For all intended uses, Tier-1 in-field and off-field HQ values are below the relevant trigger value for both indicator species. Thus, higher-Tier risk assessment and/or risk mitigation measures are not required.

**zRMS comment:**

For all intended uses including minor ones, Tier-1 in-field HQ and off-field HQ values are below the relevant trigger value for both indicator species *Typhlodromus pyri* and *Aphidius rhopalosiphi*. It may indicate for acceptable risk for non-target arthropods other than bees following the application of FGG01 (Lozzare Pro) according to the proposed use pattern for both major and minor uses.



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

### **9.8.1 Toxicity data**

Studies on the toxicity to earthworms have been carried out with Boscalid and the formulated product for Annex 1 inclusion. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of FGG01 were not evaluated as part of the EU assessment of Boscalid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results <sup>a</sup>	Reference
<i>Eisenia fetida</i>	Boscalid	Mixed into substrate 14 d, acute	LC <sub>50</sub> > 1000 mg/kg dw soil LC <sub>50 corr.</sub> > 500 mg/kg dw soil	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008 and DAR (2002)
<i>Eisenia fetida</i>	BAS 500 01 F	Mixed into substrate 14 d, acute	LC <sub>50</sub> > 1000 mg/kg dw soil LC <sub>50 corr.</sub> > 500 mg/kg dw soil	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008 and DAR (2002)
<i>Eisenia foetida</i>	BAS 500 01 F	Mixed into substrate 56 d, chronic	NOEC = 3.6 kg/ha NOEC <sub>corr.</sub> = 1.8 kg/ha corresponding to 1.197 mg a.s./kg dw soil	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008 and DAR (2002)
<i>Eisenia fetida</i>	Boscalid 500 WG = FGG01 (51.33 % a.s. w/w)	Mixed into substrate 56 d, chronic 10 % peat content	NOEC = 296.3 mg/kg dw soil EC <sub>10</sub> = 41.21 mg/kg dw soil corresponding to 21.15 mg a.s./kg dw soil <b>EC<sub>10 corr.</sub> = 10.58 mg a.s./kg dw soil</b>	New endpoint Rana (2023c) Report No. 522-3-08- 31232, UPL/2022/21325 EFSA-2022- 00011613 KCP 10.4.1.1/01
<i>Folsomia candida</i>	Boscalid 500 WG = FGG01 (51.36 % a.s. w/w)	Mixed into substrate 28 d, chronic 5 % peat content	NOEC = 556 mg/kg dw soil EC <sub>10</sub> = 72.7 mg/kg dw soil corresponding to 37.34 mg a.s./kg dw soil <b>EC<sub>10 corr.</sub> = 18.67 mg a.s./kg dw soil</b>	New endpoint Hübner (2022a) Report No. 165091016, UPL/2022/0609 EFSA-2022- 00009384 KCP 10.4.2.1/01
<i>Hypoaspis aculeifer</i>	Boscalid 500 WG = FGG01 (51.36 % a.s. w/w)	Mixed into substrate 14 d, chronic 5 % peat content	NOEC = 1000 mg/kg dw soil corresponding to 513.6 mg a.s./kg dw soil <b>NOEC<sub>corr.</sub> = 256.8 mg a.s./kg dw soil</b>	New endpoint Hübner (2022b) Report No. 165091089, UPL/2022/0610 EFSA-2022- 00009385 KCP 10.4.2.1/02
<b>Field studies</b>				
Not available.				
<b>Litter bag test</b>				
Not relevant.				

Acute endpoints shaded in grey are not used for risk assessment since more relevant chronic endpoints are available.

### 9.8.1.1 Justification for new endpoints

Acute earthworm endpoints from the EU review are no longer considered relevant for risk assessment where chronic data are available. The new endpoint from the study with FGG01 is used in the risk assessment since test design and formulation are considered most relevant for the intended GAP. In addition, new data on soil mesofauna with FGG01 is now available to cover the data requirement for the product.

### 9.8.2 Risk assessment

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

#### 9.8.2.1 First-tier risk assessment

The relevant  $PEC_{soil}$  for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 Table 8.7-4.

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group grapevine covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all intended uses in terms of maximum soil loading (see 9.1.2).

**Table 9.8-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of FGG01 in grapevine**

Intended use	Grapevine		
Chronic effects on earthworms			
Product/active substance	EC <sub>10 corr.</sub> (mg a.s./kg dw soil)	PEC <sub>soil</sub> (mg a.s./kg dw soil)	TER <sub>lt</sub> (criterion TER ≥ 5)
FGG01	10.58	0.509 <sup>a</sup>	20.8
		0.900 <sup>b</sup>	11.8
Chronic effects on <i>Folsomia</i>			
Product/active substance	EC <sub>10 corr.</sub> (mg a.s./kg dw soil)	PEC <sub>soil</sub> (mg a.s./kg dw soil)	TER <sub>lt</sub> (criterion TER ≥ 5)
FGG01	18.67	0.509 <sup>a</sup>	36.7
		0.900 <sup>b</sup>	20.7
Chronic effects on <i>Hypoaspis</i>			
Product/active substance	NOEC <sub>corr.</sub> (mg a.s./kg dw soil)	PEC <sub>soil</sub> (mg a.s./kg dw soil)	TER <sub>lt</sub> (criterion TER ≥ 5)
FGG01	256.8	0.509 <sup>a</sup>	504
		0.900 <sup>b</sup>	285

TER values shown in bold fall below the relevant trigger.

a  $PEC_{accumulation}$  ( $PEC_{act} + PEC_{soil\text{ plateau}}$ ) – FOCUS approach (refer to Section 8 (Environmental Fate))

b  $PEC_{accumulation}$  ( $PEC_{act} + PEC_{soil\text{ plateau}}$ ) – additional approach (refer to Section 8 (Environmental Fate))

For the intended use in grapevine, all Tier-1 TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exceed the relevant trigger values indicating no risk for all intended uses.

Therefore, higher-Tier risk assessment is not required.

**zRMS comments:**

The assessment for the use group grapevine covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all intended uses, including minor ones in terms of maximum soil loading.

For use in grapevine, Tier-1 TER values indicate no risk for all intended uses, including minor ones, of FGG01 (Lozzare Pro) according to the proposed use pattern.

### **9.8.2.2 Higher-tier risk assessment**

Not relevant.

### **9.8.3 Overall conclusions**

All Tier-1 TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exceed the relevant trigger values indicating no risk for all intended uses. Therefore, higher-Tier risk assessment is not required.

**zRMS comments:**

zRMS agrees with applicant's overall conclusions listed under point 9.83.

All Tier-1 TER values for earthworms and other non-target soil organisms (meso- and macrofauna) exceed the relevant trigger values indicating no risk for all intended uses, including minor ones of FGG01 (Lozzare Pro) according to proposed use pattern.

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

### **9.9.1 Toxicity data**

Studies on effects soil microorganisms have been carried out with the formulated product for Annex 1 inclusion. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of FGG01 were not evaluated as part of the EU assessment of Boscalid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process.

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

Endpoint	Substance	Exposure System	Results	Reference
C-mineralisation	BAS 500 01 F	28 d	< 25% effects at 12 kg/ha corresponding to 6 kg a.s./ha corresponding to 8 mg a.s./kg d.w. soil	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008 and DAR (2002)
N-mineralisation	BAS 500 01 F	28 d	< 25% effects at 12 kg/ha corresponding to 6 kg a.s./ha corresponding to 8 mg a.s./kg d.w. soil	Review report, SANCO/3919 /2007- rev. 5, 21 January 2008 and DAR (2002)
N-mineralisation	Boscalid 500 WG = FGG01 (51.33 % a.s. w/w)	28 d	< 25% effects at 6 kg/ha corresponding to 8 mg/kg d.w. soil corresponding to <b>4.11 mg/kg d.w. soil</b>	New endpoint Raithatha (2023) Report No. 608-3-15-31233, UPL/2022/0863 EFSA-2022-00010828 KCP 10.5/01

C-mineralisation endpoints shaded in grey are not used for risk assessment since they are no longer considered relevant.

### 9.9.1.1 Justification for new endpoints

C-mineralisation endpoints from the EU review are no longer considered relevant for risk assessment. The new endpoint from the study N-mineralisation with FGG01 is used in the risk assessment since the formulation is considered most relevant for the intended GAP.

### 9.9.2 Risk assessment

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

The relevant  $PEC_{soil}$  for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, Table 8.7-3 8.7-4 and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 9.8).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group grapevine covers the risk for soil microorganisms from all intended uses in terms of maximum soil loading (see 9.1.2).

**Table 9.9-2: Assessment of the risk for effects on soil micro-organisms due to the use of FGG01 in grapevine**

Intended use	Grapevine		
N-mineralisation			
Product/active substance	Max. conc. with effects ≤ 25 % (mg a.s./kg dw soil)	PEC <sub>soil</sub> (mg a.s./kg dw soil)	Risk acceptable?
Boscalid in FGG01	4.11	0.509 <sup>a</sup>	yes
		0.900 <sup>b</sup>	yes

a PEC<sub>accumulation</sub> (PEC<sub>act</sub> + PEC<sub>soil plateau</sub>) – FOCUS approach (refer to Section 8 (Environmental Fate))

b PEC<sub>accumulation</sub> (PEC<sub>act</sub> + PEC<sub>soil plateau</sub>) – additional approach (refer to Section 8 (Environmental Fate))

The maximum concentration of Boscalid with effects on micro-organisms ≤ 25% exceed the respective maximum PEC<sub>soil</sub> value indicating no risk for all intended uses.

### 9.9.3 Overall conclusions

The maximum concentration of Boscalid with effects on micro-organisms ≤ 25% exceed the respective maximum PEC<sub>soil</sub> value indicating no risk for all intended uses.

#### **zRMS comments:**

The risk envelope approach has been properly applied. The assessment for the use group grapevine covers the risk for soil microorganisms from all intended uses including minor ones, in terms of maximum soil loading.

Only new endpoint from the study N-mineralisation with FGG01 is used in the risk assessment since the formulation should be considered most relevant for the intended GAP.

The maximum concentration of Boscalid with effects on micro-organisms ≤ 25% exceed the respective maximum PEC<sub>soil</sub> value indicating no risk for all intended uses, including minor ones, of FGG01 (Laz-zare Pro) according to 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 the formulated product for Annex 1 inclusion. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on non-target terrestrial plants of FGG01 were not evaluated as part of the EU assessment of Boscalid. New data submitted with this application are listed in Appendix 1 and summarised in Appendix 2.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

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

Species	Substance	Exposure System	Results	Reference
6 species	BAS 510 01 F	14 d Vegetative vigour	Maximum 8.8% effect at 3.6 kg/ha	DAR (2002)
6 species	Boscalid 500 WG = FGG01 (51.36 % a.s. w/w)	21 d Seedling emergence	<b>NOER = 1000 g a.s./ha</b>	New endpoint Stürtz (2022a) Report No. 165091087, UPL/2022/0598 EFSA-2022- 00009316 KCP 10.6.2/01
6 species	Boscalid 500 WG = FGG01 (51.36 % a.s. w/w)	21 d Vegetative vigour	<b>NOER = 1000 g a.s./ha</b>	New endpoint Stürtz (2022b) Report No. 165091086, UPL/2022/0597 EFSA-2022- 00009314 KCP 10.6.2/02

m: monocotyledonous; d: dicotyledonous

### 9.10.1.1 Justification for new endpoints

Endpoints from studies with FGG01 are used for risk assessment since these are considered most relevant for the intended GAP.

### 9.10.2 Risk assessment

#### 9.10.2.1 Tier-1 risk assessment (based screening data)

Risk is considered acceptable since data on seedling emergence and vegetative vigour from six species do not indicate phytotoxic effects > 50% at the maximum intended application rate. Therefore, a Tier-2 risk assessment based on dose-response data is not required.

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

Not 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

Risk is considered acceptable since data on seedling emergence and vegetative vigour from six species do not indicate phytotoxic effects > 50% at the maximum intended application rate. Therefore, a Tier-2 risk assessment based on dose-response data is not required.

#### **zRMS comments:**

Risk for non-target terrestrial plants is considered acceptable since data on seedling emergence and vegetative vigour from six species do not indicate phytotoxic effects > 50% at the maximum intended application rate of FGG01 (Lozzare Pro).

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

Not available and not required.

### 9.12 Monitoring data (KCP 10.8)

Not available and not required.

### 9.13 Classification and Labelling according to Regulation (EC) 1272/2008

Based on the summation method and classification of Boscalid as Aquatic Chronic 2 (based on lowest chronic NOEC = 0.125 mg a.s./L<sub>nom</sub> in fish), FGG01 is classified 0.125mg Boscalid/L is equivalent to 0.25 mg FGg01/L, which results its classification as Aquatic Chronic 2, H411.

The following classification is ~~then~~ then proposed from an ecotoxicological point of view:

**Pictogram:** GHS09

**Signal word:** -

#### **H-statements:**

**H411** Toxic to aquatic life with long lasting effects.

**EUH401** To avoid risks to human health and the environment, comply with the instructions for use

#### **P-statements:**

**P273** Avoid release to the environment.

**P391** Collect spillage.

**P501** Dispose of contents/container to hazardous or special waste collection point, in accordance with local/regional/national and/or international regulation.

#### **zRMS comment:**



zRMS believes that regarding ecotoxicological properties the PPP FGG01 should be classified as:

Aquatic Chronic 2; H411 (Toxic to aquatic life with long lasting effects).

Proposed labelling is adequate to classification and consists from:

- Pictogram: GHS09,
- H-statement: H411
- EUH-statement: EUH401
- P-statements: P273, P391 and P501.

Tables considered not relevant can be deleted as appropriate.

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

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

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

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1/01	Rana, J.R.	2023a	Acute Immobilisation Study of Boscalid 500 g/Kg WG to <i>Daphnia magna</i> Report No. 502-3-07-31231, UPL/2022/21324 Jai Research Foundation, Valvada, Gujarat, India GLP Unpublished	N	UPL Europe
KCP 10.2.1/02	Rana, J.R.	2023b	Alga ( <i>Pseudokirchneriella subcapitata</i> ), Growth Inhibition Test with Boscalid 500 g/Kg WG Report No. 501-3-07-31230, UPL/2022/21323 Jai Research Foundation, Valvada, Gujarat, India GLP Unpublished	N	UPL Europe
KCP 10.3.1.1.1/01 & KCP 10.3.1.1.2/01	Knautz, T.	2022	Boscalid 500 WG: Effects (Acute Contact and Oral) on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory Report No. UPL/2022/0605, UPL/2022/0605 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.3.1.1.1/02 & KCP 10.3.1.1.2/02	Knautz, T. & Kowalczyk, F.	2022a	Boscalid 500 WG: Acute Contact and Oral Toxicity to Bumblebees ( <i>Bombus terrestris</i> L.) in the Laboratory Report No. 165091105, UPL/2022/2874 ibacon GmbH, Rossdorf, Germany GLP	N	UPL Europe

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 10.3.1.2/01	Knautz, T. & Kowalczyk, F.	2022b	Boscalid 500 WG: Chronic Oral Toxicity Test on the Honey Bee ( <i>Apis mellifera</i> L.) in the Laboratory Report No. 165091136, UPL/2022/2873 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.3.1.3/01	Colli, M.	2022	Effects of BOSCALID 500 WG (FGG01) on honeybees ( <i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure Report No. BT115/22, UPL/2022/2796 BioTecnologie BT S.r.l., Todi (PG), Italy GLP Unpublished	N	UPL Europe
KCP 10.3.2.1/01	Leopold, J.	2023a	Boscalid 500 WG: Effects on the Parasitoid <i>Aphidius rhopalosiphi</i> (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates Report No. 165091001, UPL/2022/0591 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.3.2.1/02	Leopold, J.	2023b	Boscalid 500 WG: Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates Report No. 165091063, UPL/2022/0590 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.4.1.1/01	Rana, J.R.	2023c	Reproduction toxicity test of Boscalid 500 g/kg WG to earthworm, <i>Eisenia fetida</i> Report No. 522-3-08-31232, UPL/2022/21325 Jai Research Foundation, Valvada, Gujarat, India GLP Unpublished	N	UPL Europe

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.4.2.1/01	Hübner, S.	2022a	Boscalid 500 WG: Effects on Reproduction of Collembola ( <i>Folsomia candida</i> ) in Artificial Soil Report No. 165091016, UPL/2022/0609 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.4.2.1/02	Hübner, S.	2022b	Boscalid 500 WG: Effects on Reproduction of the Predatory Mite <i>Hypoaspis aculeifer</i> in Artificial Soil Report No. 165091089, UPL/2022/0610 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.5/01	Raithatha, A.	2023	Effect of Boscalid 500 WG on Soil Microorganisms: Nitrogen Transformation Test Report No. 608-3-15-31233, UPL/2022/0863 Jai Research Foundation, Valvada, Gujarat, India GLP Unpublished	N	UPL Europe
KCP 10.6.2/01	Stürtz, S.	2022a	Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test Report No. 165091087, UPL/2022/0598 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe
KCP 10.6.2/02	Stürtz, S.	2022b	Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth Report No. 165091086, UPL/2022/0597 ibacon GmbH, Rossdorf, Germany GLP Unpublished	N	UPL Europe

The following tables are to be completed by MS

**List of data submitted by the applicant and not relied on**

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

<b>Data point</b>	<b>Author(s)</b>	<b>Year</b>	<b>Title Company Report No. Source (where different from company) GLP or GEP status Published or not</b>	<b>Vertebrate study Y/N</b>	<b>Owner</b>
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 2.1.1.1 KCP 10.1.2.1 Acute oral toxicity to mammals

##### A 2.1.1.1 KCP 10.1.2.2 Higher tier data on mammals

#### A 2.2.2 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

### A 2.1 KCP 10.2 Effects on aquatic organisms

#### A 2.1.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

##### A 2.1.1.1 Rana, 2023a (KCP 10.2.1/01)

<b>zRMS comments:</b>	<p>The study was conducted according to:</p> <ul style="list-style-type: none"> <li>– OECD guideline 202 (<i>Daphnia</i> sp., Acute Immobilisation Test, static conditions)</li> <li>– OCSPP 850.1010 (2016), .</li> </ul> <p>The validity criteria (OECD 202) were met and no deviation from this guideline was reported; the study was considered valid.</p> <p>Test media was analysed for concentration and stability at 0 and 48 h during the main study and was within the acceptable limit (&gt;80% of the nominal concentration).</p> <p>The study is acceptable for risk assessment.</p> <p>The following endpoint was derived: 48h EC<sub>50</sub> is equal to 75.683 mg/L (38.848 mg a.s./L).</p> <p>This value has been used for risk assessment.</p>
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Reference: KCP 10.2.1/01

Report Acute Immobilisation Study of Boscalid 500 g/Kg WG to *Daphnia magna*, Rana, J.R., 2023, Report no. 502-3-07-31231, UPL/2022/21324

EFSA Identification Study: EFSA-2022-00011650

Guideline(s): OCSPP 850.1010 (2016), OECD 202 (2004)

Deviations: No

GLP: Yes

Acceptability: Yes

### Executive summary

This study was performed to assess the potential toxic effects of Boscalid 500 g/Kg WG to *Daphnia magna*, under static conditions.

Based on results of the preliminary range finding studies, concentration levels selected for the main study were 8.5, 18.8, 41.3, 90.9 and 200.0 mg Boscalid 500 g/Kg WG/L, along with control. In the main study, 48-h static toxicity test procedure was followed, comprising six groups. Each group consisted of four replicates with 5 daphnids per replicate.

The stability test of Boscalid 500 g/Kg WG in the test media was performed during the method validation (JRF Study N° 228-2-13-31234). Boscalid was found to stable in the test media up to 48 h (>80% of the nominal concentration). Test media was analysed for concentration and stability at 0 and 48 h during the main study and was within the acceptable limit (>80% of the nominal concentration).

The highest tested concentration level of Boscalid 500 g/Kg WG causing no immobilisation was 18.8 mg/L and the lowest tested concentration causing 100% immobilisation within the 48-h test period was 200.0 mg/L.

The 48 h EC<sub>50</sub> of Boscalid 500 g/Kg WG was determined to be 75.683 mg/L (38.848 mg a.i./L).

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** Boscalid 500 g/kg WG (FGG01)  
**Batch no.:** ARD/BD364/50/WG/0422/59  
**Purity:** Boscalid:  $513.34 \pm 0.40$  g/kg ( $51.33 \pm 0.04\%$  w/w)  
**Date of expiry:** 21 April 2024
2. **Test concentrations:** Control: Reconstituted water  
Test item: 8.5, 18.8, 41.3, 90.9 and 200.0 mg test item/L (equivalent to 4.36, 9.65, 21.20, 46.66 and 102.66 mg a.i./L)
3. **Toxic reference:** Potassium dichromate  
**CAS No.:** 7778-50-9  
**Study No.:** 502-3-07-32856
4. **Test organism:**  
**Species:** Daphnids (*Daphnia magna*)  
**Source:** in-house culture, origin from MicroBioTests Inc., Kleimoer 15, 9030 Mariakerke (Gent), Belgium  
**Age:** Less than 24 hours old, first instar daphnids  
**Housing:** Test vessels of 250 mL capacity which was loosely covered with transparent polythene to minimize the entry of dust and other particulates into solution  
**Test medium:** Reconstituted water with a total hardness and alkalinity of 189 and 69 mg/L as CaCO<sub>3</sub>, respectively and conductivity of 370 µS/cm  
**Feeding:** No feeding during exposure

## 5. Environmental conditions:

<b>Dissolved oxygen:</b>	93.1 – 95.3 %
<b>pH:</b>	7.25 – 7.61
<b>Water temperature:</b>	20.2 – 20.4 °C
<b>Water hardness:</b>	189 mg/L as CaCO <sub>3</sub>
<b>Alkalinity:</b>	69 mg/L as CaCO <sub>3</sub>
<b>Conductivity:</b>	370 µS/cm
<b>Light:</b>	1010 - 1040 lux, 16 hours light / 8 hours dark

## B. STUDY DESIGN AND METHODS

### 1. Dates of work: 28 August – 31 December 2022

### 2. Test organism assignment and treatment

First instar *Daphnia magna* were exposed to the test item at 5 concentrations of 8.5, 18.8, 41.3, 90.9 and 200.2 mg test item/L (equivalent to 4.36, 9.65, 21.20, 46.66 and 102.66 mg a.i./L) and a control of reconstituted water for 48 hours. 4 replicates with 5 daphnids per treatment (250 mL beakers with 110 mL of test solution) were tested.

### 3. Dose preparation

Reconstituted water was used as vehicle to dissolve the test item. Five test concentrations of 8.5, 18.8, 41.3, 90.9 and 200 mg Boscalid 500 WG/L (equivalent to 4.36, 9.65, 21.20, 46.66 and 102.66 mg a.i./L) were prepared. A control was tested in parallel.

### 4. Measurements and observations

Test daphnids were observed for immobility and abnormal behaviour or appearance, at 0, 3, 24 and 48 h of exposure. Mobility of the daphnids was assessed by gently agitating each test container for 15 seconds and observing their swimming behaviour. Daphnids unable to swim, during the agitation of the test container, were recorded as immobile and removed from the test vessels at the time of observation.

### 5. Analytical verification

10 mL of test solution from each replicate were collected using a micropipette into 50 mL test tubes. The collected amount was 40 mL for each group at 0 and 48 h. The samples were divided into two portions. One portion, 20 mL of each group was sent to the Department of Chemistry, JRF for active substance concentration analysis and the second portion was stored as back up sample at  $-20 \pm 5$  °C until the study completion. Active substance concentration in each sample was determined using the validated analytical method.

### 6. Statistics

The 48 h EC<sub>50</sub> value and the associated 95% confidence limits were calculated following the Probit analysis method (Finney, 1971).

## II. RESULTS AND DISCUSSION

### A. IMMOBILIZATION AND SUB-LETHAL EFFECTS

No immobility daphnids was observed in the control group throughout the test. No immobility was observed in the test concentrations of 8.5 and 18.8 mg/L (equivalent to 4.36 and 9.65 mg a.i./L) during the exposure duration of 48 hours, however in the test concentrations of 41.3 mg/L (equivalent to 21.20 mg a.i./L), immobility was observed at 48-hour test period. Immobility was observed in 90.9 and 200 mg/L (equivalent to 46.6 and 102.66 mg a.i./L) at 24-hour test period and 48-hour test period. Detailed data is presented in the table below.



**Table A 2.1.1.1-1: Cumulative Immobility Data at 0, 3, 24 and 48 h Exposure Period**

Test concentration (mg/L)	Number of Daphnia/group	Immobility of <i>Daphnia</i>								Cumulative immobility at 48 h (%)
		0 h	%	3 h	%	24 h	%	48 h	%	
Control	20	0	0	0	0	0	0	0	0	0
8.5	20	0	0	0	0	0	0	0	0	0
18.8	20	0	0	0	0	0	0	0	0	0
41.3	20	0	0	0	0	0	0	2	10	10
90.9	20	0	0	0	0	1	5	12	60	65
200.0	20	0	0	0	0	8	40	12	60	100

## B. ANALYTICAL RESULTS

The stability test of the Boscalid 500 g/Kg WG in the test media was performed during the method validation (JRF Study N° 228-2-13-31234). Boscalid in the test media was stable up to 48 h (>80% of the nominal concentration). Test media was analysed for concentration and stability at 0 and 48 h during the main study and was within the acceptable limit (>80% of the nominal concentration).

## C. VALIDITY CRITERIA

The study was considered valid since the following criteria were met:

**Table A 2.1.1.1-2: Study validity criteria**

Parameter	Required	Observed
Immobilization in the control	≤ 10%	0%
Dissolved oxygen concentration	≥ 3 mg/L	≥ 7.80 mg/L

## D. TOXICITY ENDPOINTS

The results are presented in table below:

**Table A 2.1.1.1-3: EC<sub>50</sub> value and the associated 95% confidence limits**

Period (hours)	EC <sub>50</sub> value		Confidence limits	
			Lower	Upper
	(mg/L)	(mg a.i./L)	(mg/L)	(mg/L)
48	75.683	38.848	60.674	94.406

The highest tested concentration level of Boscalid 500 g/Kg WG causing no immobilisation was 18.8 mg/L and the lowest tested concentration causing 100% immobilisation within the 48-h test period was 200.0 mg/L.

## III. CONCLUSION

The acute immobilization of Boscalid 500 WG to the *Daphnia magna* has been investigated using a static method for 48 hours. The 48 h EC<sub>50</sub> value of Boscalid 500 g/Kg WG was 75.683 mg/L (38.848 mg a.i./L) for immobilization of *Daphnia magna*.

Immobilisation in the control was 0% at the end of the test. No sign of disease or stress, e.g., discolouration or unusual behaviour, such as, trapping at the surface of water was observed in the control group.

Dissolved oxygen concentration was ≥7.80 mg/L in the control and test vessels, at the end of the test. Thus, the validity criteria were met.

Test media was analysed for concentration and stability at 0 and 48 h during the main study and was

within the acceptable limit.

#### A 2.1.1.2 Rana, 2023b (KCP 10.2.1/02)

Comments of zRMS:	<p>The study was conducted according to:</p> <ul style="list-style-type: none"> <li>OECD Test Guideline 201 (Freshwater Alga and Cyanobacteria, Growth Inhibition Test) with minor deviations described and assessed by Study Director.</li> <li>EPC Ecological Effects Test Guidelines, OCSPP 850.4500, “Algal Toxicity”, EPA 712-C-006, January 2012 with minor deviations described and assessed by Study Director</li> </ul> <p>This forementioned deviation does not affect the outcome of the study and does not affect the ability to use the results for a risk assessment.</p> <p>The test was performed in line to GLP principles.</p> <p>Validity criteria mentioned in OECD 201 were met</p> <p>The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit (&gt;80% of nominal concentration).</p> <p>The following values were derived:</p> <p>72 h 96-h biomass IC<sub>50</sub> were 2.858 mg/L (1.467 mg a.i./L) and 2.489 mg/L (1.278 mg a.i./L) respectively.</p> <p>72 h and 96 h yield IC<sub>50</sub> were 2.877 mg/L (1.477 mg a.i./L) and 2.360 mg/L (1.211 mg a.i./L), respectively.</p> <p>72 h and 96 h growth rate IC<sub>50</sub> were 3.917 mg/L (2.011 mg a.i./L) and 3.819 mg/L (1.960 mg a.i./L), respectively.</p> <p>NOEC = 1.3 mg/L of formulation (0.67 mg a.s./L) for inhibition of biomass, growth rate and yield;</p> <p>LOEC = 2.5 mg/L of formulation (1.28 mg a.s./L) of test item for inhibition of biomass, growth rate and yield</p> <p>These values were used for risk assessment.</p> <p>Based on the result of recovery phase, it can be concluded that Boscalid 500 g/Kg WG (GPF516/FGG01) has algistatic effect on <i>Pseudokirchneriella subcapitata</i>.</p>
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Reference: KCP 10.2.1/02

Report: Alga (*Pseudokirchneriella subcapitata*), Growth Inhibition Test with Boscalid 500 g/Kg WG, Rana, J.R., 2023, Study code 501-3-07-31230

EFSA Identification Study: EFSA-2022-00011649

Guideline(s): OECD Test Guideline 201, ‘Freshwater Alga and Cyanobacteria, Growth Inhibition Test’ adopted March 23, 2006, Annex 5, corrected July 28, 2011. EPC Ecological Effects Test Guidelines, OCSPP 850.4500, “Algal Toxicity”, EPA 712-C-006, January 2012

Deviations:

As per OECD 201, for microscopic counting during main study, one cell count per replicate is performed, but for this study cells were counted as per OCSPP 850.4500, two samples were taken from each test vessel (replicate) and two counts were made of each sample.

As per OECD 201, illumination should be maintained between 4440 and 8880 lux (±15 % of mean value), but in this study it was maintained as per OCSPP 850.4500 at approximately 4300 Lux.

As per OECD 201, for main study, there should be three replicates for treatment groups and six replicates for control group(s) and for limit test, six replicates for treatment and control group(s) but in this study, replicates

were used as per OCSPP 850.4500, i.e. four replicates for treatment group(s) and control group(s).

GLP: Yes

Acceptability: Yes

### Executive summary

This study was performed to assess the inhibitory effect of Boscalid 500 g/Kg WG on the growth of alga *Pseudokirchneriella subcapitata* under static conditions. Exponentially growing cultures of *P. subcapitata* were exposed to the test concentration level 0.0 (control), 0.6, 1.3, 2.5, 5.0 and 10.0 mg test item/L. Algal cultures were assessed for the growth by visual cell count at 24 h, 48 h, 72 h and 96 h.

Recovery phase was completed for the control and the highest test concentration (10.0 mg/L), on Day 6, as normal growth was observed for the control and no growth was observed for the highest test concentration (10.0 mg/L). This indicates an algicidal effect of Boscalid 500 g/Kg WG at the test concentration 10.0 mg/L on *Pseudokirchneriella subcapitata*.

The stability test of Boscalid 500 g/Kg WG in the test media was performed during the method validation study (JRF Study N° 228-2-13-31234) and the test item in the test media was stable up to 96 h (> 80% of nominal concentration). The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit (> 80% of nominal concentration).

A concentration-effect relationship was observed and statistically analysed to obtain effect concentrations at 72 h and 96 h, respectively.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material**  
**Batch no.:** Boscalid 500 WG (FGG01)  
**Purity:** ARD/BD364/50/WG/0422/59  
**Date of expiry:** Boscalid:  $513.34 \pm 0.40$  g/kg ( $51.33 \pm 0.04\%$  w/w)  
21 April 2024
2. **Test concentrations:** Control: 0.0 mg/L medium  
Test item: 0.6, 1.3, 2.5, 5.0 and 10.0 mg/L
3. **Toxic reference:** Potassium dichromate  
**CAS No.:** 7778-50-9  
**Study No.:** 501-3-07-32855 (separate study)
4. **Test organism:**  
**Species:** *Pseudokirchneriella subcapitata*, strain ATCC 22662  
**Source:** Origin culture obtained from American Type Culture Collection, (ATCC), Manassas, USA; Alga subculture maintained at Jai Research Foundation, Ecotoxicology Section, Valvada, District Valsad, Gujarat under static condition. Pre-culture was prepared 3 days prior to commencement of study at temperature between 22°C – 24°C.  
**Test duration:** 96 hours  
**Test chambers:** 250 mL capacity sterile conical flasks
5. **Environmental conditions**  
**Temperature:** 23°C – 24°C (water temperature)  
**Test medium:** 22.2°C – 22.3°C  
**pH:** 7.25 – 7.66 (at experimental start and end)  
**Light:** 4373 to 4413 lux, continuous illumination

### B. STUDY DESIGN AND METHODS

1. **Dates of work:** 26 August – 18 December 2022
2. **Test organism assignment and treatment**

The fresh water, unicellular green alga *Pseudokirchneriella subcapitata* was exposed to the test item at five concentrations from 0.6 to 10.0 mg/L and a control for 96 hours with three replicates (sterile conical flasks of 250 mL volume with 100 mL test medium) per test concentration and six for the control. A static test was chosen. At the end of the main study, determination of algistatic and algicidal effects was performed with the following method. Aliquots of 0.5 mL from each replicate of G1 and G6 were taken and mixed, to make the volume 2 ml per group. These aliquots of 2 mL were transferred into a new test vessel with 118 mL of nutrient medium and final volume was made up to 120 mL. These cultures were incubated under the environmental conditions used in the main study for a period of 6 Days and observed for algal growth on Day 0, 2, 4 and 6 as the growth occurred by a factor of more than 100 times. Potassium dichromate is recommended as a positive control in a separate study.

#### 3. Dose preparation

Five test concentrations of 0.6, 1.3, 2.5, 5.0 and 10.0 mg test item /L were prepared in 1X AAP media as vehicle. A negative control without test item was tested in parallel. The test solutions were analysed for active ingredient and stability analysis using the validated analytical method. For the main study, four replicates for treatment and control group were prepared. 10 mL of test samples from each replicate were drawn at 0, 72, and 96 h interval and mixed together for each group.

#### 4. Measurements and observations

Cell count and cell appearance observation were made at 24 hours interval up to 96 hours by manual cell count using a haemocytometer and a microscope. During recovery phase, the cell concentration of control and highest group were determined once at 0, 2, 4 and 6 days. Microscopic observation was performed to verify the normal and healthy appearance of the inoculum culture and to observe and record any abnormal appearance of the algae.

## 5. Analytical verification

The stability test of Boscalid 500 g/Kg WG in the test media was performed during the method validation study (JRF Study N° 228-2-13-31234) and the test item in the test media was stable up to 96 h (>80% of nominal concentration). The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit (>80% of nominal concentration).

## 6. Statistics

The above-mentioned calculations were performed using in-house validated computer software. The IC<sub>05</sub>, IC<sub>10</sub>, IC<sub>20</sub> and IC<sub>50</sub> derived by this method were specified as I<sub>y</sub>C<sub>05</sub>, I<sub>y</sub>C<sub>10</sub>, I<sub>y</sub>C<sub>20</sub> and I<sub>y</sub>C<sub>50</sub>. The IC<sub>05</sub>, IC<sub>10</sub>, IC<sub>20</sub> and IC<sub>50</sub> [(0 - 72 h) and (0 - 96 h)] was calculated using the Probit analysis method (Finney, 1971) through in-house developed, validated computer software. Probit regression line was drawn for percent biomass inhibition, percent yield inhibition, and percent growth rate inhibition by plotting the probit inhibition values against the corresponding log concentrations.

NOEC and LOEC were determined by a statistical procedure for multi sample comparison. Data of biomass, specific growth rate and yield were subjected to Brown-Forsythe test and Kruskal-Wallis test before conducting Dunn's test through in-house developed, validated computer software. The IC<sub>05</sub>, IC<sub>10</sub>, IC<sub>20</sub>, and IC<sub>50</sub> were calculated using the Probit analysis method (Finney, 1971) for 72 h and 96 h.

# II. RESULTS AND DISCUSSION

## A. GROWTH AND YIELD

In the microscopic observation, the inoculum culture appeared normal and healthy. Normal cell size and shape was observed. No color differences, flocculation, adherence of algae to test vessels, or aggregation of algal cells was observed in any test concentration when compared to control during preliminary range finding study as well as in the main study.

The cell concentration in the control cultures was increased by a factor of 109.6 times within the three day test period. In treated groups, biomass was decreased with increased concentration of test item, during the 72h exposure period.

At 72 h, statistically no significant difference in the mean of biomass, growth rate and yield, was observed at concentration 0.6 and 1.3 mg/L, while statistically significant decrease was observed at concentrations 2.5, 5.0 and 10.0 mg/L.

The cell concentration in the control cultures was increased by a factor of 505.0 times within the four day test period. In treated groups, biomass was decreased with increased concentration of test item, during the 96 h exposure period.

At 96 h, statistically no significant difference in the mean of biomass, mean of specific growth rate, and yield was observed at concentration 0.6 and 1.3 mg/L, while statistically significant decrease was observed at concentrations 2.5, 5.0 and 10.0 mg/L. Hence based on statistically significant effect, NOEC and LOEC were determined as 1.3 and 2.5, respectively.

Details are presented in the tables below.

**Table A 2.1.1.2-1: Percentage inhibition of biomass, growth rate and yield**

Concentration (mg/L)	0.0	0.6	1.3	2.5	5.0	10.0
Inhibition of biomass (%) at 0-72 h	-	0.80	3.88	19.20	99.08	100.41
Inhibition of biomass (%) at 0-96 h	-	0.36	2.47	45.12	99.43	100.0

<b>Inhibition of growth rate (%) at 0-72 h</b>	-	0.15	0.61	4.60	84.51	96.93
<b>Inhibition of growth rate (%) at 0-96 h</b>	-	0.00	0.31	14.20	83.02	93.98
<b>Inhibition of yield (%) at 0-72 h</b>	-	0.84	2.82	19.54	99.01	99.85
<b>Inhibition of yield (%) at 0-96 h</b>	-	0.12	1.94	58.89	99.63	99.91

## B. ANALYTICAL RESULTS

The stability test of Boscalid 500 g/Kg WG in the test media was performed during the method validation study (JRF Study N° 228-2-13-31234) and the test item in the test media was stable up to 96 h (>80% of nominal concentration). The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit (>80% of nominal concentration).

## C. VALIDITY CRITERIA

The study was considered valid since validity criteria were met:

**Table A 2.1.1.2-2: Study validity criteria**

<b>Parameter OECD, 72 hours</b>	<b>Required</b>	<b>Observed</b>
Biomass increase at 72 h in the control	At least 16 times	109.6 factor times
Mean coefficient of variation for section-by-section growth rate for the control cultures over the test period (for 72h)	≤ 35%	2.71%
Coefficient of variation of average specific growth rate in the control (for 72h)	≤ 7%	0.64%
<b>Parameter OCSPP, 96 hours</b>		
Cell Density increase in Control Cultures (for 96h)	At least 100 times	505.0 times
Coefficient of variation of average yield and growth rate control	≤ 15%	5.17% for yield and 0.64% average specific growth rate

## D. TOXICITY ENDPOINTS

The results based on nominal test concentrations are presented in the following Table.

**Table A 2.1.1.2-3: Effective concentrations based on inhibition of biomass, inhibition of growth rate and inhibition of yield during 72- and 96-hours exposure of Boscalid 500 g/Kg WG to *Pseudokirchneriella subcapitata***

<b>Response variable based on % inhibition (0-72 h)</b>		<b>Effective concentration (mg/L)</b>	<b>95% confidence limits</b>	
			<b>Lower limit (mg/L)</b>	<b>Upper limit (mg/L)</b>
Biomass	I <sub>b</sub> C <sub>05</sub>	1.455	0.973	2.178
	I <sub>b</sub> C <sub>10</sub>	1.690	1.191	2.399
	I <sub>b</sub> C <sub>20</sub>	2.023	1.469	2.786
	I <sub>b</sub> C <sub>50</sub>	2.858	2.075	3.936
Growth rate	I <sub>r</sub> C <sub>05</sub>	1.963	1.535	2.512
	I <sub>r</sub> C <sub>10</sub>	2.286	1.786	2.924
	I <sub>r</sub> C <sub>20</sub>	2.748	2.244	3.365
	I <sub>r</sub> C <sub>50</sub>	3.917	3.396	4.519
Yield	I <sub>y</sub> C <sub>05</sub>	1.500	1.089	2.065
	I <sub>y</sub> C <sub>10</sub>	1.730	1.352	2.213
	I <sub>y</sub> C <sub>20</sub>	2.061	1.611	2.636
	I <sub>y</sub> C <sub>50</sub>	2.877	2.350	3.524

<b>Response variable based on % inhibition (0-96 h)</b>		<b>Effective concentration (mg/L)</b>	<b>95% confidence limits</b>	
			<b>Lower limit (mg/L)</b>	<b>Upper limit (mg/L)</b>
Biomass	I <sub>b</sub> C <sub>05</sub>	1.365	1.114	1.671

	I <sub>b</sub> C <sub>10</sub>	1.560	1.352	1.799
	I <sub>b</sub> C <sub>20</sub>	1.832	1.589	2.113
	I <sub>b</sub> C <sub>50</sub>	2.489	2.158	2.871
Growth rate	I <sub>r</sub> C <sub>05</sub>	1.778	1.189	2.661
	I <sub>r</sub> C <sub>10</sub>	2.104	1.483	2.985
	I <sub>r</sub> C <sub>20</sub>	2.582	1.941	3.436
	I <sub>r</sub> C <sub>50</sub>	3.819	3.119	4.677
Yield	I <sub>y</sub> C <sub>05</sub>	1.255	1.156	1.435
	I <sub>y</sub> C <sub>10</sub>	1.472	1.340	1.618
	I <sub>y</sub> C <sub>20</sub>	1.734	1.600	1.879
	I <sub>y</sub> C <sub>50</sub>	2.360	2.208	2.523

NOEC: No observed effect concentration is 1.3 mg/L (0.67 mg a.i./L) of test item at 72 h and at 96 h for inhibition of biomass, growth rate and yield.

LOEC: Lowest observed effect concentration is 2.5 mg/L (1.28 mg a.i./L) of test item at 72 h and at 96 h for inhibition of biomass, growth rate and yield.

### III. CONCLUSION

The results of the study show that Boscalid 500 g/Kg WG at tested concentrations has inhibitory effects on the growth rate and yield of *Pseudokirchneriella subcapitata* during the 72- and 96- hours exposure period. The validity criteria according to the OECD guideline 201 have been met in this study. The 72 hours and 96-hours Biomass IC<sub>50</sub> were 2.858 mg/L (1.467 mg a.i./L) and 2.489 mg/L (1.278 mg a.i./L) respectively. The 72 hours and 96 hours Yield IC<sub>50</sub> were 2.877 mg/L (1.477 mg a.i./L) and 2.360 mg/L (1.211 mg a.i./L), respectively. The 72 hours and 96 hours Growth Rate IC<sub>50</sub> were 3.917 mg/L (2.011 mg a.i./L) and 3.819 mg/L (1.960 mg a.i./L), respectively. No observed effect concentration (NOEC) is 1.3 mg/L (0.67 mg a.i./L) of test item for inhibition of biomass, growth rate and yield; Lowest observed effect concentration (LOEC) is 2.5 mg/L (1.28 mg a.i./L) of test item for inhibition of biomass, growth rate and yield. Based on the result of recovery phase, it can be concluded that Boscalid 500 g/Kg WG has algistatic effect on *Pseudokirchneriella subcapitata*.

All criteria were met. The stability test of Boscalid 500 g/Kg WG in the test media was performed during the method validation study (JRF Study N° 228-2-13-31234) and the test item in the test media was stable up to 96 h (>80% of nominal concentration). The test media was analysed for active ingredient concentration and stability at 0, 72, and 96 h, during the main study and was within the acceptable limit (>80% of nominal concentration).

**A 2.1.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms**

**A 2.1.3 KCP 10.2.3 Further testing on aquatic organisms**

**A 2.2 KCP 10.3 Effects on arthropods**

**A 2.2.1 KCP 10.3.1 Effects on bees**

**A 2.2.1.1 KCP 10.3.1.1 Acute toxicity to bees**

## A 2.2.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

### A 2.2.1.1.1.1 Knautz, 2022 (KCP 10.3.1.1.1/01)

<b>zRMS comments:</b>	<p>The studies were conducted according to OECD guidelines 213 (oral test) and 214 (contact test).</p> <p>Oral acute toxicity test was conducted as limit test with a nominal dose of 249.3 µg a.s./bee along with a control group using pure sucrose solution 50% w/v. For oral The actual dose consumed was determined as 200.7 µg a.i.s/bee.</p> <p>According to OECD 213 limit test may be performed, using 100 µg a.i./bee in order to demonstrate that the LD<sub>50</sub> is greater than this value. In performed test the limit concentration was greater than 100 µg a.s./bee.</p> <p>In case of contact test a 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet however these minor deviations do not affect the ability to use the result for a risk assessment.,</p> <p>The validity criteria from OECD 213 and 214 were met</p> <p>These studies were considered as valid.</p> <p>Based on the results of the limit test, the 48 hours acute oral LD<sub>50</sub> of test item is considered higher than 200.7 µg a.s./bee based on the actual consumed dose and the 48 hours acute contact LD<sub>50</sub> of test item is considered higher than 249.3 µg a.s./bee.</p>
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Reference:	KCP 10.3.1.1.1/01
Report	Boscalid 500 WG: Effects (Acute Contact and Oral) on Honey Bees ( <i>Apis mellifera</i> L.) in the Laboratory, Knautz, T., 2022, Study code 165091035
Guideline(s):	OECD 213: 'Honeybees, Acute Oral Toxicity Test', adopted on September 21, 1998 OECD 214: 'Honeybees, Acute Contact Toxicity Test', adopted on September 21, 1998
EFSA Study Identification.:	EFSA-2022-00009374
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Executive summary

An acute toxicity test for the effects of test item, FGG01 (Boscalid 500 WG) on honeybee, *Apis mellifera* was performed following OECD Guideline No 213 and 214 (1998).

Acute toxicity test was conducted as limit test with a nominal dose of 249.3 µg a.i./bee along with a control group using pure sucrose solution 50% w/v (oral test) and water with 0.1% Triton x-100 (contact test). For oral test, based on the diet consumed, the actual dose consumed was determined as 200.7 µg a.i./bee.

The acute oral and contact test treated groups and control groups consisted of 5 replicates, each replicate containing 10 bees per cage. The food was provided as syringes and weighed before and after the feeding duration. The bees were kept in the dark with temperature between 23°C to 25°C and relative humidity at 60 - 69 % during the test period of 48 hours with *ad libitum* sucrose solution 50% w/v (after end of dos-



ing). Observations for mortality and abnormal behaviour were made at 4 hours after start of dosing and at 24 hours and 48 hours after end of dosing.

The oral toxicity test showed no mortality in control and in the actual consumed dose of 200.7 µg a.i./bee (target oral dose 249.3 µg a.i./bee) during the 48 hours test period. No abnormal behaviour was observed in control and in test dose during the test period.

The validation test was performed using Dimethoate as positive control. The bees were treated with Dimethoate at the doses of 0.06, 0.08, 0.16 and 0.30 µg a.i./bee based on the actual consumed dose. This positive control test was conducted with 10 bees and 3 replicates. The mortalities observed were 96.7 and 100.0 % at 24 hours and 48 hours after end of dosing, respectively. Abnormal behaviours were observed in the actual consumed dose of 0.08, 0.16 and 0.30 µg a.i./bee.

The contact toxicity test showed no mortality in control and in the test dose of 249.3 µg a.i./bee during the 48 hours test period. No abnormal behaviour was observed in control and in test dose during the test period.

The bees were treated with Dimethoate at the doses of 0.10, 0.15, 0.20 and 0.30 µg a.i./for positive control. This positive control test was conducted with 10 bees and 3 replicates. The mortalities observed were 90.0 % at both 24 hours and 48 hours after end of dosing. Abnormal behaviours were observed in the actual consumed dose of 0.15, 0.20 and 0.30 µg a.i./bee.

The oral and contact LD<sub>50</sub> value of the positive control was determined statistically by probit analysis and the 95% confidence limits were calculated. From the mortality results of validation test and based on the actual consumed dose, the 48 hours oral LD<sub>50</sub> of Dimethoate was 0.11 µg a.i./bee with 95% confidence limits between 0.10 and 0.12 µg a.i./bee. The 48 hours contact LD<sub>50</sub> of Dimethoate was 0.20 µg a.i./bee with 95% confidence limits between 0.18 and 0.22 µg a.i./bee. The result were within the validity criteria as required by OECD No. 213 and 214 guideline.

Based on the results of the limit test, the 48 hours acute oral LD<sub>50</sub> of test item is considered higher than 200.7 µg a.i./bee based on the actual consumed dose and the 48 hours acute contact LD<sub>50</sub> of test item is considered higher than 249.3 µg a.i./bee.

## I. MATERIALS AND METHODS

### A. MATERIALS

- |                                |                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>1. Test material</b>        | Boscalid 500 WG (FGG01)                                                                                                                                                                                                                                                                                                                                                |
| <b>Batch no.:</b>              | 48A                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Purity:</b>                 | 513.55 ± 1.68 g/L (51.36 ± 0.17 % w/w)                                                                                                                                                                                                                                                                                                                                 |
| <b>Date of expiry:</b>         | September 14, 2023                                                                                                                                                                                                                                                                                                                                                     |
| <b>2. Test concentrations:</b> | <u>Control:</u> 50% w/v aqueous sucrose solution (oral);<br>tap water with 0.1 % Triton X-100 (contact)<br><u>Test item:</u> 250 µg a.i./bee (249.3 µg a.i./bee according to GLP certificate of analysis) as limit test<br><u>Reference item:</u><br>Oral test 0.05, 0.08, 0.15 and 0.30 µg dimethoate/bee<br>Contact test 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee |
| <b>3. Toxic reference</b>      | Dimethoate 400 g/L (DANADIM PROGRESS)                                                                                                                                                                                                                                                                                                                                  |
| <b>Batch no.:</b>              | 10214034                                                                                                                                                                                                                                                                                                                                                               |
| <b>Purity:</b>                 | 417 g/L (41.7% w/v)                                                                                                                                                                                                                                                                                                                                                    |

#### 4. Test organism:

<b>Species:</b>	Worker honeybees <i>Apis mellifera</i> L.
<b>Source:</b>	Honeybee colonies, disease-free and queen-right, bred by ibacon.
<b>Age:</b>	Adult female worker bees
<b>Housing:</b>	Stainless steel chambers with size ca. 8.2 cm × 5.9 cm × 4.2 cm with removable glass sheet in the front lined with filter paper in the inner walls. 10 bees/chamber
<b>Feeding:</b>	50% sucrose solution <i>ad libitum</i> . The feeding solution was provided in syringes that were inserted into the cages via an opening in the top of the test units and from which bees accessed the food directly.

#### 5. Environmental conditions

<b>Temperature:</b>	23°C – 25°C
<b>Relative humidity:</b>	60 – 69 %
<b>Light:</b>	24 hours dark, except during observations

### B. STUDY DESIGN AND METHODS

#### 1. Dates of work: 18-20 May 2022

#### 2. Test organism assignment and treatment

All bees used in the test derived from healthy, disease free, queen-right bee colonies and bred by ibacon. Bees were collected with plastic tubes from the outer honeycombs without the use of smoke and without anaesthetics. The bees were collected in the day before. 10 bees were randomly allocated to each labelled stainless-steel chambers, lined with filter paper in inner walls and removable glass sheet at the front. The chambers were placed under dark conditions in environmental test chambers.

##### Oral test application:

50 % w/v sucrose solution (provided as “household sugar”) *ad libitum*; was given directly after treatment. This was done with syringes that were inserted into the cages via an opening in the top of the test units and from which bees accessed the food directly. No replacement of the food was necessary during the test period (48 h).

The test item and reference item were applied in 50% w/v sucrose solution, which was used as carrier (food) in the oral test. Pure 50 % w/v sucrose solution was offered to the bees in control. The test item treated food was offered in syringes, which were weighed before and after introduction into the cages, duration of uptake was 6 hours for the test item treatment. The mean target dose levels (e.g. 249.3 µg a.i./bee nominal) would have been obtained if exactly 20 mg/bee of the treated food were ingested. In practice, uptake of the treated sugar solutions differed from the nominal 20 mg/bee and results are given based on the measured consumption.

##### Contact test application:

A single 5 µL droplet of FGG01 in an appropriate carrier (tap water containing 0.1 % Triton X-100\*) was placed on the dorsal bee thorax using a calibrated pipette. For the control one 5 µL droplet of tap water containing 0.1 % Triton X-100 was used. The reference item was also applied in 5 µL tap water (dimethoate made up in tap water containing 0.1 % Triton X-100\*). A 5 µL droplet was chosen in deviation to the guideline recommendation of a 1 µL droplet, since a higher volume ensured a more reliable dispersion of the test item; ibacon experience has proven that higher volumes are suitable and no adverse effects on the outcome of the study are to be expected.

#### 3. Dose preparation

The limit test was conducted with dose of 249.3 µg a.i./bee based on GLP certificate of analysis.

For oral test, the test solution was applied in sucrose solution 50% w/v. A positive control test was conducted at 0.05, 0.08, 0.15 and 0.30 µg dimethoate/bee. The positive control solutions containing dimethoate were prepared in sucrose solution 50% w/v.

For contact test, the test solution was applied with tap water containing 0.1% Triton X-100. A positive control test was conducted at 0.10, 0.15, 0.20 and 0.30 µg dimethoate/bee. The positive control solutions containing dimethoate were prepared in tap water containing 0.1% Triton X-100.

#### 4. Measurements and observations

Mortality was measured with the number of dead bees after 4 (± 0.5) hours (first day); 24 and 48 (± 2) hours.

Behavioral abnormalities were assessed after 4 (± 0.5) hours (first day); 24 and 48 (± 2) hours. Sub-lethal effects such as symptoms of poisoning or any abnormal behaviour in comparison to the control.

#### 5. Statistics

The oral and contact NOED values of the test item were determined using Fisher's Exact Binomial Test (pairwise comparison, one-sided greater,  $\alpha = 0.05$ ).

The oral and contact LD<sub>50</sub> values of the reference item were determined according to Probit Analysis (according to Finney 1971).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.2.1, ® ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. FOOD CONSUMPTION AND MORTALITY

The mean feed consumption and mortality after exposure of bees to a single concentration of Boscalid 500 WG and the positive control with dimethoate are presented in the table below.

No mortality occurred.

**Table A 2.2.1.1.1-1: Food consumption and mortality of honeybees treated with control and test item FGG01 (Boscalid 500 WG) in oral toxicity test**

Treatment code	Target dose	Actual consumed dose	Mortality mean (%)			Behavioural abnormalities mean (%)		
	µg a.i./bee	µg a.i./bee	4 h	24 h	48 h	4 h	24 h	48 h
Control	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0
Test item	249.3	≥ 200.7	0.0	0.0	0.0	0.0	0.0	0.0
Reference item	0.30	0.30	3.3	96.7	100.0	13.3	0.0	0.0
	0.15	0.16	0.0	83.3	93.3	0.0	6.7	0.0
	0.08	0.08	0.0	10.0	13.3	0.0	0.0	0.0
	0.05	0.06	0.0	0.0	0.0	0.0	0.0	0.0

In the positive control performed with dimethoate, mortalities between 96.7% and 100.0 % were observed at 24 hours and 48 hours after end of dosing, respectively. There was no mortality and behavioural abnormalities for the test item at test end.

**Table A 2.2.1.1.1-2: Mortality and behavioural abnormalities of honeybees treated with control and test item FGG01 (Boscalid 500 WG) in contact toxicity test**

Treatment code	Exposed dose	Mortality mean (%)			Behavioural abnormalities mean (%)		
	µg a.i./bee	4 h	24 h	48 h	4 h	24 h	48 h
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Test item	249.3	0.0	0.0	0.0	0.0	0.0	0.0
Reference item	0.30	0.0	90.0	90.0	0.0	0.0	0.0
	0.20	0.0	43.3	56.7	3.3	3.3	0.0
	0.15	0.0	6.7	20.0	0.0	6.7	0.0
	0.10	0.0	0.0	0.0	0.0	0.0	0.0

In the positive control performed with dimethoate, mortalities of 90% was observed at both 24 hours and 48 hours after end of dosing. There was no mortality and behavioural abnormalities for the test item at test end.

## B. VALIDITY CRITERIA

The test was considered valid because the following criteria were met:

**Table A 2.2.1.1.1-3: Study validity criteria**

Parameter	Required	Observed oral test	Observed contact test
Control mortality	≤10%	0%	0%
LD <sub>50</sub> -value of reference	0.10 to 0.35 µg a.i./bee (oral) 0.10 to 0.30 µg a.i./bee (contact)	0.13 µg a.i./bee	0.21 µg a.i./bee

## C. TOXICITY ENDPOINTS

The oral LD<sub>50</sub> value for Boscalid 500 WG was determine to be > 200.7 µg a.i./bee. The one tested concentration 249.3 µg a.i./bee (actual dose consumption was 200.7 µg a.i./bee) showed no mortality and no observed abnormal behaviour. Abnormal behaviours were observed in the actual consumed dose of 0.08, 0.16 and 0.30 µg a.i./bee of the reference item. LD<sub>50</sub> values were 0.13 and 0.11 µg dimethoate/bee for 24-hour and 48-hour, respectively.

The contact LD<sub>50</sub> value for Boscalid 500 WG was determine to be > 249.3 µg a.i./bee. The one tested concentration 249.3 µg a.i./bee showed no mortality and no observed abnormal behaviour. Abnormal behaviours were observed in the dose of 0.15 and 0.20 µg a.i./bee of the reference item. LD<sub>50</sub> values were 0.21 and 0.20 µg dimethoate/bee for 24-hour and 48-hour, respectively.

**Table A 2.2.1.1.1-4: LD<sub>50</sub> values for positive control (dimethoate)**

Parameter	Oral test result (µg a.i./bee)	Contact test result (µg a.i./bee)
24 hours LD <sub>50</sub> of positive control	0.13	0.21
95% confidence (lower limit)	0.08	0.20
95% confidence (upper limit)	0.19	0.23
48 hours LD <sub>50</sub> of positive control	0.11	0.20
95% confidence (lower limit)	0.10	0.18
95% confidence (upper limit)	0.12	0.22

## III. CONCLUSION

The acute toxicity of FGG01 on adult honeybees (*Apis mellifera* L.) was investigated an acute contact and an acute oral, limit study under laboratory conditions.

The contact LD<sub>50</sub>/20/10 values (24 and 48 h) of FGG01 were determined to be > 249.3 µg a.i./bee.

The oral LD<sub>50</sub>/20/10 values (24 and 48 h) were determined to be > 200.7 µg a.i./bee.

The contact and oral NOED values (24 and 48 h) were ≥ 249.3 and ≥ 200.7 µg a.i./bee, respectively.

<b>zRMS comments:</b>	<p>The study was conducted according to: OECD guidelines 246 (oral test) and 247 (contact test).</p> <p>The validity criteria from OECD 266 and OECD 247 were met.</p> <p>In case of contact test a 5 µL droplet was chosen in deviation to the guideline recommendation of 2 µL, however this deviation do not affect the ability to use the results for a risk assessment.</p> <p>The both tests were conducted with a nominal dose of 497 µg a.s./bumblebee as limit test Based on the diet consumed, the mean dose consumed was determined to be 544.4 µg a.s./bumblebee.</p> <p>The LD<sub>50</sub> value for Boscalid could not be calculated by statistical evaluation as the study was conducted as limit test.</p> <p>The study is acceptable for risk assessment.</p> <p>The contact LD<sub>50</sub>, LD<sub>20</sub> and LD<sub>10</sub> (48 h) values were estimated to be &gt; 497 µg a.s./bumblebee.</p> <p>The contact NOED (48 h) value was calculated to be ≥ 497 µg a.s./bumblebee.</p> <p>The oral LD<sub>50</sub>, LD<sub>20</sub> and LD<sub>10</sub> (48 h) values were estimated to be &gt; 544.4 µg a.s./bumblebee.</p> <p>The oral NOED (48 h) value was calculated to be equivalent to ≥ 544.4 µg a.s./bumblebee.</p>
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Reference:	KCP 10.3.1.1.1/02
Report	Boscalid 500 WG: Acute Contact and Oral Toxicity to Bumblebees ( <i>Bombus terrestris</i> L.) in the Laboratory, Knautz, T. & Kowalczyk, F., 2022a, Study code 165091105
Guideline(s):	OECD 246: 'Bumblebees, Acute Contact Toxicity Test', adopted on 9 October 2017 OECD 247: 'Bumblebees, Acute Oral Toxicity Test', adopted on 9 October 201
EFSA Study Identification:	EFSA-2022-00012189
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Executive summary

An acute oral and contact toxicity test for the effects of test item, Boscalid 500 WG (FGG01) on bumblebee, *Bombus terrestris* L. was performed following OECD Guideline No 246 and 247 adopted on October 2022 with test period of 48 hours.

Oral and contact limit test were conducted with a nominal dose of 497 µg a.i./bumblebee as limit test along with a control group using sucrose solution 50 % w/v (water control). The treated group and control group consisted of 50 replicates and 55 replicates for contact and oral toxicity test, respectively. Each replicate containing 1 bees. For the oral test, the treatment replicates were offered a volume of 40 µL test solution and control replicates were offered a volume of 40 µL sucrose solution 50% w/v for a diet consumption period of 2 - 4 hours. Based on the diet consumed, the mean actual dose consumed was determined to be 544.4 µg a.i./bumblebee.

The recovery rates of Boscalid in the test item concentration of the contact and oral test were within ± 20 % of the nominal concentrations.

The bees were kept in the dark with temperature between 24°C to 26°C and relative humidity at 56 – 61 % during the test period of 48 hours with *ad libitum* sucrose solution 50% w/v. Observations for mortality and abnormal behaviour were made at 4 hours, at 24 hours and 48 hours.

As there was no mortality in the test item treatment group in the contact test, the contact LD<sub>50</sub>, LD<sub>20</sub> and LD<sub>10</sub> (48 h) values were estimated to be > 497 µg a.i./bumblebee.

The contact NOED (48 h) value was calculated to be ≥ 497 µg a.i./bumblebee.

As there was no mortality in the test item treatment group in the oral test, the oral LD<sub>50</sub>, LD<sub>20</sub> and LD<sub>10</sub> (48 h) values were estimated to be > 544.4 µg a.i./bumblebee.

The oral NOED (48 h) value was calculated to be equivalent to ≥ 544.4 µg a.i./bumblebee.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material**  
**Batch no.:** 48A  
**Purity:** 513.55 ± 1.68 g/kg (51.36 ± 0.17 % w/w)  
**Date of expiry:** September 14, 2023
2. **Test concentrations:** Control: 50% w/v aqueous sucrose solution (oral);  
tap water with 0.1 % Triton X-100 (contact)  
Test item: 500 µg a.i./bumblebee (497 µg a.i./bumblebee according to GLP certificate of analysis) as limit test  
Mean consumed dose level of test item: 544.4 µg a.i./bumblebee for oral test  
Reference item: Oral test 4.0 µg dimethoate/bumblebee; Contact test 10 µg dimethoate/bumblebee
3. **Toxic reference**  
**Batch no.:** 10214034  
**Purity:** 394 g/kg (39.4 % w/w)
4. **Test organism:**  
**Species:** Bumblebees *Bombus terrestris* L.  
**Source:** Bumblebee colonies, healthy and queen-right, sourced from a commercial bumblebee breeding company, Koppert Deutschland GmbH, Zeppellinstr. 32, 47638 Straelen, Germany.  
**Age:** Adult female worker bumblebees  
**Housing:** Cylindrical, latticed plastic cages (Nicot queen cages) with size approx. 7.3 cm length x 2.2 cm diameter and 1.7 cm at the small opening. The test units were placed on a plate, the small opening closed by a rubber plug holding a syringe containing the feeding solution. 1 bumblebee/cage, contact test 50 for test item and control, oral test 55 for test item and control  
**Feeding:** 50% w/v sucrose solution *ad libitum*; was given directly after treatment using syringes
5. **Environmental conditions**  
**Temperature:** 24°C – 26°C  
**Relative humidity:** 55 - 61 %  
**Light:** 24 hours dark, except during dosing and observations

## B. STUDY DESIGN AND METHODS

### 1. Dates of work: 20 July – 03 August 2022 (including analytical and biological phase)

### 2. Test organism assignment and treatment

All bumblebees used in the test derived from healthy and queen-right bee colonies. The bumblebee colonies were sourced from Koppert Deutschland GmbH, Zeppelinstr. 32, 47638 Straelen, Germany. Bee colony was arranged in a plastic box, which was packed in cardboard. The bees were collected with plastic cages within one week after delivery. The bumblebees were collected from 11 colonies and were randomly allocated to the different treatment groups to avoid any colony effect within a treatment group. 1 bee per cage. Bees were handled gently by using forceps. The test units were placed on a plate, the small opening closed by a rubber plug holding a syringe containing the feeding solution and placed under dark conditions in environmental test chambers during 48 hours exposure period.

The treated/untreated food was provided *ad libitum*. Approximately 40 µL of food solution per bumblebee was provided in syringes. Syringes were weighed before and after introduction into the cages to feed the bumblebees during test period. The uptake period was 2 – 4 hours, after 4 hours the syringes containing the remaining food were removed, weighed and replaced by syringes containing fresh, untreated food. The calculation of the target dose was based on 40 mg food uptake. The ingested consumed oral doses were calculated based on the measured consumption.

The bees were fed with 50% w/v aqueous sucrose solution containing either:

- the test item (test item treatment group),
- reference item (reference item group),
- pure 50% (w/v) sucrose solution (control group)

### 3. Dose preparation

The limit test was conducted with dose of 500 µg a.i./bumblebee (equivalent to 497 µg a.i./bumblebee according to GLP certificate of analysis). The test solution was prepared in sucrose solution 50% w/v before application for oral test and tap water containing 0.1 % v/v Triton X-100 for contact test.

The nominal dose of reference item was 10 µg dimethoate/bumblebee for contact test and 4 µg dimethoate/bumblebee for oral test. The positive control solutions containing dimethoate were prepared in sucrose solution 50% v/v and tap water with 0.1 % v/v Triton X-100 for oral and contact test, respectively.

### 4. Measurements and observations

Mortality and abnormal behaviour were recorded at different timepoints, at 4-hour ( $\pm 0.5$ ) and thereafter at 24-hour ( $\pm 2$ ) and at 48-hour ( $\pm 2$ ). Sublethal effects such as symptoms of poisoning, signs of reduced coordination, cannot walk, feeble movements of legs and antennae and weak response to stimulation were recorded as abnormal behaviours.

The amount of diet consumption was assessed from weight difference of the syringes with feeding solution before and after loading treated diet and after the 4-h exposure period.

### 5. Statistics

As the test item treatment groups in the contact and oral test showed no mortality, no statistical evaluation of the LD<sub>50</sub>, LD<sub>20</sub> and LD<sub>10</sub> values was carried out. The contact and oral LD<sub>50</sub>, LD<sub>20</sub> and LD<sub>10</sub> values are considered to be higher than the tested dose rate. The contact and oral NOED values of the test item were determined using Fisher's Exact Binomial Test (one-sided greater,  $\alpha = 0.05$ ).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. FOOD CONSUMPTION AND MORTALITY

The mean feed consumption and mortality after exposure of bumblebees to a single concentration of Boscalid and the positive control with dimethoate are presented in the table below.

No mortality occurred in the test item oral and contact toxicity test results.

**Table A 2.2.1.1.1.22-1: Food consumption, mortality and behavioural abnormalities of bumblebees treated with control, reference item and test item FGG01 (Boscalid 500 WG) in oral toxicity test**

Treatment code	Nominal dose	Actual mean consumed dose	Mortality mean (%)			Behavioural abnormalities mean (%)		
	µg a.i./ bee	µg a.i./bee	4 h	24 h	48 h	4 h	24 h	48 h
Control	0.0	-	0.0	0.0	0.0	0.0	0.0	0.0
Test item	500.0	544.4	0.0	0.0	0.0	0.0	0.0	0.0
Reference item	-	4.6	27.3	100.0	100.0	95.8	-	-

No mortality showed in the test item. In the positive control performed with dimethoate, mortalities of 100 % were observed at 24 hours after end of dosing. Abnormal behaviour was observed in all test concentrations for the positive control at 4-h post-dosing.

**Table A 2.2.1.1.1.22-2: Mortality and behavioural abnormalities of bumblebees treated with control, reference item and test item FGG01 (Boscalid 500 WG) in contact toxicity test**

Treatment code	Exposed dose	Mortality mean (%)			Behavioural abnormalities mean (%)		
	µg a.i./bee	4 h	24 h	48 h	4 h	24 h	48 h
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Test item	497	0.0	0.0	0.0	0.0	0.0	0.0
Reference item	10	0.0	53.3	66.7	0.0	0.0	0.0

No mortality showed in the test item. In the positive control performed with dimethoate, mortalities between 53.3 and 66.7 % were observed at 24 hours and 48 hours after end of dosing. No abnormal behaviour was observed in all test concentrations for the positive control and test item.

### B. VALIDITY CRITERIA

The test was considered valid because the following criteria were met:

**Table A 2.2.1.1.1.22-3: Study validity criteria**

Parameter	Required	Observed
Control mortality (48 h)	≤10%	0% (48 h)
Mortality of reference (oral) Mortality of reference (contact)	≥50% at test end	100.0% 66.7%

### C. TOXICITY ENDPOINTS

The LD<sub>50</sub> value for Boscalid could not be calculated by statistical evaluation as the study was conducted as limit test.

The one tested concentration of contact toxicity test of 497 µg a.i./bumblebee showed no mortality and no observed abnormal behaviour at test end.

The actual mean consumed oral dose of the test item was 544.4 µg a.i./bumblebee for oral toxicity test and showed no mortality and behavioural abnormalities in the test item at test end.

The oral LD<sub>10</sub>, LD<sub>20</sub> and LD<sub>50</sub> were estimated to be > 544.4 µg a.i./bumblebee and NOED was calculated to be ≥ 544.4 µg a.i./bumblebee.



The contact LD<sub>10</sub>, LD<sub>20</sub> and LD<sub>50</sub> were estimated to be > 497 µg a.i./bumblebee and NOED was calculated to be ≥ 497 µg a.i./bumblebee.

### III. CONCLUSION

The toxicity of Boscalid 500 WG was tested in an acute contact and oral toxicity test using bumblebees with test period of 48 hours.

The recovery rates of Boscalid in the test item concentration of the contact and oral test were within ± 20 % of the nominal concentrations.

As there was no mortality in the test item treatment group in the contact test, the contact LD<sub>50</sub>, LD<sub>20</sub> and LD<sub>10</sub> (48 h) values were estimated to be > 497 µg a.i./bumblebee.

The contact NOED (48 h) value was calculated to be ≥ 497 µg a.i./bumblebee.

As there was no mortality in the test item treatment group in the oral test, the oral LD<sub>50</sub>, LD<sub>20</sub> and LD<sub>10</sub> (48 h) values were estimated to be > 544.4 µg a.i./bumblebee.

The oral NOED (48 h) value was calculated to be equivalent to ≥ 544.4 µg a.i./bumblebee.

#### A 2.2.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bees

For acute contact toxicity studies in honeybees and bumble bees, please refer to Points A 2.3.1.1.1.1 (Knautz, 2022; KCP 10.3.1.1.1/02) and A 2.3.1.1.1.2 (Knautz & Kowalczyk, 2022a; KCP 10.3.1.1.1/02), respectively.

#### A 2.2.1.2 KCP 10.3.1.2 Chronic toxicity to bees

##### A 2.2.1.2.1 Knautz & Kowalczyk, 2022b (KCP 10.3.1.2/01)

<b>zRMS comments:</b>	<p>The study was conducted according to : OECD guideline 245.</p> <p>The validity criteria from OECD 245 were met and no deviation from guideline was reported,</p> <p>The study was considered valid.</p> <p>The study is acceptable for risk assessment.</p> <p>The following endpoints were derived:</p> <p>LC<sub>50</sub>: 3387 mg a.s./kg  LDD<sub>50</sub>: 50.8 µg a.s./bee/day  NOEC: 2147 mg a.s./kg  NOEDD: 41.9 µg a.s./bee/day  LC<sub>20</sub>: 2265 mg a.s./kg  LDD<sub>20</sub>: 42.9 µg a.s./bee/day  LC<sub>10</sub>: 1835 mg a.s./kg  LDD<sub>10</sub>: 38.2 µg a.s./bee/day</p> <p>Only LDD<sub>10</sub> has been used for risk assessment.</p>
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Reference: KCP 10.3.1.2/01

Report Boscalid 500 WG: Chronic Oral Toxicity Test on the Honey Bee (*Apis mellifera* L.) in the Laboratory. Knautz, T. & Kowalczyk, F., 2022, Report no. 165091136

Guideline(s):	OECD (2017), Test No. 245: Honey Bee ( <i>Apis Mellifera</i> L.), Chronic Oral Toxicity Test (10-Day Feeding)
Deviations:	None
GLP:	Yes
Acceptability:	Yes

### Executive summary

Under laboratory conditions, 30 freshly hatched female worker bees (*Apis mellifera* L.) per treatment level were exposed for 10 days to 5 concentrations. The test item was administered daily to the bees in sugar solution at the following concentrations: 19886, 9469, 4509, 2147 and 1023 mg a.i./kg feeding solution. These concentrations resulted in a daily mean dose of 95.9, 80.2, 55.1, 41.9 and 25.1 µg a.i./bee/day after 10 days. An untreated control and a reference item were included in this study.

Mortality occurred in all test item treated dose levels ranging from 6.7 to 100.0 % at test end (10 days following the start of chronic exposure). There was 3.3 % mortality in the water control (50 % w/v sucrose solution) and xanthan control (50 % w/v sucrose solution containing 0.1% w/v xanthan).

Test item related behavioural abnormalities were observed on day 10 in 2 single affected bees in the test item treatment 4509 mg a.i./kg feeding solution.

The reference item (dimethoate) at a concentration of 1 mg dimethoate/kg feeding solution corresponding to an actual consumed dose of 0.013 µg a.i./bee/day caused 100 % mortality on day 7.

The analytical verification confirmed the correct preparation of all the dosing solutions of the test item.

## I. MATERIALS AND METHODS

### A. MATERIALS

<b>1. Test Item</b>	Boscalid 500 WG (FGG01)
<b>Batch No.:</b>	48A
<b>Actual content of active ingredients:</b>	Boscalid: $51.36 \pm 0.17$ % w/w, $513.55 \pm 1.68$ g/kg according to the certificate of analysis
<b>Description:</b>	Solid, light brown
<b>Expiry date:</b>	September 14, 2023
<b>2. Reference Item</b>	
<b>Toxic reference:</b>	Dimethoate 400 g/L (Danadim Progress)
<b>Batch no.:</b>	10214034
<b>Purity:</b>	394 g/kg (39.4 % w/w)
<b>3. Test organisms</b>	
<b>Species:</b>	<i>Apis mellifera</i> L.
<b>Age:</b>	Young adult female worker bees (2 days old)
<b>Source:</b>	Honeybee colonies, disease-free and queen-right, bred by ibacon
<b>Food:</b>	50% w/v aqueous sugar solution
<b>Collection:</b>	Brood combs with sealed brood from four different hives were used in the test in which bees visibly started to emerge. The combs contained pollen which was used as a first feeding source for the freshly

hatched bees.

## B. STUDY DESIGN AND METHODS

### 1. Experimental dates:

Biological  
Phase: July 19 - 29, 2022  
  
Analytical  
Phase: August 24, 2022

### 2. Test design

**Test cage description:** Stainless steel chambers 8.2 cm x 5.9 cm x 4.2 cm (length x height x width)  
**Replication:** 3 per test item dose level, controls and reference item dose  
**No. of individuals:** 10 per test unit  
**Duration of test:** 10 days

### 3. Environmental test conditions

**Temperature:** 32 - 34 °C  
**Humidity:** 50 - 62 %; average relative humidity was 61 %  
**Photoperiod:** Darkness (except during observation)

### 4. Treatments

**Test rates:** Nominal: 20000, 9524, 4535, 2160 and 1028 mg a.i./kg feeding solution  
Based on the new certificate of analysis this corresponded to: 19886, 9469, 4509, 2147 and 1023 mg a.i./kg feeding solution)  
Actual Dose Level: 95.9, 80.2, 55.1, 41.9 and 25.1 µg a.i./bee/day (based on the actual intake of treated food)  
**Controls:** 50% w/v aqueous sugar solution  
**Toxic standard:** 1 mg dimethoate/kg feeding solution (1 ppm) according to  
Actual Dose Level: 0.013 µg a.i./bee/day  
**Application method:** Oral ingestion

### 5. Procedures

This study encompassed 7 treatment groups (5 dose rates of the test item, control and 1 dose rate of the reference item) with 3 replicates each containing 10 bees.

The final concentration of sugar in the test item feeding solutions offered to the bees was in 50% (w/v) sucrose solution. The feeding solutions of the test item were prepared freshly every day.

The reference item was prepared with 50% (w/v) sucrose solution. The stock solution of the reference item was prepared once at start of the test and stored at 4°C ± 4°C over a period until day 7. 50% (w/v) sucrose solution was used for the untreated control. The feeding solutions of the reference item were prepared on days -1, 0, 3 and 7 and stored at 4°C ± 4°C in the dark. The feeding solutions of the untreated control were prepared freshly on days -1, 3 and 7 and stored at 4°C ± 4°C in the dark.

The treated and untreated feeding solutions were offered *ad libitum* to each cage in syringes. The syringes were weighed daily before introduction into the cages and after the feeding interval (before daily replacement with new syringes containing fresh test solutions).

The active ingredient Boscalid was determined in one sample of freshly prepared feeding solutions of the highest and lowest test item concentrations of day 6.

Mortalities in the water control and the xanthan control group were compared with a Fisher's Exact Binomial Test. Results obtained from the bees treated with the test item were compared to those obtained from the pooled control group.

The LC<sub>50/20/10</sub> values of the test item were determined with Probit Analysis (according to Finney 1971) and LDD<sub>50/20/10</sub> values of the test item were determined with Weibull Analysis.

The NOEDD / NOEC of the test item was determined using Rao-Scott-Step-down Cochran-Armitage Test Procedure (one-sided greater,  $\alpha = 0.05$ ). A qualitative trend analysis and Tarone's Test Procedure to test for extra-binomial variance have been performed in advance.

The LC<sub>50/20/10</sub> and LDD<sub>50/20/10</sub> calculations were carried out taking into account the mortality data corrected by control mortality using Abbott's formula (1925).

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. MORTALITY

Mortality data for the test material are summarised in the table below.

**Table A 2.2.1.2.1-1: 10 days Chronic Oral Toxicity of Boscalid 500 WG to young adult honeybees – Mortality and Abnormal Behaviors**

Treatment Group	Concentration [mg a.i./kg feeding solution]	Mean dose * [µg a.i./bee/day]	Mortality at day 10 [% Mean]	Behavior abnormalities at day 10 [% Mean]
Boscalid 500 WG	19886	95.9	100.0	0.0
	9469	80.2	100.0	0.0
	4509	55.1	73.3	6.7
	2147	41.9	13.3	0.0
	1023	25.1	6.7	0.0
Untreated control	0.0	0.0	3.3	0.0
Solvent control	0.0	0.0	3.3	0.0
Reference Item	1.0	0.013	100.0	0.0

Results are average from three replicates (ten bees each) per concentration or control

\* At the test end, 10 days following application

### B. ANALYTICAL RESULTS

The analytical recovery rates of the active ingredient Boscalid in the feeding solutions were as follows:

Feeding Solution 1023 DAA6 100 %

Feeding Solution 19886 DAA6 108 %

The analytical verification within 100 – 108 % of recovery rate of nominal values confirmed the correct preparation of the dosing solutions of the test item.

### C. VALIDITY CRITERIA

The test was considered valid since:

- after 10 days of continuous exposure, mortality in the untreated controls and solvent control were 3.3 %, thus below the threshold of 15 %.

- mortality in the reference treatment group was 100 % and thus above the threshold of 50 %.
- The recovery rates of Boscalid in the stock solution, the highest and lowest test item concentration on Day 6 were within  $\pm 20$  % of the nominal concentrations.

#### D. TOXICITY ENDPOINTS

**Table A 2.2.1.2.1-2: Summary of endpoints**

Endpoint at test termination (day 10)		
<b>LC<sub>50</sub></b>	3387 mg a.i./kg feeding solution	95 % C.I.: 2868 - 4000
<b>LDD<sub>50</sub></b>	50.8 µg a.i./bee/day	95 % C.I.: 47.3 – 54.2
<b>LC<sub>20</sub></b>	2265 mg a.i./kg feeding solution	95 % C.I.: 1758 – 2693
<b>LDD<sub>20</sub></b>	42.9 µg a.i./bee/day	95 % C.I.: 35.8 – 46.3
<b>LC<sub>10</sub></b>	1835 mg a.i./kg feeding solution	95 % C.I.: 1327 – 2244
<b>LDD<sub>10</sub></b>	38.2 µg a.i./bee/day	95 % C.I.: 29.1 – 42.5
<b>NOEC</b>	2147 mg a.i./kg feeding solution	-
<b>NOEDD</b>	41.9 µg a.i./bee/day	-

C.I. = Confidence limit

### III. CONCLUSION

The chronic oral toxicity of Boscalid 500 WG on young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

All validity criteria for the study were met. After 10 days of continuous exposure, mortality in the controls was 3.3% and thus below the threshold of 15%. Mortality in the reference treatment group was 100% and thus above the threshold of 50%.

The recovery rates of Boscalid in the highest and lowest test item concentration of Day 6 were within  $\pm 20$ % of the nominal concentrations.

The LDD<sub>50</sub>, LDD<sub>20</sub> and LDD<sub>10</sub> were determined to be 50.8, 42.9 and 38.2 µg a.i./bee/day, respectively. The LC<sub>50</sub>, LC<sub>20</sub> and LC<sub>10</sub> were determined to be 3387, 2265 and 1835 mg a.i./kg feeding solution, respectively.

The NOEDD was determined to be 41.9 µg a.i./bee/day and the NOEC was 2147 mg a.i./kg feeding solution.

#### A 2.2.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

##### A 2.2.1.3.1 Colli, 2022 (KCP 10.3.1.3/01)

<b>zRMS comments:</b>	<p>Test item: Boscalid 500 WG (FGG01).</p> <p>The study was conducted according to OECD Test Guideline 239 with minor deviation described and assessed by Study Director:</p> <ul style="list-style-type: none"> <li>– the mortality assessment on D7 was not carried out.</li> </ul> <p>This aforementioned deviation does not affect the outcome of the study and does not affect the ability to use the results for a risk assessment.</p> <p>The validity criteria from OECD 239 were met.</p> <p>The study is valid.</p> <p>The study is acceptable for risk assessment.</p>
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	<p>The following results (for adult emergence on D22) were derived:</p> <p>ED<sub>10</sub>: 82.83 [µg test item/larva]  EC<sub>10</sub>: 537.88 mg test item/kg diet  ED<sub>20</sub>: 110.45 [µg test item/larva]  EC<sub>20</sub>: 717.21 mg test item/kg diet  ED<sub>50</sub>: 170.57 µg test item/larva  EC<sub>50</sub>: 1107.60 mg test item/kg diet  NOED: 61.98 µg test item/larva; 31.84µg a.s/larva  NOEC: 402.49 mg test item/kg diet; 206.72 mg a.s/kg diet</p>
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Reference:	KCP 10.3.1.3/01
Report	Effects of BOSCALID 500 WG (FGG01) on honeybees ( <i>Apis mellifera</i> L.) 22-day larval toxicity test with repeated exposure, Colli, M., 2022, Study code BT115/22
Guideline(s):	OECD 239: 'Honey bee ( <i>Apis mellifera</i> L.) larval toxicity test, repeated exposure', adopted on 7 July 2021
Deviations:	<p>The mortality assessment on D7 was not carried out for the following reason: On D7 there are no treatments or feeding of the larvae and performing a mortality assessment would have involved opening the dryer with a consequent decrease of temperature and humidity.</p> <p>Therefore, to avoid stress to the larvae (in a very delicate stage such as the transition to pre-pupa) the assessment was performed directly on D8.</p>
GLP:	Yes
Acceptability:	Yes

### Executive summary

The 22-day larval toxicity test with repeated exposure in the laboratory was performed as a dose-response test based on OECD 239. The test item was dissolved in ultra-pure water and then in the larval food (aqueous sugar solution mixed with royal jelly) and administered daily to the larvae from day 3 (D3) to 6 (D6) of the test.

The stock solutions and the treated diet were prepared freshly each day of administration. The reference item Dimethoate was tested at a cumulative dose of 7.39 µg/larva. An untreated control was run in parallel with the royal jelly-based diet.

For each test item treatment group, for the control group and the reference item treatment group, 3 replicates of 12 larvae each were set up.

Larval mortality on Day-8 (<15% from D3 to D8), control adults emergence on Day 22 (≥ 70% across replicates) and toxicity on reference item (mortality > 50% on D8) were met.

Regarding the effects on larvae on D8, the test item BOSCALID 500 WG (FGG01) did not cause statistically significant mortality up to the T4 tested dose.

Therefore, the NOED for larvae on D8 was determined to be 136.36 µg test item/larva (70.04 µg Boscalid/larva) equivalent to a NOEC of 885.48 mg test item/kg diet (454.78 mg Boscalid/kg diet).

Regarding the effects on adult emergence on D22, the test item BOSCALID 500 WG (FGG01) caused statistically significant reduction in emergence rate respect to the control at the two highest doses.

The NOED and the NOEC for adult emergence were evaluated to be 61.98 µg test item/larva (31.84 µg Boscalid/larva) and 402.49 mg test item/kg diet (206.72 mg Boscalid/kg diet), respectively.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** Boscalid 500 WG (FGG01)  
**Batch no.:** 48a  
**Purity:** 513.55 g/kg (51.36% w/w)  
**Date of expiry:** 14 September 2023
2. **Test concentrations:** Control: ultra-pure water  
Test item: 12.81, 28.17, 61.98, 136.36 and 300.00 µg test item/larva (corresponding to 6.58, 14.47, 31.84, 70.04 and 154.08 µg a.s./larva)
3. **Toxic reference:** Dimethoate  
**Batch no.:** BCCF3993  
**Purity:** 99.4%
4. **Test organism:**  
**Species:** *Apis mellifera ligustica*  
**Source:** Healthy colony maintained at BioTecnologie BT S.r.l. (colonies no. 3, 12, 13)  
**Age:** 3 days old bee larvae  
**Test units:** Larvae were reared in crystal polystyrene grafting cells with an internal diameter of 9 mm and a depth of 8 mm. The cells were sterilized. Each cell was placed into a well of a 48-well plate. The top of the grafting cell was maintained at the level of the plate by placing below it a piece of dental roll wetted with 500 µL of sterilizing solution enhanced with 15% weight/volume glycerol. Emerging bees were fed with syrup/sucrose solution dispensed ad libitum using 2.5 mL syringes.  
**Feeding:** Diet A (D1): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 2% weight of yeast extract, 12% weight of glucose and 12% weight of fructose.  
Diet B (D3): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 3% weight of yeast extract, 15% weight of glucose and 15% weight of fructose.  
Diet C (from D4 to D6): 50% weight of fresh royal jelly + 50% weight of an aqueous solution containing 4% weight of yeast extract, 18% weight of glucose and 18% weight of fructose
5. **Environmental conditions**  
**Temperature:** 30.4 – 34.7 °C (average measured during the test 34.3 °C)  
**Relative humidity:** 85.6 – 98.9%, average 97.8% (D1 to D8)  
84.1 – 87.5%, average 85.1% (D8 to D15)  
42.9 – 71.5%, average 63.1% (D15 – D22)  
**Light:** 24 h darkness, except during observation

### B. STUDY DESIGN AND METHODS

1. **Experimental phase:** 06 – 25 July 2022 (including the analytical phase)
2. **Experiment design**

The 22-day larval toxicity test with repeated exposure in the laboratory was performed as dose-response test: the test item was dissolved in water and then in the larval food (aqueous sugar solution mixed with royal jelly) and administered daily to the larvae from day 3 (D3) to 6 (D6) of the test.

#### 3. Application

The stock solutions and the treated diet were prepared freshly each day of administration. The reference item Dimethoate was tested at a cumulative dose of 7.39 µg/larva. An untreated control was run in parallel with the royal jelly-based diet.

For each test item treatment group, for the control group and the reference item treatment group, 3 replicates of 12 larvae each were set up.

Five concentrations of test item were test along a untreated control and a reference item used for the test.

#### **4. Analytical Determination**

For analytical determination, the test items were analyzed by HPLC with DAD detector in double replicates. The analytical samples were sampled on D3 from the test solution in the test system at lowest and highest concentration.

#### **5. Measurements and observations**

Assessments of mortality and any developmental/behavioral abnormality were performed daily from D4 to D8 (except on D7) and on D15 and on D22.

Pupal mortality and the emergence rate of adults were also assessed on D22.

#### **6. Statistics**

The Software Tox Rat Pro 3.3.0 was used to perform the statistics.

## **II. RESULTS AND DISCUSSION**

### **A. MORTALITY**

The table below presents the mean mortalities on D8 and D22, the pupal mortality and the effects on adult emergence on D8 and D22.



**Table A 2.2.1.3.1-1: Mortality (M) and corrected motality (CM) of *Apis mellifera* larvae on D8 exposure to of Boscalid 500 WG (FGG01)**

Treatment group	Cumulative dose [µg test item/larva]	Concentration [mg test item/kg diet]	Larvae mortality on DB		
			Mortality mean [%]	Corr. Mortality mean [%]	Significant
Control	0	0	2.78	n.a.	n.a.
Test item (T1)	12.81	83.16	2.78	0	-
Test item (T2)	28.17	182.95	0	0	-
Test item (T3)	61.98	402.49	0	0	-
Test item (T4)	136.36	885.48	2.78	0	-
Test item (T5)	300.00	1948.05	88.89	88.57	+

n.a. = not applicable

+ = significant; - = non-significant (Step-down Cochran-Armitage test –  $\alpha = 0.05$ , one-sided greater)

Negative values are replaced with 0

**Table A 2.2.1.3.1-2: Pupal Mortality**

Treatment group	Cumulative dose [µg test item/larva]	Concentration [mg test item/kg diet]	Pupal mortality from D8 to D15*	Pupal mortality from D8 to D22**
			Mean [%]	Mean [%]
Control	0	0	2.78	5.56
Test item (T1)	12.81	83.16	0	2.78
Test item (T2)	28.17	182.95	8.33	11.11
Test item (T3)	61.98	402.49	0	13.89
Test item (T4)	136.36	885.48	5.56	28.28
Test item (T5)	300.00	1948.05	66.67	66.67

\*calculated as a percentage comparing the number of dead pupae from D8 to D15 with the number of alive pupae on D8

\*\*calculated as a percentage comparing the number of dead pupae from D8 to D22 with the number of alive pupae on D8

**Table A 2.2.1.3.1-3: Total mortality (M) and corrected mortality (CM) from D3 to D22 and emergence (E) on D22**

Treatment group	Cumulative dose [µg test item/larva]	Concentration [mg test item/g diet]	Pupal mortality from D8 to D15*			Pupal mortality from D8 to D22**	
			M – Mean [%]	CM – Mean [%]	Sign.	E - Mean [%]	Sign.
Control	0	0	8.33	n.a.	n.a.	91.67	n.a.
Test item (T1)	12.81	83.16	5.56	0	-	94.44	-
Test item (T2)	28.17	182.95	11.11	3.03	-	88.89	-
Test item (T3)	61.98	402.49	13.89	6.06	-	86.11	-
Test item (T4)	136.36	885.48	30.56	24.24	+	69.44	+
Test item (T5)	300.00	1948.05	97.22	96.97	+	2.78	+

n.a. = not applicable

+ = significant; - = non-significant (Step-down Cochran-Armitage test –  $\alpha = 0.05$ , one-sided greater)

Negative values are replaced with 0

**Table A 2.2.1.3.1-4: Reference Item (Dimethoate) – mean mortality**

Treatment group	Dose [µg a.s./larva]	Concentration [mg a.s./kg diet]	Mortality on D8 Mean [%]
Reference item	7.39	48.00	100.00

## B. ANALYTICAL RESULTS

The content of active substance was analysed in the lowest and highest test item concentrations of the stock solutions (prepared on D3) used to treat the diets and was determined to be within 20% of the nominal values for all test item treatment groups, therefore the endpoints were calculated based on nominal concentrations and doses. The highest concentration sample was named S1 and the lowest concentration sample was S5.

The control (C) was also analysed, and no contamination was detected (<LOD).

**Table A 2.2.1.3.1-5: Analytical Results**

Samples Name	Nominal Conc. [g test item/L]	Boscalid normal concentration [g a.s./L]	Sample Matrix	Sampling Date
S1D3	0.924	0.475	Ultrapure water	D3
S5D3	21.65	11.119	Ultrapure water	D3
CD3	0	0	Ultrapure water	D3

## C. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

**Table A 2.2.1.3.1-6: Study validity criteria**

Parameters	Required	Observed
Cumulative larval mortality from D3 to D8 in control	≤ 15%	2.78%
Adult emergence rate on D22 in control	≥ 70%	91.67%
Larval mortality on D8 in reference item treatment	≥ 50%	100%

## D. TOXICITY ENDPOINTS

Regarding the effects on larvae on D8 (developmental period), the test item Boscalid 500 WG caused statistically significant mortality at the highest tested doses.

Therefore, the NOED for larvae on D8 was determined to be 136.36 µg test item/larva (70.04 µg a.s./larva) equivalent to a NOEC 885.48 mg test item/kg diet (454.78 mg a.s./kg diet).

Regarding the effects on adult emergence on D22, the test item Boscalid 500 WG caused statistically significant reduction in emergence rate respect to the control at the two highest tested dose.

The NOED and the NOEC for adult emergence on D22 was determined to be 61.98 µg test item/larva (31.84 µg a.s./larva) and 402.49 mg test item/kg diet (206.72 mg a.s./kg diet), respectively at the end of the test.

**Table A 2.2.1.3.1-7: Adult emergence on D22 after repeated exposure– summary of endpoints**

Endpoint dose	Adult Emergence on D22 [µg test item/larva]	Adult Emergence on D22 [µg a.s./larva]
ED <sub>10</sub>	82.83 (57.95 – 103.45)	42.54 (29.77 – 53.13)
ED <sub>20</sub>	110.45 (84.60 – 131.69)	56.73 (43.45 – 67.64)
ED <sub>50</sub>	170.57 (145.25 – 195.57)	87.61 (74.60 – 100.45)
NOED	61.98	31.84
Endpoint concentration	Adult Emergence on D22 [mg test item/kg diet]	Adult Emergence on D22 [mg a.s./kg diet]
EC <sub>10</sub>	537.88 (376.33 – 671.72)	276.25 (193.28 – 345.00)
EC <sub>20</sub>	717.21 (549.34 – 855.14)	368.36 (282.14 – 439.20)
EC <sub>50</sub>	1107.60 (943.15 – 1269.96)	568.87 (484.40 – 652.25)
NOEC	402.49	206.72

ED/EC<sub>10/20/50</sub> evaluated by Weibull analysis: effect concentrations given with 95% confidence limits in brackets.

### III. CONCLUSION

The effects of the test item BOSCALID 500 WG (FGG01) on the larval development and subsequent adult emergence of honeybees (*Apis mellifera* L.), were tested in a GLP compliant laboratory study.

The validity criteria of the GD OECD No. 239 (2021) with regards to control larval mortality on D8, control adults' emergence on D22 and toxicity on the reference item were met. Thus, the study is valid.

The content of active substance was analysed in the lowest and highest test item concentrations of the stock solutions (prepared on D3) used to treat the diets and was determined to be within 20% of the nominal values for all test item treatment groups, therefore the endpoints were calculated based on nominal concentrations and doses.

The control was also analysed, and no contamination was detected (<LOD).

Regarding the effects on larvae on D8, the test item BOSCALID 500 WG (FGG01) did not cause statistically significant mortality up to the T4 tested dose.

Therefore, the NOED for larvae on D8 was determined to be 136.36 µg test item/larva (70.04 µg Boscalid/larva) equivalent to a NOEC of 885.48 mg test item/kg diet (454.78 mg Boscalid/kg diet).

Regarding the effects on adult emergence on D22, the test item BOSCALID 500 WG (FGG01) caused statistically significant reduction in emergence rate respect to the control at the two highest doses.

The NOED and the NOEC for adult emergence were evaluated to be 61.98 µg test item/larva (31.84 µg Boscalid/larva) and 402.49 mg test item/kg diet (206.72 mg Boscalid/kg diet), respectively.

**A 2.2.1.4 KCP 10.3.1.4 Sub-lethal effects**

**A 2.2.1.5 KCP 10.3.1.5 Cage and tunnel tests**

**A 2.2.1.6 KCP 10.3.1.6 Field tests with honeybees**

**A 2.2.2 KCP 10.3.2 Effects on arthropods other than bees**

**A 2.2.2.1 KCP 10.3.2.1 Standard laboratory testing for non-target arthropod**

#### A 2.2.2.1.1 Leopold, 2023a (KCP 10.3.2.1/01)

<b>zRMS comments:</b>	<p>Test item: Boscalid 500 WG (FGG01) - product.</p> <p>The study was conducted according to:</p> <ul style="list-style-type: none"> <li>– Mead-Briggs <i>et al.</i>: A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphii</i> (DeStephani-Perez) (Hymenoptera, Braconidae). (2000)</li> <li>– Mead-Briggs <i>et al.</i>: An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphii</i> (Hymenoptera, Braconidae). (2010)</li> </ul> <p>No deviation have been observed.</p> <p>The study was performed in line with GLP principles.</p> <p>The study is considered valid.</p> <p>The study is acceptable for risk assessment.</p> <p>Mortality: LR<sub>50</sub> &gt; 8000 g product/ha; &gt; 4108.4 g a.s./ha  NOER for mortality ≥ 8000 g product/ha; ≥ 4108.4 g a.s./ha  LOER for mortality &gt; 8000 g product/ha; &gt; 4108.4 g a.s./ha  Reproduction: ER<sub>50</sub> Value &gt; 8000 g product/ha; &gt; 4108.4 g a.s./ha  NOER for reproduction ≥ 8000 g product/ha; ≥ 4108.4 g a.s./ha  LOER for reproduction &gt; 8000 g product/ha; &gt; 4108.4 g a.s./ha  These values has been used for risk assessment Supplemental</p>
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Reference:	KCP 10.3.2.1/01
Report	Boscalid 500 WG: Effects on the Parasitoid <i>Aphidius rhopalosiphii</i> (Hymenoptera: Braconidae) in the Laboratory. A Dose Response Test on Glass Plates, Leopold, J., 2023a, Study code 165091001
Guideline(s):	<p>Mead-Briggs <i>et al.</i>: A laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphii</i> (DeStephani-Perez) (Hymenoptera, Braconidae). (2000)</p> <p>Mead-Briggs <i>et al.</i>: An extended laboratory test for evaluating the effects of plant protection products on the parasitic wasp, <i>Aphidius rhopalosiphii</i> (Hymenoptera, Braconidae). (2010)</p>
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Executive summary

An acute 48-hours toxicity study, on mortality of the parasitoid, *Aphidius rhopalosiphi*, was conducted in the laboratory based on Mead-Briggs et al. 2000 and Mead-Briggs et al. 2010. Four replicates of ten adult parasitoids (7 females and 3 males) each were exposed for 48-hours to an untreated control, the reference item Danadim Progress (nominal: 400 g dimethoate/L) and to dried residues of Boscalid 500 WG applied to glass plates at five test item application rates of 500, 1000, 2000, 4000 and 8000 g product/ha in 200 L spray volume/ha. Under worst case laboratory conditions the LR50 value of Boscalid 500 WG is estimated to be higher than 8000 g product/ha (equivalent to > 4108.4 g a.s./ha) in 200 L water/ha. The NOER (no observed effect rate) for mortality is equal to or higher than 8000 g product/ha (equivalent to  $\geq$  4108.4 g a.s./ha) and the LOER (lowest observed effect rate) for mortality is higher than 8000 g product/ha (equivalent to > 4108.4 g a.s./ha) in 200 L water/ha.

Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at all test item application rates. Reproduction was not affected up to and including the highest test item rate of 8000 g product/ha. The ER<sub>50</sub> of Boscalid 500 WG is estimated to be higher than 8000 g product/ha (equivalent to > 4108.4 g a.s./ha) in 200 L water/ha. The NOER is equal to or higher than 8000 g product/ha (equivalent to  $\geq$  4108.4 g a.s./ha) and the LOER is higher than 8000 g product/ha (equivalent to > 4108.4 g a.s./ha) in 200 L water/ha.

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

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** Boscalid 500 WG (FGG01)  
**Batch no.:** 48A  
**Purity:**  $513.55 \pm 1.68$  g/kg, ( $51.36 \pm 0.17\%$  w/w)  
**Date of expiry:** 14 September 2023
2. **Test concentrations:** Control: 200 L deionised water/ha  
Test item: 500, 1000, 2000, 4000 and 8000 g product/ha (equivalent to concentration 2.50, 5.00, 10.0, 20.0 and 40.0 g product/L)
3. **Toxic reference:** 0.3 mL Danadim Progress/ha, dimethoate  
**Batch no.:** 10214034  
**Purity:** 400 g/L (nominal), 417 g/L (analyzed)  
**Study number:** 147504103

#### 4. Test organism:

<b>Species:</b>	<i>Aphidius rhopalosiphi</i> Parasitoid (Hymenoptera, Braconidae)
<b>Source:</b>	Katz Biotech AG, Baruth, Germany
<b>Age:</b>	<48 hours, adults
<b>Test units:</b>	Exposure: Comprising of two treated glass plates (13 cm x 13 cm) which were held apart by an untreated aluminum frame (13 cm x 1.5 cm x 1 cm per side) and held together with at least 2 clamps. 3 sides of the frame had 6 ventilation holes covered with a cloth. The 4 <sup>th</sup> side of the frame had 1 small hole for inserting and feeding the test organisms. Post-exposure units: Untreated pots (13 cm in diameter) with barley seedlings ( <i>Hordeum vulgare</i> 'Sunshine'; 13 - 24 seedlings, 9 days old) infested with 100 - 200 host aphids of all developmental stages ( <i>Rhopalosiphum padi</i> ; number of aphids was estimated) were enclosed within a clear polyacrylic cylinder (30 cm high and 10 cm in diameter). The cylinder had two holes (70 x 195 mm) which were closed with a fine gauze to improve the ventilation and another hole (approximately 2 cm in diameter) closed with cotton wool for the introduction of the parasitoids. The top of the cylinder was closed with a fine gauze. The soil surface was covered with a thin layer of quartz sand.
<b>Test design:</b>	7 treatment group, 4 replicates per treatment group, 10 per unit, 15 days test duration
<b>Feeding:</b>	A solution of fructose (10 %)

#### 5. Environmental conditions

<b>Temperature:</b>	19 to 21 °C
<b>Relative humidity:</b>	73 to 82 % (acclimatization, exposure period) 73 to 82 % (post-exposure period; within the test units)
<b>Light:</b>	16:08 light-dark cycles, 1070 to 1870 lux (acclimatization, exposure, parasitisation period), 11460 to 17060 lux (post-parasitisation period)
<b>Ventilation:</b>	Exposure units were ventilated with a small pump

### B. STUDY DESIGN AND METHODS

#### 1. Experimental phase: 23 May 2022 – 08 June 2022

#### 2. Experiment treatment

Dilutions were prepared in deionised water. Treatments were applied to glass plates with spraying scheme which were left to dry and then used to construct the test units. The parasitoids were introduced to these units and their behaviour and mortality was assessed 2, 24 and 48 h later. At 48 hours, for treatment groups where the corrected mortality was  $\leq 50$  % the reproductive capacity was assessed by confining females individually over untreated barley plants infested with the host cereal aphids, *Rhopalosiphum padi*. The females were removed after 24 hours, and the aphid-infested plants left for a further 12 - 13 days before the numbers of aphid mummies that had developed were assessed.

#### 3. Analysis Determination

Analysis was done in a different study. The study code was JRF 240-2-11-31605 and conducted on 16 – 17 August 2022 with HPLC equipped with UV detector.

#### 4. Measurements and observations

Observations of mortality were recorded approx. 2, 24 and 48 hours after test initiation. The parasitoids alive, affected, moribund and dead was recorded. Moribund parasitoids were counted as dead. Mortality was determined 48 hours after exposure to the test item and the reference item, respectively and was corrected according to the corresponding results of the control group by the Abbott's formula and improvements by Schneider-Orelli.

Number of aphid mummies was counted 12 – 13 days after the 24 hours parasitisation period in all replicates where the females were alive after the 24 hours parasitisation period. The number of live, dead

or not found females after 24 hours parasitisation period was documented. The reproduction assessment was performed for the control and for those test item groups where the corrected mortality was  $\leq 50\%$ . No reproduction testing was performed with the reference item. The number of aphid mummies obtained from 18 - 20 replicates per treatment group was used to calculate the mean aphid mummies production per female ( $\pm$  standard deviation) within the 24 hours parasitisation period (post-exposure period).

## 5. Statistics

The LR50 and ER50 value could not be calculated as no mortality or effects on reproduction above 50% were noted. Mortality data obtained from the control and test item treatments were analysed for significance using the Bonferroni-Holm Fisher's Exact Binomial Test (one-sided greater,  $\alpha = 0.05$ ), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. However, a qualitative trend analysis by contrasts ( $\alpha = 0.05$ ) and the Tarone's Test ( $\alpha = 0.01$ ) had to be carried out previously to check for the presence of linear or quadratic trends and extra-binomial variance.

The two-sample comparison between the reference item and control was analysed using the Fisher's Exact Binomial Test (one sided greater,  $\alpha = 0.05$ ). Reproduction data were tested for normal distribution, homogeneity of variance and the presence of linear trends using the Shapiro-Wilk's Test ( $\alpha = 0.01$ ), the Levene's Test ( $\alpha = 0.01$ ) and a trend analysis by contrasts ( $\alpha = 0.05$ ). Because reproduction data were normally distributed and homogeneous and no linear trend was revealed, the Dunnett's t-Test (multiple comparison, one-sided smaller,  $\alpha = 0.05$ ) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH).

## II. RESULTS AND DISCUSSION

### A. MORTALITY, REPRODUCTIVE OUTPUT AND BEHAVIOUR

The mean mortality of *Aphidius rhopalosiphi* was 0.0 % in the control treatment and was between 0.0 and 5.0 % in the test item treatments, corresponding to corrected mortalities of 0.0 to 5.0 %. Mortality was not statistically significantly increased compared to the control up to and including the highest application rate of 8000 g product/ha (Bonferroni-Holm Fisher's Exact Binomial Test, one-sided greater,  $\alpha = 0.05$ ).

The reference item applied at a rate of 0.3 mL Danadim Progress/ha produced a statistically significant corrected mortality of 100.0 % after 48 hours (Fisher's Exact Binomial Test, one-sided greater,  $\alpha = 0.05$ ).

No behavioural abnormalities were observed at any test item application rate after 2, 24 and 48 hours.

Reproduction of *A. rhopalosiphi* was assessed in the control and at all test item application rates. The mean reproduction of *A. rhopalosiphi* was 42.5 mummies per female in the control treatment and was between 38.1 and 42.0 mummies per female in the test item treatments, corresponding to a reduction of reproduction of 1.1 to 10.2 %. Reproduction was not statistically significantly reduced compared to the control up to and including the highest application rate of 8000 g product/ha (Dunnett's t-Test, one-sided smaller,  $\alpha = 0.05$ ).

**Table A 2.2.2.1.1-1: Mortality and parasitisation efficiency of *Aphidius rhopalosiphi* adults after a 48-hour exposure to of Boscalid 500 WG (FGG01)**

Treatment group	Rates <sup>1</sup> [g product/ha]	Mortality <sup>2</sup> [%]		Corr. Mortality <sup>3</sup> [%]	Parasitisation Rate <sup>4</sup> [mummies/female]		Effect on reproduction <sup>5</sup> [%]
Control	0	0.0 ± 0.0	-	-	42.5 ± 19.6	-	-
Test item	500	5.0 ± 10.0	n.s.	5.0	42.0 ± 16.3	n.s.	1.1
	1000	2.5 ± 5.0	n.s.	2.5	41.6 ± 14.7	n.s.	2.1
	2000	0.0 ± 0.0	n.s.	0.0	41.3 ± 13.5	n.s.	2.8
	4000	2.5 ± 5.0	n.s.	2.5	38.1 ± 15.6	n.s.	10.2
	8000	0.0 ± 0.0	n.s.	0.0	39.1 ± 12.7	n.s.	7.9
Reference item	0.3 mL product/ha	100.0 ± 0.0	*	100.0	-	-	-

<sup>1</sup> Application rate in 200 L spray volume/ha

<sup>2</sup> Mortality: after 48 hours of exposure to spray residues on glass plates  
(Bonferroni-Holm Fisher's Exact Test, one-sided greater,  $\alpha = 0.05$ ; n.s. = not significant)

<sup>3</sup> Corrected mortality according to Abbott and improvements by Schneider-Orelli

<sup>4</sup> Reproduction: mean number of parasitised aphids/female  
(Dunnett's t-Test, one-sided smaller,  $\alpha = 0.05$ ; n.s. = not significant)

<sup>5</sup> Calculated on the exact raw data

## B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

**Table A 2.2.2.1.1-2: Study validity criteria**

Parameters	Required	Observed
Mean control mortality	≤ 13%	0.0%
Mean Reference Item Mortality	> 50%	100.0%
Mean control reproduction rate	≥ 5.0	42.5
Number of females failed to produce mummies in the control	≤ 2	0

## C. TOXICITY ENDPOINTS

The median lethal rate (LR<sub>50</sub>) for mortality and the median effect rate on reproductive output (ER<sub>50</sub>) were determined and are presented in the following table.

**Table A 2.2.2.1.1-3: Mortality and parasitisation efficiency of *Aphidius rhopalosiphi* of exposure to Boscalid 500 WG (FGG01)– summary of endpoints**

Endpoints	Rate	
	Test item [g product/ha]	Active ingredient [g a.s./ha] <sup>a</sup>
Mortality LR <sub>50</sub>	>8000	>4108.4
NOER for Mortality	≥8000	≥4108.4
LOER for Mortality	>8000	>4108.4
Reproduction ER <sub>50</sub>	>8000	>4108.4
NOER for Reproduction	≥8000	≥4108.4
LOER for Reproduction	>8000	>4108.4

LR<sub>50</sub> and ER<sub>50</sub> value could not be calculated as no mortality or effects on reproduction above 50% were noted.



### III. CONCLUSION

Under worst case laboratory conditions the LR<sub>50</sub> value of Boscalid 500 WG is estimated to be higher than 8000 g product/ha (equivalent to > 4108.4 g a.s./ha) in 200 L water/ha. The NOER (no observed effect rate) for mortality is equal to or higher than 8000 g product/ha (equivalent to ≥ 4108.4 g a.s./ha) and the LOER (lowest observed effect rate) for mortality is higher than 8000 g product/ha (equivalent to > 4108.4 g a.s./ha) in 200 L water/ha.

Reproduction of *Aphidius rhopalosiphi* was assessed in the control and at all test item application rates. Reproduction was not affected up to and including the highest test item rate of 8000 g product/ha. The ER<sub>50</sub> of Boscalid 500 WG is estimated to be higher than 8000 g product/ha (equivalent to > 4108.4 g a.s./ha) in 200 L water/ha. The NOER is equal to or higher than 8000 g product/ha (equivalent to ≥ 4108.4 g a.s./ha) and the LOER is higher than 8000 g product/ha (equivalent to > 4108.4 g a.s./ha) in 200 L water/ha.

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

#### A 2.2.2.1.2 Leopold, 2023b (KCP 10.3.2.1/02)

<b>zRMS comments:</b>	<p>Boscalid 500 WG (FGG01) – product.</p> <p>The study was conducted according to: Blümel <i>et al.</i>, 2000: Laboratory residual contact test with the predatory mite <i>Typhlodromus pyri</i> Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products</p> <p>The test was performed in line with GLP principles.</p> <p>No deviation to this guideline have been observed.</p> <p>The study is considered valid.</p> <p>The study is acceptable for risk assessment.</p> <p>NOER for mortality: 500 g product/ha, LOER for mortality: 1000 g product/ha, LR<sub>50</sub>: 1662.1 g product/ha, NOER for reproduction: ≥ 1000 g product/ha, LOER for reproduction: &gt; 1000 g product/ha, ER<sub>50</sub>: &gt; 1000 g product/ha,</p> <p>These values has been used for risk assessment</p>
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Reference:	KCP 10.3.2.1/02
Report	Boscalid 500 WG: Effects on the Predatory Mite <i>Typhlodromus pyri</i> (Acari: Phytoseiidae) in the Laboratory. A Dose Response Test on Glass Plates, Leopold, J., 2023b, Study code 165091063
Guideline(s):	Blümel <i>et al.</i> , Laboratory residual contact test with the predatory mite <i>Typhlodromus pyri</i> Scheuten (Acari: Phytoseiidae) for regulatory testing of plant protection products. (2000)
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Executive summary

An acute 7-day toxicity study, on the predatory mite, *Typhlodromus pyri*, was conducted in the laboratory according to Blümel et al. 2000. Three replicates of 20 predatory mites each were exposed for 7 days to an untreated control, the reference item Danadim Progress (nominal: 400 g Dimethoate/L) and to dried residues of Boscalid 500 WG applied to glass plates at five test item application rates of 500, 1000, 2000, 4000 and 8000 g product/ha, in 200 L water/ha.

Under worst case laboratory conditions, the LR50 value of Boscalid 500 WG for mortality is 1662.1 g product/ha, equivalent to 853.7 g a.s./ha in 200 L water/ha (95% confidence limits: 1442.1 – 1911.0 g product/ha, equivalent to 740.7 – 981.5 g a.s./ha). The NOER (no observed effect rate) for mortality is 500 g product/ha (equivalent to 256.8 g a.s./ha) and the LOER (lowest observed effect rate) for mortality is 1000 g product/ha (equivalent to 513.6 g a.s./ha) in 200 L water/ha.

Reproduction of *Typhlodromus pyri* was assessed in the control and at 500 and 1000 g product/ha. Reproduction was not statistically significantly affected up to and including the application rate of 1000 g product/ha. The ER<sub>50</sub> value of Boscalid 500 WG for reproduction is estimated to be higher than 1000 g product/ha (equivalent to > 513.6 g a.s./ha) in 200 L water/ha. The NOER for reproduction is equal to or higher than 1000 g product/ha (equivalent to ≥ 513.6 g a.s./ha) and the LOER for reproduction is higher than 1000 g product/ha (equivalent to > 513.6 g a.s./ha) in 200 L water/ha.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** Boscalid 500 WG (FGG01)  
**Batch no.:** 48A  
**Purity:** 513.55 ± 1.68 g/kg, 51.36 ± 0.17% w/w (analytic)  
**Date of expiry:** 14 March 2023
2. **Test concentrations:** Control: 200 L deionised water/ha  
Test item: 500, 1000, 2000, 4000 and 8000 g product/ha (equivalent to concentration 2.50, 5.00, 10.0, 20.0 and 40.0 g product/L)
3. **Toxic reference:** 9 mL Danadim Progress, dimethoate/ha  
**Batch no.:** 10214034  
**Purity:** 400 g/L (nominal), 417 g/L (analyzed)  
**Study number:** 147504103
4. **Test organism:**  
**Species:** *Typhlodromus pyri* Scheuten (Acari: Phytoseiidae)  
**Source:** Katz Biotech AG, An der Birkenpfehlheide 10, D-15837 Baruth  
**Age:** Protonymphs, not older than 24 hours  
**Test units:** Two cover slides (glass, 24 mm x 60 mm) fixed by gluing small cover slides (glass, 20 mm x 20 mm) to both side-ends. A glue barrier was placed on the test unit to keep the mites on this test arena. Plastic trays (11 cm x 11 cm x 6 cm) half-filled with water, with a foam rubber and a glass-plate on top covered by tissue paper, tissue paper in contact with the water. The glass slides were placed on the tissue paper.  
**Test design:** 3 replicates per treatment group, 20 per unit, 14 days test duration. Introduction of mites 25 – 40 mins after application (after drying of test units).  
**Feeding:** Pine (*Pinus sp.*) and birch (*Betula sp.*) pollen (3:1) *ad libitum* on the test start and each assessment days except for the last one *resp.* at least every four days.
5. **Environmental conditions**  
**Temperature:** 24 - 26 °C  
**Relative humidity:** 67 – 73 %

**Light:** 16:08 light-dark cycles, 310 – 380 lux

## **B. STUDY DESIGN AND METHODS**

### **1. Experimental phase: 13 - 25 May 2022**

### **2. Experiment treatment**

Spray solutions were prepared in deionised water. Treatments were applied to glass plates which were left to dry and then used to construct the test arenas. Mites were then introduced to the arenas with fine brush and spraying scheme, then their survival assessed over a 7-day period.

### **3. Analysis Determination**

Analysis was done in a different study. The study code was JRF 240-2-11-31605 and conducted on 16 – 17 August 2022 with HPLC equipped with UV detector.

### **4. Measurements and observations**

The number of living, dead and escaped mites as well as mites trapped by the glue barrier was counted on day 3 and on day 7 after test initiation. Dead mites were removed. Escaped mites were considered as dead. The mean percentage mortality (dead and escaped mites) after 7 days was calculated for each treatment and then corrected for any losses in the control treatment using Abbott's formula and improvements by Schneider-Orelli formula.

Reproduction was assessed for all test item rates between day 7 and day 14 with a maximum interval of 3 days after test initiation. Sex ratio for reproduction testing was 1 male:5 females at a minimum on day 7. All eggs laid until day 7 inclusive were removed and not counted. Three assessments were carried out on day 10, 13 and 14 after test initiation. At each assessment, eggs and juveniles were counted and removed afterwards. The mean number of eggs produced per female between 7 and 14 days after test initiation was calculated.

### **5. Statistics**

Analysis. The ER50 value for reproduction could not be calculated due to an insufficient number of test item rates available for statistical analysis. Mortality data obtained from the control and test item treatments were analysed for significance using the Step-down Cochran-Armitage Test ( $\alpha = 0.05$ , one-sided greater), which is a distribution-free test and does not require testing for normality or homogeneity prior to analysis. However, a qualitative trend analysis by contrasts ( $\alpha = 0.05$ ) and the Tarone's Test ( $\alpha = 0.01$ ) had to be carried out previously to check for the presence of linear or quadratic trends and extrabinomial variance.

The two-sample comparison between the reference item and control was analysed using the Fisher's Exact Binomial Test (one-sided greater,  $\alpha = 0.05$ ). Reproduction data were tested for normal distribution, homogeneity of variance and the presence of linear trends using the Shapiro-Wilk's Test ( $\alpha = 0.01$ ), the Levene's Test ( $\alpha = 0.01$ ) and a trend analysis by contrasts ( $\alpha = 0.05$ ). Because reproduction data were normally distributed and homogeneous and no linear trend was revealed and the number of degrees of freedoms were insufficient for the Dunnett's t-Test, the Multiple Sequentially-rejective t-Test after Bonferroni-Holm (multiple comparison, one-sided smaller,  $\alpha = 0.05$ ) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

## **II. RESULTS AND DISCUSSION**

### **A. MORTALITY, REPRODUCTIVE OUTPUT AND BEHAVIOUR**

In the control group, the mean mortality was 18.3%.

Boscalid 500 WG ranged from 20.0 to 98.3 % in the test item treatments, corresponding to corrected

mortalities of 2.0 to 98.0 %. Mortality was not statistically significantly increased compared to the control at the lowest application rate of 500 g product/ha (Step-down Cochran-Armitage Test, one-sided greater,  $\alpha = 0.05$ ). At higher rates, it was statistically significantly increased.

The reference item applied at a rate of 9.0 mL Danadim Progress/ha produced a statistically significant mortality of 96.7 % (corrected mortality 95.9 %) after 7 days (Fisher's Exact Binomial Test, one-sided greater,  $\alpha = 0.05$ ).

**Table A 2.2.2.1.2-1: Mortality and reproduction of mites (*Typhlodromus pyri*) after exposure to of Boscalid 500 WG (FGG01)**

Treatment group	Rates <sup>1</sup> [g product/ha]	Mortality <sup>2</sup> [%]		Corrected mortality <sup>3</sup> [%]	Reproduction <sup>4</sup> [eggs/female]		Effect on reproduction <sup>5</sup> [%]
Control	0	18.3		-	5.0		-
Test item	500	20.0	n.s.	2.0	4.0	n.s.	19.7
	1000	43.3	*	30.6	3.1	n.s.	39.2
	2000	68.3	*	61.2	-		-
	4000	88.3	*	85.7	-		-
	8000	98.3	*	98.0	-		-
Reference item	9.0 mL product/ha				-		-

<sup>1</sup> Application rate in 200 L spray volume/ha

<sup>2</sup> Mortality: after 7 days of exposure to spray residues on glass plates, (Step-down Cochran-Armitage Test, one-sided greater,  $\alpha = 0.05$ ; n.s. = not significant, \* = significant)

<sup>3</sup> Corrected mortality according to Abbott and improvements by Schneider-Orelli

<sup>4</sup> Reproduction: mean number of eggs/female,

(Multiple Sequentially-rejective t-Test after Bonferroni-Holm, one-sided smaller,  $\alpha = 0.05$ ; n.s. = not significant)

<sup>5</sup> Calculated on the exact raw data

## B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

**Table A 2.2.2.1.2-2: Study validity criteria**

Parameters	Required	Observed
Mean mortality in the control treatment	≤ 20%	18.3%
Mean corrected mortality in the reference item after 7 days of exposure	> 50%	95.9%
Mean control reproduction rate after 14 days	≥ 4.0	5.0

## C. TOXICITY ENDPOINTS

The median lethal rate (LR<sub>50</sub>) for mortality and the median effect rate on reproductive output (ER<sub>50</sub>) were determined and are presented in the following table.

**Table A 2.2.2.1.2-3: Mortality and reproductive output of mite *Typhlodromus pyri* of exposure to Boscalid 500 WG (FGG01)– summary of endpoints**

Endpoints	Rate	
	Test item g product/ha]	Active ingredient [g a.s./ha] <sup>a</sup>
Mortality LR <sub>50</sub> (95% CL)	1662.1 (1442.1 – 1911.0)	853.7 (740.1 – 981.5)
NOER for Mortality	500	256.8
LOER for Mortality	1000	513.6
Reproduction ER <sub>50</sub>	> 1000	> 513.6
NOER for Reproduction	> 1000	≥ 513.6

<b>LOER for Reproduction</b>	> 1000	> 513.6
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LR<sub>50</sub> was calculated with Probit Analysis; ER<sub>50</sub> value for reproduction could not be calculated due to an insufficient number of test item rates available for statistical analysis; CL = confidence limits

### III. CONCLUSION

Under worst case laboratory conditions, the LR<sub>50</sub> value of Boscalid 500 WG for mortality is 1662.1 g product/ha, equivalent to 853.7 g a.s./ha in 200 L water/ha (95% confidence limits: 1442.1 – 1911.0 g product/ha, equivalent to 740.7 – 981.5 g a.s./ha). The NOER (no observed effect rate) for mortality is 500 g product/ha (equivalent to 256.8 g a.s./ha) and the LOER (lowest observed effect rate) for mortality is 1000 g product/ha (equivalent to 513.6 g a.s./ha) in 200 L water/ha.

Reproduction of *Typhlodromus pyri* was assessed in the control and at 500 and 1000 g product/ha. Reproduction was not statistically significantly affected up to and including the application rate of 1000 g product/ha. The ER<sub>50</sub> value of Boscalid 500 WG for reproduction is estimated to be higher than 1000 g product/ha (equivalent to > 513.6 g a.s./ha) in 200 L water/ha. The NOER for reproduction is equal to or higher than 1000 g product/ha (equivalent to ≥ 513.6 g a.s./ha) and the LOER for reproduction is higher than 1000 g product/ha (equivalent to > 513.6 g a.s./ha) in 200 L water/ha.

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

#### A 2.3 KCP 10.4 Effects on non-target soil meso- and macrofauna

##### A 2.3.1 KCP 10.4.1 Earthworms

##### A 2.3.1.1 KCP 10.4.1.1 Earthworms - sub-lethal effects

##### A 2.3.1.1.1 Rana, 2023c (KCP 10.4.1.1/01)

<b>zRMS comments:</b>	<p>Test item: Boscalid 500 g/Kg WG (GPF516/FGG01)/</p> <p>The study was conducted according to OECD Test Guideline 222 (Earthworm Reproduction Test) with minor deviations described and assessed by Study Director:</p> <ul style="list-style-type: none"> <li>– the test item characterization (composition), stability, was the responsibility of the Sponsor.</li> <li>– the dose formulation were used within 2 hours of formulating, but were on subject to analytical verification which is an exception to GLP standards.</li> </ul> <p>These forementioned deviations do not affect the outcome of the study and do not affect the ability to use the results for a risk assessment.</p> <p>The validity criteria from OECD 222 were met.</p> <p>The study is valid.</p> <p>The study is acceptable for risk assessment.</p> <p>The following values were derived:</p> <p>NOEC (day 28 mortality and weight): ≥ 1000 mg test item/kg soil dry weight          LOEC (day 28 mortality and weight): ≥ 1000 mg test item/kg soil dry weight          LC<sub>50</sub>: ≥ 1000 mg test item/kg soil dry weight          NOEC (day 56 reproduction): 296.3 mg test item/kg soil dry weight          LOEC (day 56 reproduction): 444.4 mg test item/kg soil dry weight          EC<sub>10</sub> (day 56 reproduction): 41.21 mg test item/kg soil dry weight          EC<sub>20</sub> (day 56 reproduction): 71.95 mg test item/kg soil dry weight          EC<sub>50</sub> (day 56 reproduction): 209.41 mg test item/kg soil dry weight</p>
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	These endpoints were used in the risk assessment.
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Reference:	KCP 10.4.1.1/01
Report	Reproduction toxicity test of Boscalid 500 g/kg WG to earthworm, <i>Eisenia fetida</i> , Rana, J.R., 2023c, Study code 522-3-08-31232
EFSA Identification Study	EFSA-2022-00011613
Guideline(s):	OECD Guideline for the Testing of Chemicals, No. 222 (2016): Earthworm Reproduction Test in Soil; Adopted: 29th July 2016.
Deviations:	No
GLP:	Yes
Acceptability:	Yes

### Executive summary

The objective of the study was to determine the effect of Boscalid 500 g/Kg WG (FGG01), on behaviour (sub lethal), mortality, growth and reproduction of earthworm (*Eisenia andrei*). Adult earthworms were exposed to artificial soil treated with eight nominal concentrations of 58.5, 87.8, 131.7, 197.5, 296.3, 444.4, 666.7 and 1000 mg test item/kg soil dry weight in addition to untreated control for 56 days. Mortality and growth (body weight) of the earthworm were assessed after 28 days and the effects on reproduction (number of juveniles produced) were assessed after 56 days. Deionised water as control treatment.

There was no mortality of adult earthworms exposed to Boscalid 500 g/Kg WG at nominal concentrations up to and including 1000.0 mg/kg dry soil for 28 days. The LC<sub>50</sub>, NOEC and LOEC value for adult worm (28 days) based on mortality was greater than 1000.0 mg test item/kg dry weight of the artificial soil (based on nominal). The NOEC and LOEC based on change in body weight was greater than 1000.0 mg test item/kg dry weight of the artificial soil. The 56-day EC<sub>10</sub>, EC<sub>20</sub>, and EC<sub>50</sub> values for juvenile production were determined to be 41.21, 71.95, and 209.41 mg test item/kg dry weight of the artificial soil (corresponding to 21.15, 36.93 and 107.49 mg a.i./kg dry weight of soil), respectively. The 56-day NOEC, based on juvenile production, was 296.3 mg test item/kg dry weight (corresponding to 152.1 mg a.i./kg dry weight of soil) of the artificial soil and the 56-day LOEC was 444.4 mg test item/kg dry weight of the artificial soil (based on nominal concentrations).

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** Boscalid 500 WG (FGG01)  
**Batch no.:** ARD/BD364/50/WG/0422/59  
**Purity:** 513.55 ± 0.40 g/Kg, 51.33 ± 0.04% w/w (analytic)  
**Date of expiry:** 21 April 2024
- Test concentrations:** Control: deionised water  
Test item: 58.5, 87.8, 131.7, 197.5, 296.3, 444.4, 666.7 and 1000 mg test item/kg soil dry weight
- Toxic reference:** Carbendazim  
**Batch no.:** Not reported  
**Purity:** Not reported  
**Study number:** 522-3-08-31232

#### 4. Test organism:

<b>Species:</b>	<i>Eisenia andrei</i> (Earthworm)
<b>Source:</b>	In-house culture
<b>Age:</b>	Approximately 9 months old mature earthworm with clitellum with body weights ranging between 303.8 and 531.8 mg
<b>Test units:</b>	Glass beakers of 2 L capacity, each containing 600 g of artificial soil.
<b>Test substrate:</b>	Artificial soil prepared according to OECD 222
<b>Feeding:</b>	Air-dried cow dung moisture with water

#### 5. Environmental conditions

<b>Temperature:</b>	19 to 21 °C
<b>Soil pH:</b>	6.07 – 6.31 at test initiation and 6.07 – 6.44 at test termination
<b>Moisture content:</b>	23.86 % (59.65 % of WHC) at test initiation and 22.60 % (56.50 % of WHC) at test end
<b>Light:</b>	16:08 light-dark cycles, 493 to 716 lux

### B. STUDY DESIGN AND METHODS

#### 1. Experimental phase: 27 June – 27 October 2022

#### 2. Experiment treatment

Based on the result of the preliminary range-finding study, the test concentrations selected for the main study were 58.5, 87.8, 131.7, 197.5, 296.3, 444.4, 666.7, and 1000.0 mg test item/kg dry weight of artificial soil along with the control group (geometric factor – 1.5). There were four replicates for the treatment group and eight replicates for the control group, with 10 earthworms in each replicate. The percent reduction in juvenile production observed was -0.84, 26.34, 38.71, 41.90, 58.58, 75.26, 81.82, and 93.81% at test concentrations levels of 58.5, 87.8, 131.7, 197.5, 296.3, 444.4, 666.7, and 1000.0 mg test item/kg dry weight of artificial soil, respectively, when compared with that of the control group. The toxic reference, Carbendazim, was tested at least once a year at five concentrations. The most recent test relevant to this study was in April 2022.

#### 3. Measurements and observations

Worms were assessed for mortality and observed for changes in behaviour and morphology on day 28 of exposure. Body weight change (adults) was assessed at test start (Day 0) and at 28 days after application. On day 28, separated cocoons and juveniles were counted and released into the test soil for four additional weeks under the same test conditions, except that feeding only took place on one occasion at the start of this phase. On day 56, the number of juveniles was counted by hand sorting method.

#### 4. Statistics

As there was no adult mortality observed over the initial four weeks of the test, the median lethal concentration (LC50) of the test item was not calculated.

Data for percent change in body weight between day 0-28 were subjected to Bartlett's test to meet the homogeneity of variance before conducting an analysis of variance (ANOVA). Data for percent juveniles on day 56 were subjected to Kruskal-Wallis test before conducting Dunn's test. As no mortality of earthworms were observed in any of the tested concentrations statistical analysis was not performed.

## II. RESULTS AND DISCUSSION

### A. MORTALITY AND REPRODUCTIVE OUTPUT

The reference item Carbendazim (performed under the test facility Study No. 522-3-08-30130 from January to March 2022) showed statistically significant treatment related effects on reproduction at a concentration of 0.8 mg Carbendazim/kg soil dry weight and above. The EC<sub>50</sub> for reproduction was 0.971 mg Carbendazim/kg soil dry weight.

The food consumption of earthworms exposed to test rates of the test item was comparable to the control.

The results of mortality and reproductive output are presented in the table below.

**Table A 2.3.1.1.1-1: Record for reproductive assessment**

Boscalid 500 g/Kg WG [mg test item/kg sdw]	Control	58.5	87.8	131.7	197.5	296.3	444.4	666.7	1000
Mortality (day 28) [%]	0	0	0	0	0	0	0	0	0
Statistical significance	-	-	-	-	-	-	-	-	-
Body weight change (day 28) [%]	-6.66	-6.65	-3.28	-7.58	-2.33	1.68	-9.18	-7.63	-9.43
Statistical significance	-	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean No. of juveniles (day 56)	133.38	134.50	98.25	81.75	77.50	55.25	33.00	24.25	8.25
Statistical significance	-	n.s.	n.s.	n.s.	n.s.	n.s.	*	*	*
% reduction in reproductive output (day 56)	-	-0.84	26.34	38.71	41.90	58.58	75.26	81.82	93.81
Food consumption	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

sdw: soil dry weight, n.s.; not significantly different compared to the control, -; not applicable, \*; significant

## B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

**Table A 2.3.1.1.1-2: Study validity check for parameters in the control group**

Parameter	Required	Observed
Control mortality	≤ 10%	0%
Control Reproduction (Juveniles per Container):	≥ 30	124 to 147
Coefficient of Variation of the Control Reproduction:	< 30%	5.62%

## C. TOXICITY ENDPOINTS

The Lowest Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC) for mortality and reproductive output, the median Lethal Concentration (LC<sub>50</sub>) for mortality, and the Effect Concentration on reproductive output (EC<sub>10, 20, 50</sub>) were determined and are presented in the following table.

**Table A 2.3.1.1.1-3: Summary of endpoints**

	Endpoints	
	mg test item/kg soil dry weight	mg Boscalid/kg soil dry weight
NOEC (day 28 mortality and weight)	≥ 1000	≥ 513.3
LOEC (day 28 mortality and weight)	≥ 1000	≥ 513.3
LC <sub>50</sub>	≥ 1000	≥ 513.3
NOEC (day 56 reproduction)	296.3	152.1
LOEC (day 56 reproduction)	444.4	228.11
EC <sub>10</sub> Values (day 56 reproduction) (95% Fiducial Limits)	41.21 (29.17 – 58.21)	21.15 (14.97 – 29.88)
EC <sub>20</sub> Values (day 56 reproduction) (95% Fiducial Limits)	71.95 (55.46 – 93.33)	36.93 (28.47 – 47.91)
EC <sub>50</sub> Values (day 56 reproduction) (95% Fiducial Limits)	209.41 (182.81 – 239.88)	107.49 (93.84 – 123.13)

## III. CONCLUSION

There was no mortality of adult earthworms exposed to Boscalid 500 g/Kg WG at nominal concentrations up to and including 1000.0 mg/kg dry soil for 28 days. The LC<sub>50</sub>, NOEC and LOEC value for adult worm (28 days) based on mortality was greater than 1000.0 mg test item/kg dry weight of the artificial soil



(based on nominal). The NOEC and LOEC based on change in body weight was greater than 1000.0 mg test item/kg dry weight of the artificial soil. The 56-day EC<sub>10</sub>, EC<sub>20</sub>, and EC<sub>50</sub> values for juvenile production were determined to be 41.21, 71.95, and 209.41 mg test item/kg dry weight of the artificial soil (corresponding to 21.15, 36.93 and 107.49 mg a.i./kg dry weight of soil), respectively. The 56-day NOEC, based on juvenile production, was 296.3 mg test item/kg dry weight (corresponding to 152.1 mg a.i./kg dry weight of soil) of the artificial soil and the 56-day LOEC was 444.4 mg test item/kg dry weight of the artificial soil (based on nominal concentrations).

**A 2.3.1.2 KCP 10.4.1.2 Earthworms - field studies**

**A 2.3.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)**

**A 2.3.2.1 KCP 10.4.2.1 Species level testing**

**A 2.3.2.1.1 Hübner, 2022a (KCP 10.4.2.1/01)**

<b>zRMS comments:</b>	<p>Test item: Boscalid 500 WG, (Boscalid 500 g/Kg WG, FGG01).</p> <p>The study was conducted according to OECD Test Guideline 232 (Effects on Reproduction of Collembola (<i>Folsomia candida</i>) in Artificial Soil).</p> <p>There were no deviations to the guideline.</p> <p>The validity criteria from OECD 232 were met.</p> <p>The study is valid.</p> <p>The study is acceptable for risk assessment.</p> <p>FGG01 caused no statistically significant effects on mortality of <i>Folsomia candida</i> up to and including the concentration of 1000 mg test item/kg soil dry weight.</p> <p>NOEC for mortality was determined to be <math>\geq 1000</math> mg test item/kg soil dry weight.</p> <p>LOEC for mortality was estimated to be <math>&gt;1000</math> mg test item/kg soil dry weight.</p> <p>The LC<sub>50</sub> was estimated to be <math>&gt;1000</math> mg test item/kg soil dry weight.</p> <p>NOEC for reproduction of <i>Folsomia candida</i> was determined to be 556 mg test item/kg soil dry weight.</p> <p>The LOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight.</p> <p>The EC<sub>10</sub> for <i>Folsomia candida</i> in artificial soil was determined to be 72.7 mg test item/kg soil dry weight,</p> <p>The EC<sub>20</sub> was determined to be 1231.5 mg test item/kg soil dry weight.</p>
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Reference: KCP 10.4.2.1/01

Report Boscalid 500 WG: Effects on Reproduction of Collembola (*Folsomia candida*) in Artificial Soil, Hübner, S., 2022a, Study code 165091016

EFSA Study Identification.: EFSA-2022-00009384

Guideline(s): OECD Guideline for the Testing of Chemicals, No. 232 (2016): Collembola Reproduction Test in Soil; Adopted: 29th July 2016.

Deviations: No

GLP: Yes

Acceptability: Yes

### Executive summary

The objective of the study was to determine the effect of Boscalid 500 WG (FGG01), on survival and the reproductive output of the *Folsomia candida* Willem (Collembola, Isotomidae) under exposure for 28 days at nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight. Deionised water was used for the control.

Boscalid 500 WG caused no statistically significant effects on mortality of *Folsomia candida* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be  $\geq 1000$  mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) for mortality was estimated to be  $> 1000$  mg test item/kg soil dry weight. The  $LC_{50}$  was estimated to be  $> 1000$  mg test item/kg soil dry weight.

The NOEC of Boscalid 500 WG for reproduction of *Folsomia candida* was determined to be 556 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight. The  $EC_{10}$  for *Folsomia candida* in artificial soil was determined to be 72.7 mg test item/kg soil dry weight, the  $EC_{20}$  was determined to be 1231.5 mg test item/kg soil dry weight.

In a separate study, the reference item Boric acid showed statistically significant effects on reproduction at concentrations of  $\geq 78.1$  mg/kg soil dry weight. The  $EC_{50}$  for reproduction was calculated to be 777.2 mg/kg soil dry weight.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** Boscalid 500 WG (FGG01)  
**Batch no.:** 48A  
**Purity:**  $513.55 \pm 1.68$  g/kg,  $51.36 \pm 0.17\%$  w/w (analytic)  
**Date of expiry:** 14 September 2023
2. **Test concentrations:** Control: Untreated soil (moistened with deionised water)  
Test item: 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight
3. **Toxic reference:** Boric acid  
**Batch no.:** Not reported  
**Purity:** Not reported  
**Study number:** 136524016

#### 4. Test organism:

<b>Species:</b>	<i>Folsomia candida</i> Willem (Collembola, Isotomidae)
<b>Source:</b>	The synchronized individuals were bred at ibacon
<b>Age:</b>	10-12 days old juveniles at test start, adults
<b>Test units:</b>	Glass vessels (Ø: 5 cm, volume: approx. 100 mL) closed with close fitted cover. Each test unit was filled with $30 \pm 1.0$ g (equivalent to 30 g dry mass) of the test substrate.
<b>Test substrate:</b>	Artificial soil prepared according to OECD 232, maximum water holding capacity of the artificial soil, 34% of the dry weight
<b>Feeding:</b>	granulated dry yeast until test start

#### 5. Environmental conditions

<b>Temperature:</b>	18 to 22 °C
<b>Soil pH:</b>	6.2 – 6.3 at test initiation and 5.6 – 5.8 at test termination
<b>Water content:</b>	17.6 to 18.0 % (51.8 to 52.9 % of WHC) at test initiation and 15.7 to 17.0 % of soil dry weight (46.1 to 50.0 % of WHC) at test initiation
<b>Light:</b>	16:08 light-dark cycles, 400 to 800 lux

### B. STUDY DESIGN AND METHODS

#### 1. Experimental phase: 16 May 2022 – 14 June 2022

#### 2. Experiment treatment

A study was conducted to determine the effect of Boscalid 500 WG on the mortality and reproduction of Collembola (*Folsomia candida*). Eight replicates for the control and four replicates per test item group, containing ten Collembola each (total 80 individuals per control and 40 individuals per test item group) were each exposed for 28 days to nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight and to an untreated control (soil moistened with deionized water). A reference item (boric acid, at a concentration range of 30.5 to 200 mg/kg soil dry weight) is tested at least once a year to ensure the laboratory test conditions are adequate and to verify the response of the test organisms does not change significantly over time. The most recent test relevant to this study was performed in September 2021 under ibacon study number 136524016.

A stock solution was prepared by weighing 1000.1 mg of Boscalid 500 WG using an analytical balance. The test item was transferred into a glass beaker and deionised water was added to obtain a final net weight of 108.9 g. The resulting suspension contained a concentration of 9.1837 mg test item/g. A dilution series was prepared according to the dilution series. There were no significant deviations from the target concentration (<5%).

#### 3. Measurements and observations

The numbers of living adult Collembola at day 28 after application were recorded. Missing adult Collembola were recorded as dead as it was assumed that missing adult Collembola had died and degraded during the test period. Surviving Collembola were observed for any abnormal behaviour or conditions at day 28 after application. Reproduction was determined as number of juvenile Collembola at day 28 after application.

#### 4. Statistics

Mortality data were analysed for significance by using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction, one-sided greater,  $\alpha = 0.05$ ). The  $LC_{50}$  at day 28 was not determined by statistical analysis as no mortality above 50% was observed.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ( $\alpha = 0.01$ ).

Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend) the Williams t-test (multiple comparison,  $\alpha = 0.05$ , one-sided smaller) was used to

compare treatment and control values. The EC values for reproduction were calculated by Weibull Analysis.

The determination of the NOEC and LOEC values was based on the results of the statistical evaluation.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. MORTALITY AND REPRODUCTIVE OUTPUT

Mortality of *Folsomia candida* was not statistically significantly different compared to the control up to and including the highest test concentration of 1000 mg test item/kg soil dry weight. No abnormal behaviour was observed with the surviving Collembola.

There were no statistically significant effects on reproduction of *Folsomia candida* up to and including the concentration of 556 mg test item/kg soil dry weight. At the concentration of 1000 mg test item/kg soil dry weight reproduction was statistically significantly reduced compared to the control.

The reference item Boric acid showed statistically significant treatment related effects on reproduction at a concentration of 78.1 mg/kg soil dry weight and above. The EC<sub>50</sub> for reproduction was 77.2 mg/kg soil dry weight (95% confidence limits of 68.9 to 87.4 mg/kg soil dry weight).

The results of mortality and reproductive output are presented in the table below.

**Table A 2.3.2.1.1-1: Results of mortality and reproductive output of *Folsomia candida***

Test item concentration [mg/kg sdw]	Mean Mortality [%]	Statistical significance <sup>1</sup>	Mean number of juveniles	% of Control	Statistical significance <sup>2</sup>
Control	8	-	1123	-	-
16.3	8	n.s.	1111	99	n.s.
29.4	5	n.s.	1162	103	n.s.
52.9	8	n.s.	1148	102	n.s.
95.3	5	n.s.	1038	92	n.s.
171	8	n.s.	1001	89	n.s.
309	5	n.s.	973	87	n.s.
556	13	n.s.	1026	91	n.s.
1000	13	n.s.	900	80	*

sdw: soil dry weight, n.s.; not significantly different compared to the control, \*; significantly different compared to the control

<sup>1</sup> Fisher's Exact Test,  $\alpha = 0.05$ , one-sided greater.

<sup>2</sup> Williams t-test,  $\alpha = 0.05$ , one-sided smaller.

### B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

**Table A 2.3.2.1.1-2: Study validity check for parameters in the control group**

Parameter	Required	Observed
Control mortality	≤ 20%	8%
Control Reproduction (Juveniles per Container):	≥ 100	809 to 1357
Coefficient of Variation of the Control Reproduction:	< 30%	16.8%

### C. TOXICITY ENDPOINTS

The NOEC for mortality was determined to be ≥1000 mg test item/kg soil dry weight. The LOEC for mortality was estimated to be >1000 mg test item/kg soil dry weight. The LC<sub>50</sub> of Boscalid 500 WG for *Folsomia candida* in soil was estimated to be >1000 mg test item/kg soil dry weight.

The NOEC for reproduction was determined to be 556 mg test item/kg soil dry weight and the LOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight. The EC<sub>10</sub> for *Folsomia candida* in artificial soil was determined to be 72.7 mg test item/kg soil dry weight (95% confidence limits of 26.4 to 132.3 mg test item/kg soil dry weight). The EC<sub>20</sub> was determined to be 1231.5 mg test item/kg soil dry weight (95% confidence limits of 558.2 to 7181.5 mg test item/kg soil dry weight, Weibull Analysis). The EC<sub>50</sub> was not determined by statistical analysis due to mathematical reasons.

The summary of endpoints were determined and are presented in the following table.

**Table A 2.3.2.1.1-3: Survival and reproductive output of the springtail *Folsomia candida* Willem after exposure to Boscalid 500 WG – Summary of endpoints**

Endpoints [mg/kg soil dry weight]			
NOEC mortality	≥1000		
LOEC mortality	>1000		
LC <sub>50</sub> mortality <sup>3</sup>	>1000		
NOEC reproduction	556		
LOEC reproduction	1000		
EC Values reproduction <sup>4</sup>	EC <sub>10</sub> : 72.7	EC <sub>20</sub> : 1231.5	EC <sub>50</sub> : n.d.
95% confidence limits	26.4 – 132.3	558.2 – 7181.5	n.d.

<sup>3</sup> Moving Averages Computation

<sup>4</sup> Probit analysis

n.d. = not determined due to mathematical reasons

### III. CONCLUSION

Boscalid 500 WG caused no statistically significant effects on mortality of *Folsomia candida* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the No Observed Effect Concentration (NOEC) for mortality was determined to be ≥1000 mg test item/kg soil dry weight. The Lowest Observed Effect Concentration (LOEC) for mortality was estimated to be >1000 mg test item/kg soil dry weight. The LC<sub>50</sub> was estimated to be >1000 mg test item/kg soil dry weight.

The NOEC of Boscalid 500 WG for reproduction of *Folsomia candida* was determined to be 556 mg test item/kg soil dry weight. The LOEC for reproduction was determined to be 1000 mg test item/kg soil dry weight. The EC<sub>10</sub> for *Folsomia candida* in artificial soil was determined to be 72.7 mg test item/kg soil dry weight, the EC<sub>20</sub> was determined to be 1231.5 mg test item/kg soil dry weight.

In a separate study, the reference item Boric acid showed statistically significant effects on reproduction at concentrations of ≥78.1 mg/kg soil dry weight. The EC<sub>50</sub> for reproduction was calculated to be 777.2 mg/kg soil dry weight.

#### A 2.3.2.1.2 Hübner, 2022b (KCP 10.4.2.1/02)

<b>ZRMS comments:</b>	<p>Test item: Boscalid 500 WG, (Boscalid 500 g/Kg WG, FGG01).</p> <p>The study was conducted according to OECD Test Guideline 226 (Predatory mite (<i>Hypoaspis</i> (<i>Geolaelaps</i>) <i>aculeifer</i>) reproduction test in soil).</p> <p>There were no deviations to the guideline.</p> <p>The validity criteria from OECD 226 were met.</p> <p>The study is valid.</p> <p>The study is acceptable for risk assessment.</p> <p>FGG01 caused no statistically significant effects on mortality and reproduction of <i>Hypoaspis aculeifer</i> up to and including the concentration of 1000 mg test item/kg</p>
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	soil dry weight. NOEC was determined to be $\geq 1000$ mg test item/kg soil dry weight. LOEC was estimated to be $>1000$ mg test item/kg soil dry weight. The $LC_{50}$ and $EC_{50}$ values were estimated to be $>1000$ mg test item/kg soil dry weight.
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Reference: KCP 10.4.2.1/02

Report: Boscalid 500 WG: Effects on Reproduction of the Predatory Mite *Hypoaspis aculeifer* in Artificial Soil, Hübner, S., 2022b, Study code 165091089

EFSA Study Identification: EFSA-2022-00009385

Guideline(s): OECD Guideline for the Testing of Chemicals, No. 226 (2016): Predatory mite (*Hypoaspis aculeifer*) Reproduction Test in Soil; Adopted: 29th July 2016.

Deviations: No

GLP: Yes

Acceptability: Yes

### Executive summary

A study was conducted to determine the effect of Boscalid 500 WG on the mortality and reproduction of the soil mite (*Hypoaspis aculeifer*) according to OECD 226. The soil mites were exposed for 14 days to artificial soil (prepared according to OECD 226) treated with the test item to obtain the nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight and to an untreated control with added deionized water only).

Boscalid 500 WG caused no statistically significant effects on mortality and reproduction of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be  $\geq 1000$  mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be  $>1000$  mg test item/kg soil dry weight. The  $LC_{50}$  and  $EC_{50}$  values were estimated to be  $>1000$  mg test item/kg soil dry weight.

The reference item dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 2.23 mg a.i./kg soil dry weight and above. The  $EC_{50}$  for reproduction was 2.95 mg a.i./kg soil dry weight.

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** Boscalid 500 WG (FGG01)  
**Batch no.:** 48A  
**Purity:**  $513.55 \pm 1.68$  g/kg,  $51.36 \pm 0.17\%$  w/w (analytic)  
**Date of expiry:** 14 September 2023
- Test concentrations:** Control: Untreated (soil with deionized water)  
Test item: 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight

- 3. Toxic reference:** Dimethoate  
**Batch no.:** Not reported  
**Purity:** Not reported  
**Study number:** 159083089
- 4. Test organism:**  
**Species:** *Hypoaspis aculeifer* (Predatory mite, Acari:Laelapidae)  
**Source:** culture by ibacon  
**Age:** Adults, approximately 14 days after reaching the adult stage (35 days after placing adult females in clean rearing vessels over a period of 3 days)  
**Test units:** Glass vessels (Ø: 5 cm, volume: approx. 100 mL) closed with close fitted cover. Each test unit was filled with  $20 \pm 1.0$  g of the test substrate.  
**Test substrate:** Artificial soil prepared according to OECD 226, maximum water holding capacity of the artificial soil is determined to be 34%  
**Feeding:** Cheese mites (*Tyrophagus putrescentiae*) at day 0, 2, 4, 7, 9 and 11
- 5. Environmental conditions**  
**Temperature:** 18 to 22 °C  
**Soil pH:** 6.2 – 6.3 at test initiation 6.1 – 6.2 at test termination  
**Water content:** 17.6 to 18.0 % of soil dry weight (51.8 to 52.9 % of WHC) at test initiation and 16.9 to 17.6 % of soil dry weight (49.8 to 51.9 % of WHC) at test end  
**Light:** 16:08 light-dark cycles, 400 to 800 lux

## B. STUDY DESIGN AND METHODS

- 1. Experimental phase:** 16 May – 01 June 2022
- 2. Experiment treatment**

A study was conducted to determine the effect of Boscalid 500 WG on the mortality and reproduction of the predatory soil mite (*Hypoaspis aculeifer*). Eight replicates for the control and four replicates per test item group, containing ten predatory mites each (total 80 individuals per control and 40 individuals per test item group) were each exposed for 14 days to nominal concentrations of 16.3, 29.4, 52.9, 95.3, 171, 309, 556 and 1000 mg test item/kg soil dry weight and to an untreated control (deionized water only). A reference item (dimethoate) is tested at least once a year to laboratory test conditions are adequate and to verify that the response of the test organisms does not change significantly over time. The most recent test relevant to this study was conducted in January 2023 and archive under ibacon Study No. 159083089.

### 3. Measurements and observations

Number of surviving adult female predatory mites 14 days after test initiation was recorded (counted after extraction). Missing adult predatory mites were recorded as dead as it was assumed they would have degraded after death during the test period. The living predatory mites were observed for differences in morphology or any abnormalities at experimental end. Reproduction was determined by the number of juvenile mites at day 14 after application, counted after extraction.

### 4. Statistics

Mortality data were statistically analysed using Fisher's Exact Binomial Test (multiple comparison, with Bonferroni Correction,  $\alpha = 0.05$ , one-sided greater). The LC50 at day 14 was not determined by statistical analysis as no mortality above 50% was observed.

Reproduction data were tested for normal distribution and homogeneity of variance using Shapiro-Wilk's test and Levene's test ( $\alpha = 0.01$ ). Since the reproduction data were normally distributed and homogeneous and did follow a monotonicity trend (contrast trend) the Williams t-test (multiple comparison,  $\alpha = 0.05$ , one-sided smaller) was used to compare treatment and control values.

The EC values could not be determined by statistical analysis since there was no adequate concentration response. The determination of the NOEC and LOEC values was based on the results of the statistical evaluation.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. MORTALITY AND REPRODUCTIVE OUTPUT

Compared to the control group, statistically significant increase in mortality of *Hypoaspis aculeifer* was detected from the test item concentration 16.3 to 1000 mg test item/kg soil dry weight after 14 days of exposure.

The reference item dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 2.23 mg a.i./kg soil dry weight and above. The EC<sub>50</sub> for reproduction was 2.95 mg a.i./kg soil dry weight.

The results of mortality and reproductive output are presented in the table below.

**Table A 2.3.2.1.2-1: Results of mortality and reproductive output of *Hypoaspis aculeifer***

Boscalid 500 WG [mg/kg sdw]	Mean Mortality [%]	Statistical significance <sup>1</sup>	Mean Number of juveniles	% of Control	Statistical significance <sup>2</sup>
Control	1	-	256	-	-
16.3	3	n.s.	236	92	n.s.
29.4	8	n.s.	242	95	n.s.
52.9	10	n.s.	205	80	n.s.
95.3	8	n.s.	241	94	n.s.
171	10	n.s.	238	93	n.s.
309	5	n.s.	238	93	n.s.
556	5	n.s.	268	105	n.s.
1000	3	n.s.	257	100	n.s.

sdw: soil dry weight, n.s.; not significantly different compared to the control

<sup>1</sup> Fisher's Exact Test,  $\alpha = 0.05$ , one-side greater

<sup>2</sup> Williams t-test,  $\alpha = 0.05$ , one-sided smaller.

- not applicable

### B. VALIDITY CRITERIA

All required validity criteria were met. Accordingly, the study is regarded as valid.

**Table A 2.3.2.1.2-2: Study validity check for parameters in the control group**

Parameter	Required	Observed
Control mortality	≤ 20%	1%
Control Reproduction (Juveniles per Container):	≥ 50	231 to 300
Coefficient of Variation of the Control Reproduction:	< 30%	8.9%

### C. TOXICITY ENDPOINTS

The Lowest Observed Effect Concentration (LOEC) and the No Observed Effect Concentration (NOEC) for mortality and reproductive output, the median Lethal Concentration (LC<sub>50</sub>) for mortality, and the Effect Concentration on reproductive output (EC<sub>50</sub>) were determined and are presented in the following table.



**Table A 2.3.2.1.2-3: Survival and reproductive output of the Predatory mite *Hypoaspis aculeifer* after 14-days exposure to Boscalid 500 WG – Summary of endpoints**

Endpoints [mg/kg soil dry weight]	
NOEC mortality	≥1000
LOEC mortality	>1000
LC <sub>50</sub> mortality <sup>3</sup>	>1000
NOEC reproduction	≥1000
LOEC reproduction	>1000
EC <sub>50</sub> reproduction <sup>3</sup>	>1000

<sup>3</sup> estimated value

The overall No Observed Effect Concentration (NOEC) was determined to be ≥1000 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil dry weight. The LC<sub>50</sub> and EC<sub>50</sub> values were estimated to be >1000 mg test item/kg soil dry weight. Other EC values could not be determined by statistical analysis since there was no adequate concentration response.

### III. CONCLUSION

Boscalid 500 WG caused no statistically significant effects on mortality and reproduction of *Hypoaspis aculeifer* up to and including the concentration of 1000 mg test item/kg soil dry weight. Therefore, the overall No Observed Effect Concentration (NOEC) was determined to be ≥1000 mg test item/kg soil dry weight. The overall Lowest Observed Effect Concentration (LOEC) was estimated to be >1000 mg test item/kg soil dry weight. The LC<sub>50</sub> and EC<sub>50</sub> values were estimated to be >1000 mg test item/kg soil dry weight.

The reference item dimethoate showed statistically significant treatment related effects on reproduction at a concentration of 2.23 mg a.i./kg soil dry weight and above. The EC<sub>50</sub> for reproduction was 2.95 mg a.i./kg soil dry weight.

#### A 2.3.2.2 KCP 10.4.2.2 Higher tier testing

#### A 2.4 KCP 10.5 Effects on soil nitrogen transformation

##### A 2.4.1 Raithatha, 2022 (KCP 10.5/01)

<b>ZRMS comments:</b>	<p>Test item: Boscalid 500 g/Kg WG, GPF516/FGG01.</p> <p>The study was conducted according to OECD Test Guideline 216 (Soil Microorganisms: Nitrogen Transformation Test ) with minor deviations from GLP principles were described and assessed by Study Director.</p> <ul style="list-style-type: none"> <li>– the test item characterization (composition), stability, was the responsibility of the Sponsor.</li> <li>– The dose formulation were used within 2 hours of formulating, but were on subject to analytical verification which is an exception to GLP standards.</li> </ul> <p>This aforementioned deviations do not affect the outcome of the study and do not affect the ability to use the results for a risk assessment.</p> <p>The validity criteria from OECD 212 were met.</p> <p>The study is valid.</p> <p>The study is acceptable for risk assessment.</p>
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	<p>There was no significant difference in nitrate formation and rate of nitrate formation in the test concentration of 1.2 and 6.0 kg test item/ha of soil on day 0, 7, 14 and 28 when compared to control on 28 days of exposure. In test concentration of 1.2 and 6.0 kg test item/ha of soil, the percent deviation of rate of nitrate formation when compared to control on day 28 were -0.14% and +4.27%, respectively. As the difference of nitrate formation rates compared to the control were less than 25% on 28<sup>th</sup> day, the study was terminated at this point.</p> <p>Based on these results, it is concluded that the Boscalid 500 WG (FGG01) have no long-term influence on nitrogen transformation in soil.</p>
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Reference:	KCP 10.5/01
Report:	Effect of Boscalid 500 WG on Soil Microorganisms: Nitrogen Transformation Test, Raithatha, A., 2022. Study code 608-3-15-31233
EFSA Study Identification	EFSA-2022-00010828
Guideline(s):	OECD guideline for the testing of Chemicals No. 216: ‘Soil Microorganisms: Nitrogen Transformation Test’ (21st January 2000).
Deviations:	No
GLP:	Yes
Acceptability:	Yes

## Executive summary

The effect of Boscalid 500 WG (FGG01) on soil microorganisms was evaluated through a nitrogen transformation test by measuring the rate of nitrate formation in the treated soil as compared to control (untreated) soil. The study was conducted at the concentrations of 1.2 kg test item/ha of soil or 1.6 mg/ha of soil (maximum application rate) and 6.0 kg test item/ha of soil or 8.0 mg/ha of soil (5 times of maximum application rate).

The study was conducted with test soil EFM-64 (C/N) (sand: 71.49%, silt: 4.80% and clay: 16.32%), amended with 5 g of Lucerne grass per/kg soil dry weight before application, in glass containers with three replicates per treatment. On the day of treatment and on 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 28<sup>th</sup> day, the soil samples were tested for nitrate formation rate.

There was no significant difference in nitrate formation and rate of nitrate formation in the test concentration of 1.2 and 6.0 kg test item/ha of soil on day 0, 7, 14 and 28 when compared to control on 28 days of exposure. In test concentration of 1.2 and 6.0 kg test item/ha of soil, the percent deviation of rate of nitrate formation when compared to control on day 28 were -0.14% and +4.27%, respectively. As the difference of nitrate formation rates compared to the control were less than 25% on 28<sup>th</sup> day, the study was terminated at this point.

Based on these results, it is concluded that the Boscalid 500 WG (FGG01) have no long-term influence on nitrogen transformation in soil.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. Test material:	Boscalid 500 g/Kg WG (FGG01)
Batch no.:	ARD/BD364/50/WG/0422/59
Purity:	513.34 ± 0.40 g/Kh (51.33 ± 0.04 % w/w)
Date of expiry:	April 21, 2024

2. **Toxic reference:** Not reported  
**Study No.:** Not reported
3. **Test soil:**
  - Soil type:** Pasture land
  - Soil code:** EFM-64 (C/N)
  - Source:** Bank of Par River, Village Nani Vahiya, Taluka Dharampur, District Valsad, Gujarat, India, depth of collection is 0 to 25 cm
  - Soil characteristics:** Another study, 609-3-15-30729, that characterize the soil.  
Sand content: 71.49%; Silt content: 4.80%; Clay content: 16.32%; pH: 6.78; Maximum water holding capacity (WHC<sub>max</sub>): 56.76%; Cation exchange capacity (CEC): 271.74 mmol/kg; Organic carbon content: 0.59 %; Total nitrogen content (TN): 0.06%; Microbial biomass: 335.10 mg/kg soil dry weight (5.68% of TOC); Initial nitrate content of soil: 56.90 mg nitrate/kg soil dry weight
  - Details of substrate:** Lucerne grass: Supplier: Isha Agro Developers Pvt. Ltd.; Content of Lucerne grass in test concentration 5 g/kg test soil dry weight; Carbon content (C): 45.57%; Total nitrogen content (TN): 3.45%; Ratio C/N: 13:21:1
  - Test duration:** 28 days
  - Test units:** 3 replicates per treatment, 3 different treatments: Control, 1.2 (maximum single field application) and 6.0 (5 times of maximum single field application) kg/ha of soil
4. **Environmental conditions**
  - Temperature:** 20 ± 2 °C
  - Moisture:** Mean moisture content of soil: 10.29 % (WHC at 50 ± 5%)
  - Light:** Darkness

## B. STUDY DESIGN AND METHODS

### 1. Dates of work: 28 May – 30 June 2022

### 2. Soil preparation and application

The soil was collected from the Bank of Par River, Village - Nani Vahiya, Taluka - Dharampur, District - Valsad, Gujarat, India, from a depth of 0 to 25 cm. The soil sample was transported under temperature-controlled condition. The area from which soil collected was pastureland. There was no application of crop protection products, mineral fertilisers or biological materials within a year before sampling. It was neither flooded nor dried. After taking the soil to the laboratory, it was manually cleared of large objects (e.g., stones and other plants material). Then it was processed by sieving without excess drying to a particle size less than or equal to 2 mm. The soil was stored in the refrigerator at 4 ± 2 °C, and an aerobic condition was ensured during the storage of soil.

A volume of 0.5 mL of the test Formulation 1T, Formulation 5T, and Control Formulation were added to separate pre-labelled incubated containers containing 320.95 g of moistened soil in each container to achieve the dose level 1.2 kg/ha or 1.6 mg/kg dry weight soil for treatment (1T) and 6.0 kg/ha or 8.0 mg/kg of soil for treatment (5T), and control, respectively. The soil was mixed very well for a homogeneous distribution of the test item in the soil. Distilled water was added to maintain moisture level at 50 ± 5% water holding capacity. During mixing, care was taken to avoid compacting and balling of the soil. The test containers were covered with perforated polypropylene sheet to prevent water loss and maintain an aerobic condition.

### 3. Dose preparation

500 WG to 10 mL volumetric flask and the volume was made up to the mark with distilled water (Formulation 1T). The test item formulation for the dose level of 5T was prepared by transferring 40.0 mg of Boscalid 500 WG to 10 mL volumetric flask and the volume was made up to the mark with distilled water (Formulation 5T). The control sample was prepared following the same procedure using distilled water without the test item (Control Formulation).

#### 4. Measurements and observations

A sample of each replicate from each treatment was taken on the 0th, 7th, 14th and 28th day after application to determine the nitrate formation. As the difference in the mean nitrate content between the lower treatment (1T) and the control was less than  $\pm 25\%$  on day 28, the experiment was terminated on day 28, and further analysis was not performed.

A quantity of 32 g of soil (on dry weight basis of 25 g) from control and the treated soil containers were transferred into reagent bottles. A quantity of 125 mL 0.1 M potassium chloride solution was added in each bottle. To optimise the extraction, the bottles containing the soil and extraction solution were not more than half full. The mixtures were shaken at  $150 \pm 5$  rpm for 60 minutes at  $20 \pm 2$  °C. The mixtures were centrifuged at 5000 rpm for ten minutes and filtered through Whatman number 1 filter paper. From the filtrate, 100 mL liquid phase was transferred to a pre-labelled beaker and 2 mL of 2 M ammonium sulphate was added. Then these solutions were analysed for nitrate ( $\text{NO}_3^-$ ) content by pre-calibrated multi portable meter using nitrate ( $\text{NO}_3^-$ ) electrode. The calibration of multi portable meter was performed using the potassium nitrate standard solution. The nitrate ( $\text{NO}_3^-$ ) concentration was directly recorded using the multi portable meter.

#### 5. Statistics

The statistical analysis of mean nitrate content data was performed by utilizing in-house developed validated statistical computer software for the data homogeneity (F test for homogeneity of variance).

## II. RESULTS AND DISCUSSION

### A. NITRATE CONTENT

The mean nitrate ( $\text{NO}_3^-$ ) content in the control soil samples was 51.07, 58.53, 66.27, and 99.73 mg nitrate/kg dry weight soil, on day 0, 7, 14, and 28, respectively.

The mean nitrate ( $\text{NO}_3^-$ ) content at the dose level 1.2 kg/ha or 1.6 mg/kg dry weight soil (1T) was 51.47, 58.27, 66.53, and 99.87 mg nitrate/kg dry weight soil, on day 0, 7, 14, and 28, respectively.

The mean nitrate ( $\text{NO}_3^-$ ) content at the dose level 6.0 kg/ha or 8.0 mg/kg dry weight soil (5T) was 49.87, 58.67, 66.00, and 95.47 mg nitrate/kg dry weight soil, on day 0, 7, 14, and 28, respectively.

**Table Błąd! W dokumencie nie ma tekstu o podanym stylu.-1: Nitrate Content**

Treatment		Nitrate Content (mg/kg dry weight soil)			
		Day			
		0	7	14	28
Control	CR <sub>1</sub>	51.20	58.40	67.60	100.00
	CR <sub>2</sub>	50.00	59.60	66.00	99.20
	CR <sub>3</sub>	52.00	57.60	65.20	100.00
	Mean	51.07	58.53	66.27	99.73
1T	1TR <sub>1</sub>	53.20	57.20	65.60	99.20
	1TR <sub>2</sub>	51.20	58.40	66.40	100.00
	1TR <sub>3</sub>	50.00	59.20	67.60	100.40
	Mean	51.47	58.27	66.53	99.87
5T	5TR <sub>1</sub>	50.00	59.20	64.40	94.00
	5TR <sub>2</sub>	49.60	59.60	66.00	95.20

	5TR <sub>3</sub>	50.00	57.20	67.60	97.20
	<b>Mean</b>	<b>49.87</b>	<b>58.67</b>	<b>66.00</b>	<b>95.47</b>

## B. NITRATE FORMATION

Soils treated with test concentrations of 1.2(1T) and 6.0 (5T) kg test item/ha of soil were assessed for nitrogen transformation for 28 days. The difference of percent deviation of nitrate formation between the test concentration of 1.2 kg test item/ha on 0th, 7th, 14th, 28th day were -0.78%, +0.44%, -0.39% and -0.14%, respectively, whereas at the dose level 6.0 kg/ha or 8.0 mg/kg dry weight soil (5T), it was 2.35%, -0.24%, +0.41%, and +4.27%, respectively.

**Table Błąd! W dokumencie nie ma tekstu o podanym stylu.-2: Nitrate Formation Rate**

Treatment	Nitrate Formation Rate (mg nitrate/kg dry weight soil/day)		
	0-7	0-14	0-28
Control	1.07	1.09	1.74
1T	0.97	1.08	1.73
5T	1.26	1.15	1.63

**Table Błąd! W dokumencie nie ma tekstu o podanym stylu.-3: Percent Deviation of Mean nitrate Content**

Days	Percent Deviation of Mean Nitrate Content	
	Treatment (1T)	Treatment (5T)
0	-0.78	+2.35
7	+0.44	-0.24
14	-0.39	+0.41
28	-0.14	+4.27

## C. VALIDITY CRITERIA

The test was considered valid because the following criteria were met:

**Table Błąd! W dokumencie nie ma tekstu o podanym stylu.-4: Study validity criteria**

Parameter	Required	Observed
%RSD between control replicates on day 28	≤ 15%	0.46%

RSD – Relative standard deviation

## D. STATISTICAL ANALYSIS

Statistically, no significant difference in the mean nitrate content of treatment (1T) was observed on day 0, 7, 14, and 28 when compared with that of the control.

Statistically, no significant difference in the mean nitrate content of treatment (5T) was observed on day 0, 7, 14, and 28 when compared with that of the control.

### III. CONCLUSION

Results of this study revealed that the test item, Boscalid 500 WG, does not have any long-term influence on nitrogen transformation in soil, at its maximum application rate per season (1T), i.e., 1.2 kg/ha or 1.6 mg/kg dry weight soil and at its 5 times of the maximum application rate (5T), i.e., 6.0 kg/ha or 8.0 mg/kg dry weight soil.

#### A 2.5 KCP 10.6 Effects on terrestrial non-target higher plants

##### A 2.5.1 KCP 10.6.1 Summary of screening data

##### A 2.5.2 KCP 10.6.2 Testing on non-target plants

##### A 2.5.2.1 Stürtz, 2022a (KCP 10.6.2/01)

<b>ZRMS comments:</b>	<p>Test item: Boscalid 500 g/Kg WG, GPF516/FGG01.</p> <p>The study was conducted according to OECD Test Guideline 227 with minor deviation from this guideline described and assessed by Study Director.</p> <p>during the cultivation period the humidity was 40% for 2.48 hours due to failure of humidifier.</p> <p>zRMS believes that aforementioned deviation does not affect the outcome of the study result and on the possibility of using in the risk assessment.</p> <p>The study was performed in line with GLP principles.</p> <p>The validity criteria from OECD 227 were met.</p> <p>The study is valid.</p> <p>The plants of two monocot species (<i>Avena sativa</i> and <i>Allium cepa</i>) and four dicot species (<i>Brassica oleracea</i>, <i>Daucus carota</i>, <i>Glycine max</i>, and <i>Lactuca sativa</i>) were tested.</p> <p>The study is acceptable for risk assessment.</p> <p>The test item was dosed in at an amount: 1.00 kg a.i./ha (corresponding to 2.00 kg FGG01/ha).</p> <p>The NOER were 1.00 kg a.i./ha and the LOER was &gt; 1.00 kg a.i./ha for all species in the tested parameters of fresh weight, mortality, phytotoxicity and growth rate.</p>
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Reference: KCP 10.6.2/01

Report: Boscalid 500 WG: Effects on Terrestrial (Non-Target) Plants: Vegetative Vigour Test, Stürtz, S., 2022a, Study code 165091087

EFSA Study Identification: EFSA-2022-00009316

Guideline(s): OECD Guidelines for the Testing of Chemicals, Guideline 227, 'Terrestrial Plant Test: Vegetative Vigour Test', adopted on July 19, 2006

SANTE/2020/12830 Rev.1 Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes, February 24, 2021

Deviations: During the cultivation period the humidity was 40% for 2.48 hours due to failure of humidifier. No presumed effect on the study due to no symptoms observed.

GLP: Yes

Acceptability: Yes

### Executive summary

The study objective was to determine the effects of Boscalid 500 WG (FGG01) on the vegetative vigour of non-target terrestrial plant species. The effects of Boscalid 500 WG on the mortality and fresh weight of six non-target plant species representing six plant families were assessed following a single application of 2.00 kg test item/ha (corresponding to 1.00 kg a.i./ha) on *Brassica napus*, *Daucus carota*, *Glycine max*, *Lactuca sativa*, *Avena sativa* and *Allium cepa*.

The analytical recovery rate in the dosing solution was 99% of nominal concentration of Boscalid in a separate study. The analytical method of recovery was developed in the performing laboratory. The HPLC-UV determination method was implemented.

No statistically significant reduction of the fresh weight was observed for any species tested. No mortality was observed for any species tested. No phytotoxicity effects were observed for any species tested.

All validity criteria were met.

## I. MATERIALS AND METHODS

### A. MATERIALS

- Test material:** Boscalid 500 WG (FGG01)  
**Batch no.:** 48A  
**Purity:** content of a.i. (Boscalid):  $513.55 \pm 1.68$  g/kg ( $51.36 \pm 0.17\%$ )  
**Date of expiry:** 14 September 2023
- Test concentrations:** Control: deionised water  
Test item: 2.00 kg test item/ha (corresponding to 1.00 kg a.i./ha) in spray volume of 100 L/ha, prepared by diluting 20.0 g test item in 1000 mL deionized water

### 3. Test organisms:

**Plant species:** *Brassica oleraceae*, *Daucus carota*, *Glycine max*, *Lactuca sativa*, *Avena sativa* and *Allium cepa*  
**Exposure time:** 21 days  
**Test units:** Commercial plastic flower pots (Ø15 cm), filled with test soil which has been steam sterilized, delivered and analyzed by the LUFA Speyer, Germany (sandy loam, LUFA 2.3, particle size  $\leq 0.2$  cm, with a pH of  $5.9 \pm 0.4$ , and organic content of  $0.64 \pm 0.07\%$ )

### 4. Environmental conditions

**Air temperature:**  $15.6^{\circ}\text{C} - 23.6^{\circ}\text{C}$  (mean temperature  $20.4^{\circ}\text{C}$ )  
**Relative humidity:**  $40.0\% - 89.0\%$  (mean humidity 61%)  
**Photoperiod:** 16/8 h (light/dark)  
**Light intensity:**  $300 - 400 \mu\text{E}/\text{m}^2/\text{s}$  (mean light intensity  $361 \mu\text{E}/\text{m}^2/\text{s}$ )

## B. STUDY DESIGN AND METHODS

1. **Dates of work:** 29 April 2022 – 13 June 2022 (including Biological and Analytical phases)

### 2. Test design and treatment

The plants were grown until they had reached the 2 to 4 true leaf stage prior to dosing. Test rates were calculated for a water amount of 100 L/ha and were administered onto the plants using laboratory spraying equipment. At least 20 plants were tested per rate and species. The concentration of the active ingredient in the dosing solution was verified analytical. The exposure time was 21 days. The application was done using laboratory spraying equipment and in an accurate way with reproducible coverage.

### 3. Dose preparation

The spray mixture was prepared by diluting 20.0 g of Boscalid 500 WG to 1000 mL using deionised water (20.0 g test item/L corresponding to 2.00 kg test item/ha in 100 L/ha, corresponding to 1.00 kg a.i./ha in 100 L/ha). This dosing solution was checked analytically using HPLC-UV-method.

### 4. Measurements and observations

The plants of two monocot species (*Avena sativa* and *Allium cepa*) and four dicot species (*Brassica oleracea*, *Daucus carota*, *Glycine max*, and *Lactuca sativa*) were tested. Visual phytotoxicity observations and mortality counts were conducted 7 and 14 and 21 days after application. Mortality was considered if no living tissue could be found on the leaves or shoots. Growth stages were recorded according to BBCH-Monograph, at the application day and 21 days after application. The fresh weight of the above ground part of all survived plants of a pot (each pot was considered as a replicate) was determined 21 days after application.

### 5. Analytical verification

The analytical recovery rate of the active ingredient Boscalid in the dosing solution was 99% of the nominal value. In the control solution no test item ingredient was detected. For details see Appendix II: Analytical Phase.

### 6. Statistics

Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ( $\alpha = 0.01$ ) and the Levene's test ( $\alpha = 0.01$ ). The data were normally distributed and homogeneous and so the Student t-test (two-sample comparison, one-sided smaller,  $\alpha = 0.05$ ) was used.

No mortality was observed, so no statistical analysis was performed.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ToxRat Solutions GmbH.



## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL RESULTS

Mortality: 2.00 kg Boscalid 500 WG (FGG01)/ha showed no significant effect on the survivorship of the tested species.

Phytotoxicity: Test item Boscalid 500 WG (FGG01) showed none phytotoxicity symptoms for any of the tested species.

Growth stage: No differences in growth stage could be detected between the test item groups and the controls for the six tested species at any of the rates tested.

Fresh Weight: Test item Boscalid 500 WG (FGG01) showed no significant reduction of the fresh weight of the tested species.

### B. ANALYTICAL RESULTS

The concentration of the active ingredient Boscalid in the dosing solution was verified analytically using HPLC-UV-method and the analytical recovery rate in the dosing solution was 99% of the nominal value.

### C. VALIDITY CRITERIA

The study was considered valid for all species; since the following criteria were met:

**Table A 2.5.2.1-1: Study validity criteria**

Parameter	Required	Observed
Emergence in the control	≥ 70.0%	81% to 100%
Mean survival of the control plants	≥ 90.0%	100%

Moreover, no phytotoxic effects were detected in the control plants and the environmental conditions for a particular species were identical.

### D. TOXICITY ENDPOINTS

The NOER for all species were 1.00 kg a.i./ha. The LOER was > 1.00 kg a.i./ha for all species in the test parameters of fresh weight, mortality, phytotoxicity and growth rate.

**Table A 2.5.2.1-2: Toxicity endpoints**

Test species	NOER (kg a.i./ha)	LOER (kg a.i./ha)
<i>Brassica oleracea</i>	1.00	> 1.00
<i>Daucus carota</i>	1.00	> 1.00
<i>Glycine max</i>	1.00	> 1.00
<i>Lactuca sativa</i>	1.00	> 1.00
<i>Allium cepa</i>	1.00	> 1.00
<i>Avena sativa</i>	1.00	> 1.00

## III. CONCLUSION

Boscalid 500 WG was tested for effects on vegetative vigour using six plant species out of six different plant families.

The analytical recovery rate of the active ingredient Boscalid in the dosing solution was 99% of the nominal value.

No statistically significant reduction of the fresh weight was observed for any species tested.

No mortality was observed for any species tested.

No phytotoxicity effects were observed for any species tested.

The validity criteria was met.

#### A 2.5.2.2 Stürtz, 2022a (KCP 10.6.2/02)

<b>zRMS comments:</b>	<p>Test item: Boscalid 500 g/Kg WG, GPF516/FGG01.</p> <p>The study was conducted according to OECD Test Guideline 208.</p> <p>Short-term deviations (<math>\leq 2</math> hours) from the recommended ranges are partly unavoidable after activating/deactivating the lights in the growth chamber (starting phase and ending phase).</p> <p>zRMS believes that aforementioned deviation does not affect the outcome of the study result and on the possibility of using in the risk assessment.</p> <p>The validity criteria from OECD 208 were met.</p> <p>The study is valid.</p> <p>The study is acceptable for risk assessment.</p> <p>The (non-target) plants of two monocot species (<i>Avena sativa</i> and <i>Allium cepa</i>) and four dicot species (<i>Brassica oleracea</i>, <i>Daucus carota</i>, <i>Glycine max</i>, and <i>Lactuca sativa</i>) were tested.</p> <p>The NOER for all species were 1.00 kg a.i./ha. The LOER was <math>&gt; 1.00</math> kg a.i./ha for all species in the test parameters of fresh weight, mortality, phytotoxicity and growth rate.</p>
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Reference: KCP 10.6.2/02

Report: Boscalid 500 WG: Effects on Terrestrial (Non-Target) Plants: Seedling Emergence and Seedling Growth, Stürtz, S., 2022a, Study code 165091086

EFSA Study Identification: EFSA-2022-00009314

Guideline(s): OECD Guidelines for the Testing of Chemicals, Guideline 208: 'Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test', adopted on July 2006.

SANTE/2020/12830, Rev.1 (24 February 2021). Guidance Document on Pesticide Analytical Methods for Risk Assessment and Post-approval Control and Monitoring Purposes.

Deviations: No

GLP: Yes

Acceptability: Yes

#### Executive summary

The study objective was to determine the effects of Boscalid 500 WG (FGG01) on early growth of non-target terrestrial plant species. At least 20 seedlings of six different plant species were with Boscalid 500 WG (FGG01) at application rates of 1.00 kg a.i./ha in 100 L/ha spray volume. Deionized water was used for the control treatment. The plants were assessed for mortality and phytotoxicity symptoms 7, 14 and 21 days after 50% seedling emergence in the respective control. Growth stages were assessed at 14 or 21 days after 50% seedling emergence in the respective control. Fresh weight was assessed 14 or 21 days after 50% seedling emergence in the respective control.

Correct rate preparation was confirmed by analysis of the stock solution with recoveries of 99% active ingredients of the nominal value.

No statistically significant reduction of the fresh weight and of the emergence rate was observed for any species tested.

No mortality was observed for any species tested with the test item.

No phytotoxic effects were observed for any species tested.

All validity criteria were met.

The NOER was determined to be 1.00 kg a.i./ha and LOER was determined to be >1.00 kg a.i./ha for all tested species. No ER<sub>50</sub> was reported in this study.

## I. MATERIALS AND METHODS

### A. MATERIALS

1. **Test material:** Boscalid500 WG (FGG01)  
**Batch no.:** 48A  
**Purity:** content of a.i. (Boscalid):  $513.55 \pm 1.68$  g/kg ( $51.36 \pm 0.17\%$  w/w)  
**Date of expiry:** 14 September 2023
2. **Test concentrations:** Control: deionized water  
Test item: 2.00 kg test item/ha (corresponding to 1.00 kg a.i./ha) in 100 L water/ha spray volume
3. **Test organisms:**  
**Plant species:** *Brassica oleraceae*, *Daucus carota*, *Glycine max*, *Lactuca sativa*, *Avena sativa* and *Allium cepa*  
**Exposure time:** 14 or 21 days after 50% emergence in the respective control depending on the growth of the seedling  
**Test units:** Commercial plastic flower pots (Ø15 cm), filled with steam sterilized soil (LUFA 2.3, sandy loam), particle size  $\leq 0.2$  cm, with a pH of  $5.9 \pm 0.4$ , and organic content of  $0.64 \pm 0.07\%$
4. **Environmental conditions:**  
**Air temperature:**  $15.8 - 23.6^{\circ}\text{C}$  (mean temperature  $20.5^{\circ}\text{C}$ )  
**Relative humidity:**  $54.0 - 89.0\%$  (mean humidity  $64.0\%$ )  
**Photoperiod:** 16/8 h (light/dark)  
**Light intensity:**  $300 - 400 \mu\text{E}/\text{m}^2/\text{s}$  (mean light intensity  $343 \mu\text{E}/\text{m}^2/\text{s}$ )

### B. STUDY DESIGN AND METHODS

1. **Dates of work:** 29 April – 13 June 2022 (including Biological and Analytical phases)
2. **Test design and treatment**

The seeds were introduced manually into the soil. After sowing the pots were placed on saucers and watered. 4 to 10 pots per treatment group were sown one day before application. At least 20 seeds were tested per treatment group. Each pot represented one replicate. For a given test species, all uncoated seeds used in the test were from the same source and lot number.

On the day after sowing one rate of the test item was sprayed in 100 L/ha of deionised water onto the soil. At least 20 seeds were tested per rate and species. The exposure time was 14 or 21 days after 50% emergence in the respective control depending on the growth of the seedlings. The concentration of the active ingredient in the dosing solution was verified analytically.

#### 3. Dose preparation

The spray mixture was prepared by diluting 20.0 g of Boscalid 500 WG to 1000 mL using deionised water (corresponding to 1.00 kg a.i./ha in 100 L/ha). This dosing solution was checked analytically using HPLC-UV-method.

#### 4. Measurements and observations

Fresh weight, emergence and mortality of the plants treated with the test item were compared with the results of the control plants.

The fresh weight of the above ground part of all survived plants of a pot (each pot is considered as a replicate) was determined 14 or 21 days after 50% seedling emergence in the respective control. Emergence was checked daily on weekdays (except weekends) until 50% of the respective control plants had emerged. Further checks were done weekly. The number of living and dead plants was recorded 7 and 14 days or 7, 14 and 21 days after 50% seedling emergence in the respective control. A plant was considered dead if no living tissue could be found on the leaves or shoots. All other plants were considered living. Visual phytotoxicity (e.g. chlorosis, necrosis, deformation) was recorded weekly according to EPPO Standard PP 1/135 (4) after 50% seedling emergence in the respective control. Growth stages at day 14 or 21 after 50% seedling emergence in the respective control were reported according to BBCH-Monograph - Growth stages.

#### 5. Analytical verification

The stock solution was checked analytically using HPLC-UV-method.

#### 6. Statistics

Fresh weight data were tested for normal distribution and homogeneity of variance using the Shapiro-Wilk's test ( $\alpha = 0.01$ ) and the Levene's test ( $\alpha = 0.01$ ). The data were normally distributed and homogeneous and so the Student t-test (two-sample comparison, one-sided smaller,  $\alpha = 0.05$ ) was used.

For the mortality and emergence data Fisher's Exact Binomial Test (two-sample comparison, one-sided greater,  $\alpha = 0.05$ ) was used.

The software used to perform the statistical analysis was ToxRat Professional, Version 3.3.0, ® ToxRat Solutions GmbH.

## II. RESULTS AND DISCUSSION

### A. BIOLOGICAL RESULTS

No statistically significant reduction of the fresh weight and of the emergence rate was observed for any species tested.

No mortality was observed for any species tested with the test item.

No phytotoxic effects were observed for any species tested.

### B. ANALYTICAL RESULTS

The concentration of the active ingredient Boscalid in the dosing solution was verified analytically using HPLC-UV-method and the analytical recovery rate in the stock solution was 99% of the nominal value.

### C. VALIDITY CRITERIA

The study was considered valid for all species, since the following criteria were met:

**Table A 2.5.2.2-1: Study validity criteria**

Parameter	Required	Observed
Emergence in the control	$\geq 70.0\%$	80% to 95%
Mean survival of the control plants	$\geq 90.0\%$	94% to 100%

Moreover, no phytotoxic effects were detected in the control plants and the cultivation conditions for a particular species were identical.

### D. TOXICITY ENDPOINTS

The NOER was 1.00 kg a.i./ha for all test species and the LOER was >1.00 kg a.i./ha for all test species.

**Table A 2.5.2.2-2: Toxicity endpoints**

Test species	NOER (kg a.i./ha)	LOER (kg a.i./ha)
<i>Brassica napus</i>	1.00	> 1.00
<i>Daucus carota</i>	1.00	> 1.00
<i>Glycine max</i>	1.00	> 1.00
<i>Lactuca sativa</i>	1.00	> 1.00
<i>Allium cepa</i>	1.00	> 1.00
<i>Avena sativa</i>	1.00	> 1.00

### III. CONCLUSION

Boscalid 500 WG was tested for effects on seedling emergence and seedling growth of six plant species out of six different plant families.

The analytical recovery rate of the active ingredient Boscalid in the dosing solution was 99% of the nominal value.

No statistically significant reduction of the fresh weight and of the emergence rate was observed for any species tested.

No mortality was observed for any species tested with the test item.

No phytotoxic effects were observed for any species tested.

All validity criteria were met.

**A 2.5.3            KCP 10.6.3            Extended laboratory studies on non-target plants**

**A 2.6                KCP 10.7   Effects on other terrestrial organisms (flora and fauna)**

**A 2.7                KCP 10.8   Monitoring data**